STUDIES RELATED TO THE ARTIFICIAL SPAWNING AND CULTURE OF THE ABALONE, *Haliotis midae* Linne, 1785

VINCIT VERITAS

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ABSTRACT

The successful aquaculture of the abalone *Haliotis midae* requires a multi-disciplinary approach. Experiments were designed to provide insight into various aspects of abalone biology and seawater system design. A comparative evaluation of a closed and open seawater system for an *H. midae* hatchery was performed. Monthly seawater temperatures and nitrite levels were higher in the closed system. The salinity of the closed system seawater varied more than that of the open system. The pH of seawater in the closed system varied between 7.7 and 8.2. This was lower than the 7.9 to 8.35 pH range of the open system seawater. An open system is thus clearly preferable to a closed system.

Haliotis midae reproductive condition can be assessed visually by examining the shape and colour of the broodstock gonads. Spawning experiments showed that abalone should be starved for at least 24 hours prior to spawning induction. *Haliotis midae* can be induced to spawn by treatment with hydrogen peroxide when exposed to seawater at a pH of between 9.0 and 9.9 (males and females) Spawning can reliably be induced by using final hydrogen peroxide concentrations in the range of 7 to 25 mM for male and female *H. midae*. Gravid broodstock should be exposed to hydrogen peroxide for 100 to 300 minutes during spawning induction. There was no significant impact observed for prior conditioning of the brood-stock to the spawning tanks, lunar phase and timing of spawning induction relative to sunset.

Haliotis midae larval toxicity to chlorine, copper and ammonia was investigated. The LT_{50} to a chlorine concentration of 0.06 mg/l was 170 minutes. For copper the LT_{50} of larvae exposed to 0.12 mg/l was 53 minutes. The LT_{50} of ammonia at 5 mg/l was 600 minutes.

A brief synopsis of the major findings is presented in the last chapter. Some discussion on the future prospects of the abalone industry is also provided.

CHAPTER 1: INTRODUCTION

Abalone are amongst the world's most sought-after seafood culinary luxuries. These delicacies are traditionally associated with elite restaurants and hotels in Japan and other Asian countries. In Japan affluent hosts serve abalone on certain public holidays and at weddings (Watanabe, pers. comm.). The Taiwanese are also very partial to abalone and believe that consumption of abalone meat keeps their eyes bright (Lin, pers. comm.).

Abalone are marine herbivorous gastropods belonging to the genus *Haliotis* (Hahn, 1989). There are approximately 100 species distributed in shallow coastal waters throughout the world. The 10 largest abalone species are found predominantly in temperate waters, and form the target species of abalone fisheries in Japan, China, South Africa, New Zealand, Southern Australia, Mexico and the Pacific Coast of the United States of America (Bardach, *et al.*, 1972).

The large scale global exploitation of wild abalone stocks only began in the early 1950's. The bulk of the world's abalone harvest is exported to South East Asia. This catch reached a peak of 28 000 metric tons per annum in 1968 (Rudd, un publ.). By 1991 the world supply was reduced to 14 000 metric tons primarily as a result of the over

exploitation of abalone fisheries (Rudd, 1994). The major consumer countries, comprising Japan, China and other Southeast Asian countries, consume over 80 % of the world's abalone catch (Oakes & Boswell, 1994).

During prehistoric times in South Africa, Strandlopers (coastal bushmen) harvested the abalone *Haliotis midae* for food (Avery, 1974). Records of commercial harvesting of the South African abalone are only available from the early 1950's. The whole mass abalone harvest increased from 770 tons in 1953, to 1500 tons in 1954, and finally, to a record 2800 tons in 1965 (Tarr, 1989). The annual tonnage of abalone appears to have stabilised at a whole mass harvest of 640 metric tons (Tarr, 1989).

The report of Tarr (1983) implies that the fishery, at this 600 metric ton exploitation level, is not being over-fished. There are concerns however that the increasing recreational fishery combined with commercial harvesting will eventually lead to the decline of the wild abalone stock. The economic recession and the high prices that abalone reach on the black market will further threaten the continued stability of the *H. midae* resource. It should be emphasised that in terms of monitory gain per unit mass abalone is South Africa's and the world's most valuable seafood product (Wray, 1994). Exported abalone earns crucial foreign exchange for South Africa.

The ever increasing demand for abalone has unfortunately led to over-exploitation and gradual depletion of many of the wild stocks (Hooker & Morse, 1985). The natural stock depletion has increased the market value of abalone (see Table 1.1). This scenario has focused interest in the potential of abalone farming by aquaculturists, both overseas and in South Africa. The North American abalone aquaculture industry on America's West Coast have been producing abalone for the last three decades. The aquaculture of *H. midae* in South Africa is presently enjoying a great deal of attention by both the research community and the private sector.

TABLE 1.1: Live abalone prices at the Tsukiji Fish Market in Japan 14 may 1992

Description	Species	Size length (mm)	Price in $/kg
Farmed abalone	*H. rubra*	70-80	54.08
	H. gigantea	70-80	62.03
	H. discus hannai	>100	79.52 - 95.43
	H. diversicolor	40-60	38.17 - 43.74
	H. diversicolor	50-60	59.64
Wild harvested abalone	*H. discus hannai*	>100	99.40 -107.36

It should be noted that the prices shown in Table 1.1 are prices paid by consumers. There is a complicated marketing network involving various agents before the product reaches the market. The highest prices paid for abalone in Japan are for the local Japanese species (*H. discus hannai*). It would

appear that the resemblance of a foreign specie to *H. discus hannai* with regard to colour shape and texture determines the market price (Watanabe, pers. comm.). An abalone farmer exporting live abalone from South Africa could expect a price of between 25 and 35 US $/kg for live cocktail abalone.

Aquaculture is a term that includes all activities relating to the production of aquatic organisms from fresh, brackish and salt waters (Barnabe, 1990). Aquaculture has also been more narrowly defined by Hecht (1988) as: "the production of protein for human consumption in an aquatic environment under controlled or semi-controlled conditions". This last definition epitomises the goal of western aquaculture development in the early 1960's (Bardach *et al.*, 1972).

New intensive aquaculture projects are fraught with technical and financial constraints. Intensive aquaculture is likely to succeed only if a high priced product can be produced in sufficient quantities to offset the high capital input costs. The development capital of 25 million US dollars for the Abalone Farm at Cayucos provides an extreme example of high developmental costs (Wray, 1989). Abalone is an ideal species for aquaculture from a product value point of view.

The fishing industry in South Africa is a relatively mature industry with limited opportunities for expansion. Many of the existing fisheries are presently being harvested at their maximum sustainable level (Tarr, 1989; Pollock, 1989; Armstrong & Thomas, 1989; Payne, 1989). With this background, I was employed in February 1990 by Southern Sea Fishing, a division of the Premier Group, to identify suitable marine species for an aquaculture program. The idea was to direct future expansion into an intensive controlled aquaculture operation.

The South African abalone *H. midae* was identified as the most suitable marine species for aquaculture in the Western Cape for the following reasons:

1. *H. midae* is endemic to rocky reefs along the Western and Eastern Cape (see Fig. 1.1). There is at present a monitorium on importing foreign marine species into South Africa. Therefore *H. midae* is the only abalone species available for farming in South Africa. The other abalone species endemic to South Africa are relatively small and are not harvested commercially.

2. Wild harvested *H. midae* is accepted in traditional Asian markets and fetch prices in excess of US $ 30/kg. There are also high prices paid for cocktail (cultured) abalone: up to US $ 35/kg (80 to 100 mm in length).

3. There is an abundant supply of the kelp *Ecklonia maxima* along the South West Coast which can be harvested to supply the abalone with food.

4. Abalone have been successfully cultivated overseas in pump ashore flow-through seawater systems. The South African coastline is very exposed to rough seas and there are only a few protected areas where aquaculture in the sea would be viable, but shore-based aquaculture is feasible. A further advantage is that the seawater along the Cape coast is relatively unpolluted.

5. There is a surplus of unskilled labour along the Cape coast. The creation of jobs in these areas will certainly help uplift the local economies.

Important lessons can be learned from existing abalone farms situated in many parts of the world. Some of the areas where lessons could be learned would include broodstock conditioning, spawning techniques, laraval rearing and settlement, nutritional aspects, ongrowing systems and seawater system designs. Consulting with these farms would help reduce the research and development phase and associated costs. Husbandry techniques for California and Japanese abalone species have been refined over the last few

decades. Many 'bottlenecks', however, are still hampering the development of the South African abalone culture industry. Some of these problematic areas are the development of a reliable methodology for artificially inducing *Haliotis midae* to spawn, inducing larval settlement and reducing high post- settlement mortalities. Solutions to these problems will certainly improve the chances of a commercial *H. midae* farm succeeding.

Ino (1952) identified four bottle-neck areas associated with an abalone cultivation program. They are the following:

1. the lack of prior conditioning of broodstock;
2. the absence of a fertilisation technique which ensures optimal numbers of viable zygotes;
3. inability to supply adequate nutrition to the settling larvae and juveniles;
4. the lack of control over settling larvae.

The introduction of a foreign abalone species with an established culture technology is not an option at present. The introduction of a new marine species is fraught with risks which the Department of Environmental Affairs is not prepared to take.

A reliable supply of juvenile abalone seed is a primary requirement of a new abalone farm. Due to strict legislation, the only viable method of obtaining juvenile *H.*

midae required for a farming operation, is to develop an artificial breeding program. Another possibility is to buy juvenile abalone from an existing *H. midae* hatchery. This option was not available in 1990.

Carter (1991) emphasised that control of the spawning response is the most critical aspect of developing abalone culture techniques.

The main objectives of this study were:

1. To test and develop an appropriate seawater holding system for an *H. midae* hatchery.
2. To investigate a suitable method to condition and asses the gonad reproductive state of *H. midae* broodstock.
3. To develop a suitable spawning protocol to reliably induce *H. midae* to spawn.
4. Investigate the toxicity of copper, chlorine and ammonia to *H. midae* larvae.

Achieving these goals has contributed towards providing a reliable supply of juvenile abalone for the planned commercial abalone farm with which I was involved at Gansbaai (refer to Fig. 1 for location map).

The work presented here is partitioned into the following chapters:

An introduction into aspects of the reproductive and larval biology of the genus *Haliotis* is presented in chapter two. A preliminary experiment using dissection to obtain *H. midae* gametes has also been included in this chapter. The design and a comparative discussion of the two seawater systems utilised during this study are described in chapter three. In chapter four the conditioning and gonad assessment of *H. midae* is presented. Chapter five provides the results and conclusions of artificial spawning induction experiments for the abalone *H. midae*. The results of *H. midae* larval toxicity experiments are presented in chapter six. A summary of the important findings of this study are included in chapter seven.

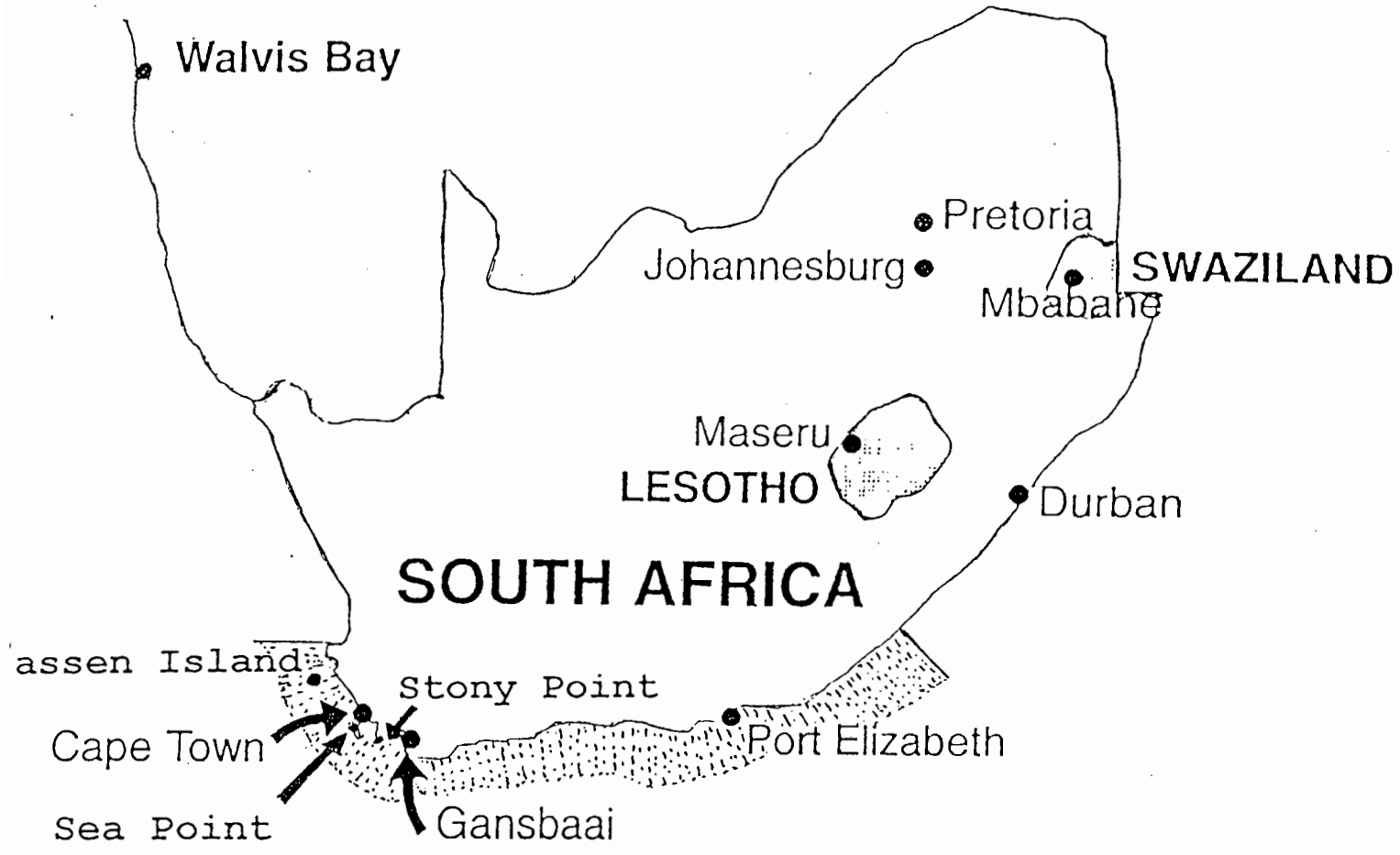


Fig. 1.1 Site locality map

Shaded area represents the distribution of *Haliotis midae*

CHAPTER TWO: REPRODUCTIVE AND LARVAL BIOLOGY OF HALIOTIDS

2.1: Introduction

Many good livestock farmers attribute their success to a thorough understanding of their animal's biology. It would therefore be especially important to review the biology of any new species identified for farming. With this philosophy in mind, an attempt has been made to present an overview of reproductive and larval biology of the genus *Haliotis*. The inclusion of larval and post-larval development is intended to compliment the work presented in chapter 6 dealing with *H. midae* larval susceptibility to common pollutants.

Abalone are sedentary, herbivorous marine gastropods with all species presently being assigned to the genus *Haliotis* (Hahn, 1989; Cropp, 1989). There are at present six known species of South African haliotids, namely: *Haliotis midae* Linnaeus, 1758; *H. quekeltei* Smith, 1910; *H. parva* Linnaeus, 1758; *H. speciosa* Reeve, 1846; *H. spadicea* Donovan, 1808 and *H. pastulata* Krauss, 1848 (Kilburn & Rippey, 1982). Of the six species only *H. midae* is harvested on a commercial scale.

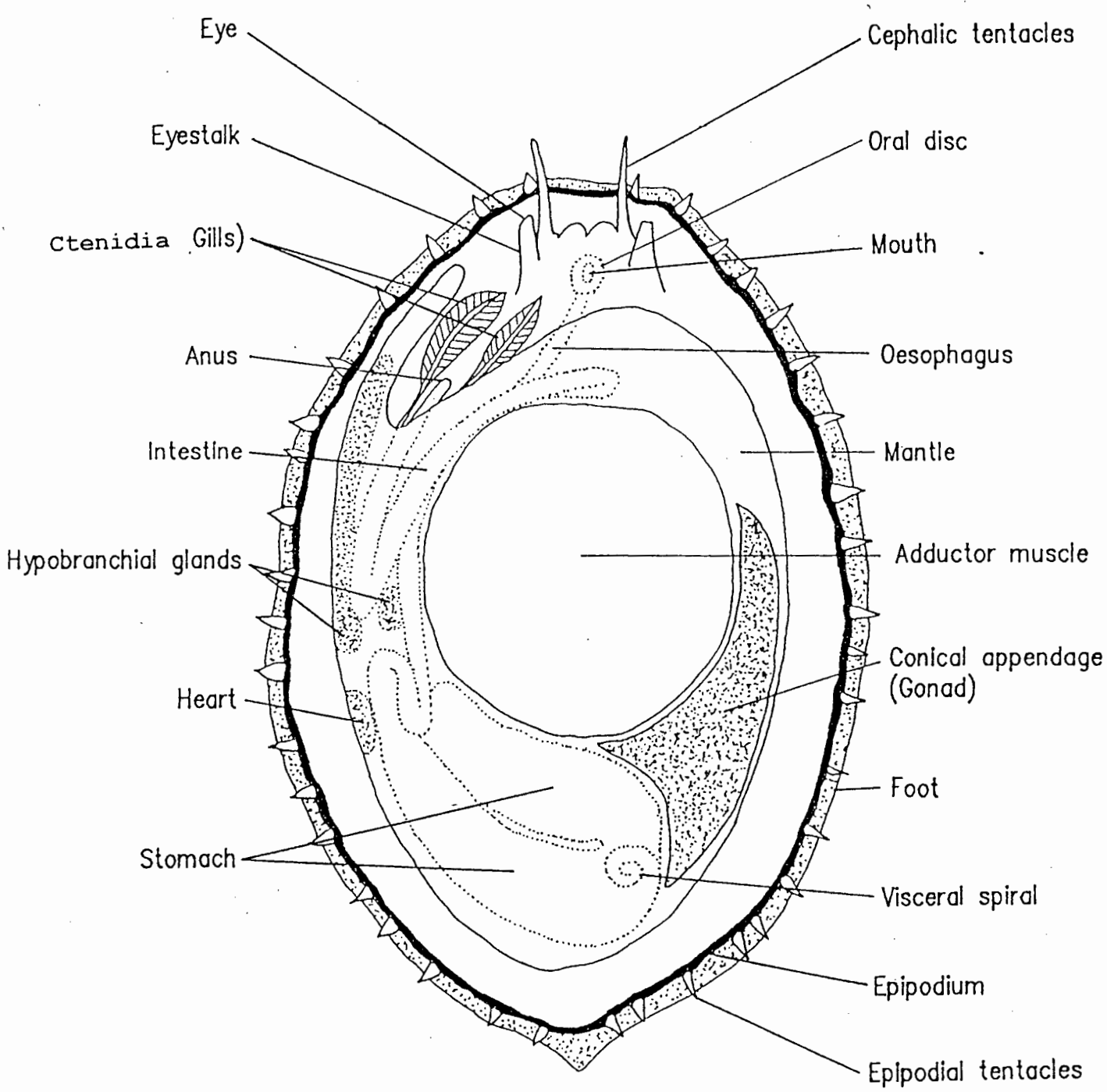


Fig. 2.1 The anatomy of a Haliotid showing the dorsal surface without the shell. After Martin *et al.*, (1983) and Fallu, (1991).

Haliotis midae is dioecious (sexes are separate), with the gonad surrounding the hepatic gland in both sexes (Newman, 1967). Together the liver and gonad form a large cone-shaped appendage which is situated around the right posterior margin of the abductor muscle (Martin, *et al.*, 1983). The gonad is cream coloured in males and green in females (Boolootian, *et al.*, 1962). The "reproductive gland" or gonad is often referred to as the conical appendage. The colour of the gonad is the only reliable way of distinguishing externally between the two sexes of *H. midae*. Haliotids are broadcast spawners where gametes are released into the environment resulting in external fertilisation (Weber & Giese, 1969).

Due to the high fecundity of haliotids, the gonads can constitute a major proportion of the soft body mass during the breeding season. Mature gonads of *H. cracheroidii*, for example, constitute between 15 and 20% of the total soft body mass (Webber & Giese, 1969). Fig. 2.1 shows the internal organs of an abalone, where the gonad's position, relative to the other organs, can be clearly seen.

The reproductive cycle is defined by Hahn (1989) as the interval between successive spawnings. There are four general phases during the reproductive cycle of a gastropod. They are: activation of gametogenesis, gametogenesis, enlargement of the gonad as the result of an increase in

the number or size of gametes and, finally, spawning followed by a resting period (Giese, 1959; Boolootian, 1966). Hahn (1989) emphasises that a distinction should be made between gametogenesis and gonad maturation. He defines gametogenesis as the developmental process of an individual gamete and gonad maturation as the developmental stages of the gonad during the reproductive cycle.

Newman (1967) noted no resorption of gametes after *H. midae* spawning and assumed that gametes that have not been spawned will be released at the next spawning. Tutschulte & Connell, (1981) found no evidence to support this hypothesis after examining *H. sorenseni* histologically. Hayashi (1982) noted that certain *Haliotis* species do not spawn all their gametes at one spawning. Tutschulte & Connell (1981), found for *H. sorenseni* that each annual reproductive cycle produces a new batch of oocytes that mature and are spawned within the same year. *H. rufescens* requires 120 days to complete a reproductive cycle in its natural environment (Ault, 1985).

Tomita (1967) has classified the development of abalone eggs into the following seven stages: (1) oogonium stage; (2) chromatin-nucleus stage; (3) yolkless stage; (4) oil drop stage; (5) primary yolk globule stage; (6) secondary yolk globule stage and (7) the mature stage.

Studying the maturation of the *H. discus hannai* testis, Tomita (1968) also classified spermatogenesis into the following three stages: (1) spermatogonium stage; (2) spermatocyte stage and (3) spermatid stage. Each reproductive stage takes a fixed length of time and, according to Hahn (1989), is controlled by different environmental or physiological factors. Tomita (1967) and Tomita (1968) divided the maturation of the testis and ovaries into the following five stages: (1) spent stage (2) recovery stage (3) pre-mature stage (4) mature stage and finally (5) spawning stage.

The anatomy of *H. rufescens* ovary is thoroughly described by Martin *et al.* (1983). It is apt, however, for this study on *H. midae* to summarise their findings. The ovary forms a series of chambers known as trabeculae that lie between the ovarian wall and the wall of the digestive gland. The outer gonad wall consists of an epidermis which is a simple glandular epithelium (Young & De Martini, 1970). Martin *et al.* (1983) further observed that the ovarian wall consists of smooth muscle cells and bundles of collagen fibrils. The three main stages of oocytes, namely presynthetic, synthetic and early postsynthetic oocytes, are enveloped in a single layer of follicle cells which attach the oocytes to the trabeculae. The follicle cells comprise bundles of microfilaments. Mature oocytes are found free in the trabecular chambers without the follicle cell layer. A

mature oocyte is surrounded by a vitelline layer, a chorion and a thick jelly coat.

The genital products of both sexes pass from the right nephropore via the cavity of the definitive right renal organ through the respiratory pores (Crofts, 1937; Newman, 1967). After activation of the spawning stimulus in female haliotids, maturation of the oocyte proceeds, with release occurring during the first meiotic reduction division (Uki & Kikuchi, 1984). The oocyte is then liberated from the trabecule into the ovarian lumen and passes to the vestibule via the right reno-pericardial canal (Uki & Kikuchi, 1984). During the elapsed time (at least 1 hour in haliotids) neurosecretery substances (Uki & Kikuchi, 1984) and prostaglandins (Morse *et al.*, 1977) effect gonadal maturation and spawning. These findings highlight the obvious limitations of gamete stripping by dissection for the purpose of obtaining viable gametes.

2.3: Gamete stripping

To evaluate the viability of gamete stripping as a source of viable *H. midae* gametes the following preliminary investigation was conducted.

2.3.1: Methods

Sperm was removed by dissection from 4 ripe males and examined under the 40X objective of a compound microscope. A sterile scalpel blade was used to make an incision through the gonad wall. The gonad was gently squeezed to help release the sperm into a 50 ml beaker with 20 ml of 1 micron filtered seawater. This sperm suspension was then used to fertilise the eggs from a spontaneously spawning female *H. midae* and also ova collected by dissection. Likewise ova were removed by dissection from 4 ripe female *H. midae* and were rinsed into separate 1 litre beakers with 1-micron filtered seawater. The ova were left in these beakers for at least one hour prior to adding dissected sperm. Spontaneously spawning males and females were observed in the broodstock tanks in September 1992. Male and female abalone were held in separate tanks. The abalone were still spawning freely when their gametes were collected for use in these experiments.

Fertilisation was initiated within 90 minutes of the release of spontaneously spawned gametes or within 90 minutes of collecting gametes by dissection. The eggs and sperm were examined under the microscope to establish if fertilisation had taken place. The fertilised gametes were gently rinsed and allowed to settle in 1 litre beakers at 16°C. The numbers of swimming larvae were recorded after 18 hours.

2.3.2: Results

The results of these preliminary gamete-stripping experiments are presented in the table below.

Table 2.1 Results of *H. midae* gamete stripping experiments

	Dissected ova (D)		Dissected ova (D)		Natural spawn ova (N)		Natural spawn ova (N)	
Beaker no	1	2	3	4	5	6	7	8
No of ova	224	196	234	170	177	185	164	198
Added N or D sperm	N	D	D	N	N	D	N	D
Observed fertilisa-tion	no	no	no	no	yes	no	yes	no
Larvae count	0	0	0	0	113	0	97	0

note: N = Spontaneous spawned gametes D = Gametes collected by dissection

2.3.3: Discussion and Conclusion

Successful fertilisation only occurred between male and female gametes derived from spontaneously spawning abalone. None of the gametes collected by dissection proved to be viable. The sperm collected from dissected *H. midae* appeared

normal when examined under the microscope, but lacked the vigorous motility of the spontaneously spawned male sperm. The lack of any viable larvae from gamete stripped *H. midae* did not justify the extra input involved with more extensive experimentation. These results are also supported by Tomita's (1968) histological studies on the maturation of *H. discus hannai* ovaries. During his investigation he did not observe ripe eggs in the ovaries which were capable of being fertilised. This implies that the ovaries may require exposure to certain maturation hormones as the eggs travel through the genital ducts during a spawning event.

2.4: Spawning process and environmental aspects

Oba (1964) described the actual spawning process of *H. diversicolor supertexta*, which begins when the abalone's posterior end touches the container and the anterior is raised. The shell is then lifted and the adductor muscle contracts rapidly, resulting in the gametes being released from the third to the sixth respiratory pores. Female abalone emit green ova [about 180 to 270 µm in diameter (Hahn, 1989)], which then sink slowly to the substratum. The males release their spermatozoa [width 1 to 1,5 µm and length 6 µm (Hahn, 1989)] from the respiratory pores in white puffs which then remain in suspension. Fertilisation of the ova by the sperm thus takes place externally in the water column. Fertilisation success of the haliotid gametes

is closely related to the sea-water temperature (Ebert & Hamilton, 1983).

The exact sequence during the natural spawning phase appears to vary among the different species of abalone. Thorson (1950) has listed 36 mollusc species, including the two haliotids, *H. gigantea* and *H. tuberculata*, which adopt the following spawning strategy: Males are first to spawn, which in turn stimulates the females of the same species to release their ova into the suspension of sperm. This strategy would appear to increase the chance of successful fertilisation. *Haliotis iris* however, does not release its gametes simultaneously during natural spawning, which may signify an irregular reproductive cycle (Sainsbury, 1982). Tutschulte & Connell (1981) found that synchrony of spawning varied, from highly synchronised for *H. sorenseni*, to less synchronised for *H. fulgens*, and least synchronised for *H. corrugata*. At this stage the exact strategy adopted by *H. midae* during the natural spawning event is not known.

Boolootian *et al.* (1962) divided the natural spawning habits of molluscs into three broad categories: (1) all-year spawners; (2) winter spawners and (3) summer spawners. Newman (1967) showed that *H. midae* spawns twice a year, the timing of spawning varying between his three study sites along the South African coastline. At Dassen Island, on the West coast, spawning took place in early spring and early

autumn; at Sea Point, also on the West coast, *H. midae* spawned in late winter/early spring; and at Stony Point, on the East coast, spawning took place during late spring/early summer and during autumn. Refer to Fig. 1.1 for the position of the various study sites.

The many species in the genus *Haliotis* have different specific temperature preferences. This is highlighted when one notes the different latitudes and oceans in which they exist (Lindberg, 1992). Even sub-populations of the same species occupy different temperature regimes (Newman, 1969).

Chen (1984) discovered that larger individuals of *H. diversicolor* had a higher optimal temperature range than did smaller abalone. An important consequence of elevated sea-water temperatures is the associated increase in abalone oxygen consumption (Sagara & Araki, 1971). This has important implications for any potential abalone culture operations.

Traditional studies on marine invertebrates emphasise the critical importance of sea-water temperatures in regulating and influencing reproductive cycles and spawning (Thorson, 1950). The importance of the influence of temperature in haliotid reproduction appears to vary among the various species.

For *H. cracheroidii*, maximal gonadal growth occurs during summer months (Webber & Giese, 1969). These authors found, however, that gonad size and initiation of gametogenesis showed no apparent relation to changes in seasonal water temperature. They reason that for broadcast spawners (including *Haliotis* species) environmental factors do not act exogenously but instead activate an internal (endogenous) control system which does not require further environmental input. Webber & Giese (1969) found that gametogenesis for *H. cracheroidii* is initiated independently of water temperature. Completion of gametogenesis only resulted in spawning when a specific temperature threshold for spawning had been reached.

Newman (1967) found that for *H. midae* reproductive changes relate closely with sea temperature changes. He concluded that the relatively low temperatures on the West coast do not rise sufficiently to promote the intense spawning which occurs at Stony Point, on the Southeast coast, where annual temperature fluctuations are large. *Haliotis diversicolour* can be artificially induced to spawn by fluctuating the sea-water temperature 3°C above and below 25°C (Oba, 1964).

Pearse (1978) found that for *H. pastulata* in the northern Red Sea, where environmental fluctuations are moderate, spawning occurs in winter. For *H. pastulata* in the tropics,

gametogenesis begins in spring during the period of increasing air and sea temperatures and spawning begins in summer. This example complements the findings of Newman (1967), which show that even among the same species, abalone can have different spawning seasons at different geographic locations. The same scenario for *H. ruber* was observed by Sherpherd & Laws, (1974).

Another example to highlight the differences between species, is that of *H. kamtschatkana*, for which spawning occurs naturally when the sea-water temperature is 9°C, with maximum growth taking place during July and August when the surface sea-water temperatures are 13 to 14°C (Paul & Paul, 1981). Underwood (1979) suggested that synchrony of spawning for cold water *Haliotis* species is associated with warmer temperatures. He reasoned that this minimises the time the lecithotrophic larvae spend in the plankton, because warmer sea-water temperatures result in accelerated larval development. Uki & Kikuchi (1984) concluded that temperature is the principle environmental factor regulating the reproductive cycle of *H. discus hannai*.

Newman (1969) found that shell length at sexual maturity varied for *H. midae* depending on the study site, implying that it was related to the relative temperature regime. The same observation was made by Prince *et al.* (1988), studying *H. rubra*. Newman (1969) showed that the percentage sexual

maturity of 75 g (shucked) body mass *H. midae* was only 20% on the colder West Coast as opposed to 96% for animals at Port Elizabeth on the southeast coast where the ambient seawater temperature is warmer.

The fecundity of a female *H. midae* can be estimated by the following equation:

$F = 0{,}000\ 4257\ B^{3{,}787}$ where

F = fecundity in millions of eggs and

B = shell breadth in cm (Newman, 1967).

A female *H. midae* of 10 cm width would therefore have a fecundity of 2,6 million ova. This would appear to be of the same order of magnitude as Giorgi & De Martini's (1977) estimates on *H. rufescens* fecundity, which varied from 619 000 to 12 million oocytes per ovary. They also found that fecundity varied significantly between *H. rufescens* individuals of the same length. The differences in fecundity might be due to variation in gonad condition. Newman (1967) established that there is a linear relationship between fecundity and body mass for *H. midae*. The fecundity of *H. rubra* is also related to size, but there is a poor relationship between age and fecundity (Nash, *et al*, 1994). Of special relevance to this study is the finding that the dietary level of mature abalone directly influences the development of the gonad, and the amount of spawning in response to artificial induction (Uki & Kikuchi, 1982).

These authors found the influence of food level to be greater in females than in males.

Growth is described by Steffens (1989) as the process that results in positive changes in the length, volume or mass of an organism, where the quantity of food ingested exceeds that required for the maintenance of the body. Peck (1983) cited in Culley and Sherman (1985) formulated the following energy budget for a haliotid:

$i = e + Pg + Pr + r + u + m$

where i = ingestion

e = egestion

Pg = somatic growth

Pr = reproductive growth

r = respiration

u = ammonium

m = mucus secreted

This formula mathematically partitions the energy from ingested food into all the vital functions in the body. Culley & Sherman (1985) were able to show from this energy budget that there is an exponential increase in mucus production with an increase in substrate particle size. Barkai & Griffiths (1988) established that 63% of the energy gained from food consumed by *H. midae* is lost as faeces. A further 32% is used for respiration. Barkai & Griffiths (1988) also showed that only 5% of the energy budget is

available for growth and reproduction. They equate this to 13% of the absorbed ration.

The feeding activity of abalone is related to sea-water temperature, the optimum varying between species (Body, 1986; McCormick & Hahn, 1983). Barkai & Griffiths (1987) found that food intake of populations of *H. midae* varied from 8,1% of wet flesh mass per day at 14°C, to 11,4% at a seawater temperature of 19°C. A commercial implication was observed by Leighton, *et al.*, (1981), who noted an improved growth rate for *H. fulgens* in culture water that is warmer than ambient seawater. In fact, this finding appears to generally hold for many abalone species (Oba *et al.*, 1968; Hooker & Morse, 1985; Tong, 1982; Leighton, 1974; Leighton *et al.*, 1981).

2.5: Larval and post larval development

The larval stages of haliotids are considered to be the geographical dispersal phase of their life cycle (Underwood, 1979). McShane, *et al.*, (1988) and Prince, *et al.*, (1988) showed, however, that recruitment of *H. rubra* is closely related to the local abundance of adults on that reef. This has major implications for managing harvesting strategies of natural abalone populations.

Seawater temperature also plays an important role in the early life stages of abalone. Ebert & Hamilton (1983) found fertilisation success of abalone gametes to be dependent on seawater temperature. The timing of hatch-out varies between *Haliotis* species and with seawater temperature. Leighton (1974) concluded from reviewing other studies that there is a species specific optimal (1 to 2 ^{o}C) temperature range for haliotid egg and larval stages. Larval development is terminated at high temperatures and slows down at low temperatures (Underwood, 1979). A sub-optimal temperature would result in a longer planktonic larval phase, increasing the probability of mortality by predation (Underwood, 1979). The rate of metamorphosis is also directly related to the seawater temperature, with warmer water inducing accelerated metamorphosis (Cuthbertson, 1978).

The trocophore larvae (about 0,2 mm) hatch some 24 hours after the egg has been fertilised. At this stage the larvae are able to swim feebly, using their cilia (Hooker & Morse, 1985). The trocophore then develops, approximately 24 hours after hatch-out, into the veliger larval stage (Hooker & Morse, 1985). Larval development is completed within 4 to 10 days, depending on species and water temperature. Haliotid larvae are considered to be lecithotrophic, i.e. they subsist on nutrients in the egg yolk and do not feed (Hooker & Morse, 1985). There is however contrary evidence to show that the larvae are capable of absorbing amino acids from

the surrounding seawater (Jaekle & Manahan, 1989). Haliotid trocophore larvae show strong negative geotaxis and concentrate at the surfaces of artificial rearing containers (Leighton, 1972).

Haliotid larvae undergo torsion during their early stages of veliger development. This process was first described by Crofts (1937) for *Haliotis tuberculata*. Leighton (1974) found torsion to be incomplete in larvae held at sub-optimal temperatures.

The post torsion larvae exhibits cephalic tentacles, stigma, foot, operculum and eye-spot formation prior to settlement (Ino, 1952). Ebert & Houk (1984), working on *H. rufescens*, found that settlement takes place after about six days and can be recognised when the veligers have four branches on their cephalic tentacles and the foot is sufficiently developed to allow locomotion by ciliary action. Moss & Tong (1992) developed a technique of counting the teeth on *H. iris* larvae's radula. They found that *H. iris* larvae with 3 or more teeth would settle but larvae with 8 teeth on the radula had a higher post settlement survival rate. In their natural environment, abalone appear to settle selectively on coralline algae (Saito, 1984; Shepherd & Turner, 1985; McShane *et al.*, 1988; Hooker & Morse, 1985).

Hooker & Morse (1985) reported that the specific crustose red algae which provide the natural cue for haliotid larval settlement are *Lithothaminion* sp., *Lithophyllum* sp. and a non-coralline red alga, *Hildenbradia* sp. Morse, *et al.*, (1979) showed that the molecule GABA (gamma-amino butyric acid) which mimics the effect of compounds derived from corallines, is capable of inducing settlement. Through a series of experiments conducted by Morse *et al.* (1980), it was concluded that contact-dependence was the method larvae used to recognise a suitable red crustose settlement substrate. Their conclusion was supported by 1) an absence of detectable chemotaxis, 2) no release of soluble inducing agent from the red crustose algae and 3) no heterologous settling occurred on adjacent non-algal surfaces. Analyses of the cephalic sensory organ of larval *H. rufescens* by Morse *et al.* (1980) indicated that it may be responsible for the contact-dependent recognition of the suitable crustose red algae settlement substrate.

An increase in the concentration of K^+ (to approximately double normal sea-water concentrations) induces settlement and metamorphosis in larvae of some marine molluscs, including *H. rufescens*. It is argued that the ability of K^+ to affect cell membrane potential is the factor responsible for its effectiveness as a settlement inducer for larvae recruiting to different habitats (Yool, *et al.*, 1986). The mucus secreted from the foot of juvenile or adult abalone

can also induce the settlement of haliotid larvae (Seki & Kan-no, 1977).

The rate of larval development is closely associated with seawater temperature. Larvae develop more rapidly at warmer seawater temperatures, but obviously their development is retarded when the seawater temperature approaches its upper tolerance level (Hahn, 1989). Larvae metamorphose into benthic creeping juveniles once the epipodal tentacles and the snout protrusions have developed (Seki & Kan-no, 1977). Larvae stop crawling once they find a suitable location; they then elevate the dorsal end of the shell so that the cephalic tentacles come into contact with the substrate (Seki, 1980). At 20°C *H. discus hannai* larval metamorphosis takes place in about 30 minutes and they then start to crawl for about eight hours, when feeding starts (Seki, 1980). During this stage a new peristomal shell is formed and 24 hours after metamorphosis the juveniles start feeding on benthic diatoms. Abalone heart beat can be detected 3-4 days after settlement (Morse, *et al.*, 1980). Recently metamorphosed *H. kamtschatkana* were found to ingest diatoms (order Pinnales) of less than 10 µm from two to six days after settlement (Norman-Boudreau, *et al.*, 1986). The first respiratory pore notch forms at about eight weeks in *H. rufescens* (Ebert & Houk, 1984).

CHAPTER 3: A COMPARATIVE DISCUSSION OF A CLOSED AND OPEN SEAWATER SYSTEM FOR A PILOT SCALE ABALONE (Haliotis midae) HATCHERY

3.1: Introduction

A seawater holding system for cultured marine organisms should ideally provide a stress-free environment. The performance of captive animals can be influenced by the type of holding system used. In order to refine culture techniques for the abalone *Haliotis midae*, the suitability of both an open and a closed seawater system was investigated.

Broadly speaking, there are only two options when designing a land-based seawater system. The first is loosely referred to as an open system, or more accurately, a semi-closed system (Huegenin & Colt, 1989). Wheaton (1972) defined an open/semi-closed system as: "a system where water from a natural body of water has only one passage through the system". The second approach is a closed system in which the seawater is replaced only at widely separated intervals (Wheaton, 1972). For the sake of clarity, this discussion will use the terms open seawater system (O.S.S.) and closed seawater system (C.S.S.).

Water parameters monitored for both seawater systems described in this study, form the criteria for assessing relative advantages and disadvantages of these systems. Temperature was chosen due to its known influence on abalone gametogenesis (Kikuchi & Uki, 1974). The optimum water temperature for *H. midae* would appear to be in the range of 13°C to 19°C (Newman, 1969). Salinity is also important because normal abalone larval development and best survival occurs in the narrow range of 27 to 39.4 mg/l (Hahn, 1989).

There are three main nitrogenous metabolites, namely, ammonia (NH_3), nitrite ($NO_2^=$) and nitrate (NO_3^-). Ammonia is the most toxic, closely followed by nitrite, with nitrate being the least harmful of the three (Haywood & Wells, 1989). Nitrite was chosen as it is cheaper and easier to measure than ammonia, and a nitrite peak closely follows an ammonia peak (Spotte, 1979). The amount of nitrite in a C.S.S. is therefore an indication of prior ammonia levels. The optimum level of nitrite in a seawater system should be below 2 mg/l (Lundegaard, 1985). Fairly high levels of nitrite are tolerated by juvenile American oyster with the LC_{50} occurring at 798 mg/l at an exposure time of 96 hours (Spotte, 1979). One can therefore assume that although some invertebrates can tolerate high levels of nitrite, its close relationship with ammonia still makes it undesirable at concentrations above 2 mg/l. The level of oxygen present in the system water influences the nitrogen dynamics. High

oxygen levels promotes the reduction of ammonia to nitrite and nitrite to the less toxic nitrate (Spotte,1992).

Finally, according to Spotte (1979) the optimum pH for most marine invertebrates is between 8.0 and 8.3. Mature abalone survive short exposures to artificially elevated pH of up to 9.3 (Morse, 1992). Fish are vulnerable to water pH outside the range of pH 5 to 9 (Randall, 1991). The toxicity of ammonia increases at higher pH levels (Spotte, 1979). For this investigation the optimum pH range of the open and closed system seawater will be considered to be 8.0 to 8.3.

This work provides new results on the impact broodstock abalone and macro algal feed have on water quality for both an open and closed seawater system. Most work regarding water quality in aquaculture has been concentrated on trout and salmon aquaculture (Spotte, 1992). This study also gives some insight as to the desirable water quality parameters which will result in successful broodstock conditioning for *H. midae*.

3.2: Materials and methods

Both systems were constructed at Gansbaai, 180 km SE of Cape Town, South Africa. The design criteria were to build a seawater facility where 40 broodstock abalone (*H. midae*), ten 20 l spawning tanks, four 40 l larval rearing tanks and

five 200 l spat growout tanks could be accommodated. I designed and built both the seawater systems described in this chapter.

The following water quality parameters were simultaneously monitored daily for both the O.S.S. and C.S.S. systems: temperature, pH, salinity and nitrite. These parameters were measured and recorded daily between 8 and 10 am. Temperature was measured using a glass mercury filled thermometer with a range of 0 to 50°C. Seawater pH was established using a Hanna portable pH meter (HI 8314 membrane pH meter). The pH meter was calibrated once a week against three standard buffer solutions with a pH of 4.01, 7.00 and 10.01. The salinity of both systems were measured using a non temperature compensating refractometer. The refractometer was calibrated with distilled water of the same temperature as the sample prior to each measurement. Nitrite was determined using a HACH DR/2000 spectrophotometer. The low range diazotization method suitable for seawater was used. The sample was diluted 10 fold prior to measuring the nitrite level. The spectrophotometer reading in mg/l was then multiplied by 10 to give the sample nitrite level.

The stocking density of abalone per litre of seawater for both the O.S.S. and C.S.S. was held at one abalone (average length 120 mm and average mass 160 g) per 30 litres of seawater. This stocking density for the broodstock abalone was the density planned for the commercial scale abalone

farm. Seawater was circulated through the tanks in both systems at a rate to exchange the volume once an hour. This ensured that the oxygen levels in the seawater of both systems never fell below 90 % saturation. The seawater oxygen levels were measured weekly. The kelp, *Ecklonia maxima*, was fed to broodstock abalone *ad lib* in both systems. The kelp was added to the tanks twice a week after removing the old uneaten kelp.

3.2.1: Closed seawater system

The construction of the closed seawater system was completed in March 1990 within pumping distance of the planned commercial site. The integral parts of the system are shown in Fig. 3.1. The seawater flowed by gravity from the header tank (1000 l) through the experimental spawning tanks, to the culture tanks and, finally, into the settlement tank (5000 l). The flow rate of water through the tanks was adjusted to allow for one exchange of the tank volume per hour. The flow rate through into each tank was therefor directly related to the volume of the tank. The water in the settlement tank was pumped by a standard one kilowatt swimming pool pump up to the biological filter, and from there back to the header tank. The temperature control system (Fig. 3.1) was used primarily as a stand-by. If the temperature exceeded 20^{o} C, the system would be activated automatically. It consisted of a refrigeration compressor

which cooled a glycol-filled reservoir. A copper coil acted as an intermediate heat exchanger. The cool glycol was then pumped through a polyethylene heat exchanger by a flooded suction pump. This pump was activated by a programmable thermostat. Any solids that settled in the settlement tank were removed manually by siphoning once a day.

In a closed system nitrification is the process where ammonia is reduced by biological oxidation to nitrite and nitrate (Spotte, 1979). In seawater *Nitrosomonas* bacteria oxidises ammonia to nitrite and *Nitrobacter* bacteria oxidises nitrite to nitrate. In both of these reduction reactions oxygen is required (Spotte, 1979). Nitrate is far less toxic than ammonia. According to Spotte (1979) the efficiency of the nitrification process is affected by the following six factors:

1. The presence of pollutants in the water.
2. Temperature
3. pH
4. Dissolved oxygen concentration
5. Salinity
6. Surface area of the biological filter material.

The general principle of a biological filter is therefor to provide maximum possible colonising surface area for the nitrosomonas and nitrobacter bacteria (Mills, 1987). The biological filter medium chosen was a glass scintered disc,

developed and manufactured by Schott Glasswerke in Germany. This material was selected for its extremely high surface area. The siporax cylinders (60 l) provided approximately 46 000 m^2 of colonising surface area for the bacteria (Dawes, 1989). A further advantage of these discs is that they offer little resistance to water flowing through the filter. The increased surface area allowed for a smaller biological filter (80 l total volume, 60 l of glass discs), which was positioned above the head tank. This configuration required only one pump set.

The biological filter was allowed to mature for 2 weeks prior to the stocking of *H. midae*. Bacterial growth was aided by introducing tidal pool fish (*Pavoclinus graminis*) to provide small amounts of nitrogenous waste products. A reverse flow protein skimmer was periodically employed to reduce the level of proteins and fats in the settlement tank. Prior to any spawning induction experiments, a 1 micron cartridge filter and uv filter were incorporated into the seawater line supplying the experimental tanks.

Every fourteen days 10% of the seawater volume in the closed system was replaced. Distilled water was used to prevent the salinity in the closed system from exceeding 34 mg/l. The addition of distilled water was achieved using a 5 mm delivery tube at a flow rate of 5 litres per minute. The slow addition of distilled water ensured that the abalone

held in the system were not subjected to sudden salinity fluctuations. This protocol also ensured that the seawater temperature was not measurably altered during the addition of distilled water. The flow rate of seawater through the abalone tanks in the closed system was adjusted to ensure an exchange rate of one volume per hour.

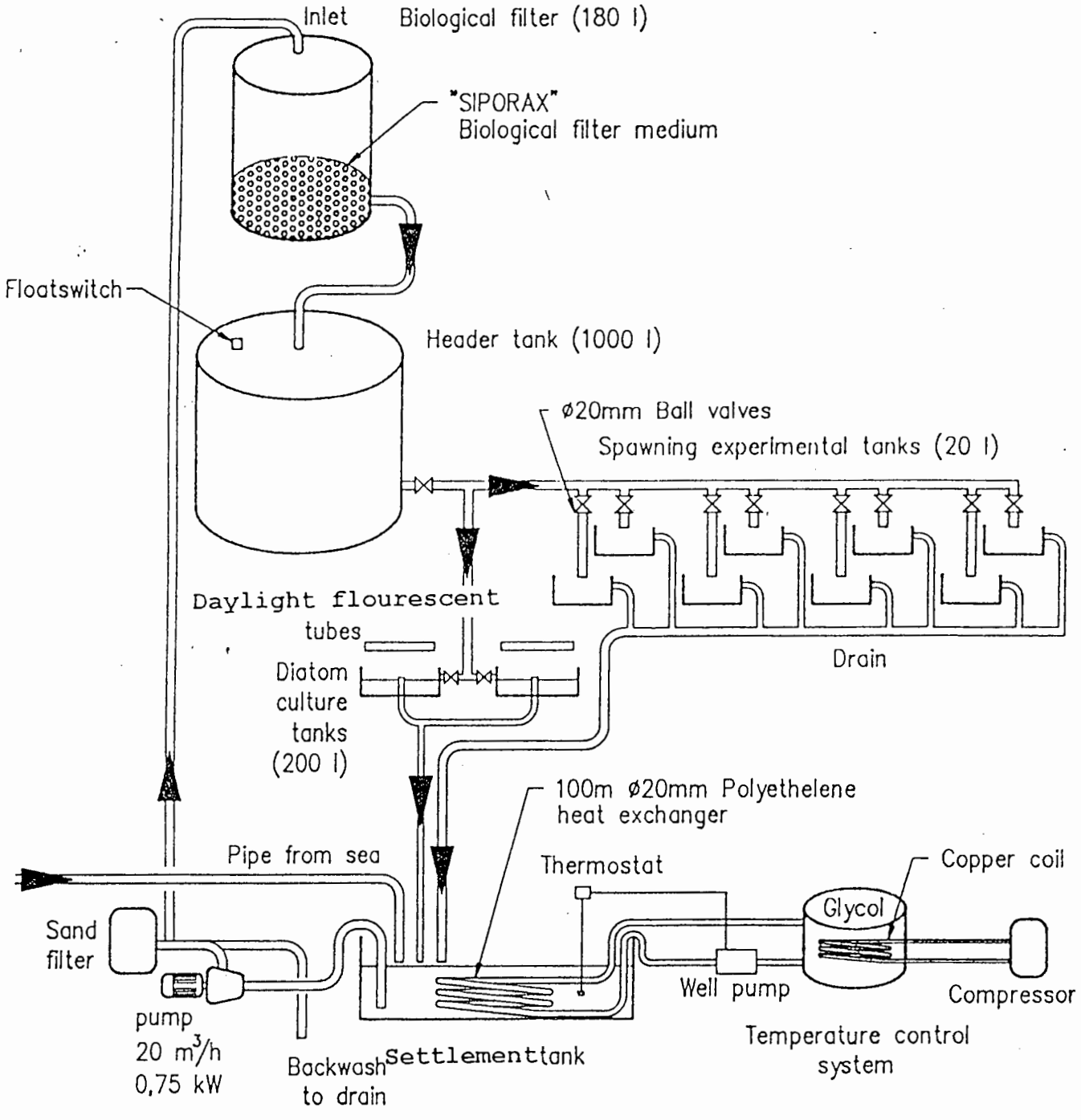


Fig. 3.1 Flow diagram of the closed seawater system

3.2.2: The open seawater system

The open seawater system was constructed in February 1992 to test the suitability of the seawater intake at the planned commercial-scale farm over a full year (Fig. 3.2). This system required a secure suction line and intake screen in the proposed pumping location. The intake consisted of a 1.5 ton concrete block with the pipe work precast into the block. A stainless steel (316 grade) mesh screen (Fig. 3.2) was then secured with precast bolts and nuts to the concrete block (Huegenin & Colt, 1989).

A 1.5 km (50 mm diameter) low density polyethylene pipeline was laid to the farm site. A positive displacement monopump of 3.5 kW provided the required 5 m^3/hr of seawater to the static head of 27 m. The seawater was pumped to the hatchery where it first entered a settlement tank, and from there a standard swimming pool sand filter was used to filter the water before being pumped into a head tank. The inlet of the header tank was screened with a 15 micron bag filter which was cleaned daily. The water then flowed by gravity through the various tanks and finally into the return drain to the sea. The open seawater system did not utilise a temperature control system.

Broodstock abalone held in both seawater systems were used in artificial spawning induction experiments. The procedure

used to induce spawning was the hydrogen peroxide method as published by Morse (1984). Fifteen separate attempts were made to spawn eight male and eight female *H. midae* from the C.S.S. A further fifteen artificial spawning induction experiments were run, using the same procedure and numbers of broodstock from the O.S.S. A spawning trial was considered successful when at least one female and one male spawned resulting in viable fertilised eggs from which normal abalone larvae hatched.

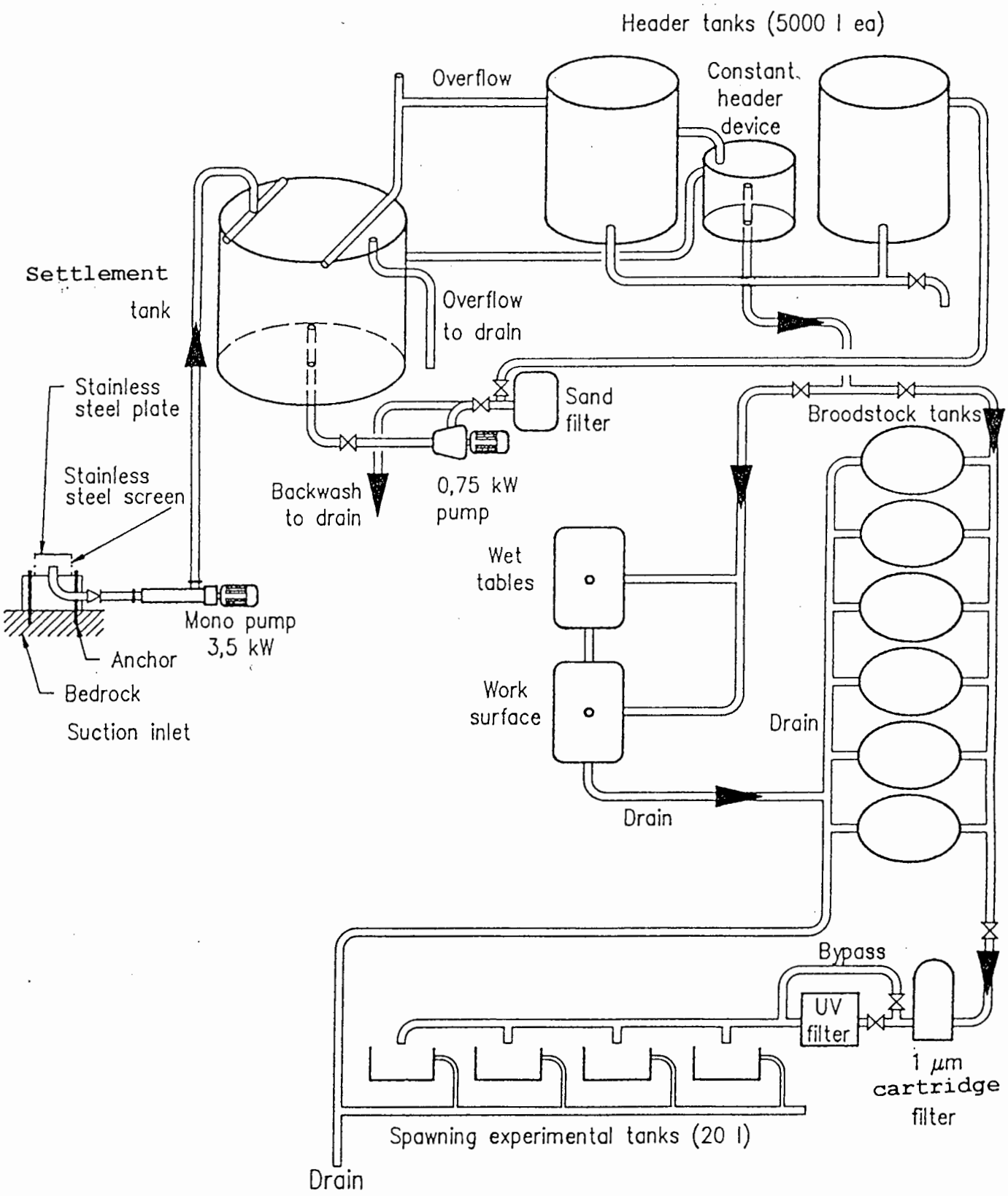


Fig. 3.2 Flow diagram of the open seawater system

3.3: Results

Mean monthly seawater temperatures in the C.S.S. were generally higher than those in the O.S.S (see Fig. 3.3). This can be attributed to high air temperatures raising the temperature of the seawater in the C.S.S. The greatest monthly temperature variation for the O.S.S. was 4.1°C while for the C.S.S. the greatest variation was 6.4°C.

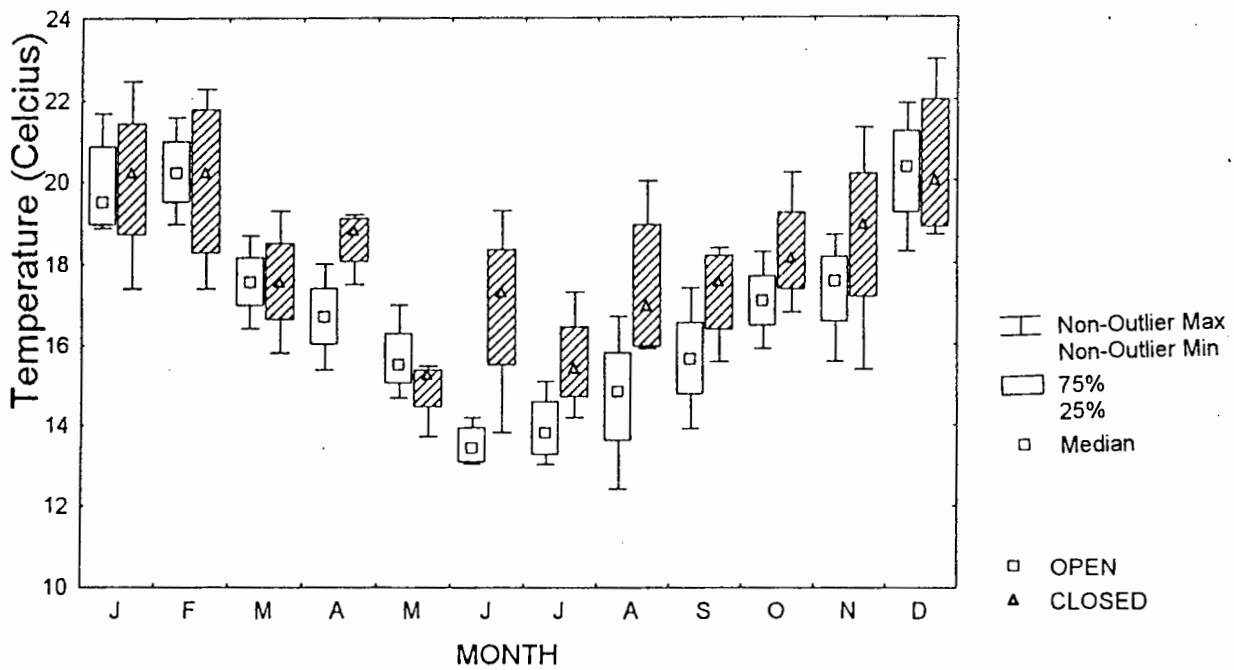


Fig. 3.3 Monthly temperatures recorded for the open and closed system

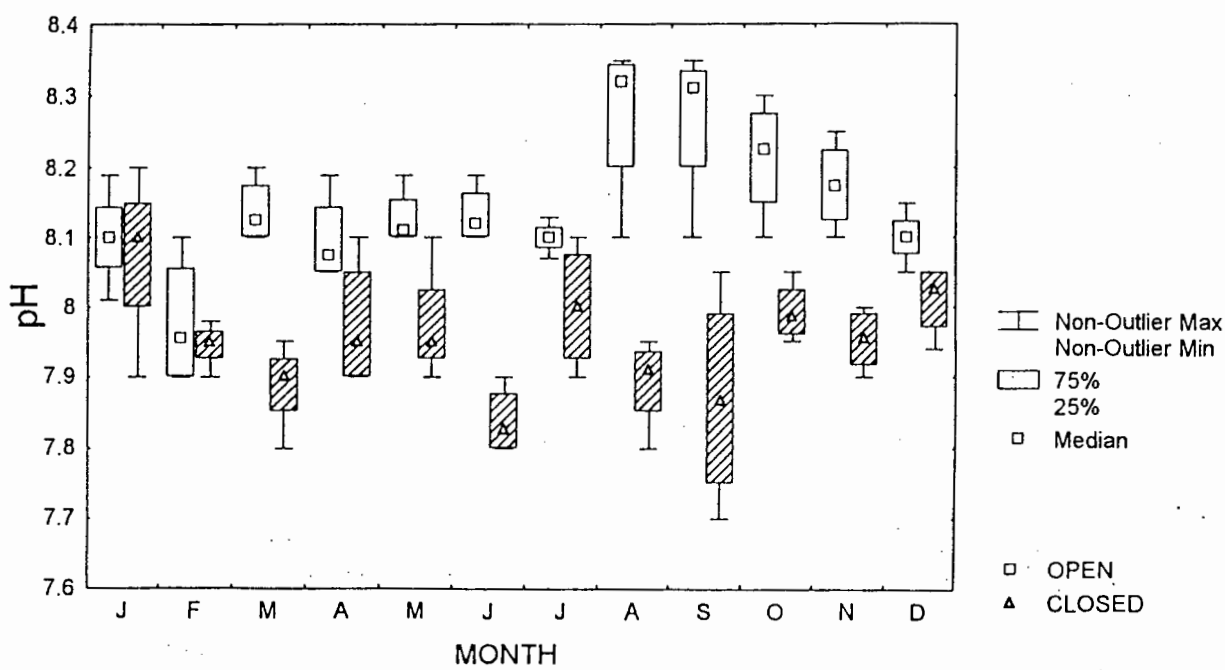


Fig. 3.4 The monthly pH for the open and closed system

The monthly pH of the C.S.S. was consistently lower than that of the O.S.S., ranging from 7.72 to 8.20 (Fig. 3.4). The monthly pH in the O.S.S. ranged from 7.9 to 8.35.

Nitrite levels in the C.S.S. were very variable, ranging from 0.5 mg/L to a high of 4.1 mg/L (Fig. 3.5). Nitrite levels in the open seawater system were below 1.5 mg/L for most of the year, except for a peak of 3.0 mg/L occurring during October to December. This was probably due to the increase in broodstock biomass as a result of rapid growth during the year.

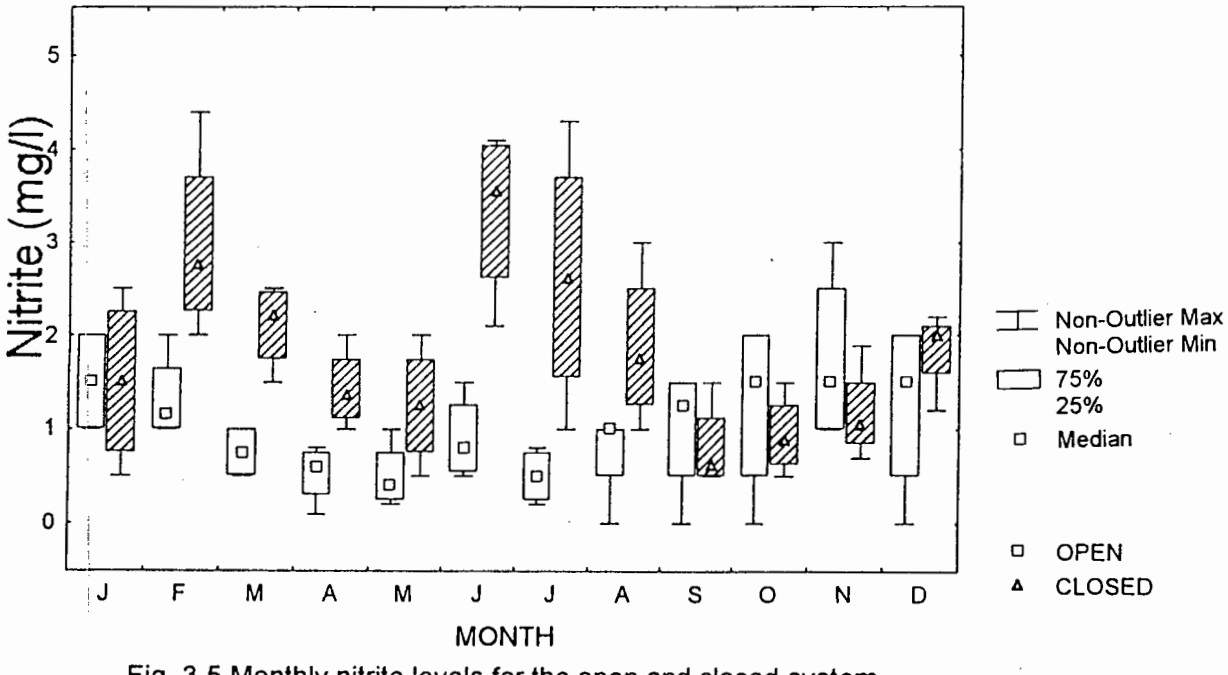


Fig. 3.5 Monthly nitrite levels for the open and closed system

The salinity data clearly highlight a difference between the closed and open system (Fig. 3.6). The variation of 2,8 mg/L for the C.S.S. greatly exceeded that of 1.0 mg/L for the O.S.S.

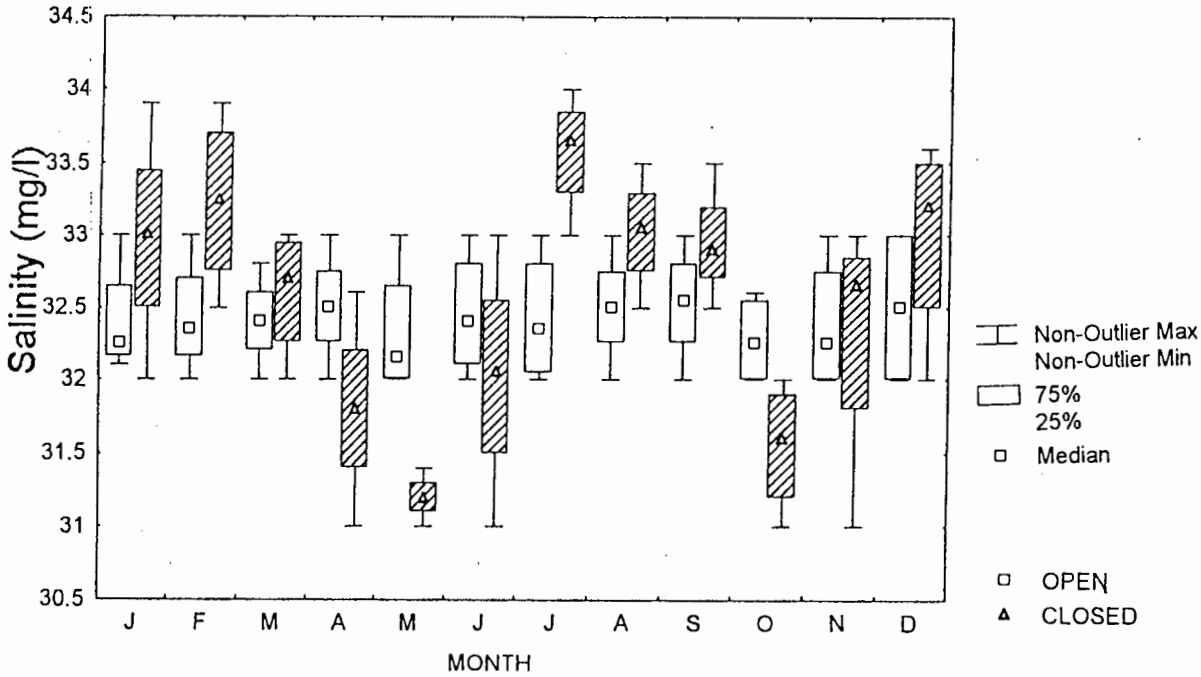


Fig. 3.6 Monthly seawater salinity levels for the open and closed system

Observations of spontaneous spawning of captive *Haliotis midae* broodstock demonstrate a major difference between the two systems. A total of seven spontaneous spawnings were observed for broodstock kept in the open system, while no spontaneous spawnings were observed for those kept in the closed system (Table 3.1). The results of the 15 attempts of spawning broodstock *H. midae* in the C.S.S. were all unsuccessful. The broodstock held in the O.S.S. were successfully spawned 7 out of the 15 attempts.

TABLE 3.1: Spontaneous spawning observations for broodstock held in open and closed seawater systems

Month	Frequency of spontaneous spawnings	
	Open seawater system	Closed seawater system
Jan	1	0
Feb	2	0
March	0	0
April	0	0
May	0	0
June	0	0
July	0	0
Aug	1	0
Sept	2	0
Oct	0	0
Nov	1	0
Dec	0	0
Total	7	0

3.4: Discussion

The variable success of spawning induction and larval survival of *Haliotis* has been attributed to seawater system design (Morse, 1992). Further Jaekle & Manahan (1989) have demonstrated that abalone larvae can absorb amino acids directly from seawater. This has an important impact on system design. Sand filters should be avoided as it is argued that bacteria colonising the filters would utilise some of the vital amino acids (Manahan & Jaekle, 1992). Jaeckle & Manahan (1988) show that larvae reared in seawater using only mechanical filtration increase in biomass, while

larvae reared in seawater that has been processed by a biological filter, decrease in biomass. These findings further highlight the potential disadvantage of using a recycling seawater system for an abalone hatchery.

This study demonstrated that the O.S.S. provided more stable temperatures and therefore a less stressful environment for broodstock abalone. The stability of the seawater temperature in the O.S.S. can partially be attributed to the influence that ground temperature played on the incoming seawater in the 1,5 km (50 mm diameter) buried delivery pipeline. The instability of water quality parameters have been observed by various researchers investigating closed water systems (Rosenthal & Black, 1993; Poxton & Allouse, 1987; Rosenthal *et al.*, 1981; Rosati *et al.*, 1993). Temperature stability would be improved in the closed system if more accurate temperature control equipment was used, such as a heat pump with titanium heat exchanging coils. However, this equipment is costly and does not reduce the fluctuations of other water quality parameters.

The biological filter, utilising heterotrophic bacteria, is the most important biochemical processor of a closed seawater system (Spotte, 1979; Lundegaard, 1985; & Mills, 1987). In spite of its existence, however, nitrite peaked at a higher level in the closed system than in the open system. The biological filter operates best when there is a constant

level of nitrogenous waste products in the water (Moe, 1989). The active nocturnal feeding of abalone, degradation of kelp in the tanks and the build up of faecal matter in the settlement tank gave rise to rapid changes in the level of the soluble nitrogenous waste products. The results clearly show that the biological filter was not capable of processing this fluctuating load. A possible improvement would be to continually filter faecal material instead of the daily siphoning from the settlement tank. An additional improvement would be to have a percentage of the seawater replaced every day as opposed to an extended interval between seawater changes. The poor performance of the C.S.S. cannot be attributed to the use of 'siporax' as the filter medium. The biological filter may however have functioned better if more filter medium had been used. The high surface area and low resistance to the seawater must make siporax an ideal biological filter medium.

The nitrite fluctuations could be considerably reduced in the open seawater system by increasing the exchange rate of the seawater. Sieswerda & Policansky (1984) observed that a flow-through system allowed rapid response to changes in water quality and permitted the use of very high food concentrations. Pump capacity prevented an increase in the flow-through rate of the seawater in the pilot-scale open seawater system. This problem was rectified in the

construction and design of the commercial scale flow-through seawater system.

Manual regulation of the salinity in the C.S.S. by periodic additions of distilled water, coupled with subsequent evaporation, could explain the less stable salinity of the C.S.S. relative to that recorded in the O.S.S. (Fig. 3.6).

The results of the artificial spawning induction experiments further emphasise the differences between the O.S.S. and C.S.S. The total lack of response to the spawning stimulus for *H. midae* held in the C.S.S. might be due to stress induced by poor seawater quality. The seven successful spawnings out of fifteen attempts for broodstock in the O.S.S. is a great improvement, but ongoing research is being conducted to improve the success rate of artificial spawning.

In conclusion, a few pertinent findings are worth emphasising. The pilot scale construction of a seawater system prior to the construction of the commercial scale aquaculture system proved to be a valuable exercise and helped reduce design errors. Water quality parameters generally fluctuated more in the C.S.S. From a biological management and operational point of view, the O.S.S. appeared to be simpler and easier to run.

Spontaneous spawning of *H. midae* broodstock were only observed in the O.S.S. Although this is not a desirable occurrence, it does indicate that the abalone were fully gravid and that gametogenesis proceeded to a stage where the slightest fluctuation in water quality would trigger spawning. The artificial spawning induction of *H. midae* was only successful for broodstock held in the open seawater system. It has been argued that abalone can be induced to spawn after being stressed by temperature fluctuations or by desiccation. In my opinion this is not as a result of stress but as a result of being exposed to a cue which could possibly be responsible for inducing wild abalone populations to spawn. Extreme tidal fluctuations could expose certain abalone species that colonise shallow rocky reefs to a period of natural desiccation. Temperature fluctuations of 3 to 4°C are also possible in the wild depending on certain weather conditions. If stressing abalone was a reliable way for inducing spawning then it follows that the *H. midae* broodstock held in the closed system should have spawned spontaneously several times as a result of greater fluctuations in seawater quality.

CHAPTER FOUR: BROOD STOCK CONDITIONING AND GONAD ASSESSMENT OF *Haliotis midae*

4.1: Introduction

In marine fish culture programs environmental manipulation and hormonal treatments are used to advance gametogenesis, resulting in synchronised spawning. The most important environmental factors regulating reproduction in fish appear to be photoperiod and water temperature (Shimizu & Hanyu, 1983). As emphasised previously abalone gonadal growth is also temperature dependant (Webber & Giese, 1969).

The hormones used in fish culture are frequently administered on several occasions over a relatively short time span (Castagnolli & Donaldson, 1981). This ultimately means that the reproductive stage of fish can be advanced prior to spawning (Fujita, *et al.*, 1986; Fortuny, *et al.*, 1988). These methods do however sometimes result in the release of gametes with lower viability (Jalabert, *et al.*, 1977). The success of spawning inducement using hormones, however, ultimately depends on prior conditioning of the fish broodstock (Saitoh, *et al.*, 1991).

A similar situation occurs when abalone are artificially induced to spawn. Due to the limited exposure of the spawning stimulus, gametogenesis needs to have progressed sufficiently so that there are well-developed gametes in the gonad prior to spawning induction. Therefore, the ability to determine accurately the real success or failure of any artificial spawning induction technique, will depend on the method used to condition and identify gravid *H. midae* broodstock. Broodstock refer to sexually mature abalone with a shell length greater than 100 mm (Newman, 1969). These abalone were used as breeding stock in the spawning induction experiments.

The successful farming of abalone relies heavily on the availability of gravid broodstock. Abalone growers should not rely on wild conditioned abalone, due to the unpredictable and irregular reproductive cycle (Sainsbury, 1982). As is the case in animal husbandry operations, parent selection and premating preparation are inherently important. Likewise the selection of breeding stock for an *H. midae* hatchery requires careful attention. Broodstock conditioning is an important prerequisite to spawning induction. Morse (1984) describes a successful method for conditioning haliotids. Conditioning requirements can be subtly different between species (Morse, 1984). General

principles of preconditioning are to supply adult abalone with large amounts of macro-algae and provide temperature conditions associated with the natural spawning season of a given species (Morse, 1984).

An important complementary aspect of broodstock conditioning is the development of a visual gonad assessment (v.g.a.) technique. The reliability of the v.g.a. technique was experimentally determined by artificially spawning abalone after they had been assessed by this technique. A visual gonad assessment technique was chosen because, unlike gonad dissection techniques, it is not necessary to sacrifice any abalone when a visual technique is used.

4.2: Methods

4.2.1: Broodstock collection

Broodstock abalone (*H. midae*) were collected by diving with scuba gear from rocky reefs in the vicinity of Gansbaai (Fig. 1.1). A total of 300 abalone were harvested for this study. The abalone were collected in the infratidal zone at depths of one to eight meters below the mean low tide mark. Special care was taken not to damage the foot when abalone were levered from the reef. A preferable method to using the standard abalone lever, was swimming up to an unsuspecting abalone and displacing it by hand before it had a chance to

secure itself firmly to the reef. This method ensured that no tissue on the foot was damaged during collection. Abalone that are hurt easily bleed to death, because they lack a blood clotting mechanism (Hahn, 1989). Less severe abrasions may not be fatal, but they do result in the loss of vital body fluids and also give access to invasive pathogens.

During collection, the adult *H. midae* were kept in anchovy net bags and immediately after the dive were placed in an open tidal pool while stowing the diving gear. The abalone were kept in their bags and placed in plastic bins with no water during transfer (approximately 15 minutes) to the broodstock tanks. This procedure resulted in a 100% survival rate of all abalone collected throughout the study period.

4.2.2: Brood stock conditioning

The broodstock utilised in this study were handled as little as possible to reduce stress levels. Stress disturbs the metabolic processes, reducing the amount of metabolic energy which could otherwise have been partitioned to reproduction (Steffens, 1989). To achieve a stress-free environment, the tank design developed by Hooker (pers. comm. 1991) was used to maintain broodstock. Water was recirculated vigorously in an 800 l fiberglass tank by an external 0.5 kW swimming pool pump. This turbulent flow performed a number of functions. Firstly, all waste products were brought to the surface

where the water flowed through a surface drain pipe. The turbulent conditions also helped keep the kelp (*Ecklonia maxima*) proximal to the sides of the tank where abalone could feed in an almost natural manner. Finally, the thorough mixing in the holding tanks ensured that the seawater quality was uniform throughout the tank. The incoming ambient seawater was adjusted to exchange the water volume once per hour. The broodstock were therefore held in a open or flow-through system.

Freshly harvested kelp *E. maxima* was added to the tanks twice a week in sufficient volumes to allow the broodstock to feed *ad lib*. An 80% shade cloth cover was secured over the top of the tank. The light meter reading gave similar light levels (120 lux) to those found at five to eight meters depth at the base of a kelp forest. Broodstock were kept under a natural photoperiod regime in these tanks for at least six weeks before attempting to artificially spawn them.

4.2.3: Assessment of gonad reproductive synchronisation for H. midae.

The sexually mature abalone collected for broodstock purposes were visually assessed to establish the stage of gonad development. The main objective was to establish whether sexually mature abalone from a specific reef showed

synchronised gonad development. The stage of gonad development was assessed, using a visual method.

4.2.4: A visual gonad assessment method

The present investigation required that artificial spawning techniques be tested on living *H. midae* specimens. Gonad condition was assessed by refining a visual gonad assessment technique first developed by Ebert & Houk (1984). The method is described in table 4.1 and relies on the colour and shape of the gonad. Observing the colour of the gonad is a reliable way of assessing the sex of *H. midae*. The gonad of the male is a creamy white, while that of the female is green.

The anatomical position of an abalone's gonad is shown in Fig. 1.2. The shape of the gonad is important in assessing the reproductive condition of abalone (Hooker, pers. comm., 1991). The gonad assessment method used by Ebert & Houk (1984), has been modified for this study (see table 4.1). The colour and shape of the gonad is observed by gently placing the abalone shell down in the palm of the hand. With the head region of the abalone facing the tips of the fingers, the left portion of the foot is carefully lifted away from the shell. This exposes the gonad which looks like an elongate kidney as shown in table 4.1. The gonad has also

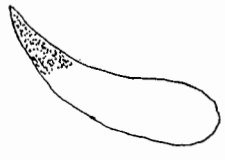
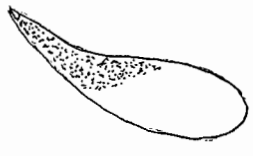
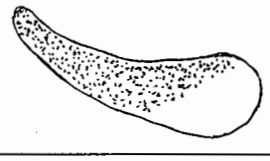
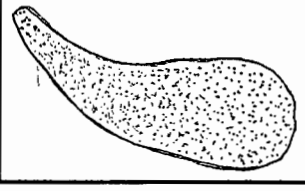
been referred to as the conical appendage (Hahn, 1989). The best way to look at the gonad is to tilt the abalone in a horizontal plane away from the gonad. The abalone should be held very still. The natural response of an upturned abalone then exposes the gonad as it extends its foot, in an attempt to right itself. This procedure allows examination of the abalone's gonadal condition without stressing or destroying the abalone.

An artificial spawning induction experiment, using the hydrogen peroxide method (Morse, 1984), was run to evaluate the v.g.a. technique. Refer to the methods in chapter five for a detailed description of the hydrogen peroxide protocol. In these experiments fresh hydrogen peroxide was added to the containers to give a final concentration of 12 mM. Abalone of each of the five gonad indices were placed in separate tanks prior to inducing spawning. The abalone were then placed in the spawning tanks after visually assessing their gonad development stage. Sufficient abalone of each gonad stage were used to provide two tanks with abalone of each gonad index. Two controls, for which no hydrogen peroxide was added, were included in the experiment. In control tanks the abalone had a gonad index of four (refer to table 4.1). A total of 16 tanks, each with four female and two male abalone, were used in this trial. The abalone

used in this experiment were pre-conditioned in the holding tanks.

TABLE 4.1: Visual gonad assessment method for *H. midae* (After Ebert & Houk, 1984 ; Grant, 1981; Neal Hooker pers. comm. 1991).

The shaded area of the gonad represents the sex specific gonad colour. The remaining clear area is the brown colour of the digestive gland.

GONAD INDEX	GONAD STAGE	VISUAL DESCRIPTION OF GONAD	SHAPE OF GONAD
0	Spent	Immature, sex indeterminate the gonad is greyish brown	
1	Recovery	Gamete development is initiated, males can be identified by cream colour gonad, female sex determination is difficult. The tip of the gonad is pointed and flaccid	
2	Premature	Gametes envelop the conical appendage. The tip of the appendage is pointed and firm. Sex determination is easy.	
3	Mature	The gonad tip is round but not extended or bulging. The entire area of the gonad does not exhibit the sex specific colour.	
4	Gravid	The gonad tip is round, extended and bulging. The entire gonad is creamy white or green depending on sex and is fully distended.	

4.3: Results

The results assessing the gonad condition of natural populations of abalone are presented in tables 4.2 a & b below. It would appear that *H. midae* collected for this investigation exhibited very little reproductive synchronisation. It is also worth noting that none of the wild abalone was found to be totally gravid (condition factor 4).

TABLE 4.2: Gonad indices for abalone collected at varying times from the same reef

Gonad Index	4.2a Female *H. midae* gonad indices during 1993											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
0	3	7	2	1	6	7	4	7	7	3	2	4
1	2	3	2	2	1	2	2	4	4	3	6	7
2	2	3	6	3	1	1	2	2	2	4	5	3
3	1	1	1	2	*	1	1	2	2	7	3	4
4	*	*	*	*	*	*	*	*	*	*	*	*
Total	9	14	1	8	8	11	9	15	22	17	16	18

Gonad Index	4.2 b Male *H. midae* gonad indices during 1993											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
0	2	3	1	2	2	3	3	1	1	4	2	3
1	2	5	*	3	2	3	3	3	3	7	2	3
2	3	4	7	2	2	2	2	5	5	3	3	2
3	1	2	5	2	1	2	2	2	2	8	4	3
4	*	*	*	*	*	*	*	*	*	*	*	*
Total	8	14	13	9	7	10	9	11	16	22	11	11

Only gravid abalone assessed to have a condition factor of 4 in the laboratory, could be induced to spawn (table 4.3). These results clearly indicate the usefulness of the visual gonad assessment technique. The results also highlight that spawning could not be induced in wild-collected abalone. No animals of condition factor four were ever found in the wild. This emphasises the vital need for pre-conditioning of broodstock in the laboratory to bring them into index factor 4.

TABLE 4.3: Results of a spawning induction experiment designed to test the visual gonad assessment method (Each tank held four animals)

TANK	GONAD INDEX	WILD/ CONDITIONED	SPAWNING % MALES	FEMALES
1	1	C	-	-
2	0	C	-	-
3	2	C	-	-
4	4	C	100	50
5	1	C	-	-
6	3	C	-	-
7	4 control	C	-	-
8	4	C	100	25
9	3	C	-	-
10	4 control	C	-	-
11	0	C	-	-
12	2	C	-	-
13	3	W	-	-
14	3	W	-	-
15	3	W	-	-
16	3	W	-	-

note: - represents no spawning observed

4.4: Discussion and conclusion

There are a number of methods utilised by researchers to determine *Haliotis* gonad indices. These procedures range from visual to more drastic dissection methods. Sheperd, *et al.*, (1985) used the following method to assess gonad condition. They made a cross sectional dissection of the conical appendage, which is then used to determine the reproductive condition. This is achieved by calculating the relative proportion of the outer gonad surface area to that of the digestive gland. Their method and other dissection methods make the assumption that adult abalone from the same reef have synchronised gametogenesis development. This assumption appears not to apply to *H. midae* where gonad development does vary between mature individuals on the same reef (refer to table 4.1). Even if they can diagnose when a particular animal is ripe for spawning, the result cannot reliably be applied to the population as a whole. It is essential that the condition of individual animals be known before they are used in an artificial spawning attempt. The number of mature abalone we were permitted to collect simultaneously was not sufficient to comment confidently on the total population's reproductive synchronisation. There was, however, enough variation in the small samples we investigated to discourse the use of destructive dissection techniques.

A more conservative method of gonad assessment and conditioning is used by Japanese abalone farmers. It relies on the quantitative temperature exposure above a critical minimum temperature to determine gonad development (Uki & Kikuchi, 1984). A relationship between gonad development and seawater temperature is calculated for each species. The biological zero point temperature is first established, at which no gonadal development takes place (Hahn, 1989). The relationship is then further developed to accurately predict how many days the abalone need to be exposed to temperatures above this zero point. The determination of this accumulative influence of seawater temperatures above the zero point temperature is a time consuming process. Furthermore, the QAT (quantitative accumulative temperature) conditioning and gonad assessment technique does not apply to all abalone species (Kikuchi & Uki, 1984). A further reason for not investigating the QAT method was the lack of seawater temperature control in the open seawater system. There was not sufficient funds available for installing a temperature control system for the flow through seawater system used in this study.

Considering the resources and time frame of this investigation it was decided to adapt, and improve and test a relatively simple visual gonad assessment method (table 4.1). The major advantage of this method is that it allows for the determination of gonadal condition without

sacrificing the animal. The technique's viability is supported by the results presented in tables 4.2 and 4.3. Only abalone assessed to be gravid (index 4), spawned when they were exposed to the hydrogen peroxide spawning induction stimulus (see table 4.3).

To summarise, the following conclusions and observations can be made:

1. Mature *H. midae* inhabiting natural reefs in the Gansbaai bay area appear not to exhibit a closely synchronised reproductive cycle. This implies that destructive methods of sampling gonadal condition could not be extrapolated to the field populations.

2. The visual method developed in this study for assessing gonad condition proved to be simple and reliable, as indicated by the fact that only adults ranked as spawners (condition 4) could be induced to spawn.

3. Conditioned abalone are more likely to spawn when exposed to hydrogen peroxide than abalone that have recently been collected from the sea. The reason for this is that wild-collected unconditioned animals are unlikely to be sufficiently gravid.

CHAPTER 5: THE REFINEMENT OF THE HYDROGEN PEROXIDE SPAWNING TECHNIQUE TO INDUCE Haliotis midae TO SPAWN

5.1: Introduction

A reliable method for inducing *H. midae* to spawn is an important requirement for the successful development of a commercial abalone hatchery. The first step towards reaching this goal was to initiate an in-depth study of documented haliotid spawning techniques. Researchers to date have successfully induced a number of *Haliotis* species to spawn artificially in captivity. Table 5.1 gives a list of *Haliotis* species and the respective methods used to artificially induce them to spawn. Gamete stripping was the first method used to obtain sperm and eggs from abalone (Hahn, 1989), but this method is usually not feasible as the gametes are often immature. The results presented in chapter two confirm this conclusion. A second method relies on desiccation: very ripe adults which are about to spawn are removed from the seawater tanks for up to one hour. Spawning begins when the abalone are returned to the water. This procedure, in isolation, has also proved to be unreliable (Hahn, 1989).

Thermal shock has been used to stimulate spawning but also often results in the release of immature gametes (Hahn, 1989). Seawater, irradiated with ultra-violet light, is a fast and reliable method for the induction of spawning for a few *Haliotis* species (Kikuchi & Uki, 1974).

TABLE 5.1: A summary of successful spawning induction methods for various species of *Haliotis*.

SPECIES	SPAWNING INDUCTION	COUNTRY	REFERENCE
H. diversicolor supertexta	Temperature fluctuation	Japan	Oba, 1964
H. discus hannai	UV irradiated seawater	Japan	Kikuchi & Uki, 1974
H. rufescens	Hydrogen peroxide	U.S.A.	Morse *et al.*, 1977
H. discus hannai	UV irradiated seawater	Japan	Seki, 1980
H. rufescens	UV & temperature increase	Chile	Owen *et al., 1984*
H. coicinea canarienus	Hydrogen peroxide	Spain	Pena, 1986
H. iris	Hydrogen peroxide	New Zealand	Tong *et al.*, 1987
H. discus hannai	Daylength manipulation	Korea	Hahn, 1989
H. diversicolor supertexta	Desiccation & UV irradiated during the breeding season; thermal shock only	Taiwan	Chen, 1989
H. gigantea & *H. discus hannai*	UV irradiated seawater with thermal shock & desiccation	Japan	Hahn, 1989
H. tuberculata	Thermal shock & desiccation	France	Hahn, 1989

The use of hydrogen peroxide to produce chemically the hydroperox free radical HOO or peroxy diradical OO is another reliable method for inducing some *Haliotis* species to spawn (Morse, *et al*, 1977).

Many of these methods cannot be applied directly to new abalone species. Genade *et al.* (1988) were first to describe a method for artificially inducing *Haliotis midae* to spawn. They exposed mature abalone to air for one hour before placing them into a tank with flowing 17°C seawater. This water was filtered to 1µm and ultra violet sterilised. Water temperature in the tank was slowly raised by 3°C over three hours and allowed to cool back to 17°C at the same rate. They reduced the water flow once spawning had commenced. Their method, in my experience, is not easily repeated.

Ultraviolet irradiated seawater and the hydrogen peroxide method's ability to induce *H. midae* spawning were evaluated in this study. Nine independent variables influence on *H. midae* spawning using hydrogen peroxide were investigated.

5.2. Ultraviolet spawning induction

The exact protocol of UV spawning induction methods is often vaguely documented in scientific publications. Seki (1980) refers only to the duration of exposure to UV treated

seawater for three hours. Uki & Kikuchi (1982) describe successfully inducing *H. discus hannai* after an exposure of only I hr 20 min for males and 1 hr 45 min for females. They exposed their abalone to 270 to 300 mWh/l UV irradiated seawater. In an earlier publication Kikuchi & Uki (1974) reported inducing *H. discus hannai* after exposing the males to 3 hrs 18 min to 800 mWh/l and 2 hrs 42 min for females.

Kan-no (1975) describes using UV irradiated seawater (200 ml/min) to induce *H. discus hannai* to spawn after a 3 hr exposure in conjunction with a temperature fluctuation. He however failed to mention the wattage of UV source. Ebert & Houk (1984) also describe using UV to spawn *H. rufescens*. They used a flow rate of 150 ml/min irradiated seawater into the spawning tanks with an exposure of 3 to 4 hours. They also do not describe the wattage of the UV source.

In an attempt to spawn *H. midae* using UV with limited information on exact protocols the following strategy was chosen.

5.2.1 Ultra violet spawning experiment method

Ten 40 l tanks, each with 4 gravid adult abalone were used in each of the three UV spawning induction experiments. These abalone were acclimatised to the spawning containers for four days and were starved for 24 hr prior to the

spawning induction trials. Aeration was provided continuously to the spawning tanks. The duration of exposure to UV was set at 4 hrs and was initiated 4 hours prior to sunset. The gametes are not harmed if spawning occurs while UV irradiated seawater is still flowing through the tanks (Uki & Kikuchi, 1982). Two tanks were used as controls, they were not exposed to UV irradiated seawater.

Four 30 watt UV water sterilisers which generated an irradiation of 2537 Amstrong were used in series. This would give a combined dosage of 120 W. The flow into each of the 8 spawning tanks was set at 0.6 l/min. This provided an exposed dosage of 250 mWh/l which is similar to that reported by Uki & Kikuchi (1982).

5.2.2 Results of the UV spawning experiments.

The results of the three UV spawning trials are provided in table 5.2. From the results presented in table 5.2 it is clear that the UV exposure trials as described in the methods were not able to induce spawning in gravid *H. midae* broodstock.

TABLE 5.2 *H. midae* spawning response when exposed to ultra violet irradiated seawater

Trial no.	UV induction	n	Percent spawning
1	males	(16)	0
	females	(16)	0
	control	(8)	0
2	males	(16)	0
	females	(16)	0
	control	(8)	0
3	males	(16)	0
	females	(16)	0
	control	(8)	0

5.2.3 Discussion on ultra violet spawning induction technique

The lack of spawning success for the abalone exposed to UV was consistent in all three trials. There are at least two possible explanations to explain these results. Firstly *H. midae* do not respond to the UV spawning induction stimulus. The second is that the intensity of the UV exposure was not sufficient to induce spawning. According to Ebert (person. comm.) a possible problem with the UV spawning technique is method of calculating the UV wattage. He suggests that it is

the effective UV wattage and not the rated wattage of the UV source that should be used in the calculations. If for example a UV source rated at 40 watts by the manufacturer was only 40% effective, then 2.5 UV units would be required to provide an effective 40 watts of radiation.

These experiments were not expanded due to the lack of spawning success and the costs involved with installing additional UV units. The use of hydrogen peroxide to induce spawning in haliotids is certainly cheaper and in some cases more effective (Morse, 1984). The following section is dedicated to *H. midae* spawning induction utilising hydrogen peroxide.

5.3 Hydrogen peroxide spawning induction methods

5.3.1 Introduction

The use of hydrogen peroxide to induce haliotid spawning, as developed by Morse *et al.*, (1977), forms the backbone of the following experiments.

Male and female *H. midae* broodstock were selected from the holding tanks and placed in 40 litre plastic bins at a density of four abalone per tank. Male and female abalone were held in separate tanks. The flow of seawater filtered to 1µm into each tank was set at 80 l/hr. All the tanks were

provided with aeration throughout the duration of the spawning trial.

The buffer tris (hydroxymethyl methylamine), was added to the static 40 litre spawning containers five to ten minutes prior to addition the hydrogen peroxide. This buffer was used to raise the ambient pH of seawater to between 9.0 and 9.5 fresh 30% reagent grade hydrogen peroxide (H_2O_2) was stored at 0 to 4°C (Morse, *et al.*, 1978).

The seawater in the tanks was decanted after the required exposure time to H_2O_2 followed by a thorough rinsing filtered seawater. The water flow into the tanks was shut off after 10 minutes of flushing. The abalone were then visually monitored for up to 5 hours to establish if spawning would take place

Separate experiments utilising the basic H_2O_2 spawning induction method were conducted to test the influence of nine variables on the controlled induction of spawning for *H. midae*. The independent variables were: (1) prior acclimatisation, (2) whether abalone were starved 24 hours before experimentation or not, (3) time of day related to sunset, (4) the influence of lunar cycle, (5) influence of pH, (6) constant temperature regime, (7) the response to a fluctuation in temperature, (8) concentration of hydrogen peroxide and, lastly, (9) the duration of H_2O_2 exposure. The

effect of each independent variable was measured in terms of the percentage of male or female abalone spawning in all the tanks of one trial.

Each trial was replicated at least three times. The reason for adopting this strategy is the following: If at best only 20 % of the abalone are likely to respond to a spawning stimulus then four abalone in one tank would not be sufficient to determine if the spawning protocol tested would be successful. Forty abalone were used in each trial which was then replicated several times. This ensured that there would be sufficient number of animals in one replication of a trial to determine if there had been any significant spawning response to the tested combination of independent variables.

For experiments evaluating the effect of a specific variable the other factors were held constant. The standard conditions were a constant temperature of 15 to 19 ^{0}C, acclimation of all the animals, starvation for 24 hours, a pH fixed during H_2O_2 exposure to between 9.2 and 9.5, and H_2O_2 exposure standardised at 12 mM for 220 minutes which was rinsed from the tanks within 30 minutes of sunset.

When abalone spawned, their gametes were microscopically investigated to establish viability. If both sexes spawned simultaneously, gametes were only considered to be viable if

fertilisation and normal larval development resulted. If only male abalone spawned, sperm was checked for motility under an inverted Olympus CK2 microscope. When only females spawned, ova were considered viable if: (1) they were released independently from each other and not in thick clumps; (2) the concentric yolk was surrounded by a vitelline layer, a chorion and a thick jelly coat; (3) the size of the ova fell in the range of 180 to 220 µm in diameter (Hahn, 1989).

Spawning percentages were converted to degrees using the angular (arcsine) transformation prior to conducting any statistical analyses (Zar, 1984). This conversion was necessary to satisfy the normal distribution assumption of the analysis of variance (Anova). Results were accepted as significant when $p < 0.05$.

5.3.2 Prior acclimatisation impact on *H. midae* spawning

Handling of broodstock in intensive aquaculture operations is a source of stress (Bray & Lawrence, 1992). The natural response of abalone when disturbed is to clamp down securely to the substrate. Experiments were conducted to evaluate whether abalone acclimatised to the 401 tanks would show an improved response to the spawning induction protocol. A period of four days was chosen as a suitable time period for the broodstock to acclimatise to the 401 spawning tanks.

During acclimation the broodstock were fed the kelp *Ecklonia maxima*. Spawning induction trials using acclimated abalone were replicated on five separate occasions. These results were compared using an Anova with the results of five spawning induction replicates where the broodstock were not acclimated.

5.3.3 *H. midae* broodstock starvation

Ebert & Houk, (1989) reported in their spawning induction methods that abalone should be starved prior to a spawning induction trial. They did not provide any data to substantiate this finding. The following set of experiments were run to explore the possible advantage of starving *H. midae* broodstock prior to spawning induction: Fifteen separate trials utilising starved broodstock were compared using an Anova to fifteen trials of abalone that had not been starved. All the abalone used in this trial were acclimated for four days prior to spawning induction.

5.3.4 *H. midae* spawning relative to sunset time

Uki & Kikuchi (1984) suggested that a peak in ultra violet induced spawning associated with nightfall was a result of dark activated biochemical reactions. The nocturnal behaviour of *H. discus hannai* as shown by their feeding patterns and oxygen consumption supports their statement

that physiological activity increases from dusk to midnight (Uki & Kikuchi, 1975; Uki, 1981).

To explore the effect of exposing abalone to H_2O_2 at different times of the day, the H_2O_2 was decanted during six different time classes relative to sunset. These times are shown in Figs. 5.3a & b. The timing of H_2O_2 decanting was varied from 300 minutes before sunset to 300 minutes after sunset. Spawning induction trials for each time class were replicated three times. The standard spawning induction conditions were used for spawning induction as described earlier in this section. A total of 18 separate spawning induction trials were run.

5.3.5 The influence of lunar cycle on *H. midae* spawning

Some invertebrates spawning time in the wild has been linked to moon phase. The spawning of the giant clam *Tridacna maxima* (Munro, 1993) and *Trochus niloticus* (Hahn, 1989) in the wild has been linked to moon phase.

The possibility exists that natural *H. midae* rhythms could influence the success of spawning in an artificial environment. Experiments designed to establish the influence of the lunar cycle were run during 2 days either side of the New, Full and Neap moons. Likewise these experiments were replicated 4 times for each moon phase using the standard

spawning conditions. These results were compared using an Anova.

5.3.6 The influence of pH on *H. midae* spawning

Morse, *et al.* (1978) described raising the pH of the seawater in the *H. rufescens* spawning containers to 9.1 using tris (hydroxymethyl) aminomethane. The objective of these experiments were to establish the optimum protocol for spawning *H. midae*. Using the standard H_2O_2 method the pH was raised between 9.0 to 9.9 (Refer to Figs. 5.5). The seven pH classes chosen in this range were all replicated three times. A total of 21 separate spawning experiments were conducted to evaluate the effect of pH on *H. midae* spawning.

5.3.7 *H. midae* spawning relative to seawater temperature

The importance of seawater temperature for invertebrate reproduction is well documented (Uki & Kikuchi, 1984; Sandifer *et al.*, 1989; Beninger & Le Pennec, 1991). The seawater temperatures range from 12 to 19°C in the Gansbaai region. Spawning induction trials were therefore evaluated at 13, 15, 17, 17, 19 and 21°C. Spawning experiments were replicated three times at each temperature. The results of these experiments were compared using an Anova.

5.3.8 The impact of temperature variation on *H. midae* spawning

Oba (1964) showed that a temperature increase of 5°C over two hours followed by a decrease to ambient within half an hour was sufficient to induce spawning of *H. diversicolor supertexta*. Thermal shock has also been used in conjunction with other methods to induce haliotid spawning (Owen *et al.*, 1984; Hahn, 1989). Six experiments were designed to evaluate the effect of temperature manipulation in conjunction with the H_2O_2 spawning method. The temperature was lowered by 5°C in one set of experiments and as high as 5°C above ambient in a further set of experiments (refer to Figs. 5.7). The temperature was raised or lowered during the 220 minutes of exposure to H_2O_2. During decanting of the H_2O_2 the seawater temperature was adjusted back to ambient over thirty minutes. Each experiment was replicated three times.

5.3.9 Hydrogen peroxide concentration impact on *H. midae* spawning

Morse *et al.*, (1977) stated that *H. rufescens* spawned after being exposed to a 5 mM H_2O_2 concentration. Morse (1984) reported that the optimum H_2O_2 concentrations required to spawn other invertebrate species varies between 1 to 50 mM. Therefore the following seven H_2O_2 concentrations were chosen to induce spawning in *H. midae*: 1.5, 3, 7, 10, 12,

20, 25 mM. These experiments were all replicated three times each.

5.3.10 Hydrogen peroxide exposure time's influence on *H. midae* spawning

The final variable considered in these experiments is the length of time the broodstock abalone are exposed to H_2O_2. Morse (1984) suggested that abalone should be exposed to H_2O_2 for 2 to 2.5 hours at the appropriate temperature. Five experiments replicated three times each were used to test the impact of exposing broodstock to H_2O_2 from 100 to 300 minutes (refer to Figs. 5.8).

5.3: Results of the spawning experiments

The results of spawning experiments for *Haliotis midae* are presented in Figs. 5.1 to 5.9. These box and whisker plots show the impact of the nine independent variables on spawning induction. The interquartile range is only displayed where the number of replicates in the analysis allow such display. The result of each variable's effect on spawning percentage will be considered separately. Control results for animals which were not exposed to H_2O_2 have been excluded from these plots. Without exception, these control abalone failed to spawn. Refer to appendix 1 and 2 for a

table of the anovas used to compare the results for male and female abalone spawning respectively.

The mean spawning for acclimatised male abalone was 43 % as apposed to 12.5 % for males that were not acclimatised. Acclimatised female abalone spawned a mean of 13 % as compared with 8 % for females that were not acclimatised (Figs. 5.1a & b). However no significant differences were found between acclimatised and non acclimatised male or female abalone (t test, males, p = 0.118 and females, p = 0.572).

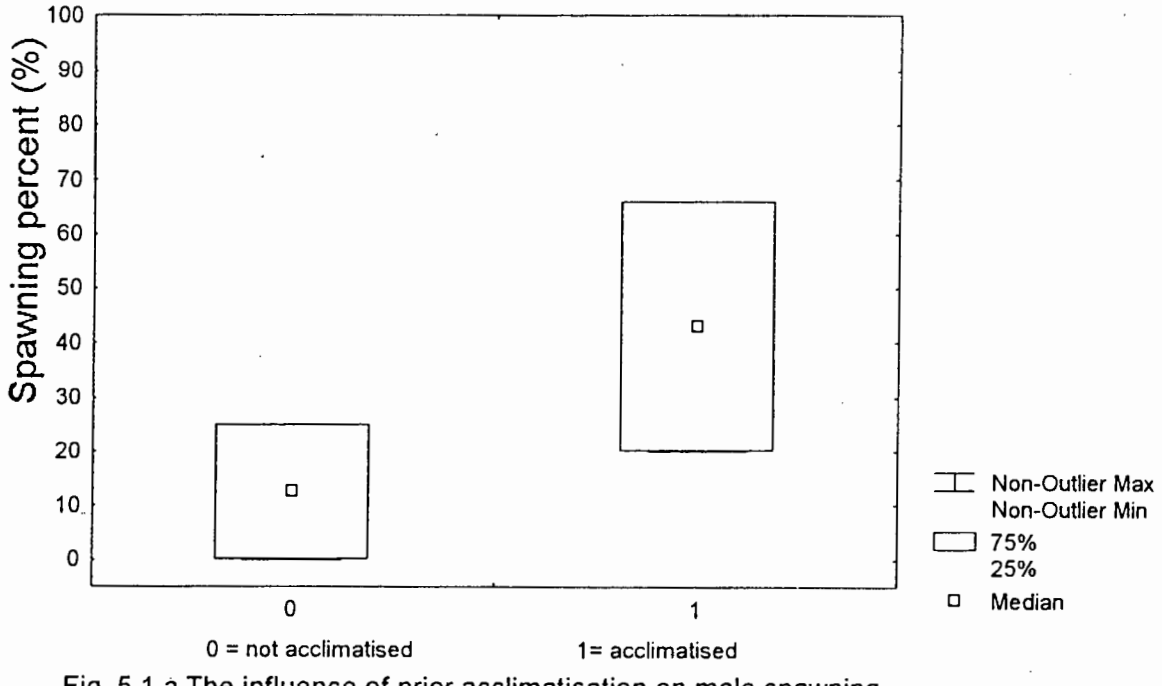


Fig. 5.1 a The influence of prior acclimatisation on male spawning

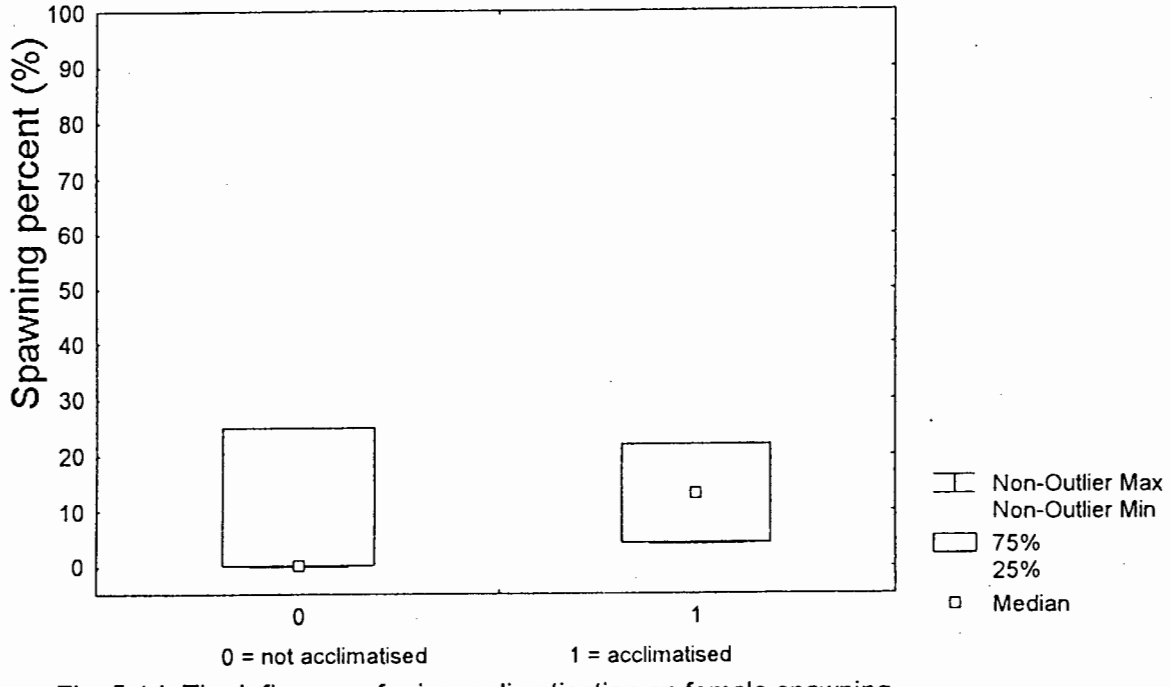


Fig. 5.1 b The influence of prior acclimatisation on female spawning

A significant improvement in spawning percentage was found for both males and females (t test, $p < 0.05$) that were starved prior to spawning (Figs. 5.2a & b). The mean spawning for starved male abalone was 27.25 % compared with 4.12 % for males that were not starved. Mean spawning of starved female abalone was 22.87 % as opposed to 4.37 % for females that had not been starved prior to spawning.

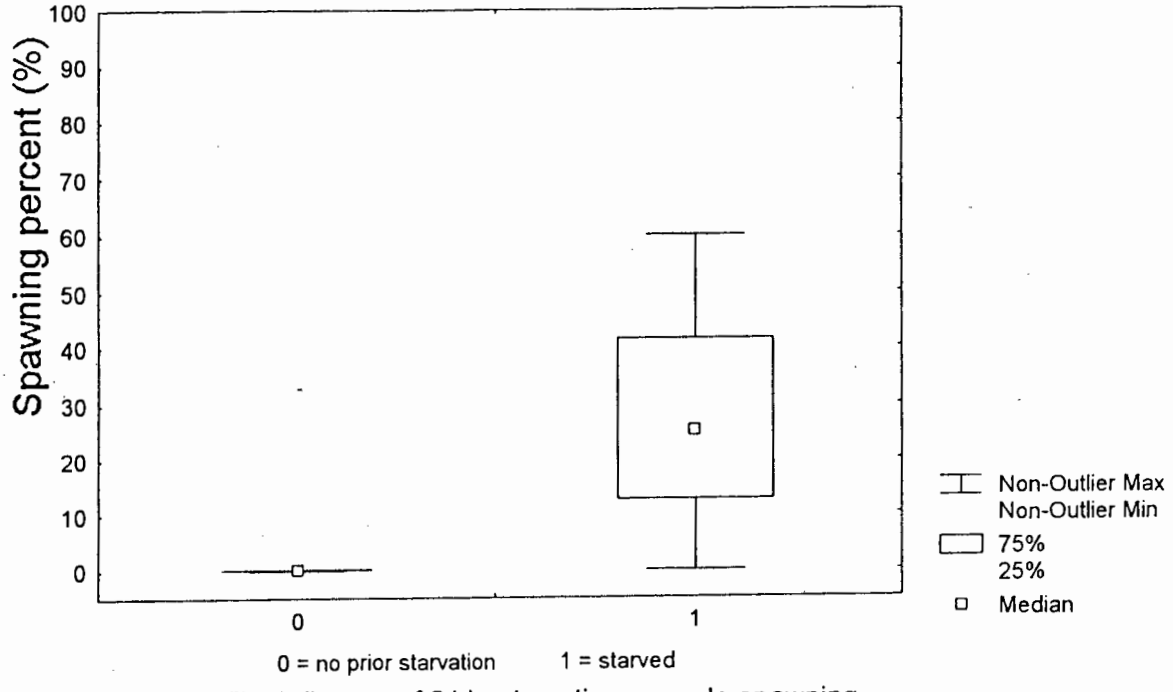


Fig. 5.2 a The influence of 24 hr starvation on male spawning

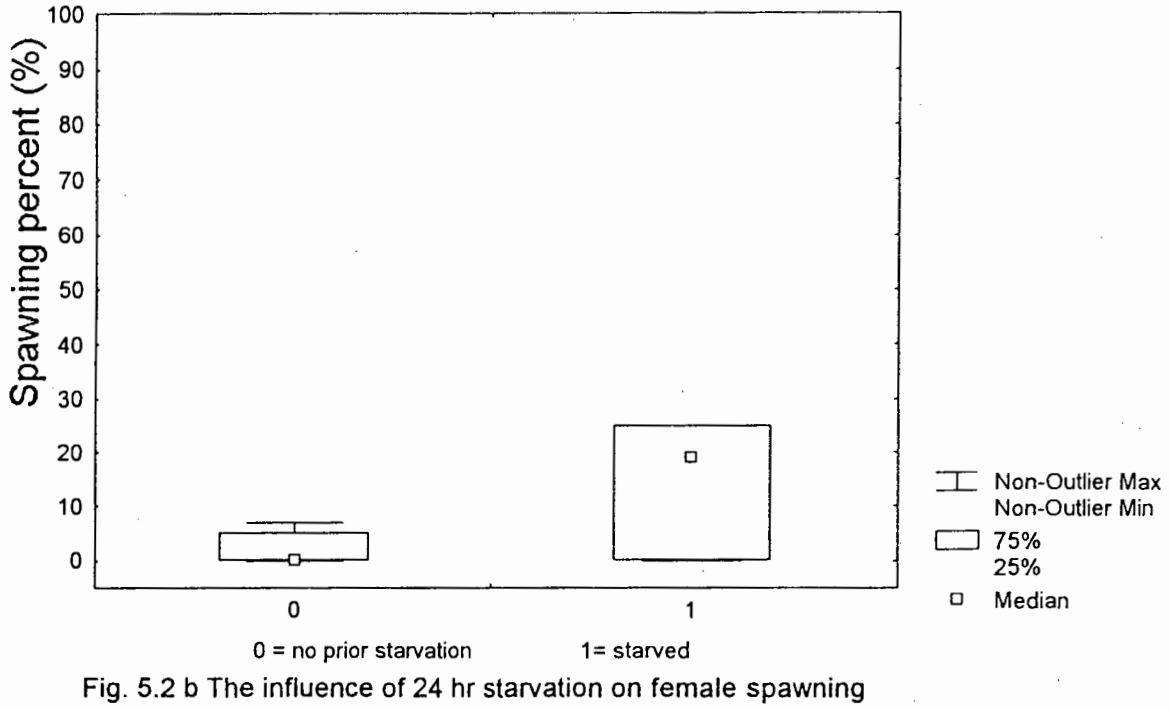


Fig. 5.2 b The influence of 24 hr starvation on female spawning

There was no significant difference in male or female *H. midae* spawning success between the various time classes ($p = 0.567$; $p = 0.437$). A debatable trend for improved spawning in males was observed for experiments conducted from -49 to 0 minutes before sunset (Fig. 5.3a). The highest percent spawning for females (25%) was observed in the time class -99 to -50 minutes prior to sunset (Fig. 5.3b).

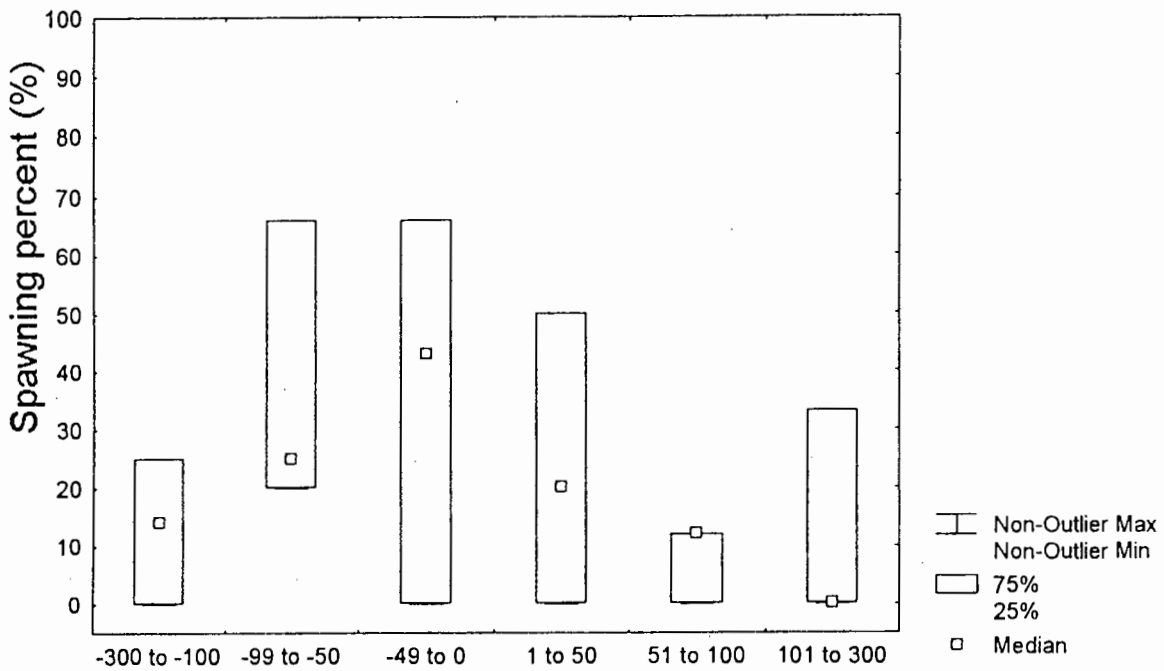


Fig. 5.3 a Male spawning relative to sunset time (0)

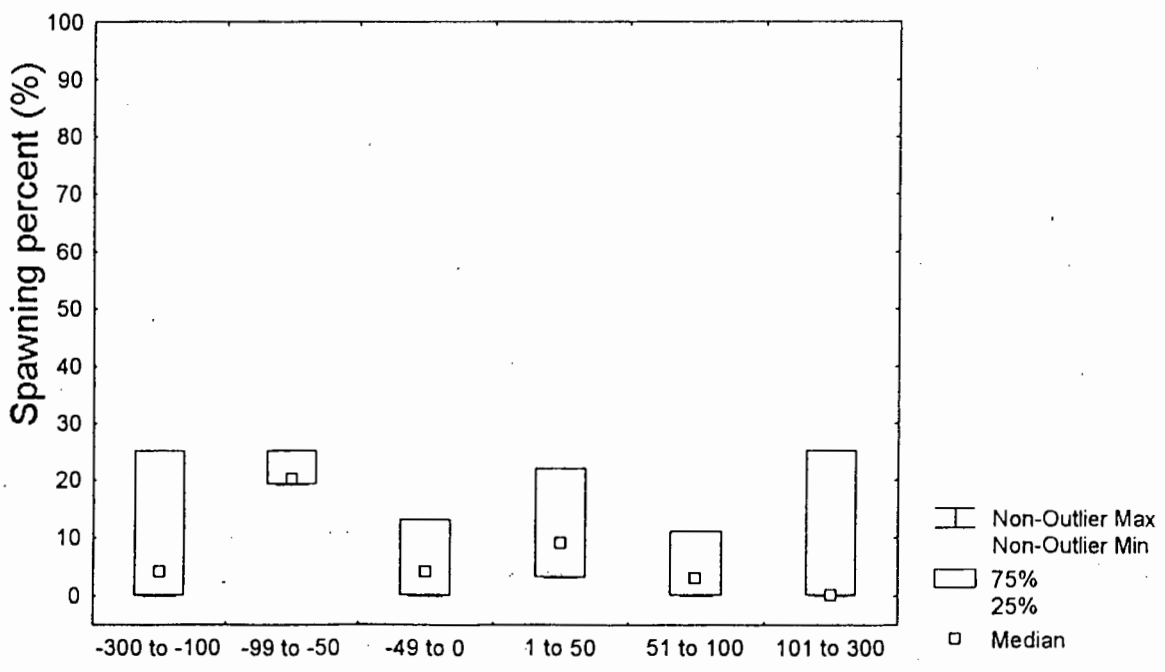


Fig. 5.3b Female spawning relative to sunset time (0)

Spawning induction experiments were compared between New (1), Neap (2) and Full moon (3) (Figs. 5.4a & b). An Anova was used to test if there were any significant improvements in spawning success between the 3 lunar phases. No difference was found for either male or female abalone (males, $f = 0.180$, $p = 0.837$; female $f = 0.408$, $p = 0.677$).

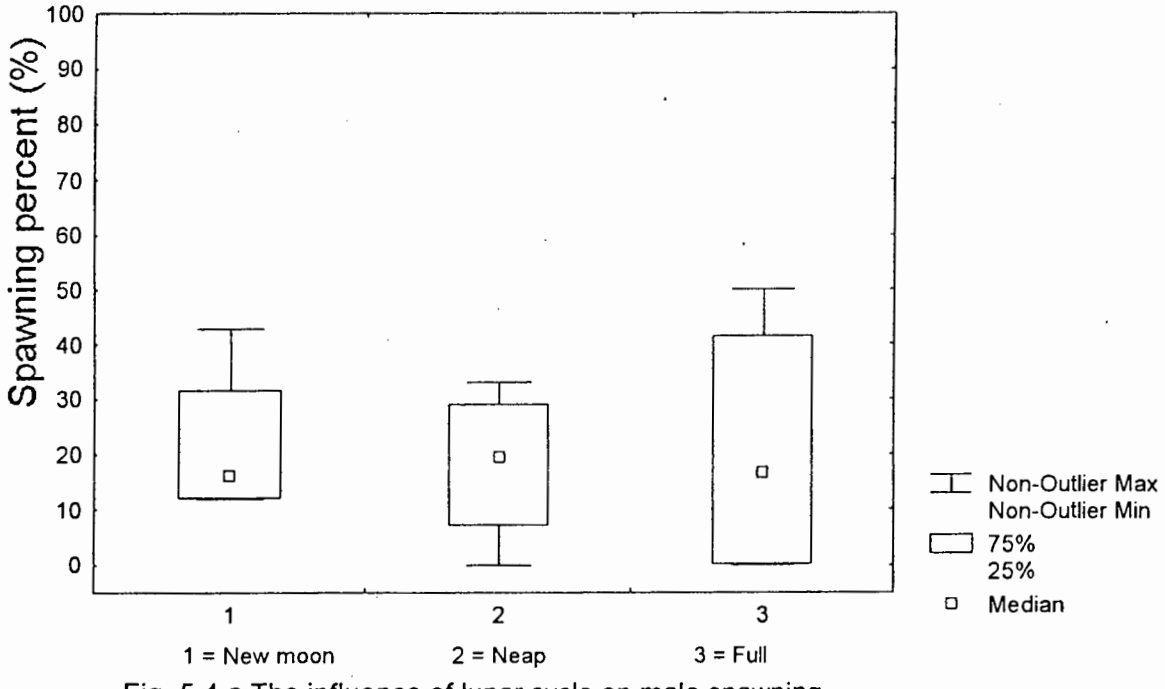


Fig. 5.4 a The influence of lunar cycle on male spawning

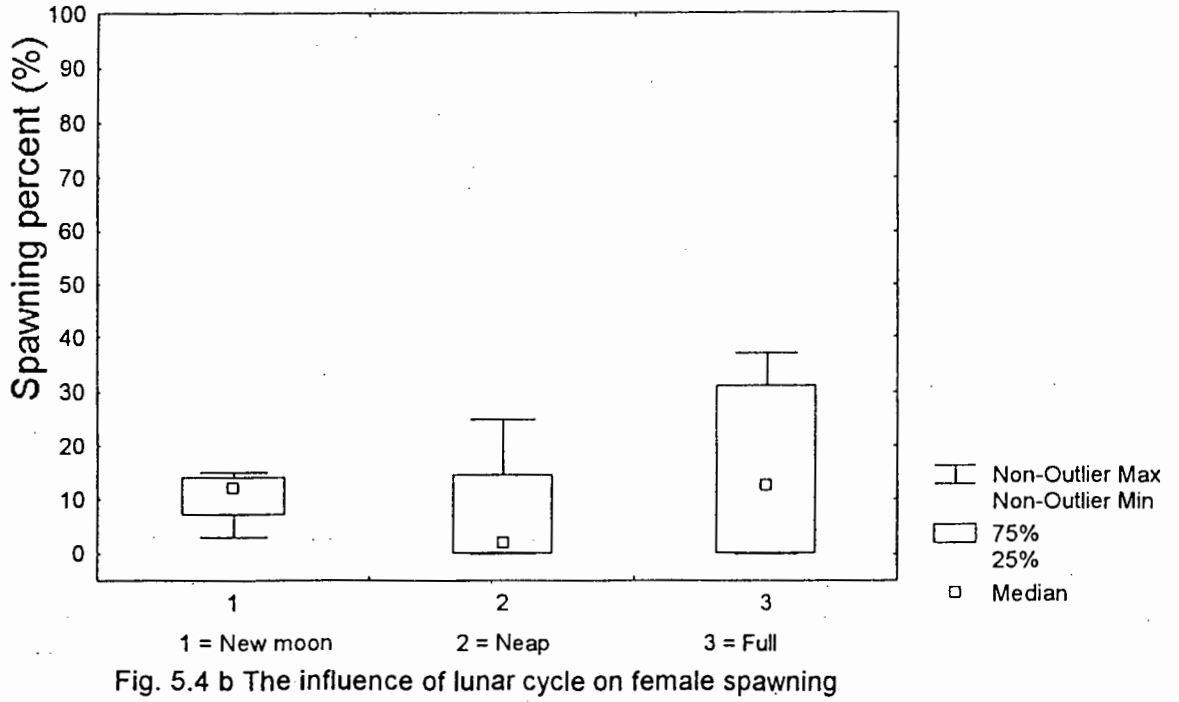


Fig. 5.4 b The influence of lunar cycle on female spawning

Figs. 5.5a and 5.5b show the relationship between pH and percentage spawning for male and female *H. midae*. An almost significant result was obtained for male *H. midae* pH results (Anova F = 2.36, p = 0.087). The highest average spawning percent was observed for male *H. midae* exposed to a pH of 9.6 (Fig. 5.6a). The results for female pH abalone trials showed no significant difference between spawning success at different levels of pH (Anova F = 1.28; p = 0.326). The trend for female spawning also appeared to improve slightly in the 9.6 pH class (Fig. 5.5b)

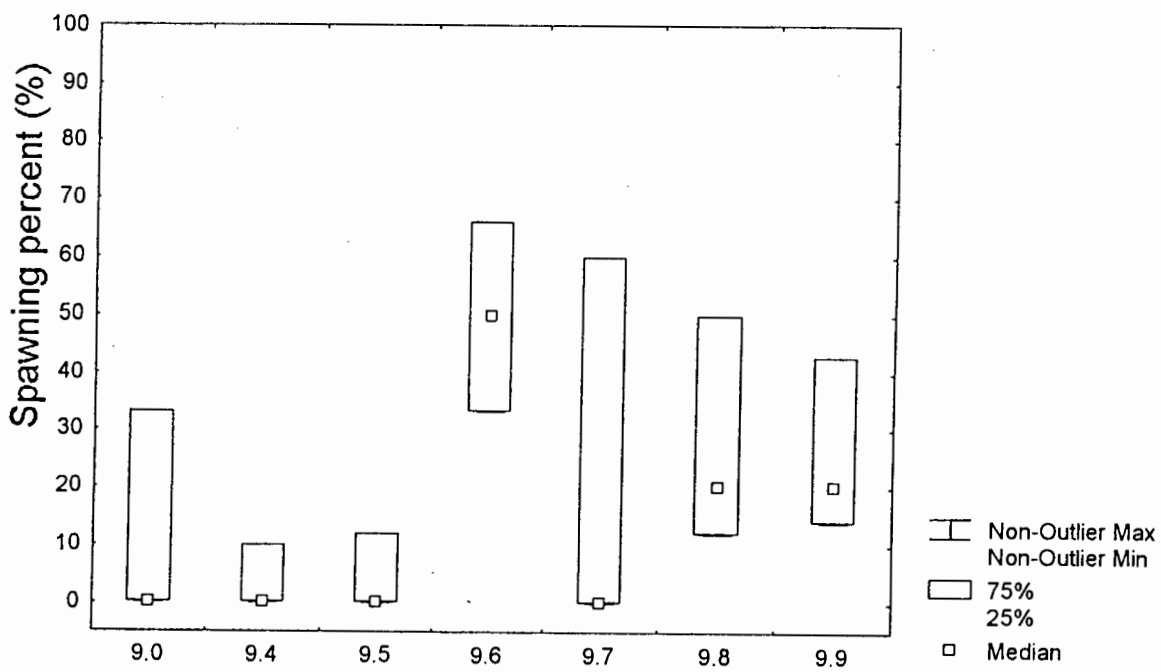


Fig. 5.5 a The influence of pH on male spawning

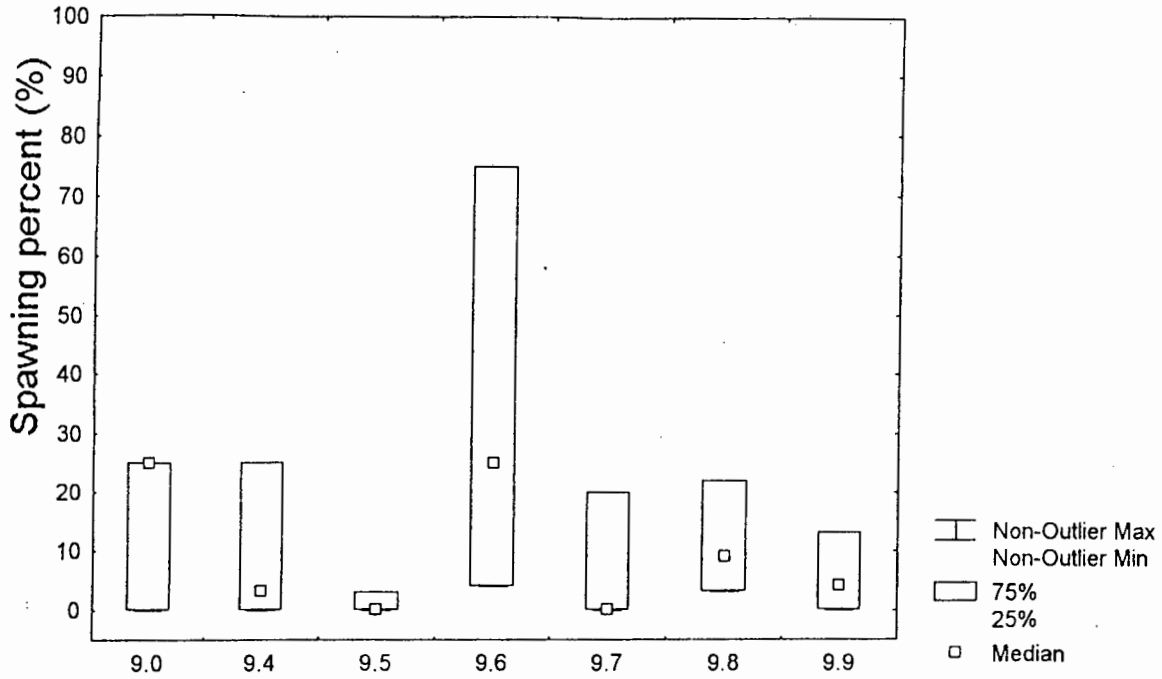


Fig. 5.5 b The influence of pH on female spawning

The relationship between a constant temperature regime and spawning success is shown in Figs. 5.6a and 5.6b. The Anova for both the males (Anova F = 2.221 p = 0.140) and females *H. midae* (Anova F = 1.829 p = 0.199) showed no significant difference for spawning success between any of the temperature classes. It is, however, worth noting that the highest average spawning percent for males and females was found in the 17°C temperature (refer to Figs 5.6a & b).

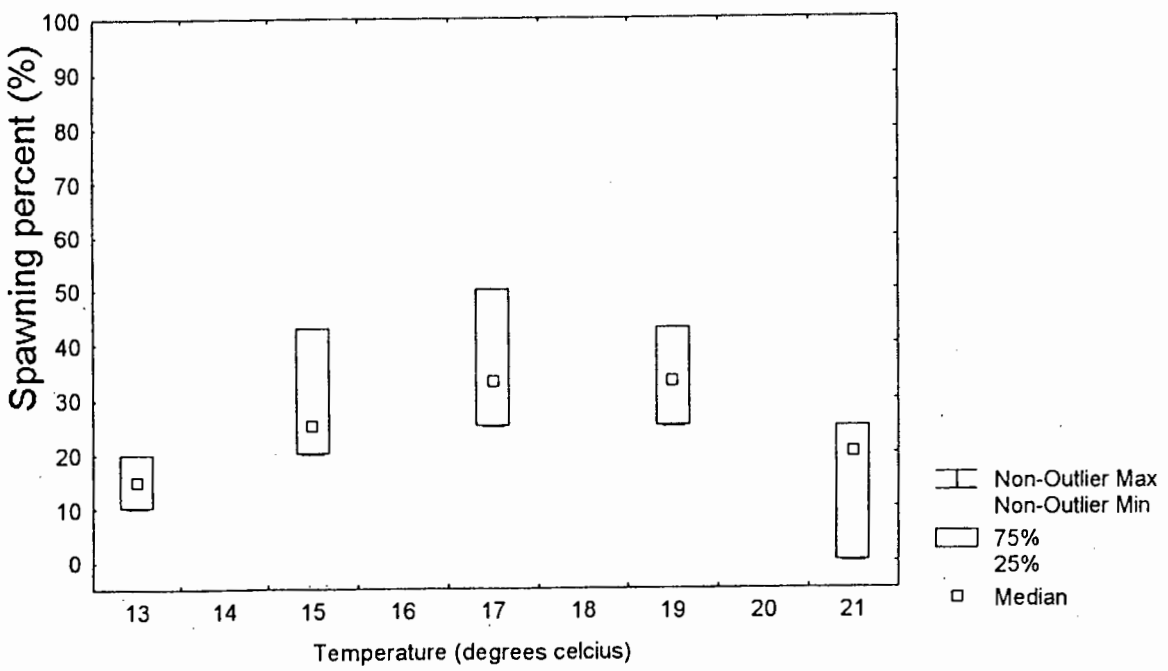


Fig. 5.6a Male spawning relative to seawater temperature

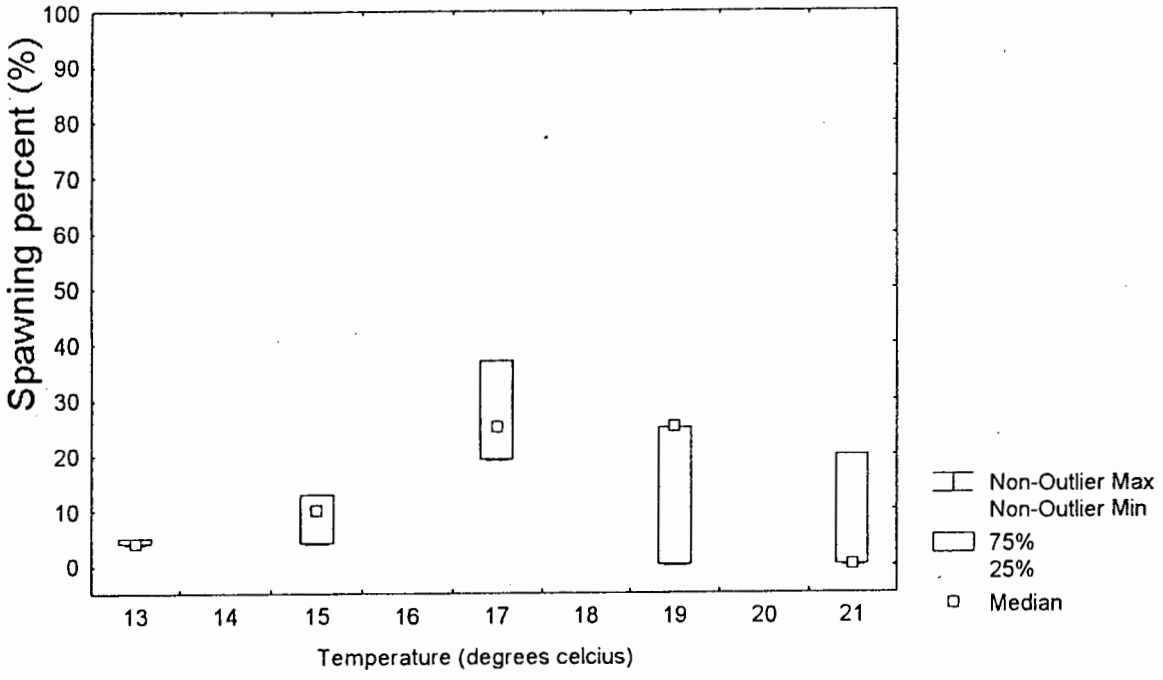


Fig. 5.6b Female spawning relative to seawater temperature

Figs. 5.7a & b shows the results of experiments designed to establish whether a fluctuation of temperature improves spawning. Anovas (for males and females separately) were used to establish if there was a significant difference in the spawning success for temperature fluctuation trials. No significant differences were found between any of these groups for both male ($F = 0.497$ $p = 0.773$) and female *H. midae* ($F = 0.272$ $p = 0.919$). The highest average spawning rate was observed for males and females in the $+3^{\circ}C$ temperature fluctuation range (Fig. 5.7a). Improved spawnings of females was observed in the -5 and $+3^{\circ}C$ temperature experiments (Fig. 5.7b).

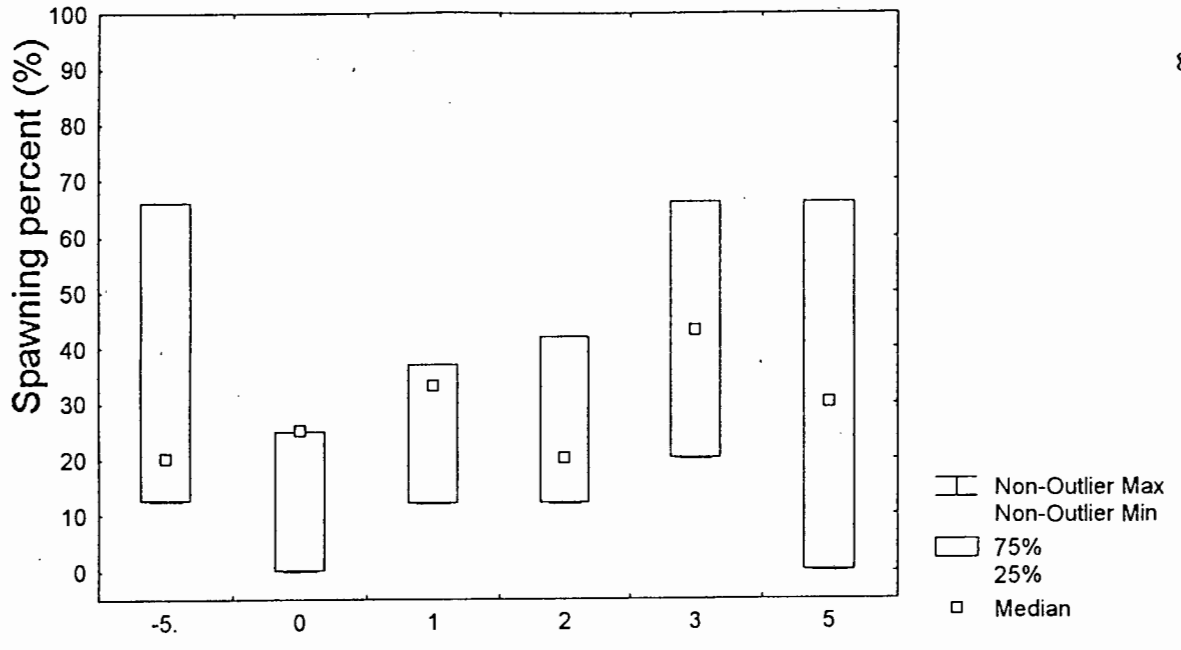


Fig. 5.7a Male spawning relative to a temperature fluctuation

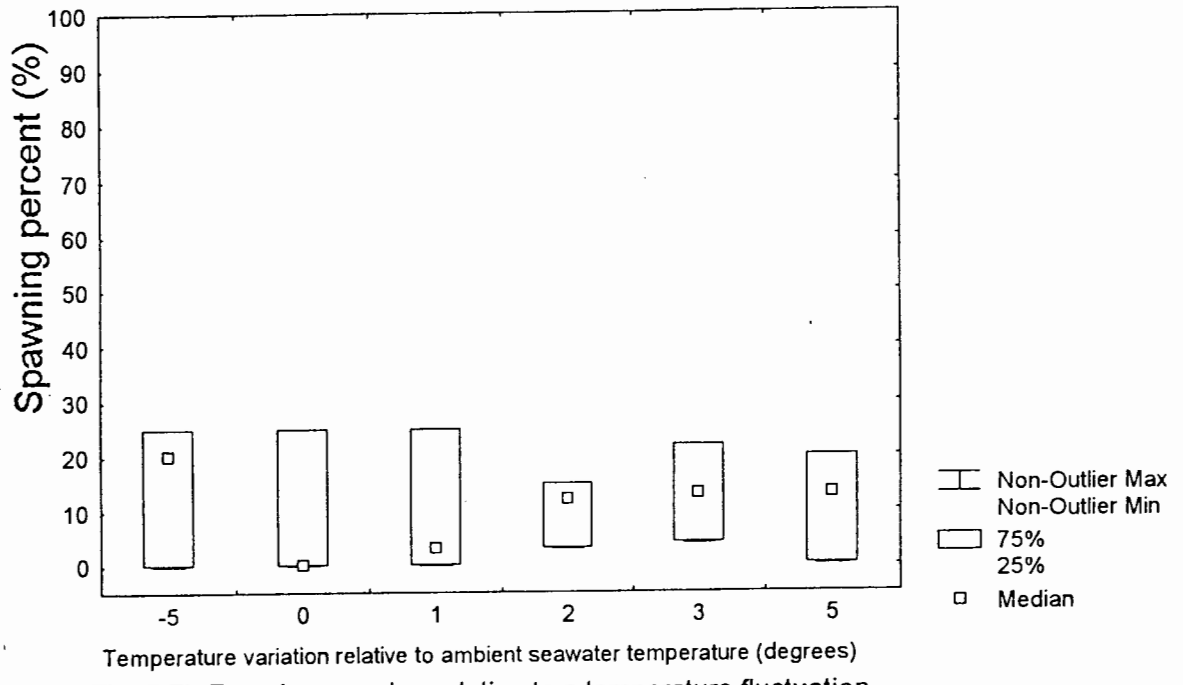


Fig. 5.7b Female spawning relative to a temperature fluctuation

The results for the experiments in which hydrogen peroxide concentration was varied are shown in Figs. 5.8a & b. An Anova gave a non-significant result for females when the hydrogen peroxide concentration groups were compared (Anova $F = 2.693$ $p = 0.059$). Similar analyses comparing the same hydrogen peroxide groups for males gave a significant difference between the groups (Anova $F = 5.353$ $p = 0.005$).

A tukey test was subsequently used to establish which of the groups were different. The 25 mM group gave the highest male spawning percentage of 55.3, followed by the 12 mM H_2O_2 group with an mean of 32.0 (see Fig. 5.8a). The results of hydrogen peroxide concentrations show that both male and female *H. midae* were successfully spawned over the 7 to 25 mM concentration (see Figs. 5.8a & 5.8b). The highest average spawning percent for female *H. midae* was in the experiments using a concentration of 12 mM hydrogen peroxide (Fig. 5.8b).

TABLE 5.9 Multiple range analysis for male *H. midae* subjected to different concentrations of hydrogen peroxide

H_2O_2	Trials	Male *H. midae* spawning		
mM	no	Tukey homogen-ous groups	Mean %	SE mean
1.5	3	1	0	0
3	3	1	0	0
7	3	2	52.3	19.3
10	3	2	25.0	17.9
12	3	2	32.0	9.4
20	3	2	20.0	28.3
25	3	2	55.3	21.8

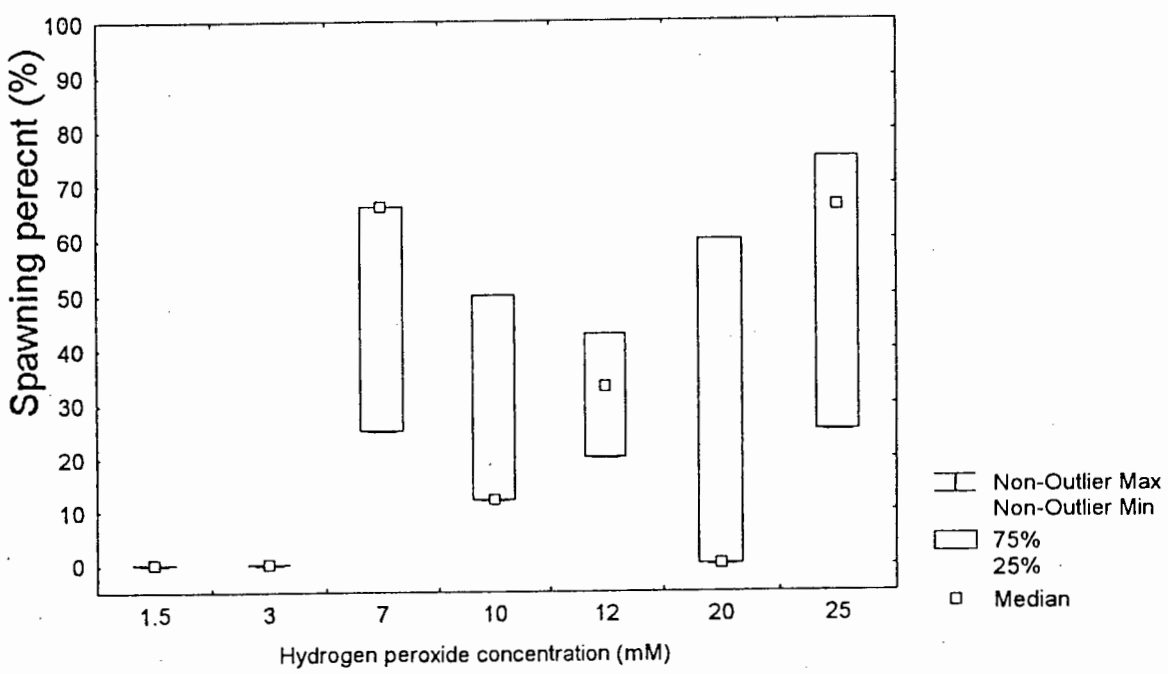


Fig. 5.8a Hydrogen peroxide (conc) impact on male spawning percent

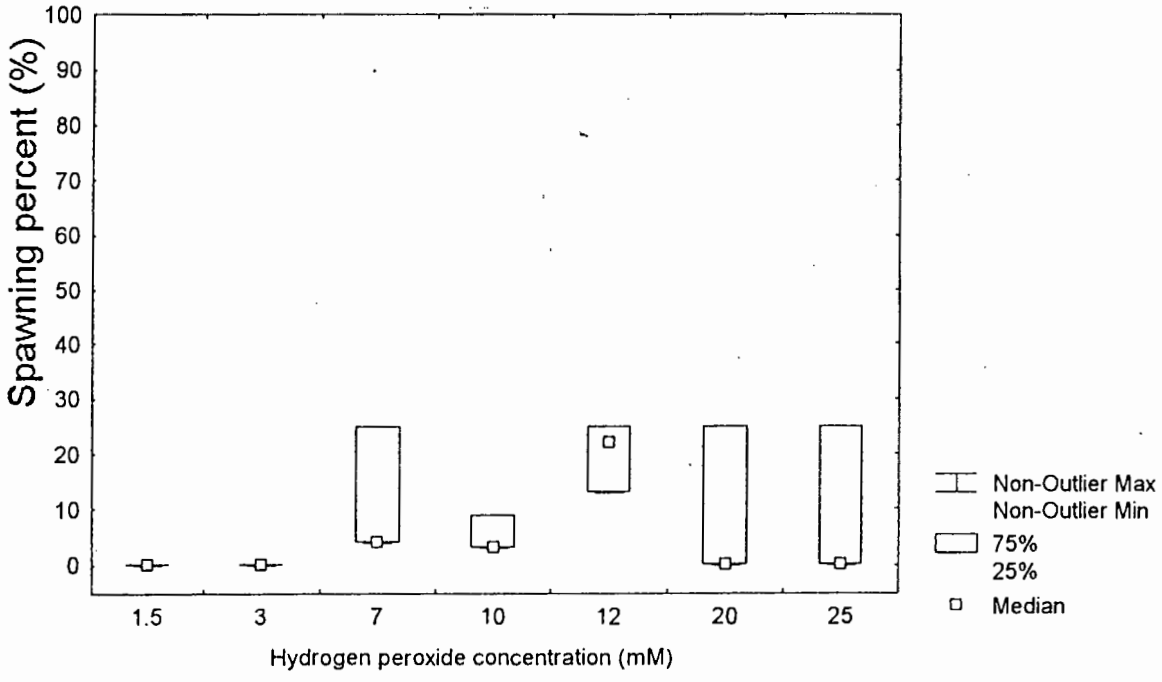


Fig. 5.8b Hydrogen peroxide (conc) impact on female spawning percent

The duration of hydrogen peroxide exposure versus male and female percentage spawning is shown in Figs. 5.9a & b. A non-significant result was obtained for both males and females when the exposure times shown Figs. 5.9a & b were compared (Anova males $F = 1.939$ $p = 0.180$ and females $F = 0.485$ $p = 0.747$). The highest average spawning for males was found in the 220 minute exposure group (Fig. 5.9a). The highest average spawning achieved for females was also obtained at a hydrogen peroxide exposure of 220 minutes (Fig. 5.9b).

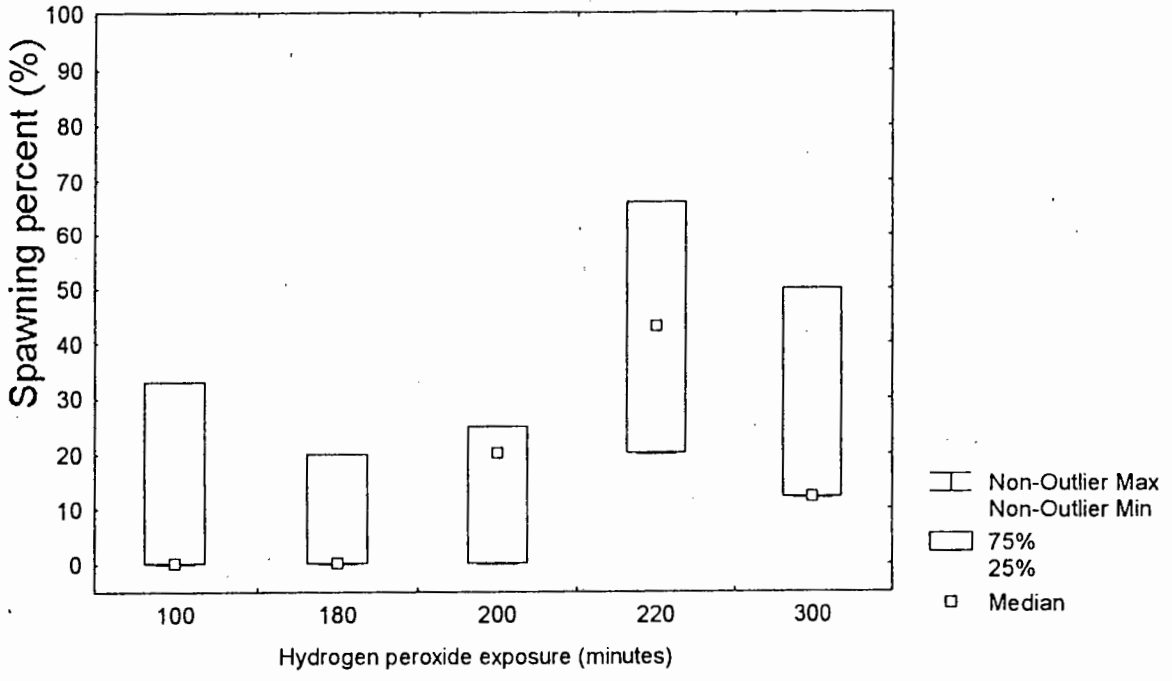


Fig. 5.9a Hydrogen peroxide exposure time relative to male spawning

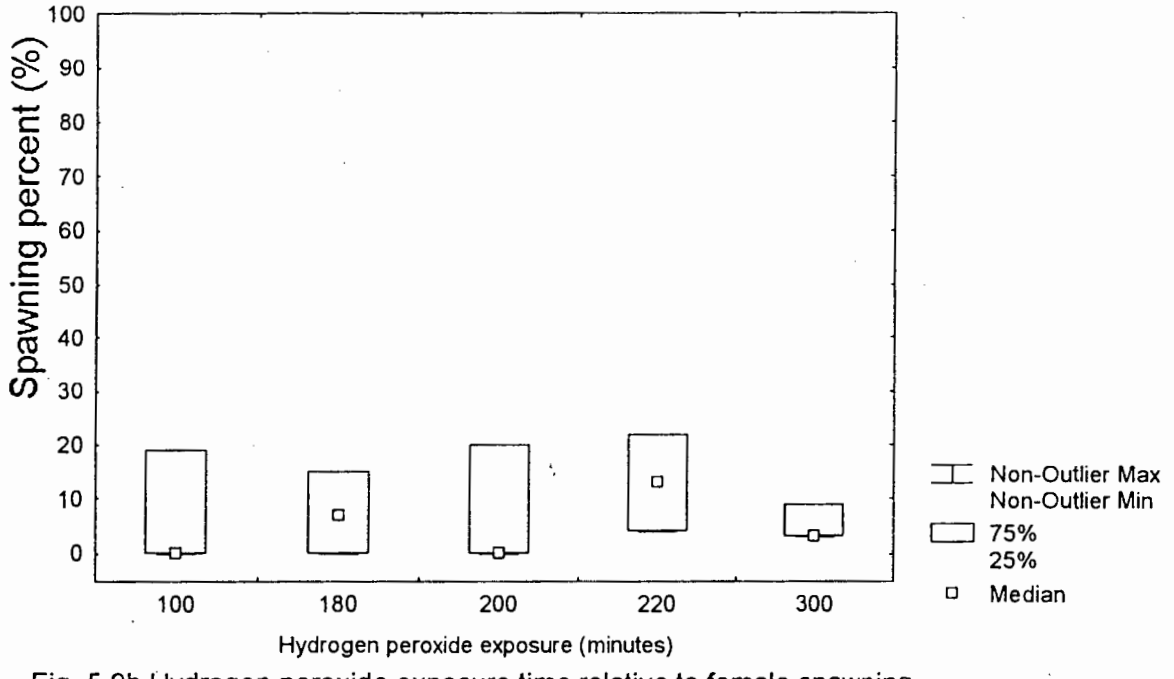


Fig. 5.9b Hydrogen peroxide exposure time relative to female spawning

5.4: Discussion

Recognising the key factors regulating reproduction is a primary concern of mariculture researchers. For a number of marine species, the potential for culture is limited by lack of scientific information on reproduction. A reliable method of inducing spawning of *H. midae* must be an important contribution towards the commercial culture of this species. The results obtained here highlight the variables that do (or do not) appear to influence the success of artificial spawning. It must be emphasised again that no spawning was accomplished by animals that were not subjected to H_2O_2 ("control" animals). The effects of all the variables thus need to be judged in terms of this impact on spawning success induced by H_2O_2.

There appeared to be no benefit of acclimatising female *H. midae* to the spawning containers (Fig. 5.1b). This was however not the case with the males (Fig. 5.1a). Although not statistically significant at the 5% level the mean spawning was higher for the acclimatised male abalone (Fig. 5.1a). The lower average spawning for females could possibly have masked the benefit of prior acclimatisation.

The significant improvement in spawning success achieved by starvation prior to spawning could be due to the reduction of food in the digestive tract. An empty gut would reduce

the possible pressure placed by the digestive system on the reproductive tract. This would then enhance the free release of gametes after exposure to the spawning stimulus hydrogen peroxide.

The physiological activity of abalone accelerates from sunset to midnight (Uki & Kikuchi, 1984). This is to be expected as abalone are nocturnal, which is apparent by their feeding patterns and daily oxygen consumption rhythms (Uki & Kikuchi, 1975; Uki, 1981). Further Uki & Kikuchi (1984) showed that optimum spawning was achieved when the ultra violet (uv) stimulus was provided 1 hr 20 min prior to sunset for males and 1 hr 45 min for females. Hahn (1989) reached a similar conclusion for inducing *H. rufescens* to spawn. He advised that uv stimulation should commence 1 hr 30 min before sunset.

Hahn (1989) also observed that induced spawning begins even if the lights are left on past the entrained dark phase. He suggests that this indicates an endogenous photo-periodic rhythm and not that the actual ending of the light phase induces spawning. Hahn (1989) reasons further that it is the beginning of the light period and not the dark period that starts the cascade to spawning in the wild. Grant (1989) found that gravid *H. rubra* also spawned at dusk even when the uv stimulation was activated 12 to 15 hours prior to sunset.

The results of experiments investigating the timing of spawning induction were not conclusive. It is interesting to note that the highest median for male spawning was achieved when H_2O_2 was rinsed from the spawning containers -49 to 0 minutes prior to sunset.

Fish belonging to the family Sciaenidae for example spotted sea trout (*Cynoscion nebulosus*) and red drum (*Sciaenops ocellatus*) are also reported to spawn at dusk in the wild and under laboratory conditions (Brown-Peterson, *et al.*, 1988). It is possible that offspring survival improves and predation of spawning adults in the wild is reduced when spawning is initiated at dusk.

From the results it is clear that, in an artificial environment, lunar cycle does not significantly influence the success of artificial spawning of *H. midae*. In the wild lunar cycles have been reported to influence the spawning of giant clams (*Tridacna* sp.) in tidal lagoons (Munro *et al.* 1993). The artificial environment in which the broodstock were held and the administration of H_2O_2 may have reduced any biological response of *H. midae* to the lunar cycles.

Morse *et al.* (1977) showed that induction of spawning is dependent on the alkalinity of the seawater and the concentration of H_2O_2. The alkalinity requirement improves

the base-catalysed decomposition of H_2O_2 to an electronically active form of oxygen.

Male *H. midae* seemed to require a pH > 9.5 for H_2O_2 to initiate spawning, but spawned at values as high as 9.9 (in highest pH tested). Females were successfully induced to spawn in the pH range of 9.0 to 9.9. This range includes the reported pH of 9.1 required to induce *H. rufescens* to spawn (Morse, 1984).

Morse (1984) suggested that H_2O_2 based spawning protocols refined for new *Haliotis* species are developed within the species temperature tolerance range. Morse *et al.* (1978) in their description of the H_2O_2 spawning technique infer that *H. rufescens* spawning can be induced in the temperature range of between 12 to 18 ^{o}C. In this study *H. midae* were successfully spawned through the temperature range of 13 to 21 ^{o}C, although the frequency of spawning by males did tend to decline at the higher end of this range (Fig. 5.6a). Tong *et al.* (1992) were able to spawn *H. iris* also using H_2O_2 over a temperature range of 12 to 18 ^{o}C. It would appear that further research aimed at identifying the exposure time above a critical biological zero would go a long way to improving the response of conditioned abalone to a spawning stimulus and elucidating the exact role temperature plays in *H. midae* gametogenesis.

Newman (1967) concluded that *H. midae* found in areas where annual temperatures fluctuate greatly, spawn more intensely than those found in colder waters along the West coast. An improved spawning success for abalone subjected to less than 3 ^{o}C thermal shock as opposed to greater temperature fluctuations has been recorded for *H. discus hannai* (Ikenoue & Kafuku, 1992). Fluctuations of temperature greater than 3 ^{o}C over a period of 30 minutes probably induce stress, rather than stimulating the spawning response. However *H. midae* were successfully induced to spawn when exposed to a drop or rise in temperature as much as 5 ^{o}C (Fig. 5.7). Owen, *et al.* (1984) noted that spontaneous spawnings of laboratory *H. rufescens* populations occurred when the ambient seawater temperature fluctuated. Temperature manipulations are commonly used to induce other invertebrates to spawn, e.g. oysters, mussels and sea-urchins (Lim, 1991).

Male and female *H. midae* were successfully spawned using similar concentrations (7 mM) as those used by Morse *et al.* (1977) to spawn *H. rufescens* (Figs. 5.8a & b). *H. rufescens* was noted to spawn at H_2O_2 concentrations of 5 and 7,5 mM (Morse *et al.*, 1978; Morse *et al.*, 1979). The broad concentration range of hydrogen peroxide successfully used to induce spawning of *H. midae*, is somewhat perplexing. These results certainly suggest that if abalone are well

conditioned and other variables are optimised, spawning can be achieved using hydrogen peroxide over a range of various concentrations between 7 to 25 mM (Figs. 5,8a & b).

Morse *et al.* (1976), using the hydrogen peroxide method to induce spawning, found that 98% of gametes released were viable. Gametes are considered to be viable when fertilisation and normal larval development occurs. Morse *et al.* (1978) emphasised that hydrogen peroxide concentrations required to induce spawning varies between different haliotids and other invertebrate species.

Morse, *et al.* (1978) advise that *H. rufescens* held at 12 to 18 ^{o}C should be exposed for 2.5 hours to H_2O_2. Further, they emphasise that the optimal concentration of H_2O_2 and the time required for induction of spawning varies for different species. Morse *et. al.* (1978) also suggest that when spawning a new species a range of H_2O_2 concentrations should be tried and the H_2O_2 should not be decanted from the containers prior to spawning. The main reason for rinsing the containers from H_2O_2 is to prevent the inactivation of the gametes by the caustic H_2O_2.

As shown in the results there was no significant improvement in spawning for male and female *H. midae* exposed longer than 100 minutes. It would appear that H_2O_2 exposure of greater than 100 minutes for males and females was sufficient time

to activate the prostaglandin endoperoxide synthetase precursors responsible for initiating the cascade of events leading to synchronised spawning (Morse *et. al.*, 1976).

The key factor of this spawning induction technique is the use of hydrogen peroxide. It is certainly worth considering closely the role H_2O_2 plays in inducing spawning of *H. midae*. Hydrogen peroxide activates the prostaglandin-dependent gamete release in haliotids (Morse, 1984). This is confirmed by results showing that the addition of Aspirin (Acetyesalicylic acid) 15 minutes prior to the addition of H_2O_2 effectively inhibits spawning (Morse *et al.*, 1976). Asipirin is known to specifically inhibit prostaglandin release (Morse *et al.*, 1976).

The relatively high concentrations of hydrogen peroxide required to consistently induce spawning of *H. midae* (7 to 25 mM from Figs. 5.8a & b) mitigates against using the uv irradiated seawater technique. Morse *et al.* (1984) speculate that uv irradiated seawater results in photolytic generation of electronically active oxygen similar, or identical, to that produced by the addition of H_2O_2 to alkaline seawater. However it would be impractical to use uv to generate sufficient electronically active oxygen to be equivalent to the yield from high concentrations of H_2O_2. Furthermore, the hydrogen peroxide method is a cheaper and more reliable method than the uv treatment protocol (Morse, 1984).

The hydrogen peroxide concentration for male *H. midae* and prior starvation for both male and females were thus the only variables for which statistically significant ($p \leq 0.05$) differences were observed between classes of a specific treatment. The high variation of spawning success observed in all the trials may be attributed to differences in gonad condition of abalone used in these trials. The success of spawning induction relies heavily on selecting gravid broodstock, and this selection is not always accurate.

Data collected from experiments which did not give statistically significant results are still of great value however. The temperature, hydrogen peroxide concentration, peroxide exposure time and pH results are important because they give a good indication of the range of each variable in which spawning will be successful.

5.5: Conclusion

These experiments provide insight into the variables regulating artificial spawning of *H. midae*. The conditions for successfully inducing *H. midae* broodstock to spawn artificially in this study are the following:

1) Application of H_2O_2 is essential to elicit reliable spawning: no abalone spawned in control tanks from which H_2O_2 was excluded. Abalone also did not spawn

in these experiments when exposed to UV irradiated seawater;

2) Abalone should preferably be starved 24 hours prior to commencing with spawning induction;
3) No significant effect on male and female spawning was observed during experiments evaluating the effect of decanting H_2O_2 relative to sunset;
4) It would appear that pH should be held between 9.6 and 9.9 for males. Females were successfully spawned over the evaluated pH range of 9.0 to 9.9.
5) Lunar cycle did not influence male or female *H. midae* spawning success in the laboratory.
6) *H. midae* can be successfully induced to spawn at least within the temperature range of 13 to 21 ^{o}C;
7) A final concentration of between 7 and 25 mM hydrogen peroxide can reliably be used to induce *H. midae* to spawn;
8) Exposure to hydrogen peroxide for between 100 and 300 minutes should induce spawning;

Finally care must be taken to select gravid broodstock, hence the importance of using the visual gonad assessment technique described in chapter four.

CHAPTER 6: THE TOXICITY OF AMMONIA, COPPER AND CHLORINE FOR Haliotis midae LARVAE

6.1 Introduction

The rearing and handling of *Haliotis* larvae is an important step in the aquaculture of abalone. The planktonic larvae are very susceptible to bacterial infections and require careful maintenance to avoid high mortalities (Hahn, 1989). The lecithotropic (not actively feeding) nature of *Haliotis* larvae greatly simplifies the rearing process (Hooker & Morse, 1985). In addition, *Haliotis* larvae can absorb amino acids from the surrounding seawater (Manahan & Jaekle, 1992). This attribute allows the abalone grower to avoid the time-consuming production of phytoplankton that is necessary for the culture of fish, prawns and other molluscs (Rojas, *et al.*, 1991). Although abalone larval rearing is relatively easy and of relatively short duration (8 to 14 days), water quality and tank hygiene are critically important.

Marine invertebrate larvae are sensitive to many common building materials and cleaning agents (Huguenin & Colt, 1989). Poor selection of hatchery materials, careless use of cleaning agents and poor tank maintenance can also result in high larval fatalities. This study attempts to identify the sensitivity of *H. midae* larvae to ammonia, chlorine and copper. Ammonia is one of the toxic nitrogenous metabolites

associated with faecal decomposition and a primary product of excretion by abalone. Chlorine is an important cleaning agent commonly used in the aquaculture industry to sterilise hatchery equipment. Finally, copper is an FDA-approved algaecide and is also used as a parasiticide (Griffin, 1992). Plumbers still commonly use copper piping in household water distribution systems.

Toxicity tests developed for marine waters often utilise marine invertebrate embryos or sperm (Martin, *et al.*, 1981; Thain, 1990, Dinnel, *et al.*, 1982) and larval stages (Sullivan & Ritacco, 1985; Widdows, 1993). *H. midae* larvae were chosen instead of using abalone embryos for these toxicity experiments. Results using larvae should be more applicable to abalone culturists.

6.2 Methods

6.2.1 Larval Rearing

The fertilised eggs collected after inducing adult abalone to spawn were siphoned onto a submerged 80 µm nylon screen. The eggs were gently rinsed for 15 minutes to remove excess sperm (Morse *et al.*, 1979). Following rinsing, the fertilised eggs were placed in clean 20 l plastic buckets, with fresh static one micron filtered seawater. After hatching out, the larvae were separated from the egg casings

by carefully siphoning the larvae near the surface of the bucket into another bucket. The larvae were also held under static conditions in a temperature controlled room (16°C) and transferred to clean containers every second day.

6.2.2 Toxicity tests

Larvae used in all the toxicity experiments were post-torsion larvae (Plate 6.1). An average of 100 larvae were placed in separate 200 ml test tubes prior to adding any of the stock solutions tested in this study.

H. midae larvae were exposed to chlorine concentrations of 5.69 mg/l, 0.569 mg/l and 0.056 mg/l. At each chlorine concentration, three replicates were used with one control. The controls received an equivalent volume of seawater with no chlorine. Chlorine in seawater can be present as free available chlorine and as combined chlorine. Free chlorine is present as hypochlorous acid and or hypochlorite ion. Combined chlorine exists as monochloramine, dichloramine, nitrogen trichloride and other derivatives. These two forms can be measured together as the total available chlorine. The method used to measure chlorine involved the addition of iodide which is oxidised by combined chlorine to iodine. The iodine reacts with DPD (N,N-diethyl-p-phenylenediamine) along with free chlorine present in the sample to form a red colour which is proportional to the total chlorine

concentration. The concentration of chlorine was determined using a Hach DR 2000 spectrophotometer. This spectrophotometer is a microprocessor controlled instrument which is precalibrated for 120 colorimetric measurements.

Larval mortalities were recorded every 30 minutes using a stereo microscope. The time taken for 50 percent of the larvae to die was recorded for the replicates. This time is referred to as the LT_{50} (Hahn, 1989). The results between replications were compared using an analysis of variance (Zar, 1984).

A similar procedure was used to establish the toxicity of copper at the following concentrations in mg/l: 3.06, 1.25 and 0.125. The results were compared using an analysis of variance. The porphyrin method was used to measure the copper content in the seawater samples. The porphyrin indicator forms a bright yellow coloured complex with any free copper in the sample. The concentration of copper was determined also using the Hach DR 2000 spectrophotometer.

The salicylate method was used to measure ammonia. This method uses the reaction ammonia compounds have with chlorine to form monochloramine. In turn monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidised in the presence of a sodium nitroprusside catalyst to form a blue coloured compound. The

blue colour is then masked by the yellow colour from the excess reagent present to give a final green coloured solution. The concentration of ammonia was also measured using the Hach DR 2000 spectrophotometer. The same strategy was used to evaluate the toxicity of ammonia at 5.0 mg/l, 0.5 mg/l and 0.05 mg/l to *H. midae* larvae.

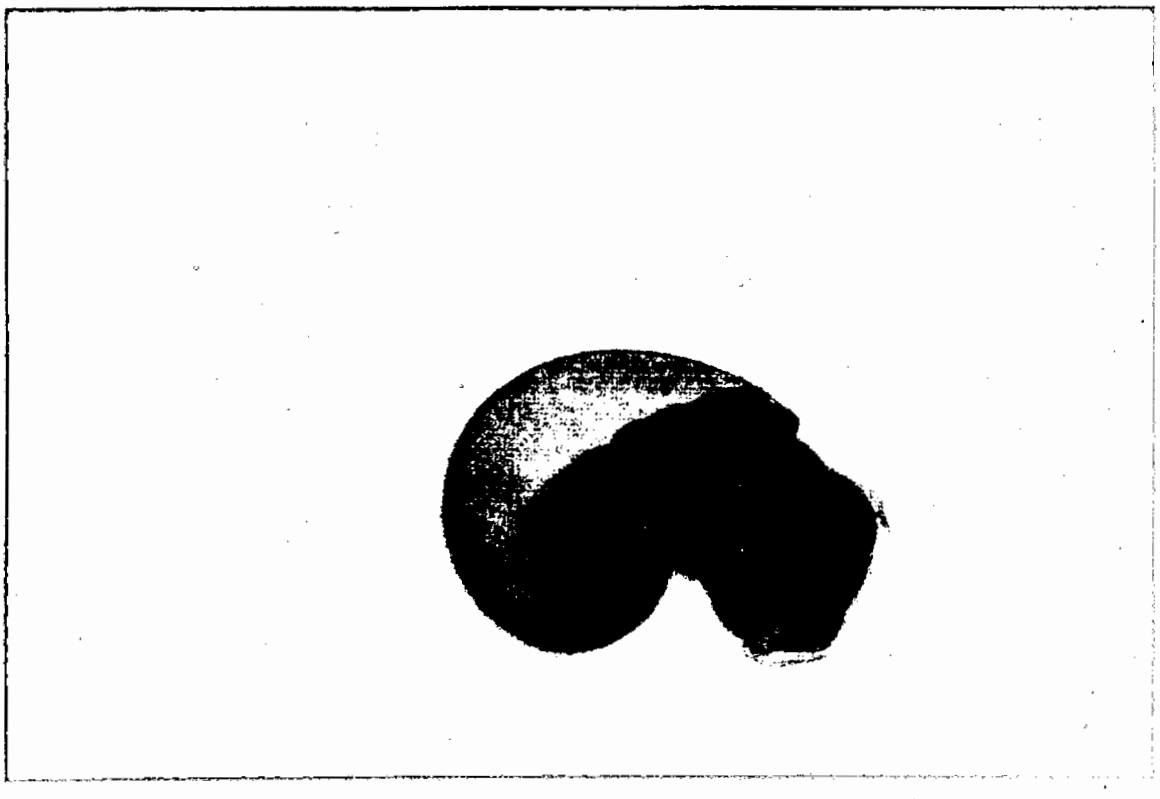


Plate 6.1. Post torsion *H. midae* larvae.

6.3: Results

The results of the experiment used to test larval sensitivity to chlorine are shown in Fig. 6.1. The mean LT_{50}'s for the three concentrations of chlorine were significantly different ($p < 0.05$). Chlorine appears to be extremely toxic for exposed abalone larvae (LT_{50} =170 minutes exposure at even 0.06 mg/l). Larvae survived only a few minutes when exposed to higher concentrations of chlorine (see Fig. 6.1). No larval mortalities were recorded for any of the controls.

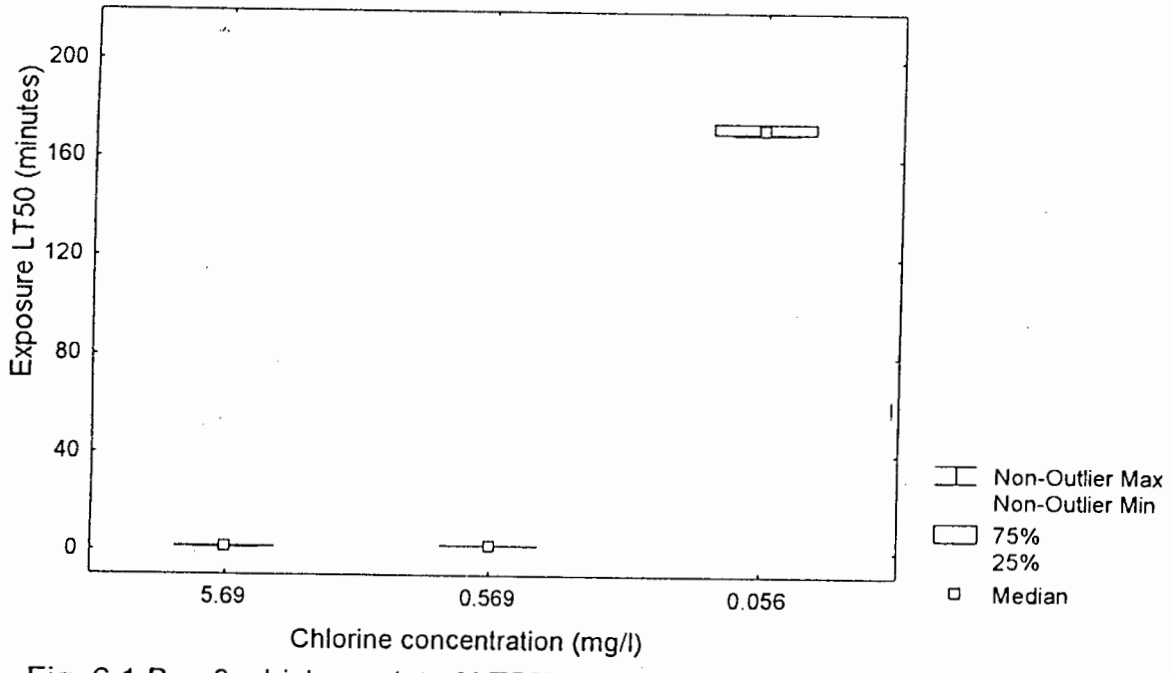


Fig. 6.1 Box & whiskers plot of LT50's for larvae exposed to chlorine

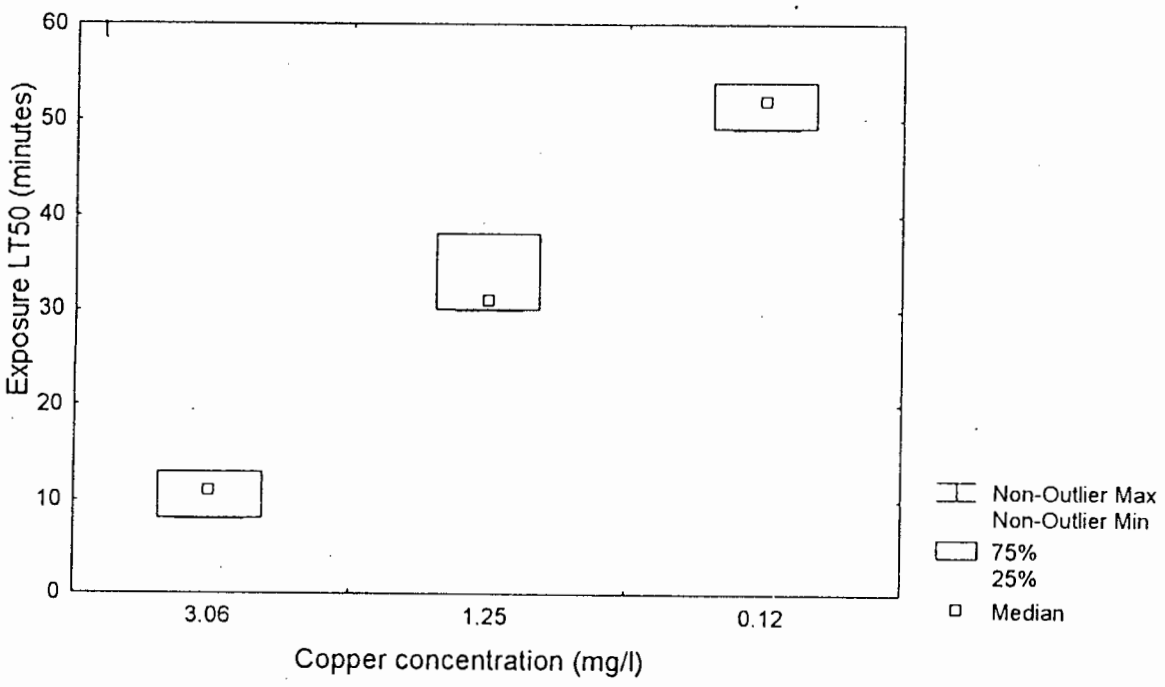


Fig. 6.2 Box & whiskers plot of LT50's for larvae exposed to copper

The results of the copper toxicity experiment are plotted in Fig. 6.2. Significant differences were also obtained when comparing the larval mortalities at the three concentrations tested (Anova, $p < 0.05$). As with chlorine, high larval fatalities occurred after a short exposure time of 53 minutes to even 0.12 mg/l copper (see Fig. 6.2).

Ammonia proved to be relatively less toxic to abalone larvae at the concentrations tested than chlorine and copper. The LT_{50} of larvae exposed to 5 mg/l ammonia was 600 minutes. No larval mortalities were observed for larvae exposed to 0.5

and 0.05 mg/l NH_3 even after 850 minutes (the maximum duration of the experiment).

6.4: Discussion and conclusions

The very fragile nature of marine larvae must make them more susceptible to adverse water quality parameters. *Haliotis midae* larvae are highly susceptible to chlorine and copper. This is evident from the high mortalities when they are exposed even to low concentrations of these chemicals (Figs. 6.1 & 6.2). The use of copper-based medication in seawater should clearly be avoided if abalone are being reared. The toxicity of copper to invertebrates is well known (Lundegaard, 1985).

To place the importance of copper toxicity to abalone larvae into context, it is worth noting that the copper content of natural seawater is less than 0.004 mg/l (Marks, 1938). Hahn (1989) reported that *H. rufescens* and *H. cracherodii* suffer from mortalities when larvae are exposed to copper concentrations as low as 50 µg/l and adults to 80 µg/l. Marks (1938) also observed that mature *Haliotis fulgens* survived for only 30 days when continually exposed to 0.05 mg/l copper. This survival was reduced to three days when the concentration was increased to 0.1 mg/l.

Marks (1938) found that many species of marine molluscs have an upper copper tolerance limit of 0.2 mg/l. This is 100 to 200 times more concentrated than the levels found in natural seawater. It is interesting to note that although abalone are very sensitive to copper it has been measured in the soft tissues of *Haliotis tuberculata* by Bryan, *et al.*, (1977). They found the highest concentration of copper (550 ppm dry tissue) in the blood and the lowest, 12 ppm, in muscle tissue.

Abalone farmers should also be aware of the extreme toxicity of chlorine to abalone larvae (refer to Fig. 6.1) The use of chlorine to sterilise hatchery equipment requires utmost caution. Chlorinated rearing vessels need to be rinsed several times with fresh water and a final rinsing with seawater, before the introduction of larvae. Any short cuts in this washing procedure could result in larval mortalities. The recommended allowable chlorine residue for shrimp larval rearing containers is less than 10 µg/l (Colt & Huguenin, 1992). Published findings on the toxicity of chlorine for haliotid larvae were not available for making comparisons. However Sano & Maniwa (1962) found that the oxygen consumption of *H. discus hannai* increased three fold when chlorine in seawater was raised to 14 mg/l.

Abalone larvae were more tolerant of the concentrations of ammonia than copper and chlorine used in this study. At

lower concentrations, no mortalities occurred during the monitored 850 minutes. The toxicity of ammonia seems to be dependent on exposure time. Larval (*Homarus americanus)* lobsters are sensitive to ammonia, and for this specie Delistraty *et al.*, (1977) suggest that ammonia concentrations be kept below 0.2 mg/l. The LT_{50} for *Macrobrachium rosenbergii* larvae exposed to 43.2 mg/l ammonia was 144 hours (Armstrong, *et al.*, 1978).

Abalone larvae appear to be less sensitive to ammonia than American lobsters, yet more sensitive than *M. rosenbergii* larvae. Pink salmon (*Oncorhynchus gorbuscha*) suffered fifty percent mortality when exposed to 0.08 mg/l ammonia over a ninety six hour period (Rice & Bailey, 1980). Salmon, with their higher metabolic rate, appear to be far more susceptible to low concentrations of ammonia even though the tolerance times vary greatly. The rapid development of *H. midae larvae* makes it unnecessary to extend toxicity experiments for periods as long as those used when testing the tolerance of salmon.

In conclusion, it is clear from this study that low concentrations of copper and chlorine are toxic to *H. midae* larvae. Ammonia appears to be toxic only if the larvae are exposed to relatively high concentrations and for a relatively long time.

CHAPTER 7: A SYNOPSIS OF THIS STUDIES FINDINGS WITH SOME COMMENT ON THE STATUS OF ABALONE FARMING

The aquaculture of abalone world-wide has still not reached its full potential. Many obstacles hinder the rapid expansion of abalone farming. In general, examples of these limitations include: a suitable reliable source of feed for abalone farming, high mortalities associated with the hatchery phase of abalone culture, methods for successfully dealing with parasites and diseases and availability of suitable culture sites. These limitations are predominantly culture-related. However the abalone markets in the East have enormous potential, mainly due to the declining natural stocks. A classical example is the Mexican abalone fishery, for which harvests have decreased from 3000 MT in the 1970's to 1000 MT (Chew, 1995).

The most important technical and biological constraints experienced by abalone farmers can be listed as follows:

A. Biological constraints

i. Difficulties in providing the optimal conditions for enhancing larval settlement.

ii. High mortalities of post larval abalone (Cesena, 1995).

iii. Larval cultivation problems associated with seawater quality (Cesena, 1995).

iv. Supplying newly settled abalone with benthic diatoms of the right size and nutritional status.

v. The slow growth rate of abalone (Hahn, 1989).

vi. The implications of the sabellid worm infestations in commercial growout farms (Oakes & Fields, 1994).

B. Farming operation constraints

i. The availability of a cheap source of feed for growout abalone (Anon, 1995).

ii. Design and construction of suitable seawater systems (Cesena, 1995).

iii. High production costs associated with land-based abalone farms (Wray, 1995).

iv. Competition for suitable culture sites with recreational activities (Anon, 1993).

v. Abalone farms are limited to isolated areas due to coastal pollution.

A possible reason why abalone farming has not reached its full potential might be the severe limitations experienced by Northern Hemisphere abalone producers. Until the early 1970's abalone farming development was mainly promoted in Japan and the United States. In these countries further expansion is limited by urbanisation, recreational

pressures, pollution, high labour and other utility costs and costly and time consuming permitting procedures. In California production costs are halved by growing abalone in sea cages (Wray, 1995).

It would appear that the challenges of developing the abalone farming industry are most likely to be met by Southern Hemisphere countries.

There are still a few unanswered questions related to abalone farming in South Africa. Examples of these challenging problem areas are:

1. Reliable spawning induction method for *H. midae* broodstock.
2. The reasons for the very high post settlement mortality.
3. How to avoid and successfully treat the sabellid boring polychaete infesting growout abalone.
4. How to improve growth rate of the abalone and and at the same time grow them at a high density.

The work presented in this study should contribute towards solving some of the constraints associated with farming *H. midae* in South Africa. The implications of the work on

seawater systems presented in chapter three supports the view that abalone hatcheries need to be provided with flow-through seawater systems.

A complementary aspect of seawater systems is seawater quality. Results of the toxic impact of certain pollutants for *H. midae* larvae are provided in chapter six. These results emphasise the importance of maintaining good water quality in an abalone hatchery. Serious mortalities of *H. midae* larvae can be avoided if water quality is optimised. These water quality experiments also have relevance to broodstock conditioning and spawning induction. A water quality stress free environment for *H. midae* broodstock would certainly be achieved if levels of possible pollutants are kept below levels that are lethal to larvae.

The UV and hydrogen peroxide experiments presented in chapter 5 emphasise the need to adapt spawning strategys for new *Haliotis* species. *H. midae* was successfully spawned using the hydrogen peroxide spawning technique. Spawning success was improved after identifying the important variables and the associated optimum range of each variable. For *H. midae* the important variables were: hydrogen peroxide concentration and exposure duration and pre spawning

starvation of the broodstock. Moon phase and pre-conditioning to the spawning tanks did not appear to influence spawning induction in the artifical environment of an abalone hatchery.

Haliotis tuberculata were reported by Hahn (1989) not to spawn when induction was attempted with the UV or hydrogen peroxide technique. However spawning induction may be possible for *H. tuberculata* if a similar strategy, where the important variables are manipulated experimentally to identify optimum levels, is adopted using the hydrogen peroxide technique. The collection of *H. midae* gametes by dissection would not appear to be a viable option. The selection of gravid broodstock using the visual method described in chapter four and the adapted hydrogen peroxide induction technique is a reliable and repeatable method for obtaining viable *H. midae* gametes.

In summary this work should help broaden the existing technology base of abalone farming in South Africa by contributing information towards:

1 Seawater system design.

2 Broodstock conditioning and selection.

3 Artificially spawning *H. midae*.

4 Water quality aspects associated with rearing *H. midae* larvae.

The results presented in this study have been successfully applied to the Premier Fishing commercial abalone hatchery and growout farm at Gansbaai. To date in excess of 100 000 abalone greater than 10mm have been produced using these methods. In conclusion these results are presently contributing to the commercialisation of abalone farming in South Africa.

Abalone farmers in Mexico and some South American countries have faced their challenges by adopting a co-operative approach. In Mexico research projects on abalone culture were initiated in the 1970's. The Japanese Overseas Corporation Foundation provided scholarships to Mexican students interested in studying abalone culture in Japan (Cesena, 1995). They were further encouraged by visiting Japanese scientists who concluded that Mexico had more potential for abalone farming than Japan.

In the late 1980's two Mexican commercial abalone farms purchased technology packages from two California based

abalone farms (Cesena, 1995). The Californian farms were able to supply the Mexicans with red abalone *H. rufescens* seed during the initial development years. Recently the World Bank (Banco Mundial) has started to provide assistance for the development of abalone aquaculture in Mexico (Cesena, 1995).

In Chile the private non-profit organisation, Fundacion Chile, has been the catalyst in developing successful abalone farms (Wray, 1994). This company utilises technology purchased from the Californian firm, Ab Lab Inc, and then transfers this technology to Chilean abalone farmers. Fundacion Chile also purchased red abalone seed to help get their farms into production earlier (Wray, 1994).

In Australia there are ten active abalone farms with a further 20 interested investor groups (Anon, 1994). The collaborative approach is also being used to help develop a suitable cheap feed for abalone farming. The Australian commercial sector, State government and Universities have contributed A $ 1.2 million to abalone feed research.

Abalone farming is also showing great promise in New Zealand. The New Zealand ministry of Agriculture and

Fisheries (MAF) were responsible for customising a protocol to produce *H. iris* (Tong & Moss, 1992). Recently a company, Aquatic Products Ltd, started building an abalone farm at Horseshoe Bay on Stewart Island (Anon, 1994). This must be the most southern abalone farm in the Southern Hemisphere. A private New Zealand feed company is developing a casein based artificial diet which is providing promising results in feeding trials (Flemming, 1994).

The South African abalone farms have not enjoyed the same level of government and overseas support or the availability of abalone seed to get new farms into production. The formation of the Abalone Farmers Association of South Africa is, however, helping to co-ordinate such resources. Joint funding of research projects will make an important contribution to the commercialisation of abalone farming in South Africa.

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Appendix 1

Anova's for male *H. midae* spawning experiments

Effect	df effect	MS effect	df error	MS error	F	p-level
Acclimatised	1	844.43	4	214.29	3.940	0.118
Starved	1	2156.9	14	250.82	8.599	0.011
Sunset time	5	312.92	12	388.38	0.805	0.567
Lunar cycle	2	53.24	9	295.14	0.180	0.838
pH	6	631.89	14	267.93	2.358	0.087
Constant temperature	4	191.56	10	86.26	2.221	0.140
Temperature fluctuation	5	138.96	12	279.77	0.497	0.773
H_2O_2 conc	6	1198.2	14	223.83	5.353	0.005
H_2O_2 exposure	4	508.46	10	262.26	1.939	0.180

Appendix 2

Anova's for female *H. midae* spawning experiments

Effect	df effect	MS effect	df error	MS error	F	p-level
Acclimatised	1	86.26	4	228.65	0.377	0.572
Starved	1	2263.6	14	184.43	12.273	0.003
Sunset time	5	142.77	12	136.91	1.042	0.437
Lunar cycle	2	92.56	9	227.07	0.407	0.677
pH	6	291.66	14	227.45	1.282	0.326
Constant temperature	4	218.67	10	119.56	1.829	0.200
Temperature fluctuation	5	51.94	12	190.78	0.272	0.920
H_2O_2 conc	6	265.15	14	98.49	2.693	0.059
H_2O_2 exposure	4	65.92	10	135.95	0.485	0.747

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