Feasibility of Enhancing Abalone Stocks by Larval Seeding

P.A. Preece, S.A. Shepherd, S.M. Clarke and J.K. Keesing

South Australian Research and Development Institute,

P.O. Box 120 Henley Beach 5022, SOUTH AUSTRALIA.

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Contents

NON-TECHNICAL SUMMARY

The decline of many of the world's fisheries has stimulated interest in the enhancement of stocks by using cultured animals. Enhancement of abalone stocks with cultured juveniles has been practised for some time (in Japan in particular), but the high cost of rearing juveniles or the high mortality of seeded stocks has created sporadic interest in seeding with larvae. Abalone larvae are relatively simple and cheap to produce in very large numbers in a basic hatchery.

In this study we examined the feasibility of seeding larvae for abalone stock enhancement. We describe methods for transporting competent-to-settle abalone larvae from the hatchery to the field. We also describe a method for pumping larvae from a small boat to a diver for seeding onto suitable habitat and we describe several experiments using different densities of seeded abalone larvae, the use of mesh "tents" to retain seeded larvae prior to settlement, and the methods we used to estimate the subsequent survival of settled larvae. We also propose a larval seeding code of practice, and give a benefit-cost economic model for assessing the economic feasibility of larval seeding.

Larval abalone seeding experiments were undertaken at 7 main sites south of Port Lincoln, South Australia. Sites were selected on their suitability for later survey and the presence of abalone in the area. We reasoned that if abalone were absent naturally, then the area may not be suitable for seeding experiments. Two commercially fished species of abalone were used in the study; *Haliotis rubra* Leach (blacklip) and *H. laevigata* Donovan (greenlip). Larvae were transported from the hatchery to the experimental sites either damp (spread thinly on mesh, in an insulated container) or suspended concentrated in hatchery seawater. They were resuspended in ambient seawater at the experimental sites prior to seeding. Some differences in settlement densities were observed at the initial survey with each transportation method. In future, transportation in hatchery seawater is recommended. Abalone were seeded at approximate densities ranging from 1600 m^{-2} to 120000 m^{-2}, either in small (1 m^2) "plots" or on a broader scale (500 m^2). Surveys were undertaken 6, 9, 27 and 39 days after seeding at various sites, and then again 6, 12, 18 and 24 months after seeding at most

sites. Survey methods in the first few months involved destructive collecting of samples of the substratum from the seeded plots and removal to the laboratory where samples were cleaned, sieved and preserved prior to sorting under the microscope. Later surveys involved non-destructive diver searching, using quadrats to measure areas searched, and also by estimating searched area with a 'standardised rock' counting method. The larval seeding code of practice policy was developed after telephone surveys of the abalone fishery and/or aquaculture managers from Tasmania, Victoria, New South Wales and Western Australia concerning their respective policies on abalone stock enhancement and farming at sea, and from several reports on these topics. The benefit cost analysis includes inputs and cost estimates from the larval supply industry and from commercial abalone divers who might consider commercial scale reseeding. It incorporates estimates of juvenile abalone mortality gained from this study and others, and allows for a range of fluctuations in costs and returns estimates.

Results of the study showed high variation in survival. We successfully enhanced stocks up to a year after seeding, but not longer. Higher release densities generally resulted in higher post-larvae densities in initial surveys, but lower survival as a proportion of number released. Mesh tents used to retain seeded larvae also generally resulted in higher larval densities but are not feasible for broad scale larval seeding. Given the likely density-dependent mortality of post-larvae after settlement, larval release densities even lower than those tried in these experiments over wider areas is likely to be the optimal seeding strategy. Our inability to detect enhancement of stocks more than one year after seeding may have been partly due to dispersal of animals out of study areas into surrounding habitat, but is more likely due to the strong density-dependent mortality. Site disturbance during surveys may also have increased slightly the predation of juvenile abalone. The experiments were all done at sites where natural populations of abalone, not depleted by fishing, occurred. Competition with naturally settling abalone larvae must have increased the density-dependent mortality observed.

The results of this study indicate that with the best estimate of seeded abalone survival, combined with current seeding costs and fishing returns larval abalone seeding is at best of marginal economic viability. Mortality rates of seeded abalone is the main factor determining profitability, while cost of

larvae is the main expense. Both of these factors may be quite variable. However, we cannot exclude the possibility that larval seeding of sites in which abalone populations have become depleted may be profitable.

A review of the stock enhancement policy and farming at sea policy from most Australian states has identified a range of issues that must be addressed by a "seeding code of practice". The main issues are the decrease in genetic diversity of seed stock, the effect of seeded stock on the genetic diversity of wild stocks and the introduction to the environment of pests, diseases or chemicals with seed abalone. Recommendations include sourcing broodstock from the immediate area to be seeded, spawning at least 25 broodstock for a larvae production run, clean and environmentally responsible hatchery practices and follow up monitoring of seeded sites.

Background

Experimental studies in Australia have established that blacklip abalone populations are recruited from local parent stock for replenishment (Prince *et al*, 1988 and McShane *et al*, 1988). This is less true for greenlip abalone (Shepherd et al. 1992). Where adult densities are low as a result of overfishing, disease or habitat disturbance, larval availability is likely to be low and consequently populations may never recover. The introduction of large numbers of cultured animals into an area (seeding) offers a way to break this cycle and rehabilitate stocks.

Stock enhancement with juvenile abalone has been attempted and is practised in some countries, but economic viability is marginal due to the high cost of production (Seki and Sano 1998), the required multiple handling of each juvenile to be seeded and the high mortality of released juveniles. Larval seeding has rarely been attempted, and where it has, results have been poorly documented (reviewed by Preece et al. 1997). Following the suggestion by Tong *et al.* (1987) for seeding larvae, Keesing *et al* (unpublished data) developed a technique to deliver larvae to the seabed from a small boat. This technique enabled a diver on the seabed to deliver a million 'competent' larvae from a small boat directly into good juvenile habitat in about 30 minutes. This study used this system to evaluate whether abalone numbers could be enhanced by seeding larvae and measuring survival rates

of the seed at different sites and with two species. We wish to assess the overall feasibility of larval seeding for abalone stock enhancement or rehabilitation.

The potential for larval seeding in South Australia has increased in recent years. The rapid expansion of abalone aquaculture and the commissioning of new farms and hatcheries in southern Eyre Peninsula will likely increase the availability of abalone larvae, while competition between farms should also see the price for larvae fall.

Need

Australia's abalone fisheries earn in excess of $100 million in exports and supply over 60% of the world's abalone catch. All Australia's abalone fisheries are fully exploited and subject to quotas which are frequently being reduced in some states in response to recruitment overfishing of some stocks. Also, the illicit catch in some areas is likely to be depleting stocks to an unknown but significant degree. Increasing recruitment by artificial means has the potential to enhance productivity and sustainability of this valuable export-orientated Australian industry.

Increasing the demand for abalone larvae is also likely to benefit the rapidly expanding abalone aquaculture industry, and assist in the development of aquaculture-wild fishery industry linkages.

Large scale seeding programmes may pose a threat to the genetic integrity and diversity of wild abalone (Benzie, 1996), so a code of practice is required covering issues such as broodstock numbers and their source in relation to sites of larval release.

Objectives

The objectives of this study were to assess the biological, economic and logistic feasibility of larval seeding for abalone stock enhancement through:

- Determining the most appropriate transport and field deployment techniques to optimise survival of seeded larvae.

- Determining whether larval seeding can enhance recruitment of abalone stocks over and above natural rates of recruitment.

- Determining the true costs and benefits of undertaking larval seeding.

- Developing a code of practice outlining larval seeding ethics.

The Victorian collaborators on this project were also to pursue site-specific objectives, but after numerous technical problems with the production of viable larvae for experimentation, withdrew from the project. Their objectives included:

- *Determine the optimal habitat requirements for larval release within the Central Zone reefs in Victoria*

- *Determine the optimal habitat requirements for larval release within the Eden reef zones in New South Wales*

- *Determine the influence of existing adult abundance on recruitment enhancement rates by larval seeding.*

Methods

Detailed descriptions of the methods used in this research are included in the draft and published papers included in the next (detailed results) section. As outlined in the original project application (Section B11 Methods), the

experimental design was modified as the project progressed. The basic experimental design changed little; however specific changes are listed:

- Six sites (instead of four) were seeded in 1994 and 1995 while none were seeded in 1996 (no net change to the total number of sites proposed for seeding).

- Proposed 3 day post-seeding surveys were undertaken at 6 or 9 days instead. Larger post-larval shell growth made identification and counting simpler.

- Proposed 1 month surveys were undertaken at approximately 1 month after seeding (27 and 39 days) due to inclement weather.

- Six month surveys were abandoned after the first year as juvenile abalone could not be reliably identified to species at that age and size.

- Post-larval abalone were removed by freezing rather than the anaesthetic technique proposed. Freezing allowed the survey samples to be temporarily stored until processed.

- A treatment factor testing different seeding densities was incorporated at the two sites chosen for "tents", adding to the number of sub-sites seeded.

- Tents were not used after the first year as originally proposed. Sub-sites were also discontinued in favour of "blanket seeding" of each site.

- Artificial settlement substrata were added to the experiments.

- Sites were pegged at 50m * 10m rather than 50m * 50m.

- The Victorian components of the study were abandoned.

Detailed Results

Detailed descriptions and discussion of the results of this research are included as a draft and a published scientific papers at the end of this section.

Determining the most appropriate transport and field deployment techniques to optimise survival of seeded larvae.

Both dry transportation (larvae spread on damp cloth in an insulated container) and wet transportation methods (concentrated in filtered, UV irradiated seawater) were tried during the course of this study. It appears from initial survey results that less settlement occurred with larvae that were transported and stored in a spherical clump overnight when compared with larvae that were transported dry but immediately resuspended in fresh seawater. Both were settled successfully in laboratory aquaria after resuspension, but no quantitative settlement data were collected.

The pressurised vessel delivery system used from the beginning of the project required only slight modification. As air entering the seed delivery hose would interrupt the flow of larvae, incorporation of a bubble trap between the pressure vessel and the larval delivery hose is one potential improvement. Exposure to possible gas supersaturation with de-pressurisation of the delivery apparatus, or other apparatus related factors did not appear to affect settlement or survival of greenlip and blacklip larvae in laboratory aquaria.

Determining whether larval re-seeding can enhance recruitment of abalone stocks over and above natural rates of recruitment.

No enhanced stocks were followed through to recruitment to the fishery; however juveniles were monitored for 24 months with enhancement achieved up to 12 months after seeding. No enhancement was demonstrated more than 12 months after seeding. Detailed results comprising this report follow in the form of two draft papers (one submitted to Can. Spec. Publn Fish. Aquat. Sci.) and one published paper:

DRAFT PAPER

Title

LARVAL ABALONE SEEDING EXPERIMENTS AT PORT LINCOLN SOUTH AUSTRALIA.

Authors

Preece, P.A.G. Shepherd, S.A. Clarke, S.M. and Keesing, J.K.

Abstract

Seeding of natural reefs around Port Lincoln, South Australia was undertaken in 1994 and 1995, with the larvae of two commercially fished species of abalone, *Haliotis rubra* and *H. laevigata*. The experimental seeding sites and unseeded control sites were sampled for settled post larvae at various times up to 39 days after seeding, and then annually for juvenile abalone. Variable post-larval density was found across sites, with generally enhanced densities of post-larvae found at seeded sites. Some enhancement of juvenile stocks was evident at some seeded sites up to one year after seeding, but not thereafter. Both semi-dry and wet larvae handling methods, larval seed delivery method and survey methods are described in detail.

Introduction

Techniques to enhance harvestable abalone stocks have long been seen as a solution to the decline in many of the worlds abalone fisheries. Attempts at stock enhancement of abalone using cultured juvenile abalone have been made in several countries including Japan, the USA and New Zealand (Saito, 1984, Tegner and Butler, 1985 and Schiel, 1993). Much of this work has been done on a small scale, and difficulties in quantifying results and comparison between studies have led to debate as to the effectiveness of using cultured juvenile abalone to enhance stocks (McCormick *et al* 1994). Tong *et al* (1987)

describe some of the problems of seeding with cultured juveniles, and tried a novel approach to seeding by using cultured *Haliotis iris* larvae. Three months after seeding with larvae they found significantly higher numbers of newly recruited juveniles less than 4 mm shell length in seeded sites than in control sites. Schiel (1992) attempted similar small scale larval seeding experiments. Larval seeding trials in Japan are referred to by Salas-Garza and Searcy-Bernal (1990) and Tegner *et al* (1986) attempted larval seeding by transplanting larvae into the field on mesh screens. Mazón-Sastegui *et al* (1996) review larval abalone seeding practices of some Baja California co-operatives, practices developed in the 1960s by Ortiz-Quintanilla (1980).

In this report we describe larval seeding experiments using two commercial abalone species, *H. rubra* Leach (blacklip) and *H. laevigata* Donovan (greenlip) at seven sites in South Australia. We describe larval handling and transportation techniques, our boat-to-sea-bed larval seed delivery system, our experimental designs and survey techniques for quantifying seeding results, a few days after seeding up to two years or more after seeding. A cost benefit analysis of larval abalone seeding is presented as well as a proposed larval abalone seeding policy.

We transported larvae from the farm to the seeding sites at high density in seawater, or carried damp on nylon mesh. We used a simple compressed air displacement method for delivering a flow of suspended larval seed from the boat to a diver on the sea bed, and we used a replicated plot design as well as non-destructive broad scale quadrat surveys to quantify results.

Results to date indicate that seed delivery can be successfully achieved using our larval transport methods and seed delivery system. Significant enhancement of post larval and juvenile abalone was shown up to a year after seeding at some sites, but then became impossible to detect using our methods. Possible juvenile dispersion out of study areas and density-dependent mortality are suggested as the causes of our failure to enhance abalone densities two years after seeding.

Materials and methods

Sites

In August 1994 (the first year of the project) three sites were selected for seeding with *Haliotis rubra* (blacklip abalone). Sites were selected in consultation with the local abalone divers, using the following criteria: the sites must be reasonably close to Port Lincoln and the abalone hatcheries, there must be an abundance of boulders of manageable size for sampling, with adequate cover of crustose coralline algae, the sites should be sheltered from the prevailing south-westerly winter winds and the sites should not be too close to existing abalone research sites. Sites would ideally be no deeper than 12 metres in order to maximise no decompression times for divers working on the sites. In November 1994 three more sites were selected for seeding with *H. laevigata* using the same criteria except that they should also be protected from the prevailing south-easterly summer winds. In November 1995 a fourth *H. laevigata* site was chosen to replace one of the 1994 sites. Backup sites were also generally prepared for the contingency of bad weather preventing access to one or more of the primary sites. A backup site was required on one occasion when the boat carrying the reseeding equipment could not be safely anchored above the site to be seeded. This site (Site 7) thereafter became one of the primary *H. laevigata* seeding sites. Control sites were adjacent to seeded sites, usually about 100 metres to the south. These sites were selected because their habitat was similar to the respective seeded site, and were tidally "upstream" of the seeded site at the time of seeding. All sites were approximately 500m^2 (50 metres long and 10 metres wide).

Site locations were kept with GPS fixes and visual sightlines, and were generally marked at each end with steel star pickets and sub-surface buoys. Table 1 shows a summary of the seeded sites and the GPS latitude and longitude coordinates. Figure 1 shows a map of site locations.

Site descriptions - blacklip

Site 2 and Site 3 are moderately exposed sites, with an estimated maximum tidal flow of about 0.5 m sec^{-1} and maximum swell height of about 1 metre. Site 1 is less exposed with less tidal flow and generally lower swell height. Site 3 is 9-12 metres deep, while the other two are 7-9 metres deep. The

bottom topography at all sites is generally a rocky shoreline then a sloping boulder field descending to the sandline. At the transition from boulder slope to sandline there is a relatively flat strip varying in width from 1 to 5 metres scattered with boulders. Dominant algae are *Ecklonia radiata*, *Seirococcus axillaris*, *Cystophora moniliformis* and *Acrocarpia paniculata*. Sandy bottom is colonised by seagrass meadows of *Posidonia* or *Amphibolis*. The main predators of small abalone include wrasses (mainly *Notolabrus tetricus*) and crabs.

Site descriptions - greenlip

Site 4 and Site 6 are moderately exposed sites, with estimated maximum tidal flow of 1m sec^{-1} and maximum swell height of about 1 metre. Site 5 and Site 7 are less exposed with less tidal flow and generally lower swell height. Sites 4, 5 and 6 are all 9-12 metres deep, while Site 7 is 5-6 metres deep. The bottom topography at these sites is generally a rocky shoreline then a boulder slope with occasional large, smooth granite sheets descending to the sandline. At the transition from boulder slope to sandline is a relatively flat strip of varying width scattered with boulders. Dominant algae are *Ecklonia radiata*, *Seirococcus axillaris*, *Osmundaria prolifera*, *Cystophora moniliformis* and *Sargassum fallax*. Generally *Posidonia* and/or *Amphibolis* colonise the sandy bottom. The main predators of small abalone include wrasses and crabs.

Site preparation

1994

Each site was prepared according to the experimental protocol designed for that site. Two main experiments were undertaken in 1994; the seeding experiments (at Sites 1 and 3 for blacklip and Sites 4 and 6 for greenlip) and the density experiments (at Site 2 and Site 4).

At the seeding experiment sites 12 * 1 $metre^2$ patches of crustose coralline algae covered boulders of suitable size for sampling were marked with a steel peg to which was attached a 100 mm numbered foam buoy on a 1 metre

rope. These 12 "sub-sites" and a belt transect area of 25m^2 marked with a short buoy in each corner were all located within each of the 50m * 10m sites and were prepared a few days prior to seeding of the sites.

At Site 2 (the blacklip density experiment site) 48 sub-sites were marked in a similar manner a few days prior to seeding the site. 24 of the sub-sites were also covered at this time with a mesh "tent", a nylon pyramid shaped structure with a basal area of 1 m^2 and a height of 400 mm. The tents were attached to the seabed at each corner by steel pegs and weighted along their basal edges with chain. The top of the pyramid shaped tent, which incorporated a sealable opening, was kept elevated with a small float. Mesh tents were removed from blacklip seeding sites a few days after seeding.

At the Taylor Island Site 4 (greenlip density experiment site) 32 numbered sub-sites were established, 16 of which were covered with mesh tents, a few days prior to seeding. Mesh tents were removed from sub-sites 24 hours after seeding.

1995

The three blacklip seeding sites established in 1994 were used again in 1995. Instead of seeding small plots it was decided to try to "blanket" the whole site with larvae. A week prior to seeding in 1995 the sites were prepared by marking out a 500 m^2 area (50 m * 10 m). The area was marked by stretching out four orange nylon lines each two metres apart and 50 metres long, and pegging them to the sea bed at each end and along their length. This gave five 100 m^2 parallel strips along which a diver could easily swim whilst concentrating on seeding.

About one week before seeding, artificial larval collector material (see Rodda *et al* 1997) was deployed at each seeding site and control site. These collectors comprise four layers of aged 20 mm corrugated black plastic biofilter material, glued face to face with the corrugations of alternate sheets running in diagonally opposite directions. This "biscuit" of corrugated plastic, with a total surface area of approximately 1m^2 , was attached to an unpainted cement roof tile with heavy duty rubber bands, and marked with a fluorescent pink surveyor's flagging tape streamer to increase visibility. The collectors were dropped onto the sites a week before seeding in order to develop a

surface film of bacteria and/or diatoms. According to Nash *et al* (1995) such conditioning encourages settlement of abalone larvae.

Two of the three greenlip seeding sites established in 1994 were used again in 1995. Site 6 was not used in 1995. A new site was established further north along the western shore of Taylor Island, and prepared along with Sites 4 and 6 with the same method as used at the three blacklip seeding sites. The new site was abandoned for a backup site on the day of seeding due to strong westerly winds which prevented safe anchoring of the boat. No collectors had been deployed at the backup site.

Receival and handling of larvae

Larvae were deemed competent to settle with the appearance of the third tubule on the cephalic tentacle (Stage 39, Hahn(1989)). On August 19, 1994, nine days after spawning, competent to settle *H. rubra* larvae were received from *South Australian Abalone Developments* (SAABDEV) for the 1994 blacklip seeding . The larvae were delivered in a spherical lump wrapped in damp nylon mesh and super-wipe dish cloth material in a small polystyrene insulated esky, and accompanied by a delivery docket stating $3.2 * 10^6$ larvae, 91.4% of which were alive. The larvae were stored in this arrangement in a refrigerator for about 18 hours at 10-12 C^o and were not sampled until resuspended in seawater immediately prior to seeding the next day.

On November 6, 1994 $7.1 * 10^6$ *H. laevigata* larvae were picked up from SAABDEV at Louth Bay. They were packed in a similar manner to the *H. rubra* larvae delivered in August. Again, due to rough weather, they were stored in a refrigerator over night, and on November 7 were resuspended in fresh seawater and released in less than ideal habitat at Cape Donnington.

Approximately $3 * 10^6$ *H. laevigata* larvae were picked up from *South Australian Mariculture* at Boston Point on November 8, 1994. These larvae were spread thinly over a piece of 125 μm nylon mesh which was then folded double (with the larvae inside) and then loosely wrapped in damp super-wipe material in an insulated esky. These larvae were immediately taken to Port Lincoln, then by boat to Taylor Island, Site 6 where they were resuspended in fresh seawater.

With all subsequent purchases of larvae the larvae were received suspended in filtered, ultraviolet irradiated seawater. Larval density was about 100 ml^{-1} (2-3 * 10^6 per container) and the seawater was gently aerated with battery powered airpumps and a single airstone.

Larvae were transported to the seeding sites as they were delivered by the hatcheries - either wrapped in damp cloth or suspended in high concentration in hatchery seawater. Once at the site all of the larvae were mixed in one 60 litre drum with fresh seawater taken from the sea surface at the seeding site. The drum was then topped up to 60 litres with more fresh seawater. Estimates of actual larvae numbers can quickly be made at this point by mixing the drum contents to ensure a homogenous larval suspension and taking a sample in a 1 ml graduated pipette. By holding the pipette horizontal against a dark background individual larvae can be seen and counted without magnification, and total number estimated by multiplication. Eight samples taken gave a coefficient of variation of 11.8%. By decanting a calculated volume of the larval suspension to a fresh drum and then topping it up to 60 litres, the required number of larvae for a complete experiment or site could be contained in a single drum. This approach was used for all experiments and sites as it avoided the need to change drums during reseeding.

Abalone Seed delivery system

The system was required to deliver abalone larvae undamaged to a diver on the sea bed. The system used throughout the project consisted of a 60 litre cylindrical plastic drum (HDPE, Atlas Plastics RD 63113) which holds the seawater-larvae suspension. The drum has a screw-on lid with neoprene O-ring seal. On the side of the drum near the base is a 17 mm threaded aperture to which a plastic ball valve is fitted. A 50 metre plastic hose 13 mm in diameter is connected to the drum ball valve at one end and has another ball valve at the other end. The 60 litre drum is pressurised in order to force the larvae down the plastic hose when both ball valves are open. Flow can be stopped at any time by the diver or surface attendant by closing one of the ball valves. Compressed air is used to pressurise the drum and is supplied from a SCUBA cylinder via a SCUBA regulator first stage, which regulates and reduces the cylinder pressure to approximately 1000 kPa (150p.s.i.). A

manually operated air flow control valve and a pressure gauge are fitted to the lid of the 60 litre drum. The SCUBA regulator first stage and the flow control valve are connected by 8 mm air hose and "Ryco" snap on fittings. The flow of compressed air can be controlled with the valve, and drum internal pressure monitored using the pressure gauge. On the downstream side of the flow control valve (on the inside of the drum lid) is a weighted 6 mm plastic hose which carries the compressed air to the bottom of the drum where it bubbles up through the seawater-larvae suspension, serving to pressurise the drum as well as both aerate and agitate the drum contents. The drum pressure was maintained at 28 kPa (4 p.s.i.) by the surface attendant during seeding and this gave a reasonably stable flow of 4 litres min^{-1}. It was important not to let air into the larval delivery hose as water pressure beyond 3 metres would then stop the flow to the diver.

Blue food colouring (*Queen Fine Foods Pty Ltd; Cake and confectionary colour*), shown by small-scale experiments to be non-toxic, was added to the larval suspension at the rate of 10 ml colouring per 60 litres seawater to make the larval suspension visible to the diver. The diver could see when flow had been interrupted by the surface attendant. The food colouring used contained colour 331 (2.1% total dyestuff) and preservative 210 (Benzoic acid, concentration unknown)

Seeding

1994

Blacklip sites

Seeding of all experimental *H. rubra* sites was undertaken on August 20, 1994. At Site 2 (the density experiment site), 48 sub-sites were seeded at one of three densities, 1 600, 16 000 or 80 000 larvae m^{-2} approximately. At the sub-sites with tents, the larval suspension was injected under the tent canopy. The different seeding densities were achieved by timing the flow of seawater with larvae in suspension, thus controlling the *volume* and hence numbers of larvae in suspension at each sub-site.

At the two seeding experiment sites, each 1 metre^2 sub-site and the 25 metre^2 belt transect area were seeded with approximately 20 000 larvae m^{-2}, again timing the flow to estimate numbers of larvae.

Greenlip sites

The *H. laevigata* seeding sites (Sites 5 and 6) were seeded on November 8, 1994 at a rate of approximately 20 000 larvae m^{-2} sub-site. The density experiment (Site 4) was seeded on December 7, 1994 with each of the 1 metre^2 sub-sites being seeded with either 2 000 or 120 000 larvae.

1995

Blacklip and greenlip sites

The three *H. rubra* seeding sites were "blanketed" with larvae at a mean density of approximately 4 000 m^{-2} on September 29, 1995. By using a broad side to side sweeping movement of the larval delivery hose, whilst swimming down the centre of each 2 metre * 50 metre laneway, it was anticipated that a reasonably even seeding rate could be achieved across the whole 500 m^2 of each site.

The *H. laevigata* sites were seeded on November 29, 1995 with the same method as that used to seed the *H. rubra* sites.

Site sampling

1994

Sampling of the sites initially consisted of a collection of the coralline algae covered boulder substrata. Divers would go back to each of the numbered subsites and collect boulders. At each subsite five boulders were placed in each of two polythene bags which were sealed underwater with elastic bands. A plastic label with the site name, subsite number, sampling date and either "A" or "B" was also included with each bag. Bags were sent to the surface several at a time using a large mesh catch bag attached to an inflatable air lift bag. Surface attendants then lifted them onto the boat with a davit. Each

polythene bag weighed 5-7 kg wet. Bags were transported back to Port Lincoln at the end of the day and frozen at -10°C for later processing. During transportation, handling and in the deep freezer prior to freezing, there was some leakage of fluid from the bags of boulders and seawater. An examination of this leaked fluid showed that it did not contain any abalone.

Blacklip seeding sites (Site 1 and Site 3) were sampled on August 26 and September 16, 1994 and the blacklip density experiment was sampled on September 9, 1994.

Taylor Island greenlip seeding sites (Site 5 and Site 6) were sampled on December 14, 1994 and the greenlip density experiment site was sampled on 13 December, 1994, and 23 January, 1995.

All sites were surveyed again one and two years after seeding with a non-destructive rock turnover method. It was assumed that seeded larvae may have dispersed from the seeded subplot areas into adjacent boulders so survey effort was not confined to subplot areas alone. The survey method involved searching 24 quadrats of 0.25m^2 across the sites. Quadrat areas were randomly selected; however only quadrats covering suitable juvenile abalone habitat were selected. Areas with no rock crevices were not surveyed. Quadrats were surveyed by turning over all the boulders within a 0.25m^2 area and measuring with a vernier calliper to the nearest millimetre the shell length of all abalone of the target size. Abalone species, size and number of rocks were recorded per quadrat. These surveys were intended to be 'non-destructive' and all boulders moved were replaced as carefully as possible.

1995

Artificial collectors were deployed at all three blacklip seeding sites on 21 September, 1995, and seeded on 29 September. They were retrieved by divers on 10 October, 1995, and individually placed in labelled polythene bags prior to freezing. The cement roof tile component was left on the sea bed at the seeded sites for future surveys. All 1995 blacklip sites were surveyed again 12 and two years after seeding with our non-destructive sampling method.

Artificial collectors were also deployed at the three greenlip reseeding sites on 23 November, 1995; seeding occurred on 29 November. The collectors were retrieved on 7 December, 1995, and labelled, bagged and frozen as for the blacklip samples. The tiles were also left at the sites. The back-up site used in 1995 (Site 7) had no artificial collectors deployed and no sampling was done. All seeded sites were surveyed six and 12 months after seeding by non-destructive sampling.

Sample processing

The frozen rock or collector samples were placed in 40 litre plastic fish bins and allowed to thaw. Samples were liberally rinsed with fresh water and the substrate removed, leaving the rinse water and residues in the fish bin. This was sieved through nested sieves of 1000μm and 125μm mesh. The contents of each sieve (screenings > 1 mm and screenings < 1 mm) were transferred to a separate 250 ml plastic screw cap jar, stained with half a rice grain of Rose Bengal and fixed in ethanol. All label details were transferred to the two jars, and the natural substrate (rocks) returned to the freezer for measuring later. Collectors were dried and stored for re-use.

Prior to sorting, preserving and staining, samples < 1 mm were further fractionated by washing through another nest of sieves with fresh water. Mesh sizes were 500μm, 250μm and 125μm, and the three fractions were rinsed into separate Petrie dishes. These fractions were individually sorted under a dissecting microscope using low power and a back lit dark field. A portion of the sample was drawn up into a Pasteur pipette and then evenly spread across another Petrie dish. This sub-sample was then searched systematically by moving it across the microscope field of view in a grid pattern until the whole sample had been covered. All abalone found were measured and their shell length recorded to the nearest 25μm. The sub-sample was then discarded and another taken until the entire fraction had been searched. This process was repeated for each of the three fractions. Samples of screenings > 1 mm were not further fractionated, but were searched in a similar manner. The post larvae from each of the samples were individually counted and measured, and then means for all samples (from that site) calculated. For each of the samples total planar surface area (p.s.a.) of

the cobbles was estimated and the post-larvae count for that sample then adjusted to give post-larvae per 1 metre^2 p.s.a. Mean number of larvae per 1 metre^2 p.s.a. for each site was then calculated and is shown in Tables 1 to 4.

(NOTE *See Results section*)

Assumptions

Shepherd & Godoy (1989) found that *H. laevigata* 6mths-2 yrs old in a boulder habitat at West Island dispersed at a mean rate of 0.5 m per month. It is likely that juvenile *H. rubra* and *H. laevigata* <6 mths old would move less than this as they are still at that age living on a crustose coralline substratum (Shepherd and Cannon, 1988). Nevertheless even a much lower rate of dispersal would require sampling outside the actual plots seeded.

Table 1. Seeding project site details.

Site	Geographic name	Latitude	Longitude	Seeding dates	Species
1	Shag Cove	34° 53.90' S	135° 58.59' E	August 20 1994 Sept 29 1995	*H. rubra*
2	Cathedral Rock tent site	34° 55.54' S	135° 59.41' E	August 20 1994 Sept 29 1995	*H. rubra*
3	Cathedral Rock	34° 55.74' S	135° 59.48' E	August 20 1994 Sept 29 1995	*H. rubra*
4	Taylor Island tent site	34° 52.85' S	135° 59.95' E	Dec 7 1994 Nov 29 1995	*H. laevigata*
5	Taylor Is tank site	34° 53.20' S	136° 00.15' E	Nov 8 1994 Nov 29 1995	*H. laevigata*
6	Taylor Is south site	34° 53.50' S	136° 00.30' E	Nov 8 1994	*H. laevigata*
7	Bill's site	34° 48.88' S	136° 00.00' E	Nov 29 1995	*H. laevigata*

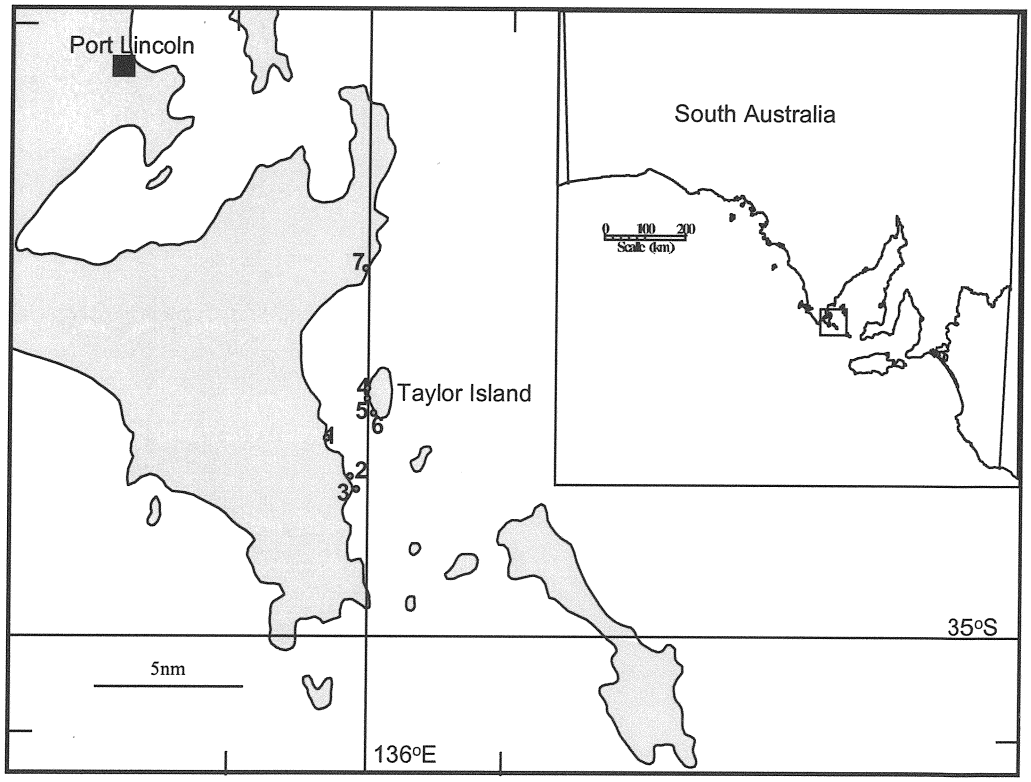

Figure 1. Map showing seeding site locations.

Results

1994

Density experiments

For the results and discussion of the *H. rubra* and *H. laevigata* density experiments see Preece *et* al (1997); a reprint of this paper is attached.

Blacklip sites

The two *H. rubra* seeding sites (Site 3 and Site 1) were sampled 6 days and again 27 days after seeding. The results are given in Tables 2,3. Note that at Site 1 at 27 days after seeding the mean density of post-larvae in the control was not significantly different from the treatment although at 6 days they differed significantly. At Site 3 the mean post-larval density at the treatment and control sites differed significantly at both 6 and 27 days after seeding.

Table 2. Site 1 data for blacklip abalone, showing mean lengths (and range) and densities (s.e. in brackets) of post-larvae found. N the is number of individual post-larvae found.

Period	Treatment				Control			
	N	Length (μm)	Density (no m^{-2})	Area searched (cm^2)	N	Length (μm)	Density (no m^{-2})	Area searched (cm^2)
6 day	12	422.5 (16.4) (415 – 500)	11.7 (6.1)	9813	2	480 (28.3) (460 - 500)	0.9 (0.9)	10363
27 day	8	619.4 (37.1) (500 – 850)	6.5 (4.0)	10479	8	600 (23.6) (475 - 675)	6.7 (3.4)	11322

Table 3. Site 3 for blacklip abalone showing mean length, size range and mean densities at treatment and control sites (standard errors in brackets). N is number of post-larvae found.

Period	Treatment				Control			
	N	Length (μm)	Density (no m^{-2})	Area searched (cm^2)	N	Length (μm)	Density (no m^{-2})	Area searched (cm^2)
6 day	32	443 (14.8) (275 – 550)	27.1 (6.9)	10850	8	470 (21.3) (375 - 575)	7.7 (3.2)	9567
27 day	43	667 (18.3) (485 – 900)	36.0 (9.4)	12053	14	626 (27.8) (475 - 775)	13.4 (5.6)	11927

Greenlip sites

The two *H. laevigata* seeding sites (Sites 5 and 6) were sampled 9 days and again 39 days after seeding. Tables 4,5 show that control site post-larval mean densities were significantly different from the treatment site mean densities at both 9 and 39 days after seeding at Site 5, and at Site 6, though very low numbers of post-larvae were found at Site 6.

Table 4. Site 5 data showing mean length, size range and mean densities at treatment and control sites (standard errors in brackets). N is number of post-larvae found.

Period	Treatment				Control			
	N	Length (μm)	Density (no m^{-2})	Area searched (cm^2)	N	Length (μm)	Density (no m^{-2})	Area searched (cm^2)
9 day	825	456 (2.8) (350 – 825)	729 (193)	12144	2	450 (0) -	2.4 (2.4)	10495
39 day	60	806 (9.1) (600 – 950)	46.1 (21.9)	12441	2	750 (100) (650 - 750)	2.1 (1.4)	10836

Table 5. Site 6 data for greenlip abalone showing mean length, size range and mean densities of post-larvae at treatment and control sites (standard errors in brackets). N is number of post-larvae found.

Period	Treatment				Control			
	N	Length (μm)	Density (no m^{-2})	Area searched (cm^2)	N	Length (μm)	Density (no m^{-2})	Area searched (cm^2)
9 day	10	463 (5.2) (425 – 475)	9.6 (3.9)	10592	1	475 -	1.0 (1.0)	10169
39 day	17	821 (32.1) (600 – 1000)	13.3 (4.9)	12480	3	787 (46.7) (700 - 850)	2.5 (1.8)	11970

Collector results

Collectors were seeded at the same time as the natural substrate at both greenlip sites. Tables 6, 7 show that the mean numbers of post-larvae found at control sites were significantly different from mean numbers found at treatment sites up to 39 days after seeding at both sites. Note that there is high variance within these means. Note also that Site 6 collectors showed higher numbers of post-larvae while natural substrata at this site showed low numbers of post-larvae.

Table 6. Site 5 collector data for greenlip abalone showing the mean numbers (N) of post larvae found per collector, mean length (standard errors in brackets) and length range, for two sampling times.

Period	Treatment		Control	
	mean N	Length (μm)	Mean N	Length (μm)
9 day	380 (210)	475 approx. (375 - 525)	2.25 (1.31)	457 (16.2) (360 - 550)
39 day	23.5 (11.9)	851 (18.4) (475 - 1075)	4 (1.68)	823 (39.2) (700 - 1000)

Table 7. Site 6 collector data for greenlip abalone showing the mean numbers (N) of post larvae found per collector, mean length (standard errors in brackets) and length range, for two sampling times.

Period	Treatment		Control	
	mean N	Length (μm)	Mean N	Length (μm)
9 day	55.8 (22.0)	462 (3.49) (325 - 550)	0.5 (0.29)	475 (475 - 500)
39 day	101 (19.2)	924 (14.7) (625 - 1175)	2.5 (1.55)	887 (63.1) (600 - 925)

1995 results

At both *H. rubra* and *H. laevigata* sites in 1995, the first post-seeding survey was done using collectors only. Tables 8, 9 show the mean number of post larvae found on treatment and control collectors 11 and 8 days respectively after seeding at six sites. Note that Table 8 shows big differences in means between sites. Bubbles were discovered in the seed delivery hose during seeding at Site 2 temporarily interrupting larval delivery. It is likely that few or no larvae were seeded in the vicinity of the collectors at Site 2, and this may account for the low numbers of post larvae found on the collectors at this site.

Table 8. 1995 Blacklip collector data showing mean number (N) of post-larvae found on treatment and control collectors at three sites (standard errors in brackets).

Site	Treatment		Control	
	mean N	Length (μm)	mean N	Length (μm)
Site 1	48.3 (27.0)	500	2.25 (0.48)	494 (6.25)
Site 2	1.8 (0.63)	500	0	-
Site 3	65 (25.4)	500	0.75 (0.75)	500

Table 9. 1995 Greenlip collector data showing mean numbers (N) (standard errors in brackets) of post-larvae found on collectors 8 days after seeding. No collectors were deployed at the backup site (site 7).

Site	Treatment		Control	
	mean N	Length (μm)	mean N	Length (μm)
Site 4	8.5 (2.9)	450	0.25	425
Site 5	7.75 (2.1)	447	0	-
Site 7	-	-	-	-

Later surveys

Non-destructive "rock turnover" surveys were undertaken at 12 month intervals after seeding at *H. rubra* sites. Independent studies on aging and growth of *H. rubra* at this site suggest a growth rate of about 28 mm yr^{-1} in the first 2-3 years (S.A. Shepherd, pers. comm.). On this assumption it is possible to decompose the length frequency data in Figure 2 into component normal distributions representing recruited cohorts. For the purposes of this study we used a knife-edge separation to estimate year-class strength, and take the size class 16-35 mm to be 1 yr old and the size class 36-60 mm to be the 2 yr old cohort. Figure 2 shows size-frequencies of abalone 16-35 mm shell length (1+ yrs) and 36-60 mm (2+ yrs) found during surveys. Note that the mean density of 1 and 2 year olds at treatment sites is slightly, but not significantly, higher than at control sites. Survey data were combined across all sites to construct these histograms as only low numbers of abalone could be detected by searching underwater. The greenlip seeding sites were searched more intensively (using five divers simultaneously) and a size frequency plot of results is shown in Figure 3. While numbers found are higher, relative densities are still low. Areas searched were estimated from numbers of rocks searched divided by mean number of rocks m^{-2}. Figure 4 shows size frequency plots of data from 1996 surveys, 12 and 24 months after seeding at greenlip sites. Again, low numbers and low densities of juvenile abalone were found. No enhancement could be detected 12 months after the 1995 seeding experiments. The enhancement detected 12 months after the 1994 seedings could not be detected 24 months after the 1994 seedings. During these non-

destructive surveys abalone found were strongly aggregated. A variance to mean (v:m) ratio or coefficient of dispersion (Sokal and Rohlf 1995) applied to numbers of abalone within the appropriate size classes was used to give a measure of aggregation. Tables 10, 11 show the v:m ratios for blacklip and greenlip abalone over the study period. They show a clear trend for aggregation by abalone to decline with increasing size. This suggests that with size, small abalone tend to go from an aggregated to a regular dispersal pattern, presumably as they disperse evenly into the under-boulder habitat.. A consequence of the aggregation of small abalone was that the variance of density estimates was very high for the sampling intensity, and the estimates of survival are very crude.

Table 10. Mean density of blacklip abalone of size found per 0.25 m^2 quadrat, variance and value for variance to mean ratios for blacklip abalone 6 days, 27 days, 12 months and 24 months after seeding. Values for variance mean ratio greater than 1 indicate aggregation.

	6 day	39 day	12 months (=25mm)	24 months (26-60 mm)
mean	4.9	5.3	0.36	0.47
variance	23.8	38.8	0.44	0.92
v:m ratio	4.9	7.3	1.20	1.96

Table 11. Mean density of greenlip abalone found per 0.25 m^2 quadrat, variance and value for variance to mean ratio of densities 9 days, 39 days, 12 months and 24 months after seeding.

	9 day	39 day	12 months (=25mm)	24 months (26-60 mm)
Mean	92.3	7.4	0.10	0.08
Variance	17291	136	0.12	0.07
v:m ratio	187	18.3	1.21	0.78

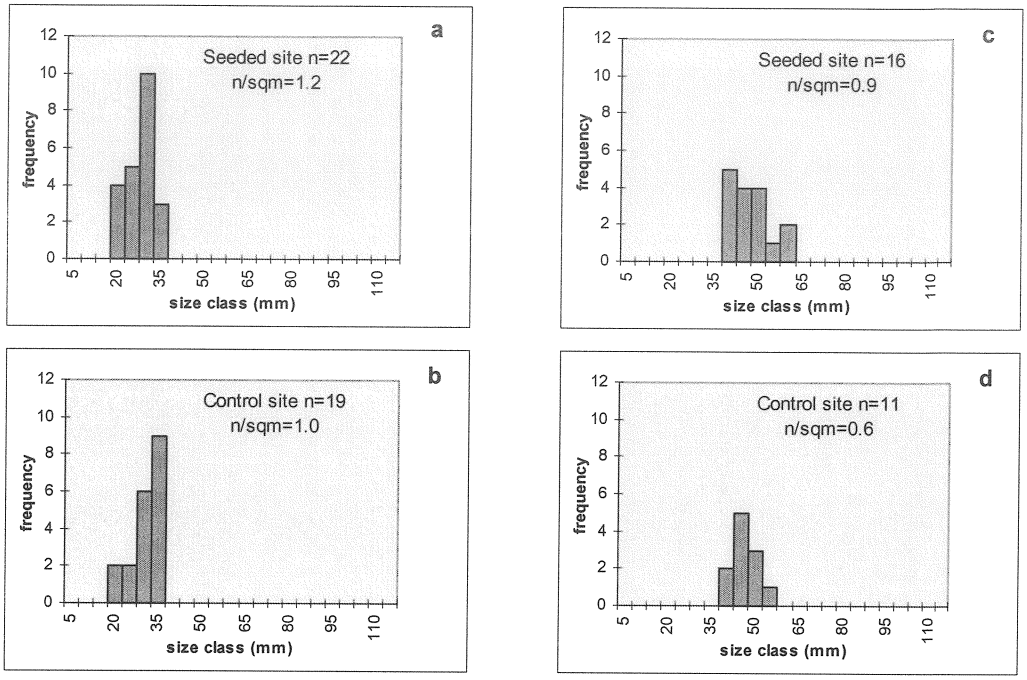

Figure 2. Size frequency plots of *H. rubra* found 12 and 24 months after seeding. Charts a and b show abalone 16-35 mm at treatment and control sites respectively, and charts c and d show abalone 36-60 mm at treatment and controls. Non-destructive surveys were undertaken in September 1995 and 1996. Data are from sites 1, 2 and 3 combined.

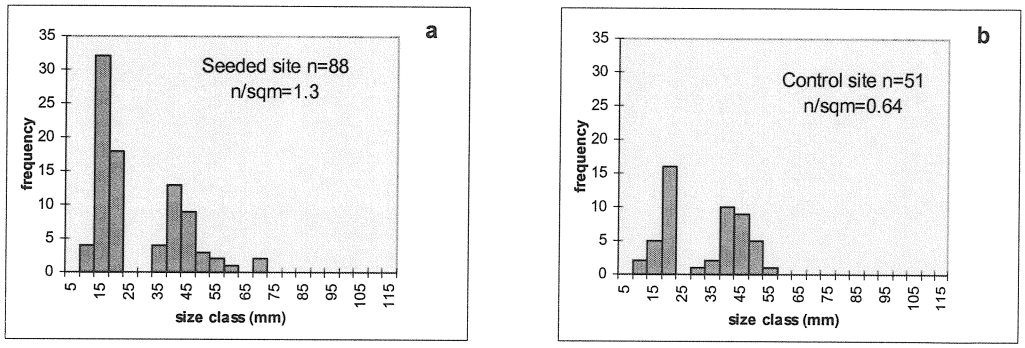

Figure 3. Size frequency of greenlip abalone found 12 months after seeding (data for Sites 4,5 and 6 combined). These sites were intensively surveyed by five divers simultaneously, and areas searched estimated by counting boulders searched. Number of one year old abalone (25 mm or less) was 54 (0.77 m^{-2}, s.e 0.15) at seeded sites and 23 (0.31 m^{-2}, s.e 0.06) at control sites. The differences were significant ($P<0.05$).

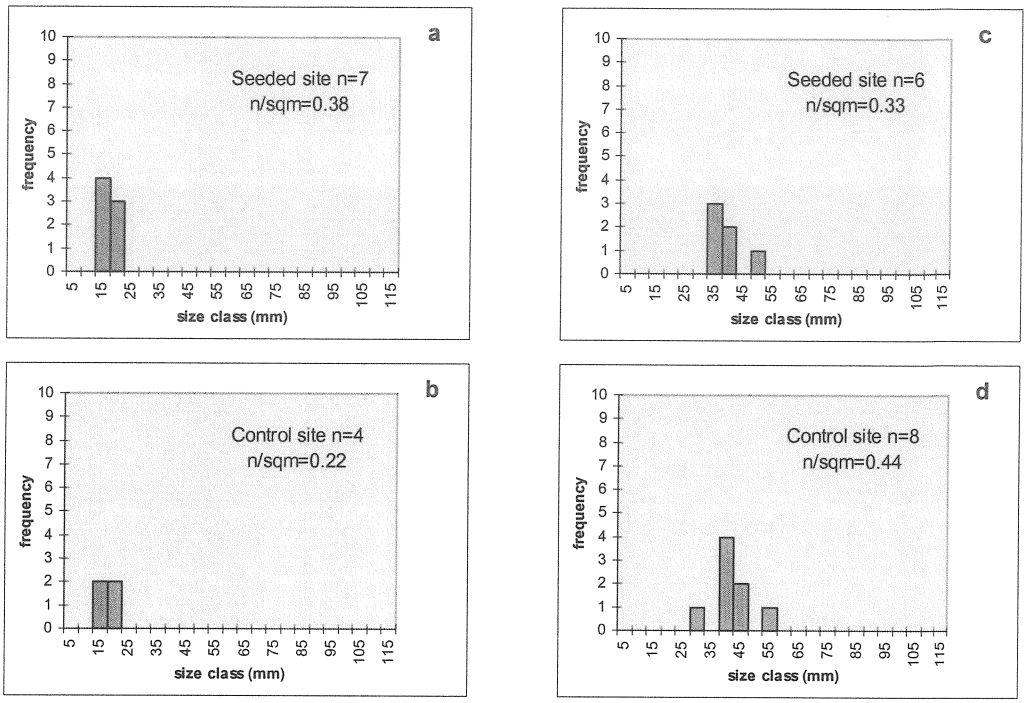

Figure 4. Size frequency plots of 1996 greenlip survey results, 12 and 24 months for greenlip abalone at seeded Sites 4-7 combined. **a, b: -** abalone 0-25 mm (1 yr old.); **c, d** : - abalone 26-60 mm (2 yr old.) at treatment and control sites.

Discussion

Post-larval blacklip abalone were enhanced by seeding on natural substrate at all sites up to six days after seeding, but at only 1 site at 27 days after seeding. Table 2 shows an increase in density of post-larvae at the control site during the period between the two samplings. This would indicate a natural spawning and settlement in the experimental area sometime between 26 August and 9 September, 1994. Rodda *et al* (1997) recorded an abalone settlement at Taylor Island (approximately 5 km north) corresponding with this period. The observation that post larval density *decreased* at the seeded site, while *increasing* at the control site to approximately the same density may indicate an optimal density of about 24 m^{-2} for settled post-larvae at this location. Tables 3, 5 show a similar increase in density of post-larvae, possibly from natural settlement at both seeded and control sites. Alternatively, the apparent increase may be an artifact of the intense patchiness. If these increases in density are real, it may indicate that the

optimum settlement density at these sites exceeded natural settlement at that time in 1994. "Topping up" of natural settlement by larval reseeding may be effective if optimal settlement density is not exceeded.

Estimates of mortality range from 0.29 mth^{-1} (Site 5 control) to 4.1 mth^{-1} (Site 5, seeded) and appear to be density dependent. Mortality estimates are confounded by the natural settlements which occurred between the two sampling dates at each site in 1994, and therefore have little meaning. Estimating mortality from time of seeding using seeding rate as initial density is also of limited use as larval settlement rates can not be measured or estimated.

With few estimates of growth rate available for these abalone species at this size, it was hoped that sampling data plotted as length frequency distributions would show clear settlement modes. This was the case with 6 and 9 days after seeding surveys (mean blacklip growth rate approximately 30 $\mu m\ d^{-1}$, mean greenlip growth rate approx 23 $\mu m\ d^{-1}$). By 4 to 6 weeks after seeding however, numbers of settled abalone recovered during sampling surveys were lower and size ranges wider, making separation of seeded and natural settlement modes difficult. Marking of larvae prior to seeding using a chemical marker (Hawkes *et al*, !997) or a genetic (allozyme) marker (Gaffney *et al*, 1996) may make later separation of seeded from natural post-larvae possible.

Use of larval settlement collectors at seeding sites to estimate seeding success may be warranted. Collectors are of uniform size, shape and structure, quick and easy to clean and sort, and lighter to handle compared with natural substrata. At both sites they gave results similar to natural substrata in terms of post-larval density changes over time (Tables 4-7). In 1995 they were used instead of natural substrate sampling and gave variable estimates of seeding success. At one site (Site 2, Table 8) post-settlement densities in collectors were much lower than at the other two sites, indicating a likely problem with the larval flow in the vicinity of the collectors, or failure of the larvae to settle at that site. At the greenlip sites (Table 9) collectors showed lower than anticipated levels of seeding success, and may indicate that the batch of larvae used to seed these sites was not ready to settle or was otherwise unfit to use. Age and degree of development of abalone larvae may have affected settlement or survival (Moss and Tong, 1992), while high

larval transport density may have reduced larval survival after release (Hahn, 1989). Settled post-larvae in collectors grew at about the same rate as those on natural substrate.

There are five abalone species found in the general area of the seeding experiments (Shepherd, 1973), and juvenile abalone found six months after seeding could not be reliably identified to species. Lower than expected densities of juvenile abalone (≥12 months old) were found at most seeded sites in annual surveys. It was anticipated that year classes would be clearly evident for the first few years after seeding, and that these cohorts would be statistically comparable with those at control sites. Clear modes were apparent in the length frequency histograms from the 1995 surveys of greenlip seeding sites, and assumptions on cohort size ranges could reasonably be made. Where clear modes were not immediately obvious, assumptions on mean annual growth rates based on data collected for these species in this area by Shepherd et al. (1992) were used to fit modes to the data. The growth rate estimates used are lower than those found by McShane (1991) for blacklip. The data in Fig. 3 show enhancement of abalone numbers by about 250% at that site at 1 year after seeding. Densities found at this and all sites were similar to those found by McShane (1991), and by Prince and Ford (1985) (using divers) and Rodda (unpublished data). Densities shown are for estimated area, with areas searched biased towards "more promising" habitat (boulders with crevices rather than smooth or part buried boulders), and manageable habitat (boulders less than 20kg). This may lead to higher estimates of density. Diver searching efficiency is likely to be considerably less than 100% (McShane, 1991, Shepherd & Godoy, 1989, Prince and Ford, 1985) leading to underestimation of abalone densities. Habitat too heavy or complex would not have been searched at all. Density of one year old blacklip abalone a year after seeding was approximately 200% that found at control sites (Figure 2 a,b), but after two years density of two year old abalone was only 120% that of control sites (Figure 1 c,d). No significant enhancement was found at combined greenlip sites one year after the 1995 seeding (Figure 4 a,b), nor two years after the 1994 seeding. Diver searching efficiency is likely to be higher, when looking for two year old abalone than when looking for one year old abalone, (McShane and Smith, 1988) but still low and variable (Prince and Ford, 1985). Small changes in observed juvenile density may reflect this diver searching efficiency variation rather than real density

differences. Finding small abalone < 50 mm shell length at any of the seeded or control sites – (all commercially productive areas) was difficult. The cryptic habit of these animals at this size and subsequent low estimates of density or survival are cause for some caution when considering the success or failure of seeding experiments. Castell *et al* (1996) found recovery of tagged and released cryptic *Trochus* to be about 50-60% after 2 -3 days, and recommended using a "sighting probability" factor to correct estimates of survival in seeding experiments.

Undertaking surveys 3 to 4 years after seeding, when juvenile abalone are less cryptic, instead of 1 and 2 years after seeding may yield more reliable results.

Shepherd (1986) found significant aggregation in larger *H. laevigata*, but discounted the idea that physical characteristics of crevices vary or influence dispersion. Of the mechanisms proposed to explain why marine invertebrates aggregate (Hutchinson, 1953 and Shepherd, 1986), it would seem that food sharing, group defence or spawning are unlikely, but that micro-habitat requirements may be. Indeed, divers undertaking the surveys suggested that they could predict with some confidence habitat concealing juvenile abalone. A narrow, tapering space between two flat or curved surfaces often with crustose coralline growth and low light, and exclusion of the main predators – crabs and wrasses, with minimum silt (indicating adequate water movement), and little or no sponge growth or other sessile fauna present was the type of habitat that the divers would predict concealed abalone. The presence of other abalone (*H. scalaris* frequently, as well as conspecifics up to 120 mm) were also commonly found with target abalone during these surveys. This supports the view that small abalone positively seek a particular micro-habitat. Given the natural habitat heterogeneity that occurs in boulder fields, then aggregation of juvenile abalone is a predicted consequence of that heterogeneity. If habitat preference influences dispersion and given that abalone have no homing behaviour nor are site attached (Shepherd and Goodoy, 1989) then it is likely that abalone will gradually disperse out of the study areas with time and that any enhancement by seeding is subsequently undetectable. Studies on dispersal and growth rates of these species at these sizes would assist interpretation of these results.

It was intended to use non-destructive survey methods during the first three years after seeding to enable repeated sampling of the sites. However it is likely that habitat disturbance during these surveys even with our non-destructive technique would have increased mortality slightly. Localised disturbance of boulders in seeded sites from our sampling was clearly evident twelve months later in some cases, and could have had some effect on juvenile abalone. Shepherd (pers. comm.) suggests that failure to replace searched boulders properly could make juvenile abalone more vulnerable to predatory wrasses and crabs. The degree to which site disturbance affects mortality rate is unknown.

The allozyme marker survey technique used by Gaffney *et al* (1996) in southern California showed that red abalone from a 1979 seeding exercise dominated samples of adult abalone collected in 1992. The technique also showed reduced genetic diversity in the enhanced abalone population and in similarly tested hatchery abalone populations. This loss of genetic diversity is potentially a major drawback of successful seeding projects, and guidelines for the best use of broodstock should be developed and implemented.

While this work has established that abalone stocks can be enhanced by larval seeding up to a year after seeding, enhancement of stocks beyond this time has not been detected possibly due to dispersion of seeded animals out of the study areas. The economic viability of larval seeding is likely to depend in large part on the price of competent larvae. As abalone larvae are not difficult to produce in culture, and as competition between suppliers increases, future prices for larvae will probably be less than that paid for larvae for these experiments.

A cost benefit analysis of larval seeding

Background

This analysis is based on estimates of all costs and returns and as such can show the break even point for economic viability of seeding according to ranges of estimates used for the various components of costs and returns,

particularly survival and growth estimates. The approach used in this analysis is to determine a profitability index as a ratio of costs:

$$profitability = \frac{(survival * weight * value) - costs}{costs}$$

For example a value of 10 for the profitability index would indicate a net return-to-costs ratio of 10:1.

Survival

Given the density-dependence of settlement and survival to 6 days of post-larvae and the density-dependent survival to one year of age, it is impossible to predict survival in general terms. The data showed that at a seeding density of 2000 m^{-2} when survival is highest, 1-6.5% (mean 2.5%) survived to 6 days, a mean of 0.25% to 36 days and a mean of 0.01% (s.e. 0.002-0.04%) to one year. For these purposes we shall assume that seeding is done in places where natural stocks have collapsed or no abalone are present, so that density-dependent effects can be ignored. Hence, an 'optimal' survival estimate from our data is about 0.03% to one year. The range of survival rates presented in this analysis comprises several orders of magnitude either side of this optimum value of 0.03% to one year, assuming thereafter a fixed M of 0.4 yr^{-1} for ages 1 to 3, and a fixed M of 0.2 yr^{-1} for ages 3 to 6 when seeded abalone are expected to enter the fishery. Hence the mean 'optimal' value of larval survival from seeding to age 6 years is 0.0074%

Weight

Weight of abalone entering the fishery at 145mm shell length is estimated at 140 g meat weight. This estimate is based on morphometric data for *H. laevigata* in South Australia (K. Rodda, unpublished data).

Value

The beach value of abalone meat is also presented as a range, from \$75 - \$125 kg^{-1}. Currently (January 1998), prices are approximately \$100 kg^{-1}.

Costs

Larval costs

Costs of producing larvae would generally be entirely absorbed by the hatchery, and incorporated into the price of the larvae. A price of $1000 per million competent-to-settle larvae for a batch of 10 million larvae was paid in the course of the project, and was still current at the time of writing (J. Morrison, SA Mariculture, pers. comm.). One hatchery manager considered that this price could increase if demand for larvae (from other abalone farms) was high. It is equally feasible that this price may fall. Costs of larvae include broodstock collection and conditioning, spawning, labour, hatchery capital costs and administrative overheads such as permits.

Seeding costs

The main seeding costs are labour and boat costs. Labour for one day's seeding was estimated at $400 per day for a casual diver, attendant and boat operator. Boat hire was estimated at $150 per day and fuel at $100 assuming a travelling range of approximately 100km. With site marking and monitoring, more than one day would normally be required to seed an area.

Profitability is relatively insensitive to costs, being more dependent on survival rates and abalone market values. Estimates for seeding costs are therefore only approximate.

Tables 12,13 show percent profitability (i.e. % return on costs) on minimum and maximum cost estimates. Minimum costs are our current estimates less 20% and maximum costs are our current estimates plus 20%. Profitability is shown as a matrix of survival rates versus abalone beach price. The matrix cell combining current beach price for abalone meat, and the 'optimal' survival to 6 years found in this study is highlighted. At current costs less 20% seeding is profitable at prices >$90/kg and at the current abalone price of $100/kg there would be a 22% return on costs(Table 12). At current costs plus 20% the break-even point is at an abalone price of about $125/kg and at current price of abalone there is a loss of 18.7%. But any improvement in survival would certainly be reflected in increased profitability.

In conclusion at current costs and prices larval seeding is unlikely to be profitable, but would become so at only slight improvement in survival or with a reduction in costs.

Table 12. Profitability matrix (% return on costs) for a range of prices of abalone ($75-$125/kg) and survival rates to 6 yrs for larval abalone. Seeding costs for this matrix are current cost estimates less 20%.

	Survival %		Costs=minimum					
Value($/kg)	0	7.43E-06	7.43E-05	0.000743	0.007425	0.07425	0.7425	7.425
75	-100.0	-99.9	-99.1	-90.8	-8.5	815.1	9050.5	91405.3
90	-100.0	-99.9	-98.9	-89.0	9.8	998.1	10880.6	109706.3
95	-100.0	-99.9	-98.8	-88.4	15.9	1059.1	11490.7	115806.7
100	-100.0	-99.9	-98.8	-87.8	22.0	1120.1	12100.7	121907.0
105	-100.0	-99.9	-98.7	-87.2	28.1	1181.1	12710.7	128007.4
110	-100.0	-99.9	-98.7	-86.6	34.2	1242.1	13320.8	134107.7
125	-100.0	-99.8	-98.5	-84.7	52.5	1425.1	15150.9	152408.8

Table 13. Profitability matrix (% return on costs) for a range of prices for abalone ($75-$125/kg) and survival rates to 6 yrs for larval abalone. Seeding costs for this matrix are current cost estimates plus 20%.

	Survival %		Costs=maximum					
Value($/kg)	0	7.43E-06	7.43E-05	0.000743	0.007425	0.07425	0.7425	7.425
75	-100.0	-99.9	-99.4	-93.9	-39.0	510.0	6000.4	60903.5
90	-100.0	-99.9	-99.3	-92.7	-26.8	632.0	7220.4	73104.2
95	-100.0	-99.9	-99.2	-92.3	-22.7	672.7	7627.1	77171.1
100	-100.0	-99.9	-99.2	-91.9	-18.7	713.4	8033.8	81238.0
105	-100.0	-99.9	-99.1	-91.5	-14.6	754.0	8440.5	85304.9
110	-100.0	-99.9	-99.1	-91.1	-10.5	794.7	8847.2	89371.8
125	-100.0	-99.9	-99.0	-89.8	1.7	916.7	10067.3	101572.5

Developing a code of practice for larval seeding

A larval seeding code of practice needs to ensure in any large scale seeding program that the genetic integrity of natural stocks is maintained, that diseased broodstock is not used and that the environmental integrity of abalone habitats is maintained. While there currently exists little information on the effect of abalone seeding, other species have been extensively studied, and similar "codes of practice" developed. This is particularly true of

Atlantic salmon (*Salmo salar* L.) which have been cultured for more than 100 years. Wild Atlantic salmon are found in local reproductively discrete populations (Verspoor, 1997) not unlike abalone (Shepherd *et al*, 1992). One study shows that more than 90% of Atlantic salmon in the Baltic Sea are of cultured origin (Hansen et al, 1997), while another showed that after altering gene frequencies in wild blowfly populations, the gene frequencies rapidly returned to normal after the experiment ceased (Foster *et al* 1985, reviewed by Benzie, 1996). For cultured fish of any species the displacement of wild stock fish by stock enhancement is to be avoided. A larval abalone seeding code of practice must address: interactions between cultured and wild abalone in terms of potential genetic impacts; impacts of diseases and parasites and ecological impacts. These aspects are reviewed for abalone seeding by Shepherd et al. (1999).

Genetic effects may be over stated. As selection pressures take over and unfavourable allelles are excluded, escaped/seeded hatchery stock may revert to wild types over several generations.

Genetic fingerprinting technologies such as analysis of minisatellite or microsatellite DNA are so powerful they can detect genetic differences that may be inconsequential, such as family differences. Where genetic differences are detected it may be prudent to assume that differences are significant and to treat stocks as different.

Use of hybrid abalone such as *H. laevigata* and *H. rubra* crosses may have some advantages in terms of identification and ownership of seeded stock and higher growth rate. However even though hybridisation does occur naturally at a low level the seeding of large numbers of hybrids may introduce novel genotypes and so adversely affect the natural gene pool.

Ownership of seeded stock when it does enter the fishery presents a difficult problem. Differentiation of seeded stock from wild stock is not possible (in situ at least) so all stock must be considered to be from natural recruitment, and therefore accessible to all fishers, including recreational and those that did not share the cost of seeding. Ownership of individual reefs by co-operatives as in Japan and Mexico is one means of assuring ownership of seeded stocks.

State Policies

South Australia

The report into Abalone farming at sea in South Australia by Benzie (1996) provides the basis on which South Australian policy for abalone stock enhancement and farming at sea are founded. The main issue identified by Benzie (1996) is the maintenance of high levels of genetic diversity in farmed stocks, as farmed abalone within 2 km of wild populations may impact on the genetic composition of that wild stock. To minimise the risks to wild stocks Benzie (1996) recommends that abalone progeny used for rearing at sea be derived from a sufficient number of parents, and that broodstock be obtained from local populations. Benzie (1996) suggests 25-30 broodstock per spawning to maintain 95% of the genetic diversity of a wild population, and warns against large scale stock translocation until more detailed information on gene flow and dispersal becomes available. In a discussion paper and draft policy statement (Ashman, 1997), Primary Industries SA (PISA) expressly prohibits the farming at sea of hybrid abalone and genetically modified stock. Genetic modification in this case includes genetic engineering as well as genetically selected stock. The same paper states that all abalone stock that are farmed in the sea must be obtained from a registered South Australian hatchery or grower. In this case the potential for "disease-free certification" exists.

Queensland

Queensland Department of Primary Industries (DPI) has published a fisheries management policy (Taylor-Moore and Retif, 1998) which requires fish stocking to be undertaken by Fish Stocking Groups (legally incorporated committees representative of the community and fishing sectors) operating under an approved DPI Fish Stocking Management Plan. It suggests that beneficiaries of any stocking contribute to the cost of that stocking. The policy also explicitly states that no translocation of marine organisms be allowed

outside of the natural range of the "species/subspecies". The policy recommends and suggests a hatchery code of practice to optimise genetic diversity, and suggests a rigorous risk assessment process. This policy is the only State policy to specifically address native title implications.

Victoria

The Victorian Aquaculture Strategy (Fisheries Victoria, 1997) does not address specific reseeding or farming at sea issues. It does however list as a "key task" towards achieving the goal of establishing a "supportive legislative, policy and administrative framework" the formation of an "independent Aquaculture Scientific Advisory Panel to advise on proposals for the use of non-indigenous species and risk management". The Strategy also states that industry must manage its own environmental impact, and lists as a task the development of "best practice environmental management guidelines" to achieve the stated goal of establishing "ecologically sustainable development". In a policy statement on native fish stocking in public waters, Fisheries Victoria will only consider stocking waters if they are within the known former natural range of the species under consideration. Fisheries Victoria also acknowledge that there may exist fish sub-species and races that should not be mixed with each other (Victorian Dept of Conservation and Environment, 1990). This policy currently relates to inland waters only. Victoria has recently granted leases of coastal bottom for abalone farming at sea but its policy for abalone farming at sea is not yet known.

Western Australia

To date Western Australia has no formal policy for either abalone stock enhancement or abalone farming at sea. However, a discussion paper and draft policy guidelines (Westaway and Norriss, 1997) have been produced and circulated within industry and recreational fishing bodies as well as in the broader community. The discussion paper raises the question of access to seeded stock and suggests that the only benefit to fishers investing in

seeding may be an improvement in catch per unit effort. It also identifies two disease and parasite introduction issues; the introduction of non-endemic, pathogenic organisms into an area by the translocation of abalone from other areas, and transfer of such organisms from the culture facility to wild populations or vice-versa. 'Genetic integrity' effects are also addressed and the Draft Policy Guidelines suggest that a minimum of 25 broodstock be used per batch of larvae produced, and that larvae for use in marine-based systems must be produced from broodstock from the same "genetic zone" as the location they are destined for. Seeding onto reefs where abalone already occur will not be considered in Western Australia.

At the time of writing New South Wales has no formal policy for either abalone stock enhancement or abalone farming at sea.

No response was received from Tasmania although it is known that limited farming abalone at sea occurs on Flinders Island, Tasmania.

National Translocation Policy

In a draft national translocation policy discussion paper (Gilliland, 1998) seven main risk factors are discussed. These are:

1. Genetic shift in wild populations
2. Establishment of feral populations
3. Environmental impacts from the release of the species
4. Translocation of disease
5. Translocation of parasites and other species associated with the target species
6. Translocation of undesirable species in the transport medium
7. Introduction of chemicals, foods and drugs to the native environment

Most of these issues have already been covered. However, the issue of accidental translocation of undesirable species in the transport medium may be particularly pertinent to any larval seeding code of practice as identification

of potentially harmful algal cells or the larvae of other invertebrates in the abalone larval suspension is difficult. No useful risk minimisation strategy (for larval seeding) is suggested in the draft policy document. The issue of introduction of chemicals - particularly broad scale antibiotics to the native environment may also be pertinent as many abalone hatcheries practise prophylactic antibiotic treatment when rearing larvae.

Summary

Several key issues arise in most of the draft policies and other literature reviewed.

- Each State requires a permit for seeding to be undertaken.
- As transportation of broodstock and larvae over some distance is not difficult, broodstock should always be obtained from the immediate area to be seeded.
- Adequate broodstock (at least 25) should always be spawned, and gametes of each parent mixed with those of as many others as possible.
- Filtration or disinfection of water to remove unwanted organisms should be routinely practised prior to the introduction of abalone larvae.
- Use of antibiotics or other chemicals that may impact on the environment should be avoided during the rearing of larvae for seeding purposes.
- Monitoring of seeded areas should be undertaken to identify any negative impacts. The use of genetic markers may provide a promising method for identifying introduced seed abalone.

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Append Preece et al (1997) *here*

Benefits

Due to the difficulties of assigning ownership of seeded abalone, any benefit derived from a larval abalone seeding exercise would be shared by all individuals with access to the abalone fishery. As commercial abalone fishers in South Australia have a total allowable quota system of management the only benefit may be in an increase in catch per unit effort. It may also be possible to rehabilitate stocks which have become depleted through overfishing. In such stocks natural recruitment levels may be inadequate to rebuild the population in what may have been a highly productive, easily accessible fishing ground for both commercial and recreational fishers. It is not easy to quantify this benefit.

Further Developments

Annual monitoring of the sites seeded in this study should be continued. Treatment and control areas should be surveyed and length frequency plots constructed and compared. As cryptic juveniles mature and emerge into more accessible habitat better estimates of survival and recruitment to the fishery will be possible. The genetic analysis of the type used by Gaffney *et* al (1996) may help establish the proportion of seeded abalone entering the fishery and the true success of this seeding exercise.

Intellectual Property

It was anticipated that some intellectual property matters may result from further development of the apparatus and techniques used to rapidly deliver large numbers of larval abalone to a diver on the seabed. As these apparati and techniques were not significantly improved upon from those employed in the pilot study, this intellectual property should be treated as being already in the public domain.

Conclusion

The objectives of this project were to assess various aspects of the feasibility of larval abalone seeding. During the course of the project the larval transport and field deployment techniques described were shown to be both simple and effective, with significant numbers of post-larvae recovered from most seeded sites at the first survey. Larvae were also successfully settled in laboratory aquaria after being transported to the field, pumped through the seed delivery system, collected and sealed in jars on the sea bed and transported back to the laboratory. The field deployment techniques described were an effective prototype and may benefit from further development. While initial post-seeding censuses at some sites showed significant enhancement of stocks over and above natural recruitment levels, no enhancement was detected more than a year after seeding. Suggested reasons explaining this disappointing result included higher density dependent mortality at seeded sites, migration of juvenile abalone out of the study areas and the insensitivity of our survey technique to detect any enhancement. In developing an outline for seeding ethics, relevant documents from several States were reviewed and issues of concern summarised. The main points were that any seeding should progress in an orderly manner, with a permit for seeding to be issued by a State authority, and that genetic integrity of wild stocks be preserved by obtaining sufficient numbers of broodstock from the immediate area to be seeded. It was also noted that pathogens, parasites and chemotheraputents of hatchery origin should not be released into the natural environment with seeded larvae. Monitoring of seeded areas should also be undertaken to identify any negative impacts. In determining the true costs and benefits of larval seeding, several matrices of costs and profitability were presented, with the outcome that in this study, larval seeding is of doubtful economic viability. However, any such analytical model will be very sensitive to survival rates for seeded larvae. The survival rates for seeded larvae found in this study were highly variable, thus precise benefit-cost analysis was not possible.

Staff

Mr Steven Clarke	Principal Investigator	20%
Dr John Keesing	Investigator	2%
Dr Scoresby Shepherd	Investigator	20%
Mr Peter Preece	Technical Officer, Port Lincoln	100%
Mr Brian Foureur	Technical Officer	5%
Ms Kate Rodda	Research Officer	5%
(Mr Ross Werner	Research Officer, Victoria	1%)