In situ time-stamping of abalone shells to determine how abalone stocks can be aged.

R. W. Day, G. P. Hawkes and V. Gomelyuk

PROJECT No. 1995/004

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OBJECTIVES:

1. To determine the timing and regularity of the layers deposited under the spire of abalone shells, through extensive field based tagging and "timestamp" marking of abalone.
2. To achieve objective 1 for populations of blacklip abalone at three sites in Victoria, three sites in Tasmania and one site in New South Wales; and also for populations of greenlip abalone at two sites in South Australia.
3. To determine how to interpret the layers in abalone shells, how reliable such interpretations are, and how layer formation may vary between localities.
4. To use this information to determine the age distribution of abalone at a number of sites.
5. To be able to predict where and how layers can be used to age abalone in stocks in southern Australia.

NON TECHNICAL SUMMARY

OUTCOMES ACHIEVED

1. Layer formation under the spire has been shown to be approximately annual over longer periods, but irregular over 1-2 years. The timing of layer formation during a year was shown to be highly unpredictable.

2. Layer formation was shown to be similar for blacklip abalone across sites in Victoria and Tasmania, and between fast and slow growth sites for greenlip abalone in South Australia.

3. The layers can be interpreted as approximately annual at all sites, but we have not been able to reliably discriminate between annual and repair layers. The reliability of ageing has been estimated, and this appears to be the same for abalone of all ages, and consistent between sites. Thus ageing data appears to be reliable enough to assist future stock assessment and modelling of state abalone resources.

4. Results suggest shell layers can be used for ageing in all stocks that are not severely bored by polychaete worms.

Introduction:
If abalone can be accurately aged, this would be a fundamental tool for more effective management of abalone fisheries. Several authors have proposed ageing abalone by grinding or cutting abalone shells, and counting the shell layers deposited beneath the spire. Other authors have cast doubt on this method. What is uncertain is how reliable these age estimates are. To construct useful models to assess and manage abalone stocks, it is important to know the accuracy of the data on which the model is based. Furthermore, it is labour intensive to collect age data, and if these data are very inaccurate, then resources are wasted in collecting it.

This project aimed to find out how reliable and accurate the ageing method was, by investigating the timing and the periodicity of layer formation in abalone shells. It seemed possible that the ageing method might work reasonably in some areas, but not in others. Thus we planned to repeat the work at many places in the hope that we could predict where ageing would be useful for managing the blacklip and greenlip abalone fisheries of Victoria, Tasmania, New South Wales and South Australia.

The layers used to age abalone are layers of the pearly nacre. Abalone shells contain two types of crystals of Calcium Carbonate. The outer, coloured part of the shell is formed of calcite. The inner nacre is made of aragonite. This is deposited as a series of thin layers of crystals over the inner surface to thicken and strengthen the shell. New layers are constantly being deposited. Most of these layers are very thin and clear, but some are dark, because they contain a lot of protein. Some of these dark layers are formed over areas where worms bore into the shell, but the remainder are thought to be laid down regularly by those who count these layers to age abalone.

To determine when and how often the protein rich layers are deposited, we have collected abalone and marked the innermost layer of the shell, using a new staining method. The abalone were then tagged and released, and subsequently collected at various times after release. We could then observe when protein rich layers were deposited after the staining mark.

The staining method was developed during a previous FRDC project. We keep the abalone in seawater with added Manganese salts, for several days. The Manganese atoms move through the abalone's body, and replace some of the Calcium in the Calcium Carbonate crystals of the nacre that the abalone is forming on the inside of the shell. When an abalone is recaptured, the shell is cut and polished. We then fire electrons at the shell to make the Manganese atoms glow, so we can see the mark.

Originally, we chose three sites in Victoria, three in Tasmania and one in New South Wales to mark blacklip abalone, and two sites in South Australia to mark greenlip. Over 500 abalone were carefully collected, stained, tagged and replaced at each site, because we intended to recapture samples at various times up to two years later. Abalone seem very sensitive to handling, and we wanted to ensure many survived. We knew that many would move out of range of our searches after one year or more.

Results.
We obtained results for blacklip abalone from sites near Portland and Mallacoota in Victoria and from Georges Reef in Tasmania, and investigated the precise timing of layers during the year in Port Phillip Bay. We obtained greenlip results from four sites near Port Lincoln, South Australia. At two sites in Tasmania we found that most of the

many abalone we marked had disappeared within a few months. At another site most abalone were infested with mudworms, and the mud blisters in the shells obliterated our manganese staining. The NSW fisheries abalone team could not arrange a suitable time for our work at Eden in New South Wales, due to management negotiations.

We found that the dark, protein rich layers were not deposited at a predictable time of year; individual abalone deposited a new dark layer at various times. As a result, abalone recaptured after periods of 3 months to 2 years showed very variable numbers of new layers. These results suggest that layers are completely unreliable for ageing. But re-analysis of ageing data obtained by Shepherd for abalone of known age showed that the number of layers was no more variable after 3-8 years than in our samples.

After the project end date, we were able to recapture marked abalone that had been more than 3 years at liberty from some of our sites, and have processed most of these. The numbers are small, but they confirm that each abalone at these sites appears to deposit about one layer per year over several years. As the data for abalone ages that is important for modelling involves adult abalone that are perhaps 4-14 years old, an error of 1-2 years in the age estimate is probably acceptable.

The fact that although abalone deposit layers at very variable times during a year, there appears to be a regular cycle of layer formation, explains why some previous researchers have concluded that layers are irregular and not useful in ageing, while others have produced circumstantial evidence supporting the use of layers in ageing.

We conclude that the estimates of age from counting spire layers in abalone are probably correct to within about 2 years, and thus ageing of abalone will be useful in stock assessment and management, providing sample sizes are high enough to provide precise enough inputs to suit the requirements of the management models.

Greenlip abalone proved very sensitive to handling, so that our marking experiments had to be repeated at two new sites. Many recaptured greenlip lacked manganese marks, perhaps because handling stress caused them to stop shell growth during the experiment. This reduced our sample sizes. The two sites were chosen to represent fast and slow growth, as Shepherd expected two layers per year in areas with rapid growth. Our results however, suggest approximately one layer per year at both sites.

We also showed that abalone deposit nacre more rapidly when their shells are bored by polychaetes, so that they have thicker shells. They also deposit more dark, protein rich layers. The dark layers cannot easily be distinguished from the annual dark layers, so that heavily bored abalone cannot be aged. Thus ageing data is unreliable if the stock is heavily parasitised by polychaetes. This is common in stunted stocks.

These results cover objectives 1. 3 and 5. We were not able to get data for all the sites listed in objective 2, or obtain age distributions of abalone at our sites, but we were able to obtain some data for layer formation over 3 year periods, which turned out to be crucial to the assessment of the value of ageing to abalone management.

KEYWORDS: Blacklip abalone, greenlip abalone, ageing, management models.

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A project of this magnitude would not have been possible without the cooperation of many sectors of the abalone fishing industry, as well as state institutions. We wish to thank in particular the Mallacoota Abalone Diver's Cooperative and Portland Ocean Trading company, and Cheetham Salt Limited Aquaculture Centre, Lara, for the use of their facilities to hold and stain the abalone we collected for marking. Our thanks to the Mallacoota abalone divers and Western Zone abalone divers, especially Vin Gannon, for recapturing abalone at Mallacoota and at Lawrence Rocks for us, and to Gary Kenyon of Souwest Seafoods, who kept tagged shells and arranged to send them to us.

In South Australia the South Australian Research and Development Institute (SARDI) provided the use of the vessel *Ngerrin*, in which the ballast tanks could be used for our manganese staining. We thank the captain and crew for assistance with our abalone tagging, and Scoresby Shepherd, Kate Rodda, Brian Davies and other SARDI staff for help in collecting and tagging, and for subsequent recapturing of the greenlip abalone at four sites. In addition, Miguel Rabi and Carol Black from Peru, and Michelle McHugh and Katie Gleeson from Melbourne acted as volunteer assistants on our staining trips.

In Tasmania Warwick Nash, Rickard Officer, and the abalone team at the Tasmanian Aquaculture and Fisheries Institute provided facilities, assistance and advice during collection and marking expeditions, and recaptured abalone for us. In Victoria Harry Gorfine and Cameron Dixon of the Marine and Freshwater Research Institute provided invaluable advice and assistance with collections. In addition numerous other volunteers and student assistants contributed to our fieldwork and laboratory work.

The processing of abalone shells was made possible by the use of a cathodo-luminescence microscope made available by Dr Malcolm Wallace at the School of Earth Sciences of The University of Melbourne. Supplementary work also involved the use of the scanning electron microscope at the Zoology Department, University of Melbourne, assisted by Joan Clark, and the cathodoluminescence scanning electron microscope at the University of Cape Town, South Africa, done by Dane Gernike.

BACKGROUND

An accurate ageing technique would provide a fundamental tool for effective management of the abalone fisheries in many countries worldwide. The management of finfish stocks throughout the world relies on ageing of fish using otoliths (Beamish 1992), and statoliths are used in ageing squid to manage squid fisheries (Jackson 1990). However, other invertebrates such as abalone and lobsters do not have equivalent structures that are known to record age. Growth checks at the growing edge of the shell can be used to age Japanese abalone (*Haliotis discus discus*, and *H. discus hannai*) (Sakai 1960), the European ormer (*H. tuberculata*) (Forster 1967), and the Omani abalone (*H. mariae*) (Shepherd *et al*.1995a), as these species show arrested growth followed by strong seasonal growth, as a result of extreme changes in water temperature. Kojima (1975, 1995) has rigorously validated this method for *H. discus discus*. Unfortunately, such growth checks cannot be seen in the commercial abalone from Australia (*H. assinina, H. laevigata, H. roei, H. rubra*), California and Mexico (*H. corrugata, H. fulgens, H rufescens, H. sorenseni*), New Zealand (*H. australis, H. iris*), or South Africa (*H. midae, H. spadicea*) (personal observations, R.D.).

Muñoz-Lopez (1976) described layers under the spire of the shell (see shell structure below) in *H. corrugata* and *H. fulgens*, and suggested these might record age in years, based on the colour of the inside of the shell in different seasons. Prince *et al.* (1988) suggested the number of spire layers was roughly annual in juvenile *H. rubra* at a site in Tasmania for which age could be predicted from size. However De Jong (1990) found a different relationship of layers to predicted age in juvenile *H. rubra*, Nash *et al.* (1994) found layers appeared to be deposited twice per year in northern Tasmania, and McShane and Smith (1992) found no relation of spire layers to age in eastern Victoria. Since this project began, Shepherd and co-workers have also related spire layers to predicted ages of juveniles in *H. fulgens* and *H. corrugata* from Mexico (Shepherd *et al*. 1995b, Shepherd and Avalos-Borja 1997) and *H. laevigata, H. scalaris* and *H. rubra* in Australia (Shepherd and Triantifillos 1997, Shepherd and Huchette 1997, Shepherd, unpublished).

But such studies involve very weak evidence, for several reasons. First, we know that estimated growth trajectories from fitting a von Bertalanffy function to tagging data are biased and also very imprecise (Day and Fleming 1992, Troynikov *et al.* 1998). Second, we know that individual abalone vary greatly in their growth rate (Day and Fleming 1992), so that finding individuals with more or less rings than their estimated age provides no evidence of how variable the number of rings per year is. Third, the growth trajectory can only provide estimates of the age of shells that are in the juvenile growth phase, and growth stops approximately at maturity (Day and Fleming 1992, Troynikov *et al.* 1998), before the minimum harvestable size. The important age range is the ages at which the abalone are harvested in the fishery. Experience with fish otoliths has shown that ageing based on juvenile data may not apply to older fish (Beamish and MacFarlane 1983), and failure to validate ageing over all ages has resulted in severe mismanagement of fish stocks (Beamish 1992).

Another commonly encountered problem in ageing abalone is that they deposit dark layers to plug holes created by boring sponges and polychaetes (Nash *et al*. 1994, Thomas and Day 1995, Shepherd *et al*. 1995b). These repair layers may obscure any regular age-related layers.

Thus it is important to rigorously validate ageing based on spire layers, especially in older abalone; to discover how to interpret the layers, and determine how accurately age can be estimated. The importance of such validation for the management of abalone fisheries was explained in Day and Fleming 1992. The objectives of this study are a direct extension of our previous FRDC project (92/40), in which we discovered that normal methods of chemically marking layers in fish otoliths did not work for the spire layers of abalone, and we developed a new method to chemically mark the shell layers, and thus "timestamp" abalone (see Methods). In this project we used this method on blacklip (*H. rubra)* and greenlip (*H. laevigata*) abalone at 10 sites.

Shell structure.

The shell contains two crystal forms of calcium carbonate. The first, "prismatic" calcite, is deposited at the growing lip in the form of columns of prism shaped crystals embedded in protein, as an outer layer of the shell (Nakahara *et al*. 1982). The pearly nacre of the shell is deposited over the whole inner surface of the shell, to thicken and strengthen it. The nacre is formed of aragonite, and usually it is deposited as thin layers (or laminae) of hexagonal, tile-like crystals (Nakahara *et al*. 1982, Mutvei *et al.* 1985). Bivalves deposit these laminae one by one, and the width of each lamina varies seasonally or tidally (Wise 1970b, Lutz and Rhoads 1980), but gastropods deposit many laminae simultaneously (Wise 1970a,b, Wilbur and Saleuddin 1983), so that similar methods to those used in bivalves are inappropriate for abalone and other gastropods.

New layers of nacre are constantly being deposited. All the shell layers contain proteins, but the stacks of thin laminae are white or semitranslucent because they are mostly aragonite crystals. The crystals contain some small proteins, and are separated by very thin protein sheets (Fritz *et al*. 1994). In the dark layers within the nacre, the crystals are not thin hexagonal tiles, but elongated, and are surrounded by a lot of very dense protein. Some of these dark layers are formed over areas where worms bore into the shell, but the remainder are thought to be laid down regularly, and are counted in the spire of the shell to estimate the age of abalone.

It is a mark of our progress that when this project application was written, it was thought (Erasmus *et al*. 1994, Shepherd *et al*. 1995b) that the dark, protein rich layers between the stacks of white laminae were formed of calcite, based largely on the work of Morse and his colleagues (Fritz *et al*. 1994, Zaremba *et al*. 1996) who found that aragonite deposition begins only after an initial protein sheet is deposited, and covered by a layer of calcite. Our work has shown that the dark layers contain aragonite, not calcite, in *H. rubra* (Hawkes *et al.* 1996), and *H. laevigata* and many other species (Hawkes and Day, unpublished data).

De Jong (1990) showed that the deposition of nacre on the inner surface of the shell occurs mainly behind the growing edge of the shell, and beneath the shell pores, which lie above the gills of the abalone. No deposition occurs where the muscle attaches to the shell. As the growing edge is extended, the muscle attachment area is moved forwards, covering some of the nacre deposited earlier. Behind the area where the muscle attaches, the shell uncovered as the muscle moves forward is covered by new, thin layers of nacre that extend over the whole inner surface of the spire. These layers must be deposited by the membrane over the animals gonad and digestive gland. A student project by S. Heady during this FRDC project has indicated that these spire

layers begin as small areas over the gonad and then spread to cover the whole area behind the muscle scar. Each new layer consists of a dark layer followed by laminae.

If the spire is ground until a hole appears, as Muñoz-Lopez (1976), Prince *et al.* (1988) and Shepherd and coworkers (e.g. Shepherd and Huchette 1997) have done, the dark layers are seen as rings. If a section is cut across the spire the dark layers are seen as bands parallel to the inner surface. This method was used by Nash *et al.* (1994) to estimate age, and is more convenient when shell sections must be mounted under a microscope, as in our study.

NEED

The abalone industry in all states would benefit greatly from methods to predict future stocks, in order to determine optimal sustainable harvests. As well as estimates of current stocks, assessments of recruitment rates, growth rates and mortality rates are essential to predict future stocks, and thus sustainable yields. Such assessments are hampered by the variability of growth, mortality and fecundity versus age within and between areas, so that the parameters must be measured at many sites. A method to age abalone is essential to do this effectively.

Fin-fish stock management throughout the world has been revolutionised by the development of accurate ageing of otoliths. However, methods to age molluscs still require development. Ageing abalone was identified as an important priority in a review of abalone research for the FRDC by Ward as long ago as 1986, and has been regarded as an important priority by the Demersal Mollusc Research Group (comprising fisheries institute abalone researchers from all southern states) since then. Widespread work on ageing abalone has been conducted in Alaska, California, Japan, Mexico and South Africa because ageing is so important to fishery management.

Abalone quota holders in all states will therefore benefit in terms of increased security of their future harvests if reliable ageing can be implemented. Similarly the processors would benefit from more long term security in the availability of product from the fishery.

OBJECTIVES

1. To determine the timing and regularity of the layers deposited under the spire of abalone shells, through extensive field based tagging and "timestamp" marking of abalone.
2. To achieve objective 1 for populations of blacklip abalone at three sites in Victoria, three sites in Tasmania and one site in New South Wales; and also for populations of greenlip abalone at two sites in South Australia.
3. To determine how to interpret the layers in abalone shells, how reliable such interpretations are, and how layer formation may vary between localities.
4. To use this information to determine the age distribution of abalone at a number of sites.
5. To be able to predict where and how layers can be used to age abalone in stocks in southern Australia.

Objective 2 was revised. The board requested we reduce costs and a site on the North Coast of Tasmania was cancelled. However, abalone returned to two of three sites in Tasmania could not be recaptured in large enough numbers to provide useful data, and we subsequently marked and replaced abalone at a third Tasmanian site (King George III reef). An expedition to Eden, New South Wales to collect, stain and replace abalone could not be arranged during the period of the project.
Objective 4 could not be achieved, as we discovered that we needed to set up marking experiments at extra sites, and also late in the project that the crucial information to achieve more important objectives (1 and 3) required long term recaptures (after 3 years or more) of marked abalone at our sites.

METHODS

"Time-stamping" to validate the ageing method:
The basic strategy of this project was to "time-stamp" abalone so as to determine the intervals over which shell layers were deposited. We collected abalone from various reefs, marked the layers of shell that the abalone were depositing at that time using a chemical, then tagged the abalone and replaced them on their reefs. Subsequently abalone were recaptured after various times, and their shells processed to investigate how many shell layers had been deposited after the chemical mark. By collecting marked abalone at various intervals after marking, we could determine when the layers were deposited, and how many layers were deposited per year.

Day and Fleming (1992) pointed out that there are several critical issues that should be investigated in deciding whether growth bands will be useful for ageing. The most obvious is whether the bands are deposited at regular intervals (e.g. annually) by all the animals. If abalone deposit layers in some years, but not in others, the ageing method would be biassed. A second issue is whether the timing of band deposition varies between animals. Bands would give a precise estimate of age if, for example, all abalone deposited bands in September (spring) each year. A third issue is whether band formation is consistent between different populations. Abalone populations are known to be genetically different due to low dispersal, and in different environments banding may be more or less regular.

We therefore time-stamped a collection of abalone in Port Phillip Bay to investigate the timing of band formation during the year, and time-stamped large numbers of abalone at many sites widely scattered across the states to investigate the regularity of band deposition between years and sites. We anticipated this would require recaptures over two years at each site, so that very large numbers of abalone had to be marked.

Abalone appear to be very sensitive to handling, for example they do not feed for many days after capture, and their growth is reduced or halted (Day *et al.* 1995). The chemical marking requires that they are actively depositing shell layers during treatment (Hawkes *et al.* 1997), and we needed to ensure that they would survive and grow well when replaced on their reefs. The collection, transport, chemical marking, tagging, and replacement of abalone therefore had to be arranged so as to maintain the abalone in very good condition.

At most sites, we needed to recapture marked animals after long periods of time, and during these periods some would die naturally, and some would emigrate from the search area. Thus we expected to recover a low percentage of those we marked, so that very large numbers of abalone needed to be collected, marked, tagged and replaced at each site. To ensure that we did not lose valuable marked abalone because tags were detached, we used two different forms of external tags: pore tags involving a round plastic tag and a plastic rivet fixed into one of the rear pore holes (Prince 1991), and tags made of copper strips with punched numbers, attached to the shell with epoxy putty. Pore tags had been used extensively by Nash in Tasmania. They can be applied rapidly, and it appears that the abalone quickly deposit shell nacre around the rivet, cementing it in place. While pore tags can be lost due to overgrowth by shell fouling over longer periods, the copper tag resists fouling. Juvenile abalone have small pore holes that the rivets do not fit, so they were tagged using copper tags only.

The manganese marking method:
The chemical marking method used was developed as a result of our previous FRDC program, and is described in the Final Report,(Day *et al.* 1998), and in Hawkes *et al.* (1996). The layer of new shell that is constantly being deposited on the inner surface of an abalone shell is marked by introducing manganese ions, in the form of manganese chloride, into the seawater. The manganese ions appear to diffuse through the abalone's tissues easily, and are presumably also carried by the blood to the sites of shell deposition. This is in contrast to traditional fluorochrome markers for calcified tissues, such as Calcein and Tetracycline, which apparently are not carried to remote shell deposition sites beneath the spire of the shell, as reported in the FRDC 92/40 final report, and by Day *et al.* (1995). We showed that the fluorochromes only mark the lip of the shell, which is in direct contact with the seawater.

The manganese ions replace some of the calcium ions in the calcium carbonate crystal lattice of the shell. When the shell is cut and polished, and the slices are put into a vacuum box, the layers containing manganese can be detected because they luminesce when a beam of electrons hits the surface (Sommer 1972). This is done using a Cathodo-luminescence microscope, in which the stage of a dissecting microscope is replaced by a vacuum box with a clear glass cover. The box is connected to a vacuum pump, and to an amplifier which forces electrons to jump from an electrode in the box onto the shell specimens. The beam of electrons can be shifted using movable magnets, and the shell specimens are on a movable tray. The colour of the light that the manganese emits depends on whether the manganese is in calcite or aragonite (Sommer 1972, Barbin 1992). This, together with the Raman laser method developed during the previous project (Hawkes *et al*. 1996) has allowed us to investigate the nature of the layers used in ageing (see the section on shell structure in "Background").

The manganese is toxic to abalone at very high concentrations. Extensive preliminary trials, reported in the Day *et al.* (1998) and in Hawkes *et al.* (1997), were done on *Haliotis rubra* to determine the optimum concentration of manganese, and the time required to immerse abalone in seawater with manganese, so as to obtain easily visible marks in the shell. We were particularly concerned with the spire region of the shell, which is used to estimate age. In all our field marking, Manganese chloride tetrahydrate was added to the water to produce a concentration of the Manganese ion of 202 mg/L, which results in a 1:10 ratio of Mn:Ca. We discovered during this project that *Haliotis laevigata* appeared to be more sensitive to the treatments, so that further laboratory trials had to be undertaken to determine the optimum method for this species.

Shell processing:
The manganese marks in the abalone shells were examined under a cathodo-luminescence microscope (Herzog *et al.* 1970, Marshall 1988) at the School of Earth Sciences of The University of Melbourne, courtesy of Dr M. Wallace. The setup consisted of a dissecting microscope with a long focal length objective, over the window of a vacuum box (at approximately 50 Φtorr) containing the shell sections. A cold, low intensity beam of electrons (8 ke-v beam energy, 0.6 ma current) was produced by an electrode in the box, and directed at the sections using a movable magnet. The luminescence from the manganese was viewed at magnifications of 20-35X.

If abalone were recaptured alive, they were frozen and shucked. The shells were dried in a 60EC oven, and any fouling was brushed off the shell. A roughly 10 by 30 mm piece of the shell that included the spire was cut out of the shell with a 0.25 mm diamond tipped lapidary saw blade, and embedded in polyester non-fluorescing embedding resin (RF Services 61-283). The resin blocks were then cut using the same saw so as to produce a cross section of the shell spire, and this surface was polished using a series of wet and dry sandpapers, down to 800 grade, and then polished using A1 powder, to produce a very smooth surface. The blocks were then trimmed into small thin blocks to fit into the vacuum box of the microscope. Thin blocks and sections also reduce the problem of absorbed moisture, which delays the production of a sufficiently good vacuum.

When the recaptured shells were examined, the position of the luminescent timestamp mark was determined in the spire under the electron beam, and then the number of subsequent dark layers was assessed, by increasing the illumination until the dark layers could be seen.

Fieldwork:
The initial field marking was carried out at Mallacoota in 1995. Between January and March 1996, we collected, stained with manganese, double tagged, and replaced large numbers of blacklip abalone (*Haliotis rubra*) in the Western zone of Victoria and at two sites in Tasmania. In addition we marked large numbers of greenlip abalone (*Haliotis laevigata*) at two sites in South Australia. However, for reasons described below, we did not achieve good results at the Tasmanian or South Australian sites, and therefore in 1997 we marked abalone at one more site in Tasmania, and two more sites in South Australia. We also marked large numbers of abalone at Flinders Reef in central Victoria in 1997, in addition to smaller numbers marked at Point Cook in Port Phillip Bay in August 1996.

Field work in New South Wales was initially postponed to 1997, when we were advised by the NSW Fisheries Research Institute that tank facilities in Eden would not be available in 1996. Later, as explained in our milestone reports, we were forced to cancel such work, as no arrangements could be made for our work at Eden in 1997, and later staining would be too late to get useful recaptures after a sufficient period to deposit ageing layers.

Victoria:
The initial time-stamping in the field was carried out at Mallacoota as part of our previous project, with the assistance of the Mallacoota Abalone Cooperative and several of the Mallacoota abalone divers, as well as the Victorian Department of Conservation and Environment. 500 abalone were collected from the east side of Gabo Island, where growth rates are fairly rapid. They were transported in wet sacks (for 1.5 hr in the boat) back to large plastic bins of seawater on pallets in the cool room of the Mallacoota Abalone Cooperative (14 degrees C). We used a trailer mounted tank and pump to fill these bins with seawater. The abalone were stained over three days (72 hours) using a 1:10 ratio of Manganese to Calcium, and the bins were stirred with pumps and strong aeration. We then double tagged. each abalone with pore and copper tags and packed between layers of wet hessian into the University boat, *Nerita*.

We intended to return the abalone to an isolated reef at Gabo Island, but our vessel lost steering at sea, and we had to put into Gabo Harbour on the auxiliary outboard.

Consequently the plastic boxes were lowered to the bottom, and the abalone then placed into a sheltered reef near Gabo Harbour. Most immediately moved nto crevices in the reef. Abalone divers visit this site regularly, but avoided harvesting our tagged animals.

We marked abalone at a site north of Point Cook, Port Phillip Bay, to investigate the timing of band formation during the year, as we could access this site regularly. 300 abalone, ranging in size from 80-110 mm, were collected in late August 1996, and transported to four 10 m by 0.5 m seawater tanks at Cheetham Salt Limited Aquaculture Centre, Lara. They were given flow through seawater and red and green algal food for 10 days to recover from handling. Manganese staining continued over 9 days. with a fresh change of seawater and manganese every 3 days. After staining, they were left in flowing seawater for 4 days, and then tagged and allowed to recover for a further 7 days. The abalone were transported back to the reef in wet sacks and replaced on the reef in early September. Underwater markers and GPS coordinates were used to identify the site. Samples of these abalone were recovered at 1-2 month intervals, depending on the weather, over 12 months.

Lawrence Rocks, near Portland in western Victoria, was chosen as a site with fast growing animals, after consultation with divers and processors from Portland. Our fieldwork was carried out using the *Nerita*, and with help from the Portland office of the Department of Conservation and Natural Resources. We are glad to acknowledge help from Portland Ocean Trading Co. Pty Ltd.,who allowed us to use their large abalone holding tanks on the quay at Portland. The abalone were collected from crevices immediately beneath the east face of the northern rock, and carried between wet sacks to the holding tank. Prior to staining they were provided with constant sea water flow for 14 days for acclimatisation and recovery from the shock of collection. During that period they were fed with large quantities of green and red algae. The abalone were tagged with both tag types and measured, prior to staining.

Staining using Manganese added to the seawater necessarily involves holding abalone in water that is not flowing. Our laboratory experiments had shown that the time required for sufficient shell marking was at least 96 hours, and not all abalone had been marked during 72 hours of staining at Mallacoota. We therefore stained the abalone for 6 days. During this time, the large tank was strongly aerated, and stirred with submersible pumps. Abalone were given flowing seawater for 1 day after staining, then packed into fish boxes between wet sacks and returned to Lawrence rocks, by placing them in the crevices where we had found them, until they adhered to the rock.

Abalone divers collect abalone from Lawrence rocks near Portland. Through the major processors, Portland Ocean Trading Co. in Portland and Souwest Seafoods Pty. Ltd. in Port Fairy, we provided local divers with information about our project and its purpose, and requested that if divers find they have collected tagged shells, the empty shells be sent to us. Many of the shells recovered from Lawrence rocks were collected in this way.

In 1997, we marked large numbers of abalone on a reef complex off Flinders, in the central zone, by arrangement and with the assistance of Harry Gorfine at MAFRI. This site was chosen because MAFRI was using this site to investigate the effects of fishing on abalone movements, size distribution and abundance estimates; and had negotiated with Victorian central zone commercial divers for the use of this site. We collected,

stained, tagged and replaced 503 abalone at this site in September 1997, using aquarium facilities at MAFRI in Queenscliff.

Tasmania:
In Tasmania, with the help of Warwick Nash and his abalone team from the Tasmanian Department of Primary Industry and Fisheries, we chose Ninepin Point - a small marine reserve area with slow abalone growth, and Stinking Bay, where abalone have a very fast growth rate. Both are localities where the Tasmanian Fisheries abalone research team have carried out trials on abalone migration, growth and mortality, and commercial diver are in agreement with such work and do not fish either site.

With the help of the Tasmanian abalone research team, more than 600 abalone were collected from Ninepin Point, and 650 from Stinking Bay, packed between wet sacks, and transported by truck to the the excellent seawater tank facilities of the Taroona Research Laboratories. Abalone from each site were put in 4-5 separate tanks to provide enough water volume per abalone. As at Portland, flowing sea water was provided and the abalone were fed with large quantities of green and red algae for 14 days prior to staining. We stained the abalone for 6 days (144 hr), but as this involved keeping abalone amongst their excretions, which is known to reduce growth in mariculture, the seawater and the manganese solution in the staining tanks was changed within each 48 hour period. The temperature, oxygen and ammonia content of the water was measured in each tank on day 3. We measured and double tagged the abalone immediately after staining, when the tanks were emptied of manganese solution, and placed them into flowing seawater.

We packed and transported the 584 stained and tagged Ninepin Point abalone on the day following tagging. Due to extremely heavy floods, there was a lens of brown fresh water at this site, and abalone are known to be sensitive to reduced salinities. We therefore took them down under the lens inside the bins, and released them onto rocks at 5-9 m depth, even though under normal conditions most abalone are found among kelp at 1.5-3 m depth at this site.

The 634 stained and tagged Stinking Bay abalone were packed, transported and placed on the reef on the next day. As abalone are normally most abundant on rocks close to shore, inside a dense band of kelp, we placed the abalone in this area. This site is on the east of the Tasman Peninsula, and thus was not subject to a fresh water lens. We found, however, that large wrasses were abundant, and quickly attacked any abalone that we did not protect until they were safely inside a crevice or well attached on a rock. A number of abalone were seen to be severely damaged or killed during the diving operation, and many empty shells were found one week later.

As a result of very poor recaptures of abalone at these two Tasmanian sites, we decided to stain a further set of abalone in 1997, from King George 111 Reef (Georges Reef), a more extensive reef south of Hobart that is more isolated from poaching. This site is also regularly used as a research site by the Tasmanian abalone research team, by agreement with Tasmanian abalone divers. 585 abalone were collected in late April 1997, stained and tagged from 8-10 May 1997, and replaced on the reef on 12 May 1997. To reduce stress during staining and tagging, which may have led to increased mortality at Stinking Bay, we reduced the staining time to 72 hours, and tagged abalone with only one tag each, pore tags for larger abalone, and epoxy putty copper tags for smaller abalone.

South Australia:
In March 1996, two sites were chosen in the Port Lincoln area, after consultation with Scoresby Shepherd, and the abalone research team from SARDI. The first was at McLaren Point, with a moderately slow growth rate, and the second was the area between Thistle and Hopkins Islands, which has a fast growth rate. Both sites are near survey area regularly visited by the SARDI team, and commercial divers are aware of tagging work in these areas, and do not fish there. 525 Greenlip abalone from McLaren Point and 473 abalone from between Thistle and Hopkins Islands were stained with manganese, measured, double tagged and released back to their natural habitat.

Staining was carried out in large portable plastic bins aboard the SARDI research vessel *Ngerrin*, which minimised transport time after collection, but abalone were stained immediately after collection, as ship time was limited. We stained the abalone for 6 days, and changed the seawater and the manganese solution in the staining tanks within each 48 hour period. As the days were hot, we surrounded the bins with water and ice to keep the abalone cool. Nevertheless there was a high mortality (>20%) of the greenlip during both acclimation and staining.

This first field marking exercise in South Australia was fortunately attempted ahead of schedule, as we decided it was necessary to repeat field marking of greenlip at these sites, because the high mortality encountered meant that our chances of recapturing enough abalone later were low. We also decided that further laboratory experiments were required to estimate the concentration of Manganese chloride and volume of water per abalone that are optimal for greenlip abalone, as our previous work had been on blacklip abalone (see "Observations during staining" in Results).

We stained and tagged abalone again in January 1997, at two further sites neighboring to our previous slow and fast growth sites. These were at off Frog Rock near McLaren Point, and off Taylor Island (a faster growth site). As we believed our previous poor results were caused by the high numbers of abalone we had kept in the tanks on the deck of the Ngerrin as compared to the volume of the tanks, we purchased large pumps, and used these to cycle seawater from the very large ballast tanks of the Nggerin through the deck tanks where the abalone were kept (see Figure 1). We added large quantities of manganese chloride to the ballast tanks, so as to obtain the correct concentration of manganese in this large volume. We also kept fewer abalone in the tanks. 218 abalone were marked and replaced at Frog Rock, and 161 at Taylor Island. At the second site we tagged the abalone with either pore or putty tags, not both, to further reduce the apparent stress of these greenlip abalone.

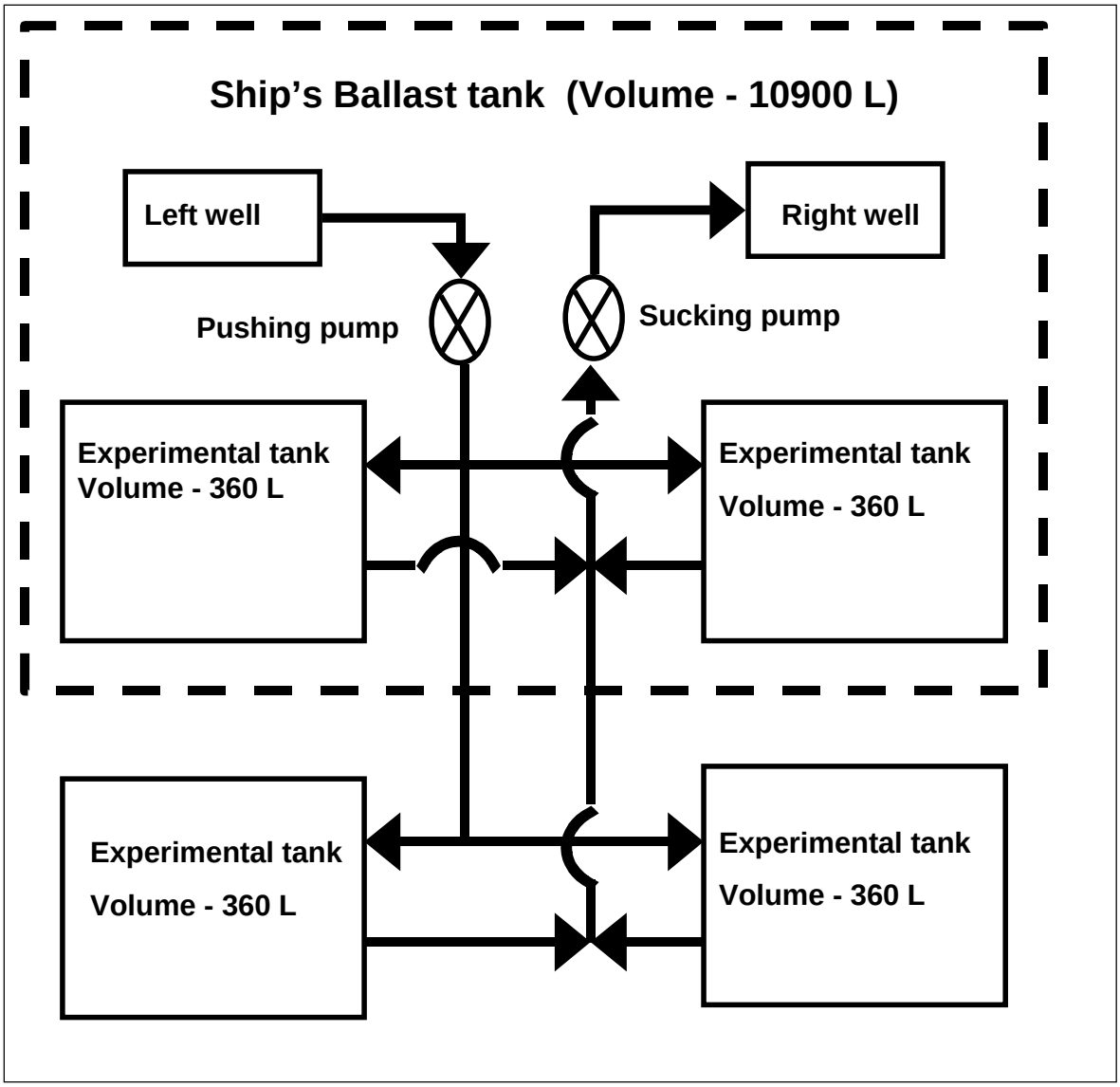

Figure 1:
Diagram of the system used to circulate seawater dosed with manganese chloride over abalone held in the deck mounted tanks on the vessel *Ngerrin*, from the large ship's ballast tank.

RESULTS

Observations during staining:
We discovered that the volume of water provided per abalone appears to be a crucial factor in maintaining abalone in good condition during staining. Although we did not change the water in the large holding tank at Portland Ocean Trading, there was almost no mortality during staining (Table 1), whereas some mortality occurred at the other sites, where the tanks were smaller, even though we had changed the water and manganese within each 48 hour period.

The Tasmanian abalone may have been more stressed during collection, or less tolerant of artificial conditions, as some mortality (1-3%) occurred during acclimatisation. The numbers dying during staining varied greatly between the 9 tanks used, suggesting that once some abalone die, others become more stressed. The average temperature in these tanks was 15.3 ^{0}C, mean O_2 was 8.52 and mean Ammonia was 0.55. These conditions would not be expected to stress abalone in themselves.

Greenlip abalone appeared to be the most sensitive subjects in our field experiments. Despite frequent water changes, cooling of tanks using a water jacket with ice around the tanks, rapid aeration and stirring with pumps, these experiments had the highest mortality (Table 1).

Table 1. Conditions and mortality in field experiments with manganese.

Location, Species	No. of abalone stained	$MnCl_2.4H_2O$ concentration (mg/L)	Immersion time (hr)	Water Vol. per abalone	Mortality (%)
***Haliotis rubra*:**					
Portland, Western zone, Victoria	572	272	96	10 L	<0.1
Stinking Bay, Tasmania	634	272	144	7 L	<10
Ninepin Point, Tasmania	580	272	144	7 L	<10
***H. laevigata*:**					
McLaren Point,	525	272	96	4 L	>20
Thistle & Hopkins Islands	573	272	96	4 L	>20

Laboratory experiments with *Haliotis laevigata*:
As greenlip abalone appeared to be more sensitive to the Manganese treatments, we carried out additional laboratory studies on the effects of $MnCl_2$ concentration, immersion time and the volume of water provided per abalone, to test the effects of

these factors on greenlip mortality, and the manganese marking achieved. Greenlip abalone were collected at Pt. Lonsdale, Port Philip Bay, Victoria. The factors varied in the experiments are shown in Table 2

Table 2. Conditions used in experiments with greenlip abalone (*H. laevigata)*

Water volume per abalone (L)	No. abalone per tank	No. of tanks	$MnCl_2$ concentration	Immersion time (hours)
5	18	2	142	96
5	18	2	202	96
5	18	2	202/101	48/48
9	10	2	202	96
3	10	2	202	96
7.5	5	3	202/101	48/48
7.5	5	3	202	96

The first three experiments tested the effects of manganese concentration, and whether an initial high dose "pulse" followed by a lower dose would be as effective in staining. The next two experiments tested the effects of the volume of seawater per abalone. The last two experiments tested the effects of a concentrated "pulse dose" followed by lower concentrations, versus a high dose over the full period, with larger water volumes.

After staining the abalone were placed in tanks with flowing seawater, and were fed with an excess of red algae. After 40 days the abalone were sacrificed, and the shells processed using standard methods. A thin section of each shell was analysed using the cathodo-luminescence microscope. The brightness of the staining was scored, and the results analysed using one-way ANOVA.

It appeared that a concentration of 202 mg/L $MnCl_2$, in more than 5 L of water per abalone gave the best staining results. The worst marks were from the treatment with 3 L of water per abalone ($F = 3.90$, $df = 8$, $P = 0.001$). However, no significant differences were found between the full dose (202 mg/L) for 96 hours and the ‘pulse’ dose treatments (202 mg/L for 48 hours + 101 mg/L for 48 hours).

Recaptures of marked abalone from field sites:
The numbers of abalone recaptured from the field sites at various times during the project are shown in Table 3. We deliberately did not collect large numbers during collections soon after marking, as we wanted to maximise collections after periods longer than one year. At a number of sites we found that recaptures were so low as to be useless, and this appeared to be a result of different factors at each site, as discussed below. We were forced to mark at more sites. At some sites, however, we were able to achieve much longer term recaptures than we had planned for, as shown later. This turned out to be very important for our overall success (see the discussion below).

Table 3: Tagging and recapture times, and numbers processed during the project (blacklip abalone).

Site & Recapture time	Release Period	No. recaptured
Gabo Is. Mallacoota, Vic. (460 tagged Jan. 1995)		Total: 148
February 1995	27	97
August 1995	210	15
December 1995	343	16
December 1996	701	14
Lawrence Rocks, Victoria (564 marked 7 Feb. 1996)		Total: 67
1 May, 1996	84	20
17 August, 1996	192	19
20 October, 1996	256	4
1 November, 1996	268	4
13 March, 1997	400	20
Flinders Reef, Victoria (503 tagged 4 Sept 1997)		Total: 0
No recaptures .		
Stinking Bay, Tasmania (634 tagged 20 Mar. 1996)		Total: 21
24 October, 1996	218	14
6 May, 1997	414	7
Ninepin Point, Tasmania (580 tagged 19 Mar. 1996)		Total: 10
23 October, 1996	218	1
6 May, 1997	417	9
Georges Reef, Tasmania (585 tagged 8 May 1997)		Total: 52
25 November, 1997	201	28
21 April, 1998	347	24

Table 3, Continued (greenlip abalone).

Site & Recapture time	Period until recapture	No. recaptured
McLaren Point, S.A. (525 tagged 6 Mar. 1996)		Total: 129
35354	225	22
35437	308	29
35574	384	44
35677	548	5
35808	679	29
Thistle & Hopkins Is, S.A. (473 tagged 7 Mar. 1996)		Total: 68
35354	224	21
35437	307	1
35574	444	42
35808	678	4
Frog Rock, S.A. (slow) (218 tagged 21 Jan. 1997)		Total: 90
35572	122	10
5 Sept 1997	227	43
35808	358	37
Taylor Island, S.A. (fast) (161 tagged 25 Jan. 1997)		Total: 29
35574	119	13
5 Sept 1997	222	14
35808	353	2

Mallacoota:
Abalone divers assisted us in making many collections. The survival of abalone long after staining demonstrates any long term effects of the manganese or tagging are minimal. In the first of these sets of collected abalone 60% of the 50 shells examined had strong marks in the spire region. In a number of others fainter marks could be discerned. These results demonstrated that our shore based field marking process was successful, but as we needed a higher marking rate, we increased the staining time in subsequent experiments, to increase the average width of the shell layer with a mark.

Lawrence Rocks, Portland, Victoria:
The survival of black lip abalone at this site appeared to be quite high. Animals after release apparently settled down and were observed at the same place several times. The only problem in collecting tagged abalone from this habitat was that the crevices were so extensive and abalone so abundant that long searches were needed. Our double tagging seemed to be effective: we lost only perhaps 20% of the pore tags and less than 10% of the copper tags attached with epoxy putty, and even if one of the tags was lost the abalone could still be identified. Many tagged shells were collected with the help of Souwest Seafoods and Vin Gannon.

Flinders Reef, Victoria.
Despite extensive searches on many occasions by ourselves and MAFRI researchers, we have not recovered our tagged abalone. MAFRI researchers tagged several thousand abalone underwater at this site as part of an investigation of the effects of fishing, and have found very few of all these tagged abalone. It appears that this site has very extensive cryptic habitat into which marked animals may disperse. This entirely unexpected result suggests that the numbers of adult and legal sized abalone in cryptic habitats may be very large on some reefs. This is an important factor to consider in evaluating catch over time data in stock assessment models.

Ninepin Point and Stinking Bay, Tasmania:
After intensive searching by several divers 7 months after their release, only a small number of tagged abalone was recaptured at each site. At Stinking Bay there would have been a high mortality initially because the weakened abalone were attacked by large wrasses immediately they were replaced. Judging from the dead shells seen, however, this is not sufficient to explain the low returns, and we suspect poaching.. At Ninepin Point only 6 dead shells were found, even after prolonged searches, so that high mortality can probably be excluded. However, as we had returned the abalone during a period after heavy rain, and had placed them on deeper rocks, we probably encouraged them to migrate. The few recaptures were found in shallow water some distance from the return site, although extensive searches of the area were carried out. We believe that either the bulk of the tagged abalone emigrated and dispersed some distance down the coast, or that many of our abalone were poached from the reserve.

16 abalone were recovered from Stinking Bay, and two from Ninepin Point, 7 months after release (Table 3). A further extensive search at Ninepin Point recovered 9 abalone, 14 months after the release. The processing of the shells from Stinking Bay also revealed that a high percentage had been parasitised by the mudworm, *Boccardia* sp., which creates a large blister under the spire, and so leaves the shell unusable for ageing or age validation. These results led to our decision to stain another collection of abalone in in January 1997, from Georges Reef.

Georges Reef site:
As abalone were marked so late in the project, and we were reliant for recapture collections on research trips by the Tasmanian abalone team to this isolated site for other projects, only two recapture collections were possible by the end of the 3 year cycle of the project, on 25 November 1997 and 21 April 1998. We found, unfortunately, that a lower percentage of these abalone had staining marks than at other sites, presumably due to the reduced staining time we used. However, no dead shells were found, suggesting the reduced stress led to high survival.

South Australian sites:
Recaptures of our stained abalone were carried out by SARDI staff. While those greenlip abalone that survived at the McLaren Point and Thistle Island sites appeared to have retained their tags well, there was a high initial mortality of the replaced abalone when the sites were examined by SARDI divers soon afterwards. We suspect the abalone were overcrowded in the small deck mounted tanks of the *Ngerrin* during staining, and this probably also was responsible for a low percentage of visible manganese marks in the spires of recovered abalone. Only 10 to 20 % of abalone were successfully stained in the spire in 1996, although 80% of the abalone recaptured had a mark at the shell edge, where shell deposition is more rapid. These results and the consequent laboratory experiments on greenlip described above, were the basis for our decision to repeat this staining experiment at the same sites in 1997, using a much larger volume of water per abalone. Survival after the 1997 staining at Frog Rock and Taylor Island appeared to be far better.

Dark layers deposited by abalone after the staining mark:
Table 3 shows that the recapture rates at all sites were much lower than expected, but the numbers collected from most sites would be expected to be sufficient for analysis . The results from abalone recaptured over longer periods at Mallacoota are summarised in Table 4, and for other sites in Table 5. The results from Mallacoota showed a very high percentage of abalone were stained, and the layers are surprisingly consistent. These data suggest a slow accumulation of layers, approximately one per year. Note that the standard deviation (variability) of the annual rate declines in the longer term recaptures. In contrast, at most sites the percentage of shells collected in which we could both find the manganese mark, and reliably count subsequent dark bands was disappointingly low. In blacklip shells this was due mainly to boring of shells, which led to thick black protein repair layers. These sometimes obscured the manganese mark, and often made counting ageing layers impossible. Greenlip suffered from boring far less, but they appear much more sensitive to stress, and many of them apparently did not deposit any shell while in the manganese stain, so that they had no discernable mark.

Table 4. Results from Mallacoota: interval before recapture and counts of dark layers after the manganese mark (SD = Standard Deviation)

Days after release	Years after release	No. of ageable abalone shells	Mean layers after mark (+/-SD)	Annual rate of deposition (+/- SD)
210	0.6	13	0.3 +/- 0.4	0.5 +/- 0.7
343	0.9	13	0.9 +/- 0.7	0.9 +/- 0.7
701	1.9	12	1.3 +/- 0.6	0.7 +/- 0.3

Table 5: Recapture periods, and dark spire layers after staining. Data obtained during the project

Period	Days	Years	No. usable	Av. layers	SD layers	Av. layers/yr	SD/yr
Lawrence Rocks							
Feb-May	84	0.23	11	1.82	0.75	7.91	3.26
Feb-Aug	192	0.53	10	1.60	1.35	3.02	2.55
Feb-late Oct	268	0.73	7	1.57	0.53	2.15	0.73
Feb96-Mar97	400	1.10	14	2.43	0.85	2.21	0.77
Ninepin Point, Tas							
Mar 96-Oct 96	218	0.6	1	0	-		
Mar 96-May 97	417	1.14	6	1.5	0.55	1.32	0.48
Stinking Bay							
May 96-Oct 96	218	0.6	12	1.08	0.79	1.81	1.32
Georges Reef							
May - Nov 97	201	0.55	18	1.00	0.69	1.82	1.25
May 97-Apr 98	347	0.95	16	2.44	1.36	2.57	1.44
McLaren Point, S.A.							
Mar-Oct 96	225	0.62	8	1.25	0.71	2.02	1.14
Mar 96-Jan 97	308	0.84	10	2.3	0.67	2.74	0.8
Mar 96-May 97	384	1.05	18	2.6	0.97	2.49	0.93
Mar 96-Sep 97	548	1.50	3	3	2	2	1.33
Mar 96-Jan 98	679	1.86	6	4	2.2	2.15	1.23
Thistle and Hopkins Islands							
Mar - Oct 96	225	0.62	9	1.55	1.13	2.51	1.82
Mar 96-Jan 97	308	0.84	2	2.5	0.71	2.98	0.84
Mar 96-May 97	444	1.22	26	2.38	1.02	1.95	0.84
Mar 96-Jan98	678	1.86	2	3	1.41	1.61	0.76
Frog Rock							
Jan-May 97	122	0.33	0	-			
Jan-Sep 97	227	0.62	2	2	0	3.23	0
Jan 97-Jan 98	358	0.98	14	1.86	1.17	1.9	1.19
Taylor Island							
Jan-May 97	119	0.33	4	1.25	0.5	3.79	1.52
Jan-Sep 97	222	0.61	2	2.5	0.71	4.1	1.16
Jan 97 - Jan 98	353	0.97	1	3	-	3.1	-

It is clear that boring is far more of a problem for blacklip than greenlip, and that the number of layers is much more variable for blacklip. We think this is due to extra layers being deposited after boring, even in lightly bored shells. To overcome this problem a method to distinguish repair and annual growth layers is needed. A project student was set the task of developing methods to examine growth layers under the scanning electron microscope. While this did not prove fruitful, we were able to show that abalone produce nacre more rapidly after being bored, with more layers. These results are reported in Marshall and Day (2001).

Table 5 also shows that the number of bands deposited in fixed intervals is extremely variable at these sites. This would suggest that the ageing method based on these bands is very unreliable. Furthermore, the average band deposition per year is often

more than two, in contrast to the claims of one band per year by Prince *et al.* (1988) for blacklip in Tasmania, and one or two layers per year for greenlip by Shepherd and Triantafilos (1997). Close inspection of the data also shows that larger numbers of layers in abalone shells are not limited to abalone that had been heavily bored by polychaetes, which might have inflated the averages. Interestingly, there is a general trend in Table 5 towards lower annual rates of layer deposition in the longer term recaptures. Before considering this issue further, several other sources of information should be considered: the timing of bands during the year, data obtained by Scoresby Shepherd on layers in known age abalone, and recaptures we obtained after the end of the project.

Timing of band formation during the year:
Identifiable manganese marks were found within the spires of over 90% of the abalone recaptured from Port Phillip Bay, in the monthly collections. This suggests the methods to minimise handling stress during the collection, staining and replacement process at this site (see Methods) were effective. Such methods are only possible where field time is not limited, as it was at most sites. The results are summarised in Table 6

Table 6. Timing of dark band deposition in abalone released in September in Port Phillip Bay.

Month collected	Days after release	No. ageable shells	% with one dark band	% with two dark bands	Mean no. dark bands
November	54	25	8	0	0.08
December	82	11	18	0	0.18
January	126	25	32	4	0.36
February	149	9	67	11	0.78
March	181	8	88	0	0.88
May	227	16	75	25	1
June	252	21	95	19	1.14
August	308	17	94	29	1.23
September	354	14	100	21	1.21

What these results suggest is that abalone may deposit a dark layer at almost any time of year, from November through to August, although there is an increased rate of dark layer formation in summer. Taken at face value this result is very disappointing, as they suggest there is no fixed environmental cue that triggers layer formation. Without any fixed cue, there is no obvious reason why layers would be deposited regularly on any fixed cycle, and therefore useful in ageing. But if this were true, one would expect the variability in the number of layers deposited to increase very rapidly as the recapture period increases. At all sites (Tables 4, 5) the variability in total number of bands does increase, but not markedly, and the variation in the annual rate decreases in longer term recaptures.

If layer deposition was irregular, it is also hard to explain why Prince *et al.* (1988) would find a roughly annual rate of deposition in juvenile blacklip abalone and Shepherd has found fairly regular rates of deposition in various abalone species (Shepherd *et al*. 1995b, Shepherd and Avalos-Borja 1997, Shepherd and Huchette 1997). Furthermore, while we were evaluating our data at the end of the project, Shepherd provided data suggesting that layer deposition was consistently related to age in blacklip and greenlip abalone.

Evidence from work by Shepherd:

Shepherd has recently investigated the relation between the size of juvenile abalone and the number of rings visible in the spire after grinding it to expose a hole (see 'Background; Shell structure), in blacklip (Shepherd unpublished) and greenlip abalone (Shepherd and Triantifillos 1997).

Shepherd and Triantifillos (1997) counted rings in greenlip abalone that were collected from a number of sites where von Bertalanffy parameters of the growth rate had been estimated from tag recapture data. This type of data has also been obtained to a lesser extent for blacklip abalone. It is however, very weak evidence, as discussed in the "Background" section. They also counted the number of rings deposited in two groups of greenlip abalone reared in an aquarium for 20 months and 25 months. These groups had different growth rates in culture. The number of rings recorded in the 20 month group ranged from 4 to 7, with a mean of 5.5 (3.2 per year). In the 25 month group, 5 to 8 rings, averaging 6.6 (3.3 per year) were deposited.

These data show the same large variation over short periods as in our data. However, there was evidence that the number of layers deposited increased with growth rate, fast growing abalone depositing 4 layers per year, while slow growing animals deposited about 2 rings per year. Growth of some of the abalone in a batch of juveniles in culture is much faster than recorded in the field, so that these data fit with their field evidence, and suggest that over longer periods, the number of layers deposited per year in *Haliotis laevigata* varies between 2 and 3 depending on how fast the abalone has grown. This in turn indicates that the variation in the frequency of layers is not merely a reflection of the timing of layer deposition, and does not bode well for accurate ageing in greenlip. However, both these sources of data refer to juveniles, which grow rapidly. The question of how frequently adults deposit layers remains open.

Shepherd and Triantafillos attempted to extend the ring versus size relation to adults, by counting rings in animals that were tagged while juveniles, and recaptured some time later. This method relies on an estimate of how many rings were present when the abalone was tagged, based on the relation of ring number to size in juveniles from the same site. Thus the method suffers from the problems described in the "Background" section, plus problems related to the timing of layers over short recapture times (as explained later), and false layer responses to borers. Also, as in our data, there are small numbers of suitable recaptures.

The data again suggests 2-3 layers per year on average, but with some abalone depositing 1 or 4 layers. However what is interesting is that the four abalone recaptured over long periods (>4 years) had deposited 1-2 layers per year. Shepherd (unpublished) also investigated greenlip abalone that had been planted onto reefs at Flinders Island and recovered a number of years later. There were 5-7 rings in 3 year

olds (n=18), 6-8 rings in 4 year olds (n=4), and 17-20 rings in 9 year olds (n=16). This suggests the deposition of layers over long periods may be less variable.

Shepherd (unpublished) also counted rings in maricultured juvenile blacklip abalone up to 5.5 years old from Tas-Univalve, and has kindly supplied these data. He concluded that 4 minor rings were laid down in the first 2.5 years (which corresponds to the earlier work of Prince *et al.* (1988) if the last minor ring is reclassified as a major ring), and found the number of rings deposited thereafter was close to one. But it is more instructive to examine the variation in the data.

Table 7 shows the means and standard deviations. Another approach is to consider the frequencies of counts. Of 19 shells aged 3.5 years, 12 (63%) had 5 rings, 4 (21%) had 4 rings, 2 had 6 rings and one had 3 rings. Out of 19 shells aged 4.5 years, 12 had 6 rings, 4 had 5 rings, 2 had 7 rings and one had 8 rings. In a much larger sample of 54 shells aged 5.5 years, 59% had 7 rings, 22% had 8 rings, 11% had 6 rings, 4% had 5 rings, and one (2%) had 9 and one had 10 rings.

Table 7: Dark rings in *Haliotis rubra* from culture. (Courtesy of Shepherd)

Age	**3.5 yr old**		**4.5 yr old**		**5.5 yr old**	
sample size:	19		19		54	
	Layers	**No/yr**	**Layers**	**No/yr**	**Layers**	**No/yr**
Average	4.79	1.37	6.00	1.71	7.13	2.04
Standard Deviation	0.71	0.20	0.75	0.21	0.85	0.24

Consideration of both the Table 7 and the frequencies of counts suggests the distribution of deviations in ring counts remains constant with increasing age. This is not what would be expected if there was simply chance variation each year, as such variation would add together year by year. It seems that ring counts become more reliable over longer periods. What this suggests is that the variability between abalone is almost all due to when, in any given year, they deposit a dark layer in the shell. Figure 2 demonstrates how this might work.

Abalone at many of our sites were collected, stained, tagged and replaced in early 1996 (see the Staining arrow in Figure 2). If the timing of dark layers varies over 10 months each year (represented by the grey zones), then abalone collected after about 9 months (Sample 1) might have no dark bands if the abalone had deposited a dark layer before they were stained, one band if they laid down a dark layer between March and August 1996 (but no more before they were collected in Sample 1), or even perhaps two bands if they deposited a band after March in 1996, and another at the beginning of the next grey zone, in late 1996. Sample 2, after about 1.4 years, would also contain abalone that had 0, 1 or 2 bands. Samples 3 and 4, after about 1.8 and 2.3 years, would both have abalone with 1, 2, or 3 bands. A long term recapture sample, obtained after our project cycle after 3.2 years, would have 2, 3 or 4 bands.

The variability in our data, and in Shepherd's data, cannot all be explained by this theory. Both we and Shepherd found the band counts varied by more than 3 in abalone of the same age. But some extra variation is to be expected, because it is sometimes difficult to distinguish the "true" shell bands from layers that are deposited to repair damage by boring polychaetes.

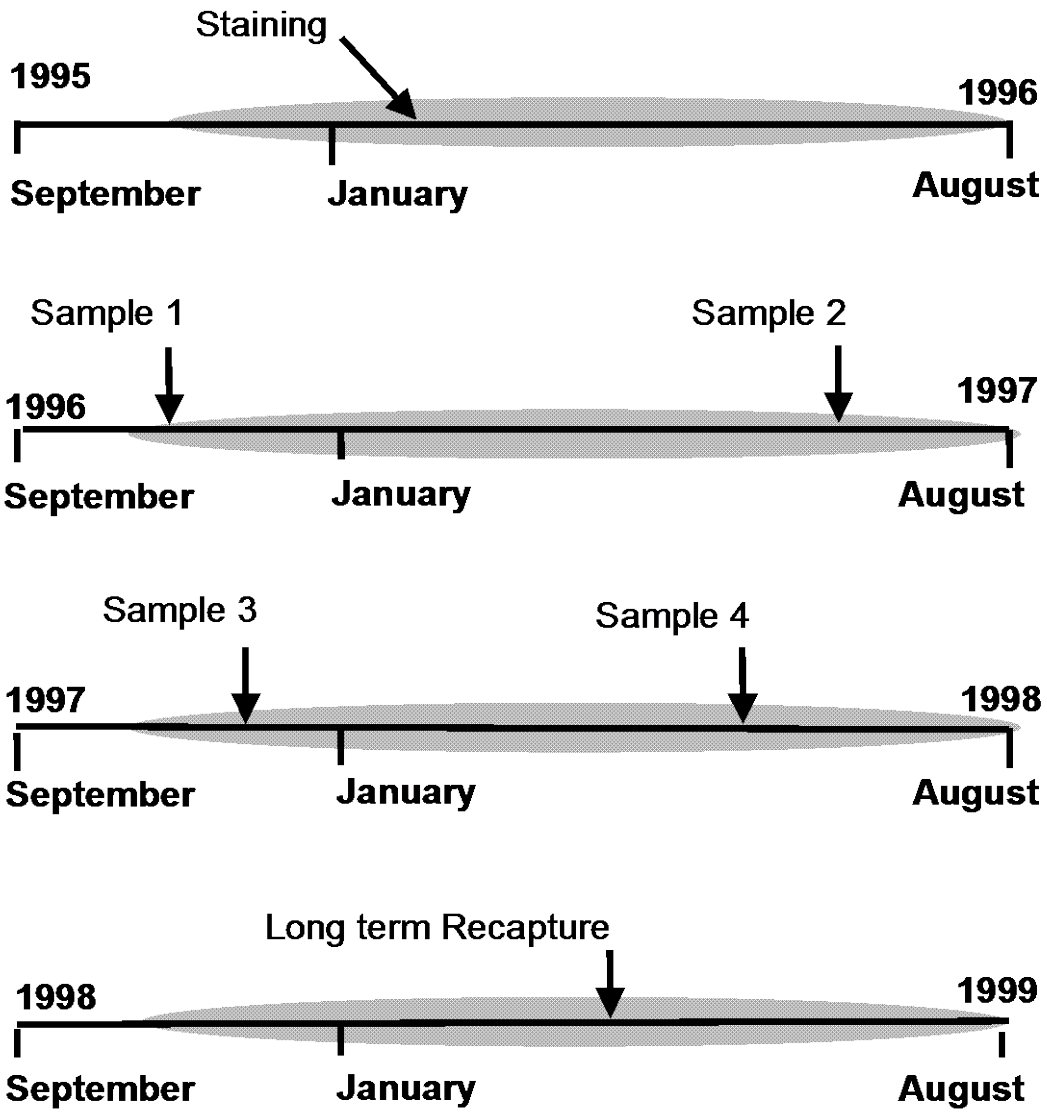

Figure 2:
Diagram showing how variable timing of dark layer deposition in abalone would affect the number of layers found in the staining - recapture experiments. Each line represents a year from July to the following June during our project. The period over which individual abalone may deposit dark layers is represented by the grey zones. The time at which abalone were time stamped with stain, and the recapture sample times are shown by arrows.

Our data has standard deviations that are often larger than those of Shepherd. In some cases this may well be due to the small sample sizes of ageable abalone in our recapture samples, but it seems likely that the method of counting rings in ground-down spires that Shepherd used is more likely to distinguish and thus discount repair layers.

Shell repair has been studied by Thomas and Day (1995). The abalone responds to a hole penetrating the inner surface of the shell by depositing a very thick, dark layer of protein over and around the hole, which is visible on the inner surface over an area of about 15-20 mm diameter. This protein layer thins towards the edges, and may extend over a wider area as a dark layer of the same thickness as "annual" dark layers.

Figure 3 shows the relationship between the bands counted in spire sections and the rings counted after grinding down the spire. If a section slices through the area of thick repair protein, the repair layer can be easily identified. But if the section passes the edge of a repair zone, the repair layer appears like any other dark layer. In contrast, when the spire is ground down, repair layers can be often seen to extend for only a limited distance around one part of the shell around the spire, and so can be more easily identified.

Long Term Recaptures (obtained after the end of the project):
Even Shepherd's data from known age animals relates mostly to juveniles. The hypothesis arising at the end of the project cycle meant that we could only draw a reasonable conclusion about the value of shell layers for ageing if we could obtain long term recaptures of stained abalone. It seemed worth delaying the reporting of this project until we could obtain and process long term recaptures.

Fortunately, we were able to obtain further recaptures from several sites more than two and a half years after they had been stained. One was found after 4.6 years at Mallacoota by a Victorian fisheries research diver (S. Cameron). We have also obtained more of our tagged animals from Georges Reef, found 3.5 years after release, during other work by the TAFI abalone research team at this site, but they have not yet been processed.

Furthermore, a surprisingly high percentage of these long term recaptures showed manganese marks. Perhaps these were largely abalone that remained in very good condition during our staining. This is fortunate, as our experiments had been designed to provide sufficient recaptures after about 2 years only. Over longer periods the marked abalone disperse over a wider area. Thus even the fairly small samples we obtained over long periods are a mark of unexpected success at some sites.

The results from all sites (Table 8) suggest a long term annual rate of about 1.2 layers per year, but more importantly, the standard deviation of layer numbers remains at about 1.4, so that the standard deviation of the rate of layer formation per year is much lower in these data than in Table 5, and is lower for the longer term data in Table 8. The high standard deviation of 1.84 at Lawrence rocks may be the result of boring in some abalone, as boring was very common at this site. Layer formation does appear to be fairly regular over longer periods. While the sample sizes from each site and time are small, the data are remarkably consistent, so that it seems reasonable to draw a fairly firm conclusion for all sites together.

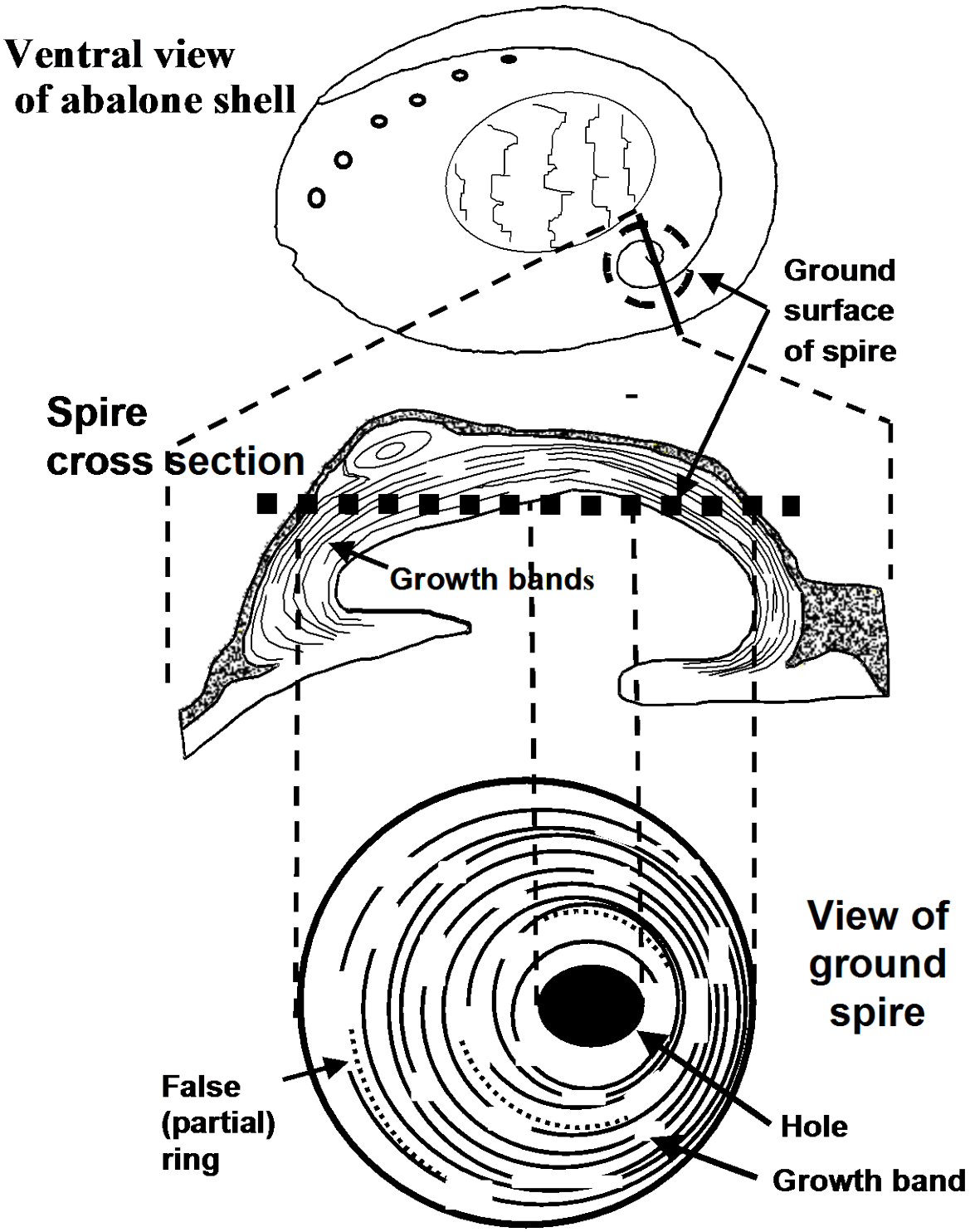

Figure 3:
Diagram showing the relation between the layers seen in cross sections of the spire and rings seen when the shell spire is ground until a hole appears. Note the false rings due to shell repair appear as partial rings.

Table 8: Long term recaptures of stained abalone, obtained after the project period.

Site	Period (yr)	No. Recaptured	No. ageable	Av. layers	SD layers	Av /yr	SD /yr
Mallacoota	2.5	5	3	3.25	1.26	1.36	0.43
(*H. rubra*)	4.6	1	1	3.7	-	1.24	-
Lawrence Rocks							
(*H. rubra*)	3.1	8	7	4.21	1.84	1.36	0.59
	3.7	8	6	4.55	1.08	1.23	0.29
	4.0	1	1	4	-	1	-
Frog Rock, S.A.							
(*H. laevigata*)	2.8	14	3	3.55	1.38	1.29	0.5
McLaren Point, SA							
(*H. laevigata*)	3.6	12	8	2.69	1.18	0.75	0.33

Our standard deviations for long term recaptures (Table 8) remain higher than those of Shepherd (Table 7), presumably due to the ring count method he used. The overall standard deviation for the ring count method in adult abalone might be expected to lie between our average of about 1.4 (lower if we discount the 1.84 from the 3.1 year Lawrence Rocks data) and the value of 0.75 from Shepherd's data. A standard deviation of 1 from ring counts would mean that 60% of the abalone would have an age within one year of the estimated age, and 90% would be within 2 years of the estimated age. As the data for abalone ages that would be used as inputs for management models would involve abalone that are perhaps 4-14 years old, an error of 1-2 years in the estimates for individual abalone is probably acceptable.

The overall average number of rings per year of 1.2 may also be inflated by the number of repair layers incorrectly included in our counts. Thus it seems that layers are deposited approximately annually at all the sites we investigated. This includes both blacklip and greenlip, and both fast and slow growing stocks of greenlip abalone.

General Discussion of Results:

A major disadvantage of our data is that our estimates of the variability in long term rates of dark layer formation, which are the crucial results in evaluating the value of ageing data, are based on very small sample sizes. This is an unavoidable consequence of the fact that tagged animals disperse and die over time. It is a mark of the scale and effectiveness of our experiments that we were able to recover any tagged animals after more than 2 years at any of the sites. The original design of the experiments was based on a need to obtain recaptures for 2 years. It was only when we found the unexpected result that the timing of layers was unpredictable, yet dark layer formation appeared to be periodic, that it became clear that samples over long periods (3 years or more) were crucial to achieve the overall objective of this project.

Fortunately, we had marked large enough numbers at many sites, and our methods led to long term survival of both the abalone and the tags, so that we were able to obtain such data. These recaptures were obtained after the normal period of the project, when the project funds had been expended, so that trained personnel to process the samples were not available, and this led to long delays in obtaining the required information. We still have a number of abalone from Georges Reef to process, and these should contribute substantially to the sample size of our long term recaptures, and thus to how well we can estimate the uncertainty in age estimates in blacklip abalone.

BENEFITS

Both the blacklip and the greenlip abalone fishing industries will benefit in the longer term through more secure predictive management. Management decisions at present rely on assessments of stocks that are based on models that incorporate:
(a) diver surveys of the abundance and size distributions of abalone at selected sites.
(b) estimates of recruitment rates based on these surveys.
(c) estimates of growth rates derived from tag recapture studies at a limited number of sites
(d) an even more restricted set of estimates of natural mortality

The methods to obtain growth and mortality estimates are costly, and the abalone fishery is known to be based on a large number of somewhat independent substocks, with highly variable growth rates, and probably very variable mortality rates too. Our results show that estimates of growth and mortality based on ageing are feasible, and the error in such estimates can now be predicted. Subject to such errors the ageing technique can extend growth and mortality estimates to a large number of substocks. In addition, age distributions of abalone at sites can reveal age at maturity and age specific fecundity, and in conjunction with abundance surveys can reveal the rate at which recruitment to the fishery is occurring at that site.

These data would contribute markedly to more comprehensive and useful predictions from models, and eventually to models that provide separate predictions for substocks. Such models should lead to better management and more security in future stocks for the industry. Security of stocks appears to be an important factor in determining the value of the industry, as it determines investor confidence.

FURTHER DEVELOPMENT

As discussed previously, the crucial long term estimates of how precise ageing is when based on counts of dark layers, are based on very small sample sizes. This is a disadvantage, as it leads to uncertainty in our estimates. We have received further recaptures from Georges Reef, but a lack of funds and trained personnel after the project was ended have delayed processing. We hope to have these data available soon. The abalone shells have been set in resin and polished for examination. The results will be reported to the abalone modelling group as soon as they are available.

We have decided to process the extra Georges Reef long term recaptures in a new way, in the light of the difference in variability between our results and those of Shepherd. We have first ground the spires, and then sectioned a neighbouring part of the spire, in order to compare the results for the two methods. This has involved developing a way to set the ground surface of the spire into a resin block in such a way as to obtain a thin section that can be placed in the vacuum box of the cathodoluminescence microscope. It remains to be seen whether this is successful. We hope however, to demonstrate that ring counts of ground down spires are less variable and thus more reliable.

The results of this project have been disseminated, as the project progressed, to those involved in the current FRDC abalone modelling project, which is developing a

framework for modelling abalone stocks in all states, and management oriented models for their fisheries. The initial results of this study led to an expectation that age estimation based on dark layers would be inaccurate and of little value in modelling. The present version of the model has been developed as a size-structured model, to make best use of the information available from surveys, catch records, and tagging experiments to measure growth.

However our later results and analysis were presented in preliminary form at the last International Abalone Symposium in Cape Town, South Africa, and subsequently to meetings of the modelling project advisory group, which includes researchers from all states. This has encouraged further work to obtain age distributions of abalone from samples throughout the Tasmanian fishery, and from many sites in Victoria. Currently there is debate as to how such ageing data should be used, informed by the results of this project. As our results show, age estimates based on layer counts are inaccurate, as any estimate must be to some extent. The value of this project is that we now have an estimate of how precise age estimates are, and that the precision apparently does not depend on the age of the abalone.

We now know that samples to determine growth rates by ageing and measuring abalone, random samples of abalone at sites to determine the age distributions of stocks, and estimates of mortality based on random samples will be useful. But this work is time consuming and expensive, and our project has also shown that there is a limited but important error in these age estimates. Further work is now required by modellers to evaluate the costs and benefits of large scale ageing work to stock assessments, and management models, and recommend to the states to what extent resources should be directed to obtaining such data for their stocks.

Our results are also important internationally, as many important abalone fisheries in other nations appear to deposit layers in a similar way. Once all our long term recaptures are analysed we will publish the results in international journals. We have shown what form of validation is required for other species and stocks, and how this can be achieved. Dark layer formation might be expected to follow the same pattern in other species and stocks, but there is no guarantee of this. For example we cannot predict whether the deposition of dark layers is regular over the long term in blacklip abalone from New South Wales, where temperatures are warmer and the habitats rather different. One might speculate that the presence of the black urchin, *Centrostephanus rogersi* in these habitats, could conceivably lead to periodic episodes of starvation and thus changes in shell layer formation. Thus validation of the kind achieved in this project is needed in other fisheries before ageing data can be used with any confidence to support management.

PLANNED OUTCOMES

In our application, we stated that the project would be successful if it provides a method of producing age-frequency estimates for abalone populations, or if it can demonstrate that this method is not usable for abalone. The expected output was that the timing and regularity of layers would be found to be good at some sites, but not in others, so that our second objective was to carry out experiments across a number of sites in important areas of the blacklip and greenlip fisheries.

What we found was more complex. While the timing was irregular, and the number of layers deposited over 1-2 years was very variable, the long term regularity of layer formation appears to be reasonable. Furthermore, layer formation was shown to be consistent across a number of sites in Tasmania and Victoria for blacklip abalone, and in both fast and slow growing sites for greenlip abalone in South Australia. This output must now be evaluated by modellers to determine how much effort should be put into obtaining estimates of growth, mortality, age-specific fecundity and age distributions from sites across the state fisheries. The outcome here is that the states now have a basis for evaluating the value of obtaining age based estimates. As discussed in "Further Developments", the results have been communicated to modellers and state abalone researchers at the Victorian abalone modelling meetings, so that they can influence current planning and model development.

We stated in our application that an important outcome would be a knowledge of which sites could be used for ageing. Our data show consistency in layer formation across sites, which is very useful, but our data are based on abalone that were not highly bored. We found that boring both obscured the chemical mark, and reduced our ability to count dark layers.

We have not found a unequivocal way to discriminate between the regular layers and repair layers, which would have been a useful extra output, as it would allow the ageing method to be used more generally - a very important outcome. For some sites, where boring is common, the use of shell layers to estimate age will not be useful, because extensively bored abalone shells will not be ageable, and there is a real risk that these would be the older abalone, and also possibly the slower growing abalone. This would bias any results obtained from the age estimates. Often areas with stunted stocks have many very bored abalone, so that these sites are not likely to be usable for ageing work.

This does not detract from the importance of being able to age abalone at other sites. It is the faster growing sites that have the highest productivity to the fishery, so that ageing should be useful on the most productive and important reefs in the fisheries. The error in age estimates is probably acceptable for most modelling purposes.

We stated in the risk analysis of our application that the major risk to our ability to discover the relation between layers and age (as opposed to the risk of unageable abalone) was that we might recover too few time-stamped abalone at the end of our experiments to work out how regularly the layers are deposited, because we did not know how fast the marked abalone would disperse or die at each site. Our insurance policy was to mark as many abalone as possible in these experiments, so that we could obtain sufficient data. In fact as a result of this policy we were able to obtain data from long term recaptures, which unexpectedly were a vital output from the project. Without these we could not have achieved our overall objective.

CONCLUSION

Our longer term results have achieved the overall objective of the project, to answer the question: how reliable are estimates of age based on the method of counting shell layers? Our estimates of the reliability of age estimates can now be used by state management committees to determine the value of ageing work for future management.

Our first listed objective was to determine the timing and regularity of spire layers in abalone. While we found the timing of layer formation was very variable, the long term regularity of layer formation appears reasonably good. We were only able to achieve this objective, however, by obtaining and processing marked abalone recaptured long after the project 3 year period. This final report was delayed for this reason.

Our second listed objective was to obtain such results for blacklip at three sites in Victoria, three in Tasmania and one in New South Wales, and for greenlip at two sites in South Australia. While we set up experiments in more sites than envisaged in our application, we found that there are many unexpected ways such experiments can fail. But we have obtained data from a range of sites for blacklip in Tasmania and Victoria, and for greenlip in South Australia. Unfortunately we were not able to make arrangements for work in New South Wales, and the timing and regularity of layers in that fishery may well be different, although our results from Mallacoota suggest that useful ageing may be achievable there also.

Our third objective was to determine how to interpret layers in abalone shells, how reliable such interpretations are, and how layer formation may vary between sites.. We have not found a way to distinguish between layers laid down on a regular basis and the layers deposited to repair shell boring, although we attempted to find differences under the Scanning electron microscope. We suggest that interpretations of layers may be better when counting rings in ground down spires, although we have not yet any good data to demonstrate this. We hope that shells currently being processed from Georges Reef will provide such data.

The most important aspect of our third objective was the reliability of age estimates based on layer counts. Our long term results and those of Shepherd suggest the standard deviation of the number of layers in shells of a given age lies between 0.75 and 1.4. The variation would be at the lower end of this range for sites where abalone are not bored, and higher when abalone are extensively bored. Lower variation is also expected if the ring count method is used, and more variation if the shell section method is used. We expect that the error in age estimates will be sufficiently small to make the method useful in most circumstances.

In relation to the last part of our third objective, our results show that the rate of layer formation is remarkably consistent across sites. This is a very encouraging result, as it suggests that layer formation will be consistent at many other sites.

Our fourth objective, to obtain age distributions of abalone at a number of sites, proved unachievable due to the extra marking experiments we had to do at new sites when we could not recapture many abalone at several sites. The achievement of this objective would have facilitated the use of ageing data in models and management, but such data

needs to be collected at many sites throughout the fishery, so that we could only have provided example data for the model. This was our least important objective.

Our last listed objective was to be able to predict where and how layers can be used to age abalone in southern Australia. The consistency of our results allows us to predict that the interpretation of layers will be the same for all sites, except those subject to heavy boring. We demonstrated that when they are bored, abalone deposit more nacre and extra repair layers. This will apply particularly to stunted stocks, but these are less important to the fishery (in terms of recruitment to legal size).

In summary, we have found that the dark layers in the spire of adult blacklip and greenlip abalone shells appear to be deposited on a regular schedule over the long term, at a range of sites for both species. We have found that the error in age estimates of large abalone based on dark layers is likely to be almost independent of age, and that the estimates are probably precise enough for most purposes, provided heavily bored sites are avoided. We recommend that state research advisory groups should consider obtaining age distributions of abalone from a range of reefs within each fishing zone. These data should provide useful data on growth, mortality, age specific fecundity, and age distribution and thus contribute substantially to better stock assessments and management models.

REFERENCES

Barbin, V. (1992). Fluctuation in shell composition in *Nautilus* (Cephalopoda, Mollusca): evidence from cathodoluminescence. *Lethaia* 25: 391-400.

Beamish, R. J. (1992). The importance of accurate ages in fisheries sciences. In: 'The measurement of age and growth in fish and shellfish'. Australian Society for Fish Biology Workshop. (Ed D. A. Hancock) pp 8-22 (Bureau of Rural Resources: Canberra)

Beamish, R. J., and McFarlane, G. A. (1983). The forgotten requirement of age validation in fisheries biology. *Transactions of the American Fisheries Society* 112: 735-43.

Day, R. W. and Fleming, A. E. (1992). The determinants and measurement of abalone growth. In: 'Abalone of the World: Biology, Fisheries and Culture.' (Eds: S. A. Shepherd, M. J. Tegner and S. A. Guzmán del Proó.) pp. 141-168. (Blackwell Scientific: Oxford).

Day, R. W., Williams, M. C., and Hawkes., G. P. (1995). A comparison of fluorochromes for marking abalone shells. *Marine and Freshwater Research* 46: 599-605.

Day, R., Hawkes, G. and Williams, M. (1998). Development of a new chemical marker in abalone, for age validation and other applications. FRDC final report, Project 92/40. (University of Melbourne: Parkville, Vic) 72pp.

de Jong, J. (1990). 'The relationship between age and growth of the blacklip abalone, *Haliotis rubra* (Leach) in Port Phillip Bay, Victoria.' BSc. Hons. thesis, University of Melbourne, 81 p.

Erasmus, J., Cook, P. A. and Sweijd, N. (1994). Internal shell structure and growth lines in the shell of the abalone, *Haliotis midae*. *Journal of Shellfish Research* 13: 493-501.

Forster, G. R. (1967). The growth of *Haliotis tuberculata*: results of tagging experiments in Guernsey 1963-65. *Journal of the Marine Biological Association of the U.K.* 47: 287-300.

Fritz, M., Belcher, A. M., Radmacher, M., Hansma, P. K., Morse, D. E., Stucky, G. D. and Mann, S. (1994). Flat pearls from biofabrication of organized composites on inorganic substrates. *Nature* 371: 49-51.

Hawkes, G. P., Day, R. W., Wallace, M. W., Nugent, K. W., Bettiol, A. A., Jamieson, D. N. and Williams, M. C. (1996). Analysing the growth and form of mollusc shell layers, in situ, using cathodoluminescence microscopy and Raman spectroscopy. *Journal of Shellfish Research* 15: 659-666.

Hawkes, G. P., Day, R. W., Wallace, M. W. and Harriden, T. D. (1997) Optimal conditions using manganese as a shell marker for abalone age validation studies. *Molluscan Research*, 18: 169-181

Herzog, L. F., Marshall, D. J. and Babione, R. F. (1970). The luminoscope - a new instrument for studying the electron-stimulated luminescence of terrestrial, extra-terrestrial and synthetic materials under the microscope. In: 'Space Science Applications of Solid State Luminescence Phenomena'. (Eds: J. N. Weber and E. White) pp. 79-98. (MRL pub. 70-101. State College, Penn State University: Pennsylvania).

Jackson, G. D. (1990). The use of tetracycline staining techniques to determine statolith growth ring periodicity in the tropical loliginid squids *Loliolus noctiluca* and *Loligo chinensis*. *Veliger* 33: 389-393.

Kojima, H. (1975). A study on the growth of the shell of *Haliotis discus discus* (Gastropoda: Haliotidae) - 1. Age and growth. *Aquaculture* 23: 61-66.

Kojima, H. (1995). Abalone stock enhancement through the release of hatchery-reared seeds. *Marine and Freshwater Research* 46: 689-695.

Lutz, R. A. and Rhoads, D. C. (1980). Growth patterns within the molluscan shell: an overview. In: 'Skeletal Growth of Aquatic Organisms'. (Eds: D. C. Rhoads and R. A. Lutz). pp. 203-254. (Plenum Press: New York).

Marshall, D. J. (1988). 'Cathodoluminescence of Geological Materials'. Unwin Hyman: Boston, Massachusetts. 146 pp.

Marshall, D. J. and Day, R. (2001) Change in the rate of shell deposition and shell microstructure in response to shell borers in the abalone *Haliotis rubra*. *Marine and Freshwater Behaviour and Physiology* 34: 189-195.

McShane, P. E., and Smith, M. G. (1992). Shell growth checks are unreliable indicators of age of the abalone *Haliotis rubra* (Mollusca: Gastropoda). *Australian Journal of Marine and Freshwater Research* 43: 1215-1219.

Muñoz-Lopez, T. (1976). Resultados preliminares de un método para determinar edad en abulones (*Haliotis* spp.) de Baja California. *Memorias del Simposio sobre Recursos Pesqueros Masivos de México Ensenada B. C.* pp. 281-301.

Mutvei, H., Dauphin, Y., and Cuif, J-P. (1985). Observations sur l'organisation de la couche externe du test des *Haliotis* (Gastropoda): un cas exceptionnel de variabilité mineralogique et microstructurale. *Bulletin de Museum National d' Histoire naturale, Paris* 7: 73-91.

Nakahara, H., Bevelander, G., and Kakei, M. (1982). Electron microscopic and amino-acid studies on the outer and inner shell layers of *Haliotis rufescens*. *Venus* 41: 33-46.

Nash, W. J., Sellers, T., Talbot, S., Cawthorn, A., Ford, W. (1994). The population biology of abalone (*Haliotis* species) in Tasmania. 1. Blacklip abalone (*H. rubra*) from the North coast and the islands of Bass Strait. *Tasmanian Division of Sea Fisheries Technical Report 48*. 69 pp.

Prince, J. D. (1991) A new technique for tagging abalone. *Australian Journal of Marine and Freshwater Research*. 42: 101-106.

Prince, J. D., Sellers, T. L., Ford, W. B., and Talbot, S. R. (1988). A method for ageing the abalone *Haliotis rubra* (Mollusca: Gastropoda). *Australian Journal of Marine and Freshwater Research* 39: 167-175.

Sakai, S. (1960). On the formation of the annual ring on the shell of the abalone, *Haliotis discus* var *hannai* Ino. *Tohoku Journal of Agricultural Research* 11: 239-244.

Shepherd, S. A., Al-Wahaibi, D., and Al-Azri, A. R. (1995a). Recruitment and growth of the Omani abalone *Haliotis mariae*. *Australian Journal of Marine and Freshwater Research* 46: 575-582.

Shepherd, S. A., Avalos-Borja, M., and Quintanilla, M. O. (1995b). Toward a chronology of *Haliotis fulgens* with a review of abalone shell microstructure. *Australian Journal of Marine and Freshwater Research* 46: 607-616.

Shepherd, S.A. and Avalos-Borja, M. (1997). The shell microstructure and chronology of the abalone *Haliotis corrugata*. *Molluscan Research*, 18:197-207.

Shepherd, S. and Huchette, S. (1997). Studies on southern Australian abalone (genus *Haliotis*) XVIII. Ring formation in *H. scalaris*. *Molluscan Research*, 18:247-252.

Shepherd, S. and Triantifillos, T. (1997). Studies on southern Australian abalone (genus *Haliotis*) XVII. A chronology of *H. laevigata*. *Molluscan Research*, 18: 233-245.

Sommer, S. E. (1972). Cathodoluminescence of carbonates, 1. Characterization of cathodoluminescence from carbonate solid solutions. *Chemical Geology* 9: 257-273.

Thomas, M. and Day, R. W. (1995). Site selection by a small drilling predator: why does the gastropod *Haustrum baileyana* drill over muscle tissue of the abalone *Haliotis rubra*? *Marine and Freshwater Research.* 46: 647-656.

Troynikov, V.S.; Day, R.W.; Leorke, A.M. (1998): Estimation of seasonal growth parameters using a stochastic gompertz model for tagging data. *Journal of Shellfish Research*. 17: 833-838.

Ward, T. J. (1986). 'Abalone biology and fisheries in Australia: a review of research.' Report to the Fishing Industry Research and Development Committee, June 1986.

Wilbur, K. M. & Saleuddin, A. S. M. (1983). Shell formation. In: 'The Mollusca, Vol. 4, Physiology, Part 1'. (Eds: A. S. M. Saleuddin and K. M. Wilbur.) pp. 235-287. (Academic Press: New York).

Wise, S. W. (1970a). Microarchitecture and deposition of gastropod nacre. *Science* 167: 1486-1488.

Wise, S. W. (1970b). Microarchitecture and mode of formation of nacre (mother-of-pearl) in pelecypods, gastropods, and cephalopods. *Eclogae Geologia Helvetica* 63: 775-797.

Zaremba, C. M., Belcher, A. M., Fritz, M., Li, Y., Mann, S., Hansma, P. K., Morse, D. E., Speck, J. S. and Stucky, G. D. (1996). Critical transitions in the biofabrication of abalone shells and flat pearls. *Chemical Materials* 8: 679-690.

APPENDIX 1
INTELLECTUAL PROPERTY

No intellectual property was expected from this project, as it did not involve the development of new products. The benefits of this project, as discussed above, lie in the fact that it will enable future stock assessments to be based on a better knowledge of stock productivity at a large number of sites across the fisheries in each state.

The expertise and knowledge developed as a result of this project may, however, be useful in developing intellectual property in future projects. For example, during this project we have discovered that the manganese staining can be detected under a scanning electron microscope, and may be useful in investigating the nature of the process of shell deposition. A number of researchers overseas are funded to investigate this process, due to the enormous strength of the composite material that molluscs produce. This strength appears to rely on the nano-scale structure of the nacre, in which proteins and aragonite crystals are precisely positioned and bound together during the process of shell deposition.

Publications arising from this project:

Bettiol, A. A., Yang, C., Hawkes, G. P., Jamieson, D. N., Malmqvist, K. G. and Day, R. W. (1999). The identification of growth lines in abalone shell using a nuclear microprobe. *Nuclear Instruments and Methods in Physics Research B.* **158/1-4**, pp 299-305

Marshall, D. J. and Day, R. (2001) Change in the rate of shell deposition and shell microstructure in response to shell borers in the abalone *Haliotis rubra*. *Marine and Freshwater Behaviour and Physiology* 34: 189-195.

Conference Presentations arising from this project:

Day, R. Hawkes, G.P., and Wallace, M.: *The value of research to age abalone, and how to timestamp them.* Poster, World Fisheries Congress, Brisbane, July 1996.

Day, R.: *Age estimation of bivalves and gastropods.* Presentation to ageing workshop, World Fisheries Congress, Brisbane, July 1996.

Gerneke, D. Hawkes, G. and Day, R. The use of cathodoluminescence, backscatter and EBSD in the SEM to microcharacterize shell layers of the black-lip abalone (*Haliotis rubra).*Presented at the 15th Australian Conference for Electron Microscopy, Hobart February 1998.

Hawkes, G.P., Day, R., Wallace, M. and Harriden, T: *The use of manganese as a shell marker for ageing abalone, and a new underwater marking tank.* Presented at Australasian Conference on abalone biology, culture and fisheries, Wellington, New Zealand, October 1996.

Hawkes, G. and Prince, K: The use of SIMS in the study of abalone shells to determine age. Presented at SIMS Applications Workshop, Sydney, April 1997.

Day, R., Seddon, S. and McHugh, M.: *Sex change in the epibiotic limpet Hipponix australis.* Presented at Fourth International Temperate Reefs Symposium, Santiago, Chile, July 1997.

Gleeson, K. and Day, R.: *Growth rates and shell repair after damage by boring polychaetes in the shells of Haliotis rubra.* Poster presentation at Third International Abalone Symposium, Monterey, California, October 1997.

Hawkes, G., Day, R. Gerneke, D. and Bettiol, A.: *High resolution microstructure analysis of cathodoluminescent bands within the shell of black-lip abalone.* 3rd International Abalone Symposium, Monterey, California, October 1997.

APPENDIX 2
STAFF

1995/6: Gerard Hawkes and Victor Gomelyuk were employed as part time research assistants. Timothy Harriden was employed on a casual basis during fieldwork expeditions.

1996/7: Victor Gomelyuk was employed as a part time research assistant. Gerard Hawkes was given a research scholarship stipend, with permission from the FRDC. In addition the following research assistants were employed for the fieldwork and laboratory processing of shells:
Michelle Love, Adrian Marantelli, David Rawlins, Craig Shaw, Rodney Treble, Nicholas Yee.

1997/8: Victor Gomelyuk was employed as a part time research assistant. Gerard Hawkes received the scholarship stipend. Funds unspent after June 1998 were used to employ Victor Gomelyuk on a casual basis, with permission.

Numerous volunteers assisted us on fieldwork expeditions. These included many abalone divers and processors, and many employed by the fisheries Research Institutes in South Australia, Tasmania and Victoria. In addition many students from the Zoology Department, University of Melbourne assisted us, particularly Katie Gleason, Steven Heady, Dustin Marshall, Michelle McHugh and Matthew Reardon. Miguel Rabi and Carol Forster from IFREMER in Peru (the Peruvian Fisheries Research Institute) also assisted us on our first South Australian marking experiment.

APPENDIX 3
ABALONE RECAPTURE DATA

Table A1. Abalone (*H. rubra*) recaptured from Lawrence Rocks, Victoria

Abalone No	Floyd tag No	Putty tag No	Abalone length	Recap Date	Recap Length	Mark?	Bored?	Layers after the mark
18	D.972	593	142.1	1/5/96	142	n		
155	D,842	362	118.8	1/5/96	122.9	n		
196	D,928	198	122.2	1/5/96	130	y	bored	1
228	Ao27	291	130.2	1/5/96	130.2	n		
245	A051	519	122.2	1/5/96	124	n		
305	A210	240	115.7	1/5/96	118.9	n		
335	A002	615	139.8	1/5/96	145	y	bored	1
360	B479	280	121.4	1/5/96	128.2	y	bored	2
423	B411	515	114.2	1/5/96	115.8	y		2
425	A177	493	127	1/5/96	129	y		1
439	A066	858	125.3	1/5/96	128	n		
442	A201	259	129.5	1/5/96	132.7	n		
488	A147	153	129.8	1/5/96	130.1	y	bored	2
492	A142	495	113.2	1/5/96	118.4	y	bored	3
520	A156	158	94.3	1/5/96	112.1	y		2
534	AO41	585	124	1/5/96	124.3	n		
561	A124	283	132	1/5/96	132	y		2
562	A,445	921	115	1/5/96	123.2	y		1
563	AA01	10	113	1/5/96	120	y		3
564	A112	224	100	1/5/96	115	n		
54	D.982	350	112.3	17/8/96	127.1	y		5
93	D,885	612	133.5	17/8/96	135	y		1
119	D,874	320	140	17/8/96	140	n	bored	
132	D,812	949	117.7	17/8/96	123.5	y		1
142	D,861	323	118.1	17/8/96	123	n		
149	D,819	589	116.7	17/8/96	124	y	bored	2
217	D,873	222	150	17/8/96		n	bored	
266	A030	343	129	17/8/96	130	y	bored	1
296	A225	308	127.5	17/8/96	133	n		
311	D894	281	119.4	17/8/96	125.2	y		1
316	D878	235	125.5	17/8/96	130.1	n		
321	A060	600	127.3	17/8/96	132	y		0
340	A128	958	132.1	17/8/96	133	y		2
370	B428	313	121.4	17/8/96	127	y		1
373	B405	346	125.4	17/8/96		y		2
432	B412	378	127.6	17/8/96		n		
478	A135	478	118	17/8/96	126	n		
532	A065	266	146.4	17/8/96		n		
559	A133	298	122	17/8/96	133	n		
52	D.948	244	118.8	20/10/96	125	y	bored	1
170	D,788	374	118.3	20/10/96	123.3	y	bored	2
317	A003	154	133.3	20/10/96	135	n		
324	D795	262	138.5	20/10/96	138.3	y		1
41	D.964	460	127	1/11/96	129	y		2
61	D,916	none	114.7	1/11/96	125.3	y		1
62	D,952	400	89.7	1/11/96	102	y		2
222	Ao94	297	105.5	1/11/96	115	y	bored	2
43	D.970	120	128	13/3/97	136	n		
66	D,906	785	104.7	13/3/97	103.4	y		2
90	D,823	285	106.7	13/3/97	126.5	y		3

137	D,836	356	121.7	13/3/97	123.1	y		3
192	D,850	321	127.4	13/3/97	130.4	y		1
221	D,814	455	118	13/3/97	120	y		2
250	A043	255	97.1	13/3/97	107	n		
275	A092	179	98.4	13/3/97	109	y		2
313	A023	324	118.5	13/3/97	123.4	n		
343	B480	553	104	13/3/97	117	y		3
406	A231	119	126.3	13/3/97	129.4	n		
417	B454	611	131	13/3/97	132	y	bored	1
420	B458	280	118.4	13/3/97	119	y		2
465	B410	329	102	13/3/97	114	y	bored	4
490	A137	947	133.7	13/3/97	133.7	y		2
506	A113	337	104.6	13/3/97	114	y	bored	3
523	A117	283	108.7	13/3/97	115.5	y		3
567	AA02	906	131	13/3/97	133	y		3

Table A2. Abalone (*H. rubra*) recaptured from Ninepin point, Tasmania.

Abalone No.	Floyd tag No.	Putty tag No.	Ab length (mm)	Recap. Date	Recap Length	Remarks	Mark?	No layers after mark
94	VB0964	646	82	24/10/96	98	putty tag lost	y	0
506	BB1503	629	107	28/3/96	-	dead shell		
44	none	24	49	6/05/97	67		y	1
62	O917	155	126	6/05/97	130		n	
117	BB0772	301	112	6/05/97	132		y	2
220	B0960	601	104	6/05/97	107		y	1
456	B1786	513	90	6/05/97	98		y	2
527	BB1538	127	130	6/05/97	134	dead shell, putty tag lost		
531	BB1605	945	117	6/05/97	121		y	2
534	BB1700	27	125	6/05/97	127	dead shell	y	1
562	BB1543	340	112	6/05/97	123		n	

Table A3. Abalone (*H. rubra*) recaptured from Stinking Bay, Tasmania.

Abalone No.	Floyd tag No.	Putty tag No.	Ab length (mm)	Recap Date	Recap Length	Remarks	Mark?	No layers after mark
4	B1803	937	137	28/3/96		dead shell		
10	B1862	987	134	28/3/96		dead shell		
11	B0518	565	113	28/3/96		dead shell		
15	B1851	269	125	28/3/96		dead shell		
38	B1833	317	108	28/3/96		dead shell		
47	B0568	608	107	28/3/96		dead shell		
51	B0550	143	79	28/3/96		dead shell		
65	B0647	914	99	28/3/96		dead shell		
69	BO588	216	132	28/3/96		dead shell		
71	B0633		140	28/3/96		dead shell, putty tag lost		
73	B0643	544	127	28/3/96		dead shell		
80	B0543	426	105	28/3/96		dead shell		
95	B0541	778	118	28/3/96		dead shell		
109	B0665	448	128	28/3/96		dead shell		
137	B0534	873	101	28/3/96		dead shell		
141	BO583	35	110	28/3/96		dead shell		
152	B0514	243	130	28/3/96		dead shell		

156	B0839	700	80	28/3/96		dead shell		
162	B0650	357	99	28/3/96		dead shell		
164	B0800	605	135	28/3/96		dead shell		
178	B0870	123	131	28/3/96		dead shell		
206	B0715	89	92	28/3/96		dead shell		
235	B0867	364	122	28/3/96		dead shell		
242	B0614	180	136	28/3/96		dead shell		
253	B0854	360	122	28/3/96		dead shell		
263	B0751	491	136	28/3/96		dead shell		
274	B0733	94	120	28/3/96		dead shell		
305	B0855	812	122	28/3/96		dead shell		
312	B0872	436	137	28/3/96		dead shell		
320	B0888	223	115	28/3/96		dead shell		
326	B0632	750	76	28/3/96		dead shell		
353	AA1049	759	111	28/3/96		dead shell		
367	AA1064	897	132	28/3/96		dead shell		
385	AA1030	213	133	28/3/96		dead shell		
390	AA1090	247	125	28/3/96		dead shell		
393	BB1679	560	136	28/3/96		dead shell		
413	B1873	none	143	28/3/96		dead shell		
425	B1869	406	133	28/3/96		dead shell		
431	B1894	502	142	28/3/96		dead shell		
438	B1997	138	122	28/3/96		dead shell		
440	B1958	546	136	28/3/96		dead shell		
441	B1963	57	90	28/3/96		dead shell		
442	B1952	121	80	28/3/96		dead shell		
447	B1965	405	118	28/3/96		dead shell		
448	B1927	749	134	28/3/96		dead shell		
451	B1857	955	134	28/3/96		dead shell		
455	B1854	386	71	28/3/96		dead shell		
460	B1900	45	128	28/3/96		dead shell		
467	B1855	560	124	28/3/96		dead shell		
473	B1967	938	111	28/3/96		dead shell		
474	B1992	853	133	28/3/96		dead shell		
482	B1859	742	125	28/3/96		dead shell		
531	B1904	988	94	28/3/96		dead shell		
533	B1871	307	139	28/3/96		dead shell		
535	B0544	708	130	28/3/96		dead shell		
539	B0651	1020	136.5	28/3/96		dead shell		
543	B1959	none	145	28/3/96		dead shell		
550	B1804	902	143	28/3/96		dead shell		
557	B0648	382	105	28/3/96		dead shell		
558	B0602	837	125	28/3/96		dead shell		
561	B0560	408	97	28/3/96		dead shell		
567	B1812	9	140	28/3/96		dead shell		
573	B1866	899	105	28/3/96		dead shell		
575	B0530	885	137	28/3/96		dead shell		
583	B0574	10	104	28/3/96		dead shell		
589	AA1047	748	121	28/3/96		dead shell		
591	BB1693	842	108	28/3/96		dead shell		
599	AA1095	503	113	28/3/96		dead shell		
605	AA1076	946	125	28/3/96		dead shell		
610	AA1021	675	131	28/3/96		dead shell		
612	AA1050	860	117	28/3/96		dead shell		
631	AA1092	444	110	28/3/96		dead shell		
3	B1684	400	94	24/10/96	106		y	1
25	B1991	509	110	24/10/96	116	Putty tag lost	y	1
27	B1802	45	113	24/10/96	120		y	1
34	B1964	741	108	24/10/96	120	Putty tag lost	y	1

64	B0508	322	107	24/10/96	120		y	1
101	B0593	764	130	24/10/96	132		y	2
105	B0613	566	116	24/10/96	121		y	3
241	B0840	104	130	24/10/96	132	Pore tag lost	bored	
281	B0773	860	112	24/10/96	119	putty tag lost	n	
295	B0864	417	93	24/10/96	109		y	1
317	B0832	508	110	24/10/96	116		y	0
414	B1947	401	139	24/10/96	140	Pore tag lost	y	1
479	B1816	815	102	24/10/96	115		y	0
489	B1905	461	88	24/10/96	100		y	1
116	BB0503	none	120	6/05/97		dead shell		
223	B0836	887	123	6/05/97		dead shell		
269	B0853	909	100	6/05/97	101	dead shell		
276	B0838	922	137	6/05/97		dead shell		
520	B1929	577	76	6/05/97	78	dead shell		

Table A4. Abalone (*H. rubra*) recaptured from Georges Reef.

Abalone No.	Pore tag No	Colour of tag	Putty tag No	Ab length (mm)	Recap Date	Recap Length	Mark?	Layers after mark	Bored ?
1	17	B		141	25/11/97	142	y	1	not b
8	55	B		147	21/4/98	148	n		
15	99	B		138	21/4/98	145.7	y	5	bored
39	191	O		143	21/4/98	145	y	1	not b
40	254	W		148	21/4/98	156	y	4	bored
42	281	W		144	21/4/98	144	n		
54	250	W		129	25/11/97	129	n		bored
59	268	W		129	25/11/97	135	y	1	not b
60	263	W		142	21/4/98	147	y	1	not b
63	294	W		149	21/4/98	149	n		
66	267	W		156	21/4/98	156	n		
81	162	O		153	21/4/98	153	n		
83	242	W		143	21/4/98	143	y	2	bored
88	210	W		141	25/11/97	157	y	0	bored
127	195	O		128	21/4/98	137	y	2	not b
128	238	W		146	21/4/98	150	y	2	not b
134	286	W		143	21/4/98	143	n		
140	53	B		157	21/4/98	158	n		
147	95	B		143	21/4/98	144	y	2	not b
151	93	B		144	21/4/98	144	y	2	not b
153	205	W		139	21/4/98	141	y	4	bored
161	256	W		147	21/4/98	147	n		
167	261	W		152	21/4/98	152	y	1	bored
190	64	B		95	21/4/98	132	y	1	bored
204	5	B		144	21/4/98	145	n		
207	1	B		142	25/11/97	142	y	1	not b
210	74	B		136	25/11/97	136	n		
214	25	B		112	25/11/97	115	y	1	bored
256	199	P		140	21/4/98	138	y	4	bored
264	122	P		150	21/4/98	150	y	4	bored
270	200	P		155	25/11/97	155	n		
328	211	O		130	25/11/97	130	y	1	not b
409	328	W		128	21/4/98	150	y	1	not b
421	Nil		270	89	25/11/97	99	n		bored
426	Nil		407	114	25/11/97	120	y	1	not b

427	Nil			468	99	25/11/97	109	y	3	bored
430	Nil			740	124	25/11/97	124	y	0	bored
433	Nil			419	102	25/11/97	110	n		
440	257	O			129	21/4/98	155	y	3	bored
445	226	O			127	21/4/98	154	n		
458	223	O			112	21/4/98	139	n		
489	239	O			145	25/11/97	146	n		
535	Nil			216	73	25/11/97	109	y	0	not b
548	355	W			123	25/11/97	149	n		bored
554	354	W			110	25/11/97	110	y	1	bored
561	Nil			272	108	25/11/97	115	n		
566	Nil			583	104	25/11/97	111	y	1	bored
567	Nil			266	87	25/11/97	88	y	1	not b
570	Nil			351	101	21/4/98	114	n		
572	Nil			97	123	25/11/97	123	y	1	bored
573	Nil			339	102	25/11/97	113	y	1	not b
574	Nil			204	96	25/11/97	105	n		
577	Nil			502	106	25/11/97	114	y	1	not b
578	Nil			654	90	25/11/97	95	y	1	not b
579	Nil			373	81	25/11/97	85	y	?	bored
581	Nil			274	104	25/11/97	114	y	2	not b

Table A5. Abalone (*H. laevigata*) recaptured from McLaren Point, S. A.

Abalone No.	Floyd tag No	Putty tag No.	Ab length (mm)	Recap Date	Recap Length	Mark?	Layers after mark
9	C695	817	133.1	17/10/96	131	n	
12	B289	726	142.8	17/10/96	141	n	
51	C650	282	152	17/10/96	153	n	
59	B281	121	143.5	17/10/96	146	y	1
97	B299	219	162	17/10/96	164	y	1
132	C724	155	139.4	17/10/96	138	y	2
139	C708	55	151	17/10/96	153	y	0
193	C503	477	150	17/10/96	151	y	1
194	B348	559	144.9	17/10/96	153	n	
225	C588	545	145.3	17/10/96	145	n	
246	C657	188	142	17/10/96	145	n	
260	B361	511	146.2	17/10/96	146	n	
305	B275	424	125.8	17/10/96	129	n	
327	BB941	47	160	17/10/96	160	n	
336	B379	423	124.6	17/10/96	133.7	n	
415	B315	248	125	17/10/96	127.6	y	1
424	B311	172	136.1	17/10/96	147	n	
430	B323	797	126.1	17/10/96	126.6	n	
441	B347	513	134.2	17/10/96	135	y	2
460	BB993	264	90.3	17/10/96	103.5	y	2
468	BB905	78	119.6	17/10/96	124.1	n	
475	BB952	420	152	17/10/96	155	n	
3	C630	134	134.3	8/1/97	147	n	
222	B316	490	142.5	8/1/97	143	y	2
48	C613	269	127.1	8/1/97	135	y	3
82	B271	845	135	8/1/97	141	y	3
87	C519	500	165	8/1/97	165	n	
90	A179	535	109.7	8/1/97	119	n	
111	B406	800	141.4	8/1/97	142	n	
116	C596	540	147	8/1/97	152	y	2

123	C525	818	141.2	8/1/97	159	n	
131	C744	35	147	8/1/97	153	n	
134	C738	390	152	8/1/97	153	n	
136	C742	163	145.9	8/1/97	163	n	
157	C712	191	150	8/1/97	150	n	
220	C533	859	133.1	8/1/97	149	n	
228	C678	238	149.1	8/1/97	149	n	
250	C693	193	100.5	8/1/97	113	n	
251	C690	216	135.3	8/1/97	148	n	
275	B392	503	96.8	8/1/97	117	n	
290	C626	528	117	8/1/97	134	n	
295	C683	714	128.4	8/1/97	139	y	2
296	B309	219	153	8/1/97	153	n	
405	B378	214	140.7	8/1/97	142	y	3
412	B375	29	144.3	8/1/97	147	n	
416	B332	19	97	8/1/97	115	n	
418	B349	508	123.6	8/1/97	132	y	2
442	B398	357	108.9	8/1/97	121	n	
443	B394	59	145	8/1/97		y	1
457	BB913	496	117	8/1/97	134	n	
471	BB933	130	128.5	8/1/97		y	2
506	BB983	155	91	8/1/97	112	y	3
10	C647	921	102	25/5/97	124.6	n	
35	C696	991	99.2	25/5/97	123.8	n	
42	B280	860	146.4	25/5/97	150.4	n	
45	AO84	144	114.4	25/5/97	141.7	n	
49	C687	594	144.1	25/5/97	148.1	n	
63	B284	222	148	25/5/97	158.2	y	2
83	B256	309	139.5	25/5/97	139.6		
88	C729	none	125.6	25/5/97	137.8	n	
94	B262	311	150.5	25/5/97	151.3	n	
101	B255	83	150	25/5/97	152	n	
110	A208	90	144.9	25/5/97	152.1	y	4
129	C704	247	159	25/5/97	159	n	
143	C737	962	150	25/5/97	165	n	
146	C740	209	108.6	25/5/97	135.2	y	1
166	C709	145	131.3	25/5/97	139.8	y	2
181	C567	147	99.7	25/5/97	123.8	n	
183	C505	465	145.4	25/5/97	150	n	
192	B346	96	160	25/5/97	160	y	2
221	C568	159	139.4	25/5/97	154	n	
234	C681	436	142.9	25/5/97	152.1	y	2
238	C685	252	96.1	25/5/97	116.4	n	
291	B371	595	124.9	25/5/97	156.3	n	
308	B264	295	74.3	25/5/97	107.8	y	3
334	BB960	11	89.8	25/5/97	115.8	n	
399	B322	34	134.4	25/5/97	136	n	
406	B372	815	118.8	25/5/97	124.5	y	4
414	B380	291	103.7	25/5/97	125.4	n	
426	B334	264	143.1	25/5/97	153	y	2
433	B328	251	138.4	25/5/97	142	n	
437	B305	139	138.3	25/5/97	142.1	n	
444	B350	359	147	25/5/97	147	y	3
455	BB989	887	98.2	25/5/97	121	y	2
505	BB911	737	79.8	25/5/97	105.8	y	3
522	B257	620	80.1	25/5/97	101.6	y	4
14	C638	304	110	23/5/97	123	y	3
89	C581	254	132	23/5/97	138	n	
128	C706	146	148	23/5/97	155	n	

359	C520	127	143.5	23/5/97	155.4	y	1
397	B383	857	131.7	23/5/97	134	n	
409	B333	238	107.7	23/5/97	130	y	4
432	B320	517	127.6	23/5/97	137	y	3
490	BB982	202	84.9	23/5/97	125	y	2
28	C631	716	116.9	5/9/97	136	y	3
93	C726	62	97.7	5/9/97	?	n	
103	B279	238	161	5/9/97	167	y	5
133	C586	58	152	5/9/97	162	y	1
306	B258	233	112.4	5/9/97	137	n	
13	C611	272	120.8	14/01/98	142	y	4
23	C639	730	132.3	14/01/98	145	n	
41	C643	336	135.9	14/01/98	138	n	
80	B283	533	97.3	14/01/98	132	y	6
175	C557	516	128.8	14/01/98	136	y	4
179	C561	711	140.3	14/01/98	150	n	
188	C559	197	146.2	14/01/98	146.4	n	
195	C577	907	130.4	14/01/98	141	y	7
400	B306	528	130.6	14/01/98	147	n	
436	B317	306	140.6	14/01/98	145	y	2
446	B330	462	162	14/01/98	170	n	
456	B285	910	98.6	14/01/98	104.8	y	1

Table A6. Abalone (*H. laevigata*) recaptured from between Thistle and Hopkins Islands, S. A

Abalone No.	Floyd tag No	Putty tag No.	Ab length (mm)	Recap Date	Recap Length	Mark?	Layers after mark
12	BB0635	279	131.4	17/10/96	146	y	2
16	BBO795	79	118.2	17/10/96	131	y	3
29	BBO871	852	83.2	17/10/96	104.2	n	
34	BBO861	225	117.8	17/10/96	133.1	n	
38	BBO869	237	130	17/10/96	145.4	n	
41	BBO885	197	129	17/10/96	138.1	n	
75	BBO815	223	127.2	17/10/96	147.6	n	
95	BBO707	189	139.5	17/10/96	147	y	1
113	BBO786	613	117.7	17/10/96	138.9	y	2
148	BB514	194	124.7	17/10/96	141.1	n	
156	BB508	67	161	17/10/96	170	y	0
166	BB529	273	146.3	17/10/96	156	y	1
219	AA482	none	113.3	17/10/96	132.6	y	3
282	BB589	905	113	17/10/96	117.3	n	
287	BB537	47	102.3	17/10/96		?	
347	BB563	172	135.2	17/10/96	142	?	
355	BB931	296	154	17/10/96	154	n	
369	BB561	253	136.7	17/10/96	151	y	0
382	AA331	none	102	17/10/96	122.6	y	2
386	AA330	none	134	17/10/96	146.8	n	
122	BBO701	780	144	8/1/97	151	y(b)	3
188	AA1338	236	178	8/1/97	178	y	2
10	BBO620	946	125.1	25/5/97	143.1	y	3
31	BBO878	123	130.5	25/5/97	153	y	1
36	BBO868	873	105.2	25/5/97	126.3	y	3
45	BBO891	451	88.5	25/5/97	126	y	1
47	BBO890	229	155	25/5/97	157.3	y	4
52	BBO800	607	147.1	25/5/97	160	y	3
53	BBO866	22	94.5	25/5/97	122.1	y	2
62	BBO834	261	97	25/5/97	132.3	y	2

72	BBO787	441	111.1	25/5/97	138.2	y	1
76	BBO813	309	93	25/5/97	126.3	y	2
87	BBO814	876	139.8	25/5/97	159.4	y	2
90	BBO860	54	110.1	25/5/97	133.8	y	1
91	BBO857	44	120.6	25/5/97	136.6	n	
92	BBO873	519	145.1	25/5/97	159.5	n	
97	BBO747	242	105.8	25/5/97	142.8	y	2
99	BBO767	310	136	25/5/97	143.9	n	
107	BBO765	710	117.2	25/5/97	133.7	n	
114	BBO702	547	137.2	25/5/97	150	n	
115	BBO770	186	139	25/5/97	155.5	n	
121	BBO730	461	132.9	25/5/97	146.2	?	
126	BBO751	210	143.7	25/5/97	157.4	n	
129	BBO773	218	132.3	25/5/97	156	y	2
133	BBO734	327	115	25/5/97	141	y	3
134	BBO752	161	106	25/5/97	126.4	y	1
223	AA474	none	160	25/5/97	169.5	y	3
325	AA312	none	163	25/5/97	167.2	n	
370	BB562	191	112.2	25/5/97	134.5	y	2
30	BBO852	828	83.7	23/5/97	132.1	y	5
46	BBO835	28	72	23/5/97	125.4	y	3
48	BBO858	199	81.7	23/5/97	136.7	y	2
73	BBO853	523	145.1	23/5/97	161	y	3
94	BBO823	554	89.3	23/5/97	133.5	y	2
111	BBO709	46	128	23/5/97	156.5	n	
117	BBO775	186	145.1	23/5/97	165	?	
124	BBO778	80	103	23/5/97	139.9	y	2
125	BBO790	948	149	23/5/97	172.5	n	
130	BBO706	71	136.8	23/5/97	157.6	n	
139	BBO799	27	133	23/5/97	157.5	n	
227	AA1310	143	170	23/5/97	171.8	n	
276	AA1345	269	102	23/5/97	150.3	y(b)	4
319	BBO844	351	114	23/5/97	151	y	3
324	BBO900	466	142	23/5/97	142	?	
9	BB0627	192	106.7	23/5/97	125.2	?	
131	BBO715	601	148.5	14/1/98	156	y	2
299	AA1318	282	152	14/1/98	155	y	4

Table A7. Abalone (*H. laevigata*) recaptured from Frog Rock, S. A

Abalone No.	Pore tag No.	Putty tag No.	Ab length (mm)	Recap Date	Recap Length	Mark?	Layers after mark	Bored?
5	13	275	157	5/9/97	158	y	2	not b
19	64	550	134	5/9/97	147	y	2	not b
31	77	157	126	5/9/97	129	?		
37	48	741	129	5/9/97	139	n		
39	4	493	147	5/9/97	148	n		
52	67	57	151	5/9/97	156	n		
56	79	104	151	5/9/97	158	n		
75	7	234	156	5/9/97	160	n		
83	97	227	128	5/9/97	132	n		
88	2	433	154	5/9/97	154	n		
91	32	445	153	5/9/97	157	?		
93	29	308	146	5/9/97	148	?		
97	6	18	142	5/9/97	151	n		
132	404	520	160	5/9/97	163	n		
133	486	246	143	5/9/97	144	n		

139	500	481	144	5/9/97	148	?			
147	428	512	146	5/9/97	147	n			
159	413	504	154	5/9/97	157	n			
161	412	273	146	5/9/97	147	n			
168	450	777	158	5/9/97	156	n			
175	494	431	155	5/9/97	155	n			
200	482	202	170	5/9/97	170	n			
206	99	356	155	5/9/97	156	n			
207	496	524	145	5/9/97	145	n			
71	1	639	139	23/5/97	140	?			
94	84	794	161	23/5/97	?	?			
78	83	642	150	21/7/97	149	?			
129	405	629	147	14/1/98	148	n			
15	3	672	128	14/01/98	135	n			
68	21	396	163	14/01/98	163	?			
86	8	200	154	14/01/98	159	n			
107	91	632	151	14/01/98	155	n			
108	429	479	156	14/01/98	159	n			
113	440	197	141	14/01/98	145	n			
123	420	98	111	14/01/98	127	n			
124	447	217	141	14/01/98	147	n			
131	484	779	155	14/01/98	157	n			
135	468	300	167	14/01/98	167	y	1		not b
138	434	181	131	14/01/98	132	y	1		not b
143	416	335	140	14/01/98	156	y	1		not b
144	410	788	122	14/01/98	132	y	2		not b
148	478	421	162	14/01/98	163	n			
149	451	623	159	14/01/98	163	n			
152	463	596	95	14/01/98	126	?			
153	460	778	115	14/01/98	138	y	3		not b
154	474	613	140	14/01/98	148	y	2		not b
158	437	619	148	14/01/98	157	y	2		not b
160	471	98	107	14/01/98	133	n			
162		700	88	14/01/98	116	y	1		not b
167	443	137	125	14/01/98	138	y	3		not b
174	436	182	120	14/01/98	141	y	1		not b
177	426	286	149	14/01/98	158	n			
182	456	20	157	14/01/98	158	y	5		not b
184	414	547	136	14/01/98	135	y	1		not b
186	438	198	120	14/01/98	142	y	2		not b
190		169	95	14/01/98	120	y	1		not b
198	490	466	145	14/01/98	146.5	dead			
201	464	682	122	14/01/98	142	n			
203	461	580	122	14/01/98	141	n			
205	485	621	129	14/01/98	143	n			
217	2		140	14/01/98	140	dead			

Table A8. Abalone (*H. laevigata*) recaptured from Taylor Island, S. A.

Abalone No.	Pore tag No.	Putty tag No.	Ab length (mm)	Recap Date	Recap Length	Mark?	layers after mark	Bored?
16	35		152	25/5/97	152	n		
21	174		160	25/5/97	160	n		
28	171		157	25/5/97	159	n		

73	25		135		25/5/97	137		n			
83	22		153		25/5/97	155		n			
121	65		132		25/5/97	134		n			
140	430		111		25/5/97	119		y	2		not b
144		13	104		25/5/97	119		y	1		not b
147		909	78		25/5/97	99.2		y	1		not b
148		194	107		25/5/97	121		n			
149		242	100		25/5/97	108.3		n			
152		316	108		25/5/97	124		y	1		not b
158		135	103		25/5/97	104.1		n			
47	455		153		5/9/97	158		y	3		bored
48	159		164		5/9/97	166.5		n			
61	74		137		5/9/97	145		?			
94	6		135		5/9/97	140		n			
95	156		180		5/9/97	180		n			
96	36		152		5/9/97	158		n			
99	99		147		5/9/97	147		n			
115	100		131		5/9/97	141		n			
120	82		121		5/9/97	131		n			
126	64		122		5/9/97	127		?			
133	97		115		5/9/97	136		n			
135	46		115		5/9/97	133		?			
136	26		120		5/9/97	133		n			
141	46		117		5/9/97	121		y	2		not b
116	67		129		14/1/98	139		?			
143	110		110		14/1/98	138		y	3		not b
150		473	102		14/1/98	134		n			
63	75		169		?	168		?			