STOCK EVALUATION AND RECRUITMENT MEASUREMENT IN THE W.A. PEARL OYSTER FISHERY.

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NON-TECHNICAL SUMMARY

The Western Australian fishery for the pearl oyster (*Pinctada maxima*) provides the foundation for the production of most of Australia's cultured pearls, with a value for 1994 estimated at about $200 million. This project was aimed at improving the scientific basis for the management of the Western Australian pearl oyster stock by improving the understanding of the biology of pearl oysters, by improving the ability to properly interpret catch and effort statistics and by examining whether there was a practical means of forecasting recruitment to the fishery.

The biological studies focussed on the growth of pearl oysters in the Eighty Mile Beach area and extended studies commenced under a previous FRDC grant (88/93). This part of the study involved the tagging and release of pearl oysters over a five year period and resulted in 1853 single year at liberty recaptures, with the data gained providing the first scientific record of pearl oyster growth under natural conditions. Through data on spat settlement derived from the previous FRDC study, which allowed the age of small oysters to be determined, it was possible to use the growth data to develop a size-age relationship. This indicated that, for the Eighty Mile Beach area, pearl oysters would reach the minimum legal size of 120 mm in their third year of life. They would then be exposed to fishing mortality for three to four years before they grew to a size which was no longer useful for round pearl culture. The data also indicated that large pearl oysters (around 200 mm shell height) would be 15 to 20 years old.

Techniques were also examined to see if pearl oyster shells could be independently aged using the macroscopically-visible banding in pearl oyster shells or by using possible temperature-dependent incorporation of strontium into the calcium carbonate of the shell. This would provide a method of independently ageing pearl oysters similar to the way tree rings are used to age trees. The method involved putting either a chemical (elevated strontium level) or visible (fluorescent oxytetracycline) 'time-stamp' in the shell of the oyster and then leaving it to grow for a further one or two years. Both techniques of inserting a 'time stamp' into pearl oyster shells were successful, but the macroscopically-visible banding was not able to be properly imaged at the higher levels of magnification required to view the oxytetacycline mark. However, further work on the recaptured shells may produce a method to resolve this problem. In the case of the chemical mark, the strontium 'time-stamp' was generally clearly detectable, but no temperature-dependent variation in strontium levels was apparent.

The key parameter examined to aid in the interpretation of catch and effort statistics was catchability (or diver efficiency). Catch rate, the product of the catchability of a species and its abundance or density in the area of action of the fishing gear, is commonly used as an indicator of abundance or density of animals, particularly in quota fisheries where total catch cannot be used as an indicator of stock size. Having an estimate of catchability provides a means of

interpreting the relationship between catch rates and the abundance or density of the species being fished.

The technique used to estimate catchability (or diver efficiency) was the DeLury method. This technique involves repeatedly fishing the animals within a closed or defined area, causing the population to decline and, in parallel with the decline in the population, a decline in catch rate. If the catchability of the animals is high the population, and the catch rate, will decline quickly while, if catchability is low, the population and the catch rate will decline slowly. The rate of decline in the catch rate provides the estimate of catchability. The area to be fished was defined and the position of the vessel kept within the defined area by using precision electronic navigation methods. A standard commercial pearl fishing vessel and pearling industry divers were used so that the results were relevant to standard industry fishing methods.

The tests of the method were carried out on two different bottom types - "Garden" bottom and "Potato" bottom, as these represent the two main bottom types in the fishery. The experimental method appeared to work very well, and obvious impacts on the catch rate were evident with each bottom type by the time each area had been fished twice. The catchability over all culture-sized oysters on "Garden" bottom was estimated at 37% and for "Potato" bottom at 24%, although with only four complete sweeps of the fishing area the confidence limits of the estimates are fairly wide. There were no differences in catchability between large and small culture-sized oysters on the "Garden" bottom, but larger culture-sized shell appeared to be less catchable than smaller shell on "Potato" bottom. This apparent difference in catchability probably arises because larger oysters are more heavily encrusted with "potatoes" (ascidians) than smaller, younger shell and it are more difficult for divers to see.

The use of piggyback spat as a possible means of measuring recruitment was also examined. Piggyback spat are pearl oyster spat which are attached to larger pearl oysters, presumably as a result of having settled-out from the plankton onto the oyster at metamorphosis. Previous attempts to develop a method of recruitment monitoring for the pearl oyster fishery under FRDC 88/93 were focussed on the potential of spat collectors, but the results of that work suggested that spat collectors would not be a practical or efficient technique for monitoring recruitment to pearl oyster stocks. However, it was considered that piggyback spat may provide a practical alternative for recruitment measurement, on the basis that the rate at which piggyback spat were found on larger pearl oysters in a locality was likely to be proportional to the total number settling in the locality.

Analysis of the data found that variations in piggyback spat rates were significantly affected by a range of factors, but that the factors were often confounded. Patchiness in pearl oyster and piggyback spat abundances was a further factor which limited the usefulness of the data. The use of a random or structured sampling design would have minimised some of the problems encountered, but such sampling regimes were not possible when using commercial vessels as platforms. However, the use of commercial pearling vessels is intrinsic to the method, as it is only through their use that realistic numbers of shell can be sampled. Consequently, the options for determining the crucial factors affecting piggyback spat rates appear limited. The outcome of the study was that the sampling of piggyback spat rates from commercial vessels does not appear to provide a useful broadscale index of recruitment, although it may be useful in the determination of local recruitment rates.

In parallel with the main programme, but capitalising on the field presence provided by that programme, a small sub-programme was also carried out to examine the possibility of translocating one of the smaller pearl oyster species from Shark Bay to Broome to provide an alternative culture species with possible potential for training Australian technicians. The Australian pearl industry is currently highly dependent on Japanese-trained technicians, who initially develop their skills on the small Japanese akoya oyster (*Pinctada fucata*). The larger Australian *P. maxima* is more highly valued because of the large pearls it can produce and the Australian pearling industry is understandably reluctant to allow unskilled trainees to use these valuable oysters. However, the small Australian pearl oyster *P. albina* is highly abundant in Shark Bay and, although also present in the Broome area, it is at a much lower abundance. Translocation of *P. albina* to Broome could provide a ready source of low-cost, low-value oysters for use in the training of pearl oyster technicians.

However, because of the genetic and disease issues involved in translocating animals from one area to another, the thrust of this sub-programme was to identify whether there were genetic or disease risks involved in any possible translocation. The examination of the disease risks sought to determine whether there were any diseases of *P. albina* in Shark Bay which did not also occur in *P. albina* or *P. maxima* in the Broome area. The outcome was that no serious pathogens were detected in *P. albina* from either locality, and that the other organisms associated with *P.albina* in both localities were common to a range of tropical bivalves. The genetic studies were based on samples taken from populations of *P. albina* at several points across northern Australia and provided a broad overview of genetic variability over this part of the species' range. The outcomes of the genetic studies indicated only small genetic differences between *P. albina* from Shark Bay and Broome, but even smaller differences between *P. albina* from Shark Bay and Townsville, while populations from the Coburg Peninsula in the Northern Territory were genetically divergent from the other three sites. Thus, although the genetic differences between Broome and Shark Bay were small, the even smaller differences between Shark Bay and Townsville, with an intervening population which is genetically different, point to the limitations of genetic data. Where there are genetic differences it can be concluded that the populations are isolated from each other but, where the genetic differences are only small, it cannot necessarily be concluded that the populations are genetically connected.

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BACKGROUND

The fishery for the pearl oyster *Pinctada maxima* provides the foundation for the cultured pearl industry - Australia's most valuable aquaculture industry, with a value for 1994 estimated at about $200 million. However, the quota management regime for the fishery is not based on a formal analysis of the stock or a comprehensive understanding of the biology of pearl oysters. This project was aimed at improving the scientific basis for the management of the W.A. pearl oyster stock by improving the understanding of the biology of pearl oysters, by improving the ability to properly interpret catch and effort statistics and by examining whether there was a practical means of forecasting recruitment to the fishery. A previous FRDC grant (88/93) provided the basis for some initial studies of the stocks and biology of *P. maxima* and the current project (FRDC 92/147) extended some of the studies commenced under FRDC 88/93 as well as initiating some new studies.

The project was composed of two sub-programmes. Sub-programme A was directed at stock evaluation and recruitment measurement in the W.A. pearl oyster fishery. This sub-programme embodied two follow-on studies from the earlier FRDC project (88/93), as well as a new segment on the measurement of pearl diver efficiency (catchability of pearl oysters). Sub-programme B was directed at an evaluation of translocated *Pinctada albina* as an alternative culture species, with particular regard to its potential for use as a training species for pearl oyster technicians. Despite having quite different aims, the two sub-programmes were teamed together to capitalise on the benefits of field time available under sub-programme A.

NEED

The need for the two sub-programmes, as set out in the original application, was:

Sub-programme A

i. The ability to properly interpret pearl oyster catch and effort statistics to allow for the rational management of the pearl oyster stock.

ii. The understanding of recruitment processes in pearl oyster populations.

Sub-programme B

i. The development of an alternative culture species with potential for the training of Australian technicians.

OBJECTIVES

The objectives of the two sub-programmes, as set out in the original application (with an assessment of whether they were achieved), were:

Sub-programme A

1. To develop techniques for the assessment of pearl diver efficiency and selectivity in taking pearl oysters. (Achieved)

2. To consider the impact of fishing on the reproductive potential of wild stock pearl oysters. (Partially achieved through the development of mean density measurements during the pearl diver efficiency work)

3. To develop a database on recruitment to commercially fished grounds. (Achieved)

4. To examine geographic variability in pearl oyster growth rates and age at recruitment to the fishery. (Partially achieved. Concentrated on the completion of the growth/ageing data for Eighty Mile Beach)

Sub-programme B

1. To examine the degree of genetic difference between populations of *Pinctada albina*. (Achieved)

2. To examine the disease status of Broome and Shark Bay stocks of *P. albina*. (Achieved)

METHODS

Sub-programme A

1. Evaluation of pearl diver efficiency and selectivity; Estimation of pearl oyster density

The method tested was based on a technique developed previously for the measurement of catchability of saucer scallops (Joll and Penn, 1990). The technique uses precision navigation equipment to electronically mark out an area of sea bed to be fished. This area is then repeatedly fished and the catch retained or returned to the sea away from the fishing area. As the accumulated catch taken increases, the catch rate declines as the remaining stock in the area is reduced. The rate of decline of the catch rate is a function of the catchability of the animals (or the gear efficiency) (Ricker, 1975). The more catchable the animals the more rapidly the catch rate declines, as the stock in the area is removed more quickly. Provided that catchability does not change as the abundance decreases, and that all animals are catchable, then the catch rate will be zero when all the stock is taken. In practice it is not feasible to fish the stock to extinction within the fishing area, but extrapolation of the fitted regression of catch rate on accumulated catch to its intersection with the X-axis (zero catch rate) gives an estimate of the total abundance in the fished area. The slope of the fitted regression is the estimate of catchability or gear efficiency.

Application of the method to the measurement of pearl diver (gear) efficiency in the pearling industry required only a minor adaptation of the method developed for scallops. The major difference is that, unlike trawl nets used for the capture of saucer scallops, the gear used to take pearl oysters involves direct human activity by divers. In order to avoid changes in the efficiency of divers as the available catch of pearl oysters declined, the pay rate of divers was varied from the usual commercial practice of a fixed amount per shell for each shell caught by a diver. Instead, a fixed total amount for the day was set for the whole diving team, with the total payment being shared between divers on the basis of the percentage of the total catch taken by each diver. Thus, the incentive to catch shell remained constant over the period of fishing.

Two trials of the technique were undertaken to test the efficiency of divers on two different bottom types. Both trials were carried out using the pearling vessel "Dalumba" (Paspaley Pearling Company) and a team of six experienced pearl divers, with four divers of the six being common in the two trials. Navigational equipment was supplied and operated by the Coastal Information and Engineering Services section of the Western Australian Department of Transport. The first trial was undertaken in November 1993 on "Light Garden" bottom in La Grange Bay (Fig. 1a) and the second was conducted in July 1994 on "Light Potato"

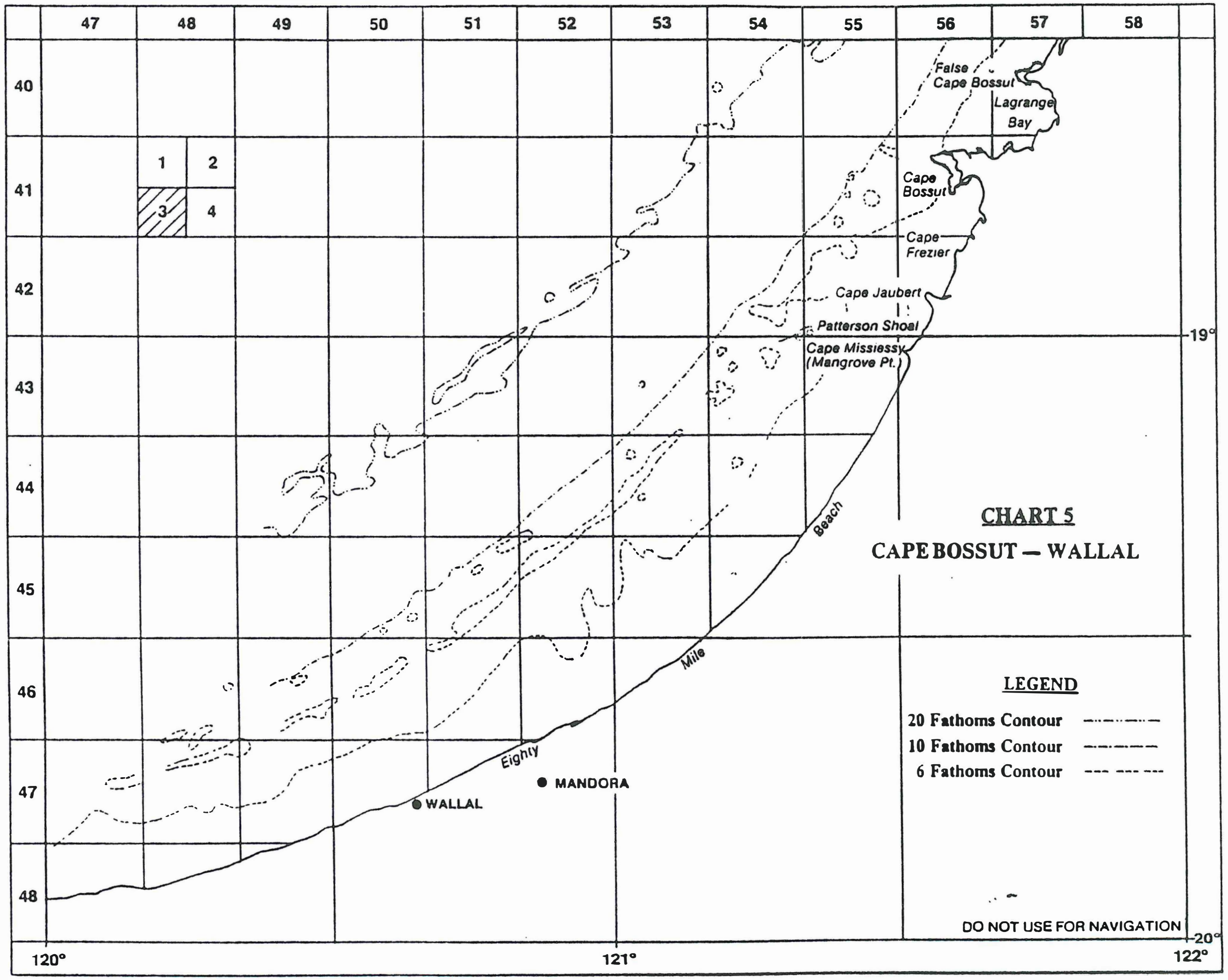
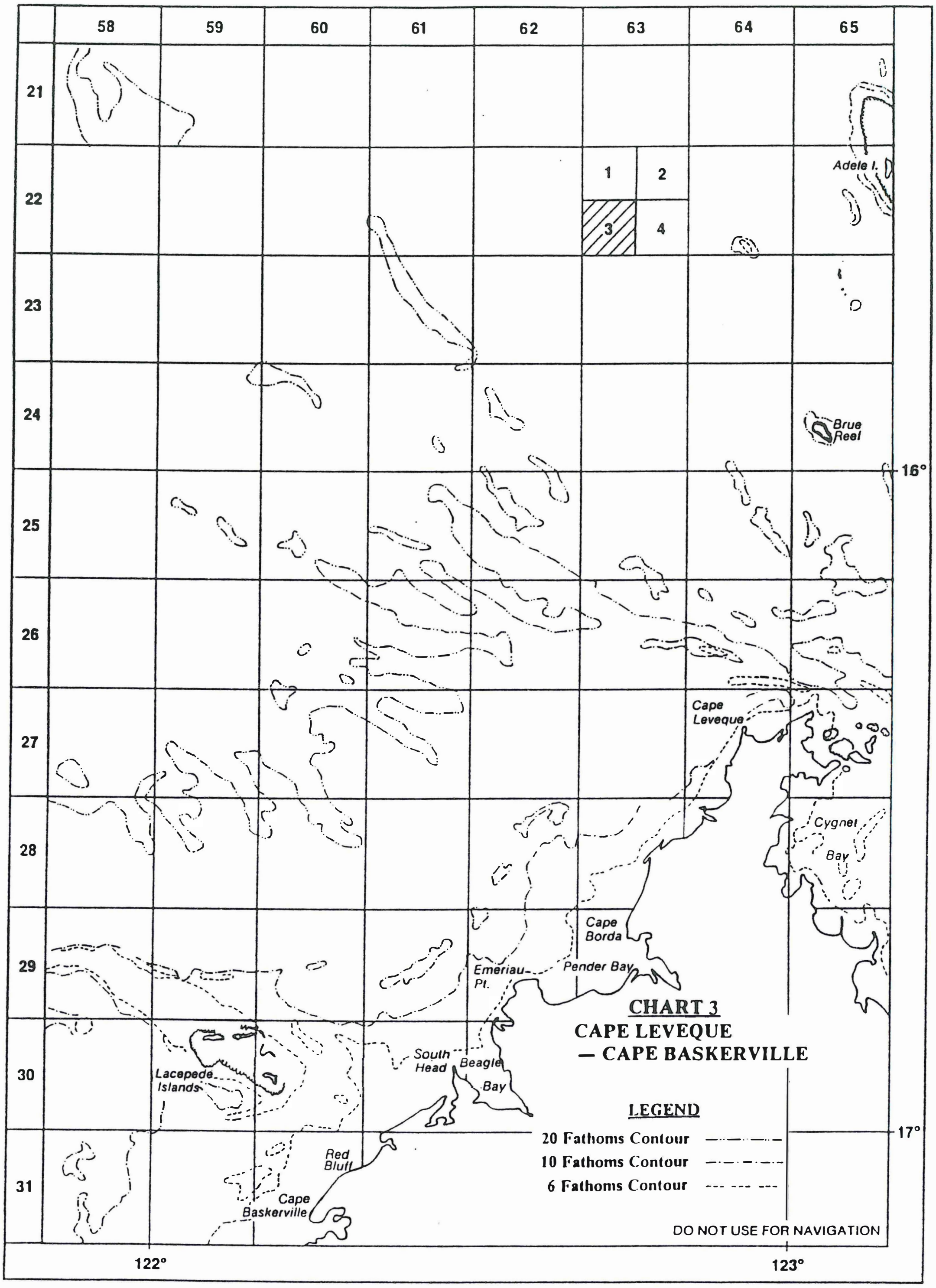


Fig. 1a Map sheet from the Pearl Oyster Fishing Daily Log book showing coastal features referred to and grid squares used to record fishing locations. Cape Bossut to Wallal.

Fig. 1b Map sheet from the Pearl Oyster Fishing Daily Log book showing coastal features referred to and grid squares used to record fishing locations. Cape Leveque to Cape Baskerville.



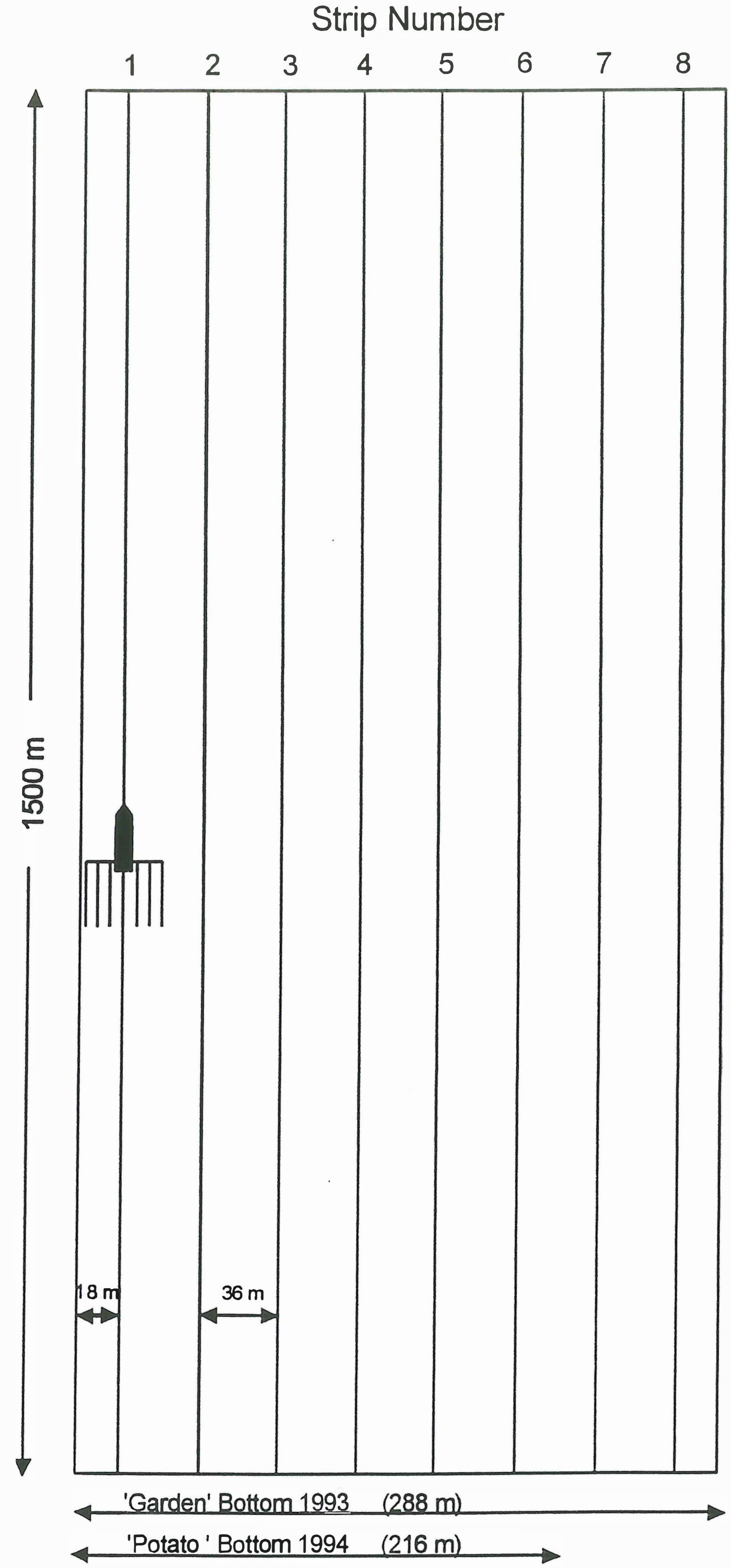
bottom in an area of the Eighty Mile Beach approximately 27 miles south west of Cape Missiessy (Mangrove Point) (Fig. 1b). These two areas were selected as they contained the two common bottom types which industry believed were likely to have very different levels of diver efficiency. "Garden" bottom refers to a community of sedentary benthic organisms such as hard and soft corals, sponges and bryozoans which, according to the density of these organisms, is graded as light, medium or heavy. "Light Garden" is generally considered to be the easiest to fish because oysters are usually only lightly encrusted by other sedentary organisms and are fairly visible on the sea bed between the other sedentary organisms. "Potato" bottom refers to a community of ascidians (which resemble potatoes) and is similarly sub-divided into light, medium and heavy according to the abundance and density of ascidians. Pearl oysters on this bottom type are usually located in amongst clumps of ascidians and are sometimes heavily encrusted by them, making them quite difficult to see.

The navigational equipment used in each of the trials was quite different, although the final output of navigational information was basically the same. The first trial used line-of-sight microwave position fixing equipment similar to that described by Joll and Penn (1990). This technique uses three microwave transmitters at fixed, surveyed-in locations on the shore around the fishing area and allows the position of the vessel to be determined with an accuracy of about 1m. The position of the fishing vessel is calculated at 1 second intervals by an on-board receiver-computer, by triangulation from the known positions of the transmitters. Information on vessel speed and position within the marked out fishing area was displayed in the wheelhouse at two second intervals, allowing course and speed adjustments to be made, and the vessel's position was written to disk every 10 seconds.

The second experiment used differential GPS equipment for position fixing, as the straight coastline in the area south of Mangrove Point was not suitable for the placement of microwave beacons in positions which would allow accurate triangulation of vessel position. The differential GPS technique used a single shore-based GPS receiver/transmitter stationed at a surveyed-in reference site and an on-board GPS receiver which received data from GPS satellites as well as from the shore-based reference station. The shore-based reference station is able to determine correction factors for the GPS satellite data because it is at a known location. This correction data is then transmitted to the on-board GPS unit, allowing it to make corrections to the GPS data which it receives, and produce a position fix with an accuracy of about 1m. As with the previous method, information on vessel speed and position within the marked out fishing area was displayed in the wheelhouse, allowing course and speed adjustments to be made and the vessel's position was written to disk at 10 second intervals.

Each of the fishing areas was marked out in fishing 'strips' which were the width of the fishing path of the vessel (Fig. 2). This was calculated on the measured width from boom-tip to boom-tip of the vessel of 30 m plus an allowance of 3 m on each side for the outside diver to

Fig. 2 Schematic diagrams of the fishing 'strips' used in the DeLury experiment to estimate catchability/pearl diver efficiency.



swim beyond the line of the boom-tip, giving an overall path width of 36 m. Total length of the strips was set at 1500 m, which approximates the length and duration (35 - 40 minutes) of a standard commercial dive. The first trial ("Garden" bottom) used a fishing area eight strips wide while, because of time limitations, the second trial ("Potato" bottom) used an area six strips wide. The fishing areas were laid-out along the axis of flow of the tidal stream (approximately north-west/south-east), with the pearling vessel fishing ('drifting') in its normal pattern of moving with the tidal stream. Strips were all fished in the same direction while the tide was flowing in that direction, with the direction of the strips being reversed when the tide changed. The timing of fishing particular strips was varied so that most strips were fished alternately in a 'tide-in' and then a 'tide-out' direction, as this allowed oysters on the bottom to be seen by divers from different directions on alternate drifts. Each fishing area was completely covered four times (i.e. 32 strips in the first trial and 24 strips in the second trial).

Divers were instructed to catch only the same size range of oysters as caught in the commercial fishery for round pearl culture shell, so that the catchability/efficiency measure was compatible with commercial fishery operations. In their normal catch, however, divers usually take some small shell (< 120 mm shell height (S.H.)) which is legally undersize and some larger (> 170 mm S.H.) shell which is unsuitable for round pearl production. Final acceptance of product and the discarding of under- and over-size shell is done by the skipper, who grades the landed catch. Oysters brought on-board by divers in these trials were also graded for size by the skipper using the normal commercial fishery methods and only acceptable grade product was retained. The size of each retained oyster was measured and the total catch and sizes of oysters graded as commercially acceptable was recorded. However, small pearl oysters have prominent growth processes ("fingers") around 5 - 8 mm long around the ventral margin ("lip") of the oyster and the legal size of oysters is usually measured by industry to the tip of these fingers. Because of their relatively fragile nature, however, these fingers are sometimes broken in the catching and on-board handling process and all measurements of shell height were therefore made to the base of the fingers. This resulted in the some culture shell being recorded at sizes less than 120 mm S.H., although they were legally acceptable sizes when measured to the tip of the fingers.

2. Recruitment monitoring

The rate of "piggyback" spat found on culture-sized pearl oysters was examined for its potential as a method of monitoring and measuring recruitment to the pearl oyster stock. Piggyback spat are pearl oyster spat which are attached to larger pearl oysters, presumably as a result of having settled-out from the plankton onto the oyster at metamorphosis (see Joll, 1994 - Fig. 12). Previous attempts to develop a method of recruitment monitoring for the pearl oyster fishery under FRDC 88/93 (Joll, 1994) were focussed on the potential of spat collectors, although several years of data collection on piggyback spat were also undertaken. The results of the spat collector work suggested that spat collectors would not be a practical or efficient technique for monitoring the recruitment to pearl oyster stocks, but that piggyback spat may provide a practical alternative. The method was based on the proposition that the rate at which piggyback spat were found on larger pearl oysters in a locality was likely to be proportional to the total number settling in the locality.

The piggyback spat method offered a number of possible advantages over artificial spat collectors. By using pearl oysters caught by pearl divers operating from commercial pearling vessels spat settlement could, potentially, be examined over a much wider area and at more localities than could reasonably be examined by spat collectors. Also, by using the shell catch of commercial divers, the source material for examination was obtained at minimal cost and in a quantity which could not reasonably have been provided by research diving.

To determine piggyback spat rates, research staff travelled aboard commercial pearling vessels for the duration of a neap (the standard fishing period of low tidal rise and fall) and examined all legal-sized oysters collected by divers on each dive. The examination was restricted to the divers normal catch of legal-sized oysters suitable for round pearl culture (culture shell) to avoid bias which may result from any tendency which divers may have to collect under-sized and large mother-of-pearl (over-sized) oysters which had piggyback spat attached. Pearl oysters collected by the divers were graded by the skipper into legal-size shell suitable for culture (greater than 120 mm S.H. and generally less than 170 mm S.H.). These oysters were then individually examined for the presence of piggyback spat before being passed to the shell cleaners, who remove the encrusting fouling organisms from the shell before it is placed in pearl oyster "panels" which are used to retain pearl oysters after capture.

The encrusting material removed from the oysters was then examined by placing it in graded sieves, with a final mesh size of 6 mm square (nominal). The encrusting material was washed thoroughly on the graded screens using the vessel's deck hose and examined closely for spat which may have escaped detection in the initial examination. Each piggyback spat shell found was measured (shell height) and the number of spat and the number of culture-sized pearl shell caught on each drift were recorded. Data on depth, time of day, date and vessel location for each drift were taken from the vessel's daily fishing log. Location was always provided at the level of the 10 x 10 n. mile grids (Fig. 1 a, b), although some skippers provided data at the level of sub-grids (5 x 5 n.mile) and a few skippers provided more detailed GPS position data.

Under the previous FRDC project (88/93), some preliminary data on "piggyback" spat were collected from the Eighty Mile Beach in 1990, with more extensive sampling conducted in 1991 and 1992. Sampling in the Lacepede Islands area, north-west of Broome, was also carried out under FRDC 88/93 in 1992. Sampling in these two areas was continued under the current project, providing five years of data for the Eighty Mile Beach area and four years for the area around the Lacepede Islands northwest of Broome.

Analysis at this stage was confined to an examination of the factors associated with variations in the number of piggyback spat per number of culture shells collected (spat rate) and possible trends in recruitment from year-to-year. More complete analysis of the relationship between recruitment (as measured by piggyback spat) and the abundance of pearl oysters in the fishery (as measured by catch rate) will need to wait until there is a full sequence of fishing years to match the recruitment data. Also, because the fishery catch rate results from the abundance of a standing stock of at least three year classes, data on the catch rate of the various size (age) classes will also be required.

The examination of the hypothesised factors which may be associated with variations in the spat rate was carried out using analysis of variance on the log-transformed spat rate ($\log_e$ (rate + 0.002)). The factors considered were year, month, grid and depth (2 m classes). However, because the depth recorded at the time of sampling included tidal effects, the depth data were adjusted to remove this effect. The depth data were transformed to depth at mean low water springs using tidal data provided by the Coastal Information and Engineering Services section of the Western Australian Department of Transport for the area and the time and date of each drift.

3. Growth and ageing

i. Growth of tagged shell in the Eighty Mile Beach area.

This section of the project built on tagging work commenced under the previous FRDC project (88/93) and extended the period at large for oysters tagged and released in that study, as well as adding to the group of tagged shell by further releases.

Pearl oysters were tagged using plastic (PVC and polyethylene) shellfish tags (Hallprint, South Australia), which were applied to the surfaces of the left and right valves using a cyano-acrylate glue (see Joll, 1994 - Fig. 4). For all but the smallest oysters a tag measuring approximately 15 x 7 mm was used, with a smaller tag (7 x 3 mm) being used for small (< 50 mm S.H.) oysters. Each pair of tags was printed with a 4 character individual identifier.

Oysters to be tagged were obtained from two main sources:

i. Rejected by-catch material from commercial pearling vessels, both undersize shell (<120 mm S.H.), including piggyback spat and other small shell, and oversize shell (> ≈170 mm S.H.).

ii. Shell caught by research diving in the area where tagged shell had been released. Shell from this source covered the full size range and included culture-sized shell.

Oysters caught on pearling vessels were held in aerated or flowing seawater in small tanks on-board the commercial vessels and then moved to flowing seawater tanks on the Fisheries Department's research vessel "Flinders", where all tagging was carried out. All research diving was conducted from "Flinders" and shell from this source was brought onto "Flinders" deck and placed directly in the flowing seawater tanks.

After tagging each shell was measured using the five different measures described by Hynd (1955) (Dorsoventral measurement, Anteroposterior measurement, Hinge line, Heel depth and Thickness). Dorso-ventral measurement (= shell height) was to the base of the "fingers", as for shell measured in the pearl diver efficiency study. Oysters were held in the deck tanks for 2 - 12 hours after tagging, before being released on an area of "light garden" seabed approximately 1000 m long x 150 m wide, in about 15 m depth approximately 8 miles south of Cape Missiessy. This area was marked by GPS co-ordinates and industry agreed not to fish this area, so that recapture of tagged shell could be made at pre-determined intervals. Very small oysters (< 60 mm) were placed in a localised area around an underwater marker to increase the chances of their recapture. Recaptures were made using standard pearl diving techniques, with all releases and recaptures being conducted during a single neap in June in each year. Recaptured oysters were re-measured using the five measures noted above and then re-released in the same area. Any new pearl oysters taken during the recapture were tagged, measured and released along with the recaptured oysters.

Some oysters were caught one year after release, while others were caught 2, 3 and 4 years after initial release. Also, some oysters were caught several times, providing sequential growth data on individuals. In the analysis presented here, only single year at liberty recapture data were used, but planned analyses of the data will make use of the multi-year at liberty data and the sequential nature of some measurements where oysters were recaptured over several years Data for single year periods at liberty were fitted to a von Bertalanffy growth equation, using an estimate for T_o of 5 months at 30 mm S.H. This estimate was derived from the sizes of spat captured in the spat collector trials conducted under FRDC 88/93 (Joll 1994).

ii. Ageing

Several methods were tested for their use in independent ageing of pearl oyster shells. The aim of these methods was to put a 'time-stamp' into pearl oyster shells and then release them back into the natural environment for one to two years. The shell growth in the one and two year periods after the insertion of the 'time-stamp' would then be clearly defined as the shell between the 'time stamp' and the inner margin of the nacre. This would provide a part of the shell with a known time calibration, which could be used to calibrate shell features which may have a time cycle. The particular features for which calibration was sought were possible variations in the chemical composition of the shell, and in particular the strontium-calcium ratio, and the frequency of banding visible at the macroscopic level (Sagara and Takemura, 1960) in pearl oyster shells cut dorso-ventrally.

a). Marking of pearl oyster shells with chemical markers.

The calcium carbonate of which pearl oyster shells is composed consists of two crystal forms - calcite (as prismatic crystals) and aragonite (as flat crystals of nacre). Incorporated within the structure of the crystal lattices are various other trace metals such as strontium, iron, zinc and aluminium. Other workers (e.g. Smith *et al.* 1979, Kalish 1989, Radke and Shafer 1992) have suggested that the incorporation of strontium into the crystal lattice of aragonite produced by various aquatic organisms is primarily a function of temperature, and that the fluctuations in strontium-calcium ratios can be used to interpret the thermal history of the animal. This section aimed to determine whether a 'time-stamp' could be inserted into pearl oyster shells by exposure to elevated strontium levels, so that the variation in natural strontium-calcium ratios over a defined 1 or 2 year period could be examined to determine whether natural strontium-calcium ratios had a cycle which reflected the natural temperature cycle. As the natural temperature cycle is an annual cycle, determination of the number of cycles in the strontium-calcium ratio would provide an independent estimate of age.

Live pearl oysters collected as part of the tag and release study of growth were treated by being soaked in a solution of strontium chloride-dosed seawater. The strontium chloride was solution was prepared by making a 1 molar solution in freshwater and adding this to the seawater to make a final solution strength in seawater of about 0.01 molar. Pearl oysters were then kept in an aerated tank of this solution (volume approximately 40 l) for approximately 18 hours. Twenty nine of the strontium chloride-soaked pearl oysters were then tagged using the normal plastic shellfish tags and released into a localised area on the bottom around an underwater marker.

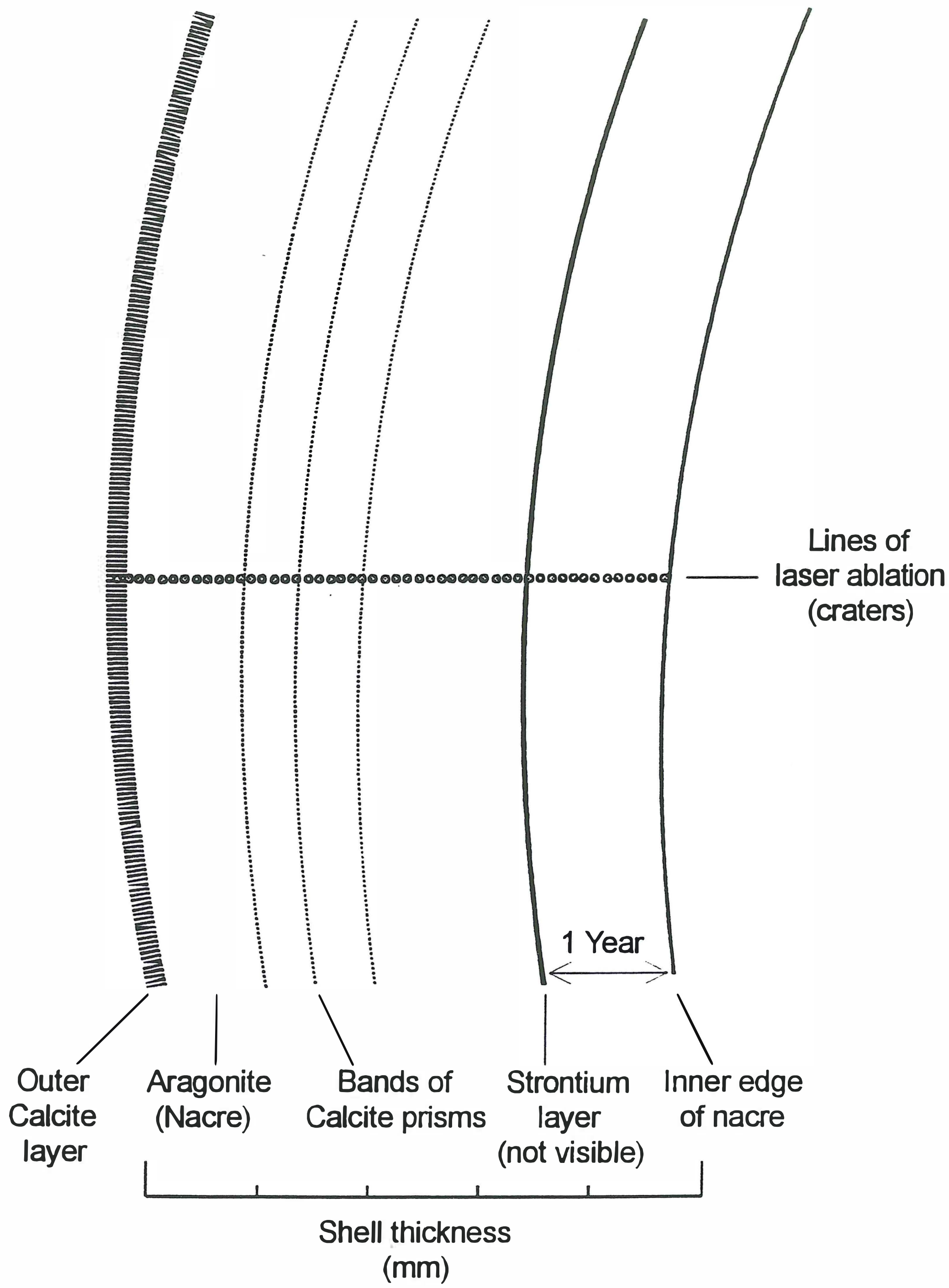
Oysters were then recaptured at one and two year intervals after release. The oysters were killed and the valves sectioned along the dorso-ventral axis using a diamond saw. The cut surface of the shell was then scanned using laser ablation inductively coupled plasma mass spectrometry (ICPMS). These analyses were conducted by Dr John Edmonds (W.A. Fisheries Department, Fisheries Research Division) while on study leave at the National Institute for Environmental Studies in Tsukuba, Japan. The ICPMS analyser was tuned to record the relative abundance of particular isotopes, in this case ^{88}Sr and , because of the high levels of calcium in the material analysed, the less common isotope of calcium ^{42}Ca. A less common isotope of calcium was used as the calcium reference to avoid "swamping" the detector. This technique provides data on the relative abundance of these elements (or at least these isotopes of the two elements) within the area of impact of the laser beam (approximately 30 μm diameter), giving a profile of the different elements across the thickness of the shell at approximately 100 μm intervals (Fig. 3). The data for the strontium-calcium ratio was then plotted by position across the shell thickness, with the distance between the point of elevated strontium and the inner edge of the nacre being a period of one or two years, depending on the period of release.

b). Marking of pearl oysters shells with visual markers.

The two types of visual markers tested were the fluorescent chemicals Alizarin Red and Oxytetracycline. Alizarin Red has previously been used to put markers into the calcium carbonate structures of corals, fish and molluscs (e.g. Stearn *et al.* 1977, Blom *et al.* 1993, Sire 1984), and Oxytetracycline has been used to put fluorescent markers in the otoliths and hard structures of fish, molluscs and sea urchins (e.g. McFarlane and Beamish 1987, Pirker and Schiel 1993, Dawe *et al.* 1985, Gage 1992). Initially both stains were tested during June 1992 for direct uptake by live oysters, by placing oysters in aerated tanks (volume approximately 40 l) containing solutions of one or other of the two stains (Alizarin Red approximately 1 g per 40 l, Oxytetracycline approximately 250 mg per litre). The shell were then released on the bottom in a localised area around an underwater marker for subsequent recapture. Trial recaptures four months later showed that exposure to these stains by placing live animals into solutions did not result in successful staining of the shell.

Preliminary trials were then commenced with injecting oxytetracycline. Trials carried out in April 1993, with shell released for 1 month, demonstrated that oxytetracyline (10 mg/ml filtered seawater) injected into the adductor muscle at approximately 0.1 ml per 10 gram of estimated wet tissue weight [based on a previously determined shell height-wet tissue relationship] (i.e. 0.1 mg tetracycline per g wet tissue weight) produced a readily visible band when the sawn face of the shell was viewed under ultraviolet light. A large scale mark and release was carried out in conjunction with the June 1993 tagging programme, with 298 oxytetracycline-marked shell being released into a localised area on the bottom around an underwater marker. These shells were then recaptured at one and two year intervals after marking.

Fig. 3 Schematic diagram of the methodology of ICPMS sampling across the sawn face of pearl oyster shell.



Sub-programme B

1. Genetic status of populations of *Pinctada albina*
(In association with M. Johnson, Dept. of Zoology, University of Western Australia)

Samples of *P. albina* for genetic analysis were examined from five areas: Shark Bay, Broome (Roebuck Bay), the Lacepede Channel (northwest of Broome), Coburg Peninsula area (N.T.) and Townsville (Qld). Sample sizes were 40 to 50 individuals from each area, except the Lacepede Channel, for which only 20 individuals were available. The samples from the Coburg Peninsula were provided by Ian Knuckey (N.T. Dept of Primary Industries and Fisheries) and the Townsville samples were provided by John Norton (Queensland Dept. of Primary Industry).

Enzymes were extracted from either the adductor muscle or digestive gland using the method described in Johnson and Joll (1993). A preliminary search for variation revealed eight enzymes that were sufficiently polymorphic to allow statistical comparison among samples. The various allozymes were separated by standard horizontal starch-gel electrophoresis. At each locus alleles were labelled according to the rate of migration of their respective allozyme relative to the most common allele, which was given a value of 100.

For each locus the departure of the frequency of heterozygotes from the expected frequency under Hardy-Weinberg equilibrium was calculated and the statistical significance determined as described by Johnson and Joll (1993). Variations in allelic frequencies between samples were quantified by F_{ST}, the standardised variance in allelic frequencies. F_{ST} was calculated for each locus as well as for the entire set of samples and for each pair of samples. Finally, to summarise the geographical patterns of variation across all eight loci, the pairwise values of F_{ST} were subject to nonmetric multidimensional scaling.

2. Disease Status of Shark Bay and Broome *Pinctada albina.*
(In association with M. Hine, Fish Pathology Section, W.A. Fisheries Department)

Specimens of *P. albina* were collected from Shark Bay, from waters around the Lacepede Islands and from spat collectors on a pearl farm in Roebuck Bay (Broome). One hundred and fifty specimens from Shark Bay and 171 specimens from the Lacepede Islands area were preserved in 10% formalin-seawater, while another 150 specimens from Shark Bay and Broome were incubated on thioglycolate medium for *Perkinsus* testing. The specimens were forwarded to the Fish Health Section of the W.A. Fisheries Department where the thioglycolate media were examined and the fixed material subjected to routine inspection and histological examination.

DETAILED RESULTS

Sub-programme A

1. Evaluation of pearl diver efficiency and selectivity; Estimation of pearl oyster density

As the vessel moves over the bottom the skipper steered the vessel to follow the centre line of the strip as closely as possible. However, there is inevitably some variation in the position of the vessel from the centre line, with the vessel steering a 'snake-like' course along the strip (Figs. 4, 5). In most cases the vessel was able to maintain station within a few metres of the centre line with the variations on one line largely being cancelled out by the variations on adjacent lines. Also, because the divers are towed behind the vessel on ropes, it is likely that they follow a track which is actually straighter than that achieved by the vessel. On a few drifts (e.g "Garden" bottom - La Grange, Sweep 1 [Fig. 4a]) the vessel was caught by the wind or tide changes during the drift and, at the speed at which the vessel is able to properly tow divers over the bottom (around 1.2 - 1.3 kts), it was not possible to correct the vessel's position. Overall, however, the positioning of the vessel was maintained within narrow limits (Tables 1, 2).

Table 1. Navigational results from the "Garden" bottom DeLury.
Data in the table show mean deviations (in m port or starboard) from the planned steering line. Value shown is the difference between the sum of the deviations to port and to starboard from the fixes recorded at 10 second intervals.

	Strip No.							
Sweep No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	2.4 P	8.5 P	17.2 P*	0.1 P	2.1 S	10.1 P	23.2 P*	69.6 P*
2	0.6 S	4.2 S	1.1 S	6.3 S	1.8 S	3.2 P	0.4 P	1.7 P
3	0.4 P	1.2 S	0.1 S	5.6 S	2.9 S	5.4 S	0.6 P	4.2 S
4	1.3 P	3.0 P	0.0 P	2.2 S	1.4 S	2.7 S	1.6 P	2.5 S

* Drifts where vessel pushed offline by wind or tide.

Fig. 4a Vessel tracks for the DeLury experiment on "Garden" bottom. Sweep 1

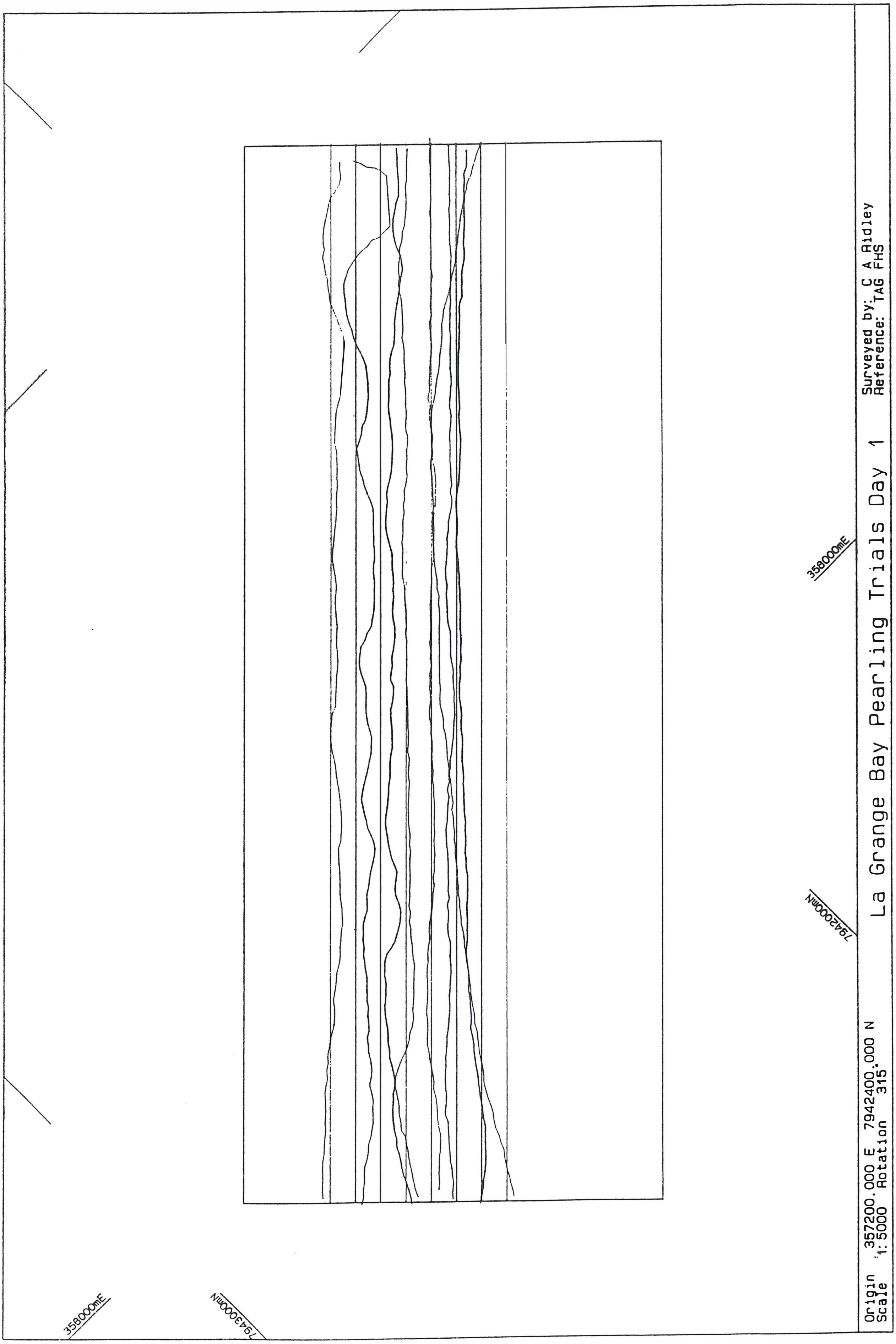


Fig. 4b Vessel tracks for the DeLury experiment on "Garden" bottom. Sweep 2

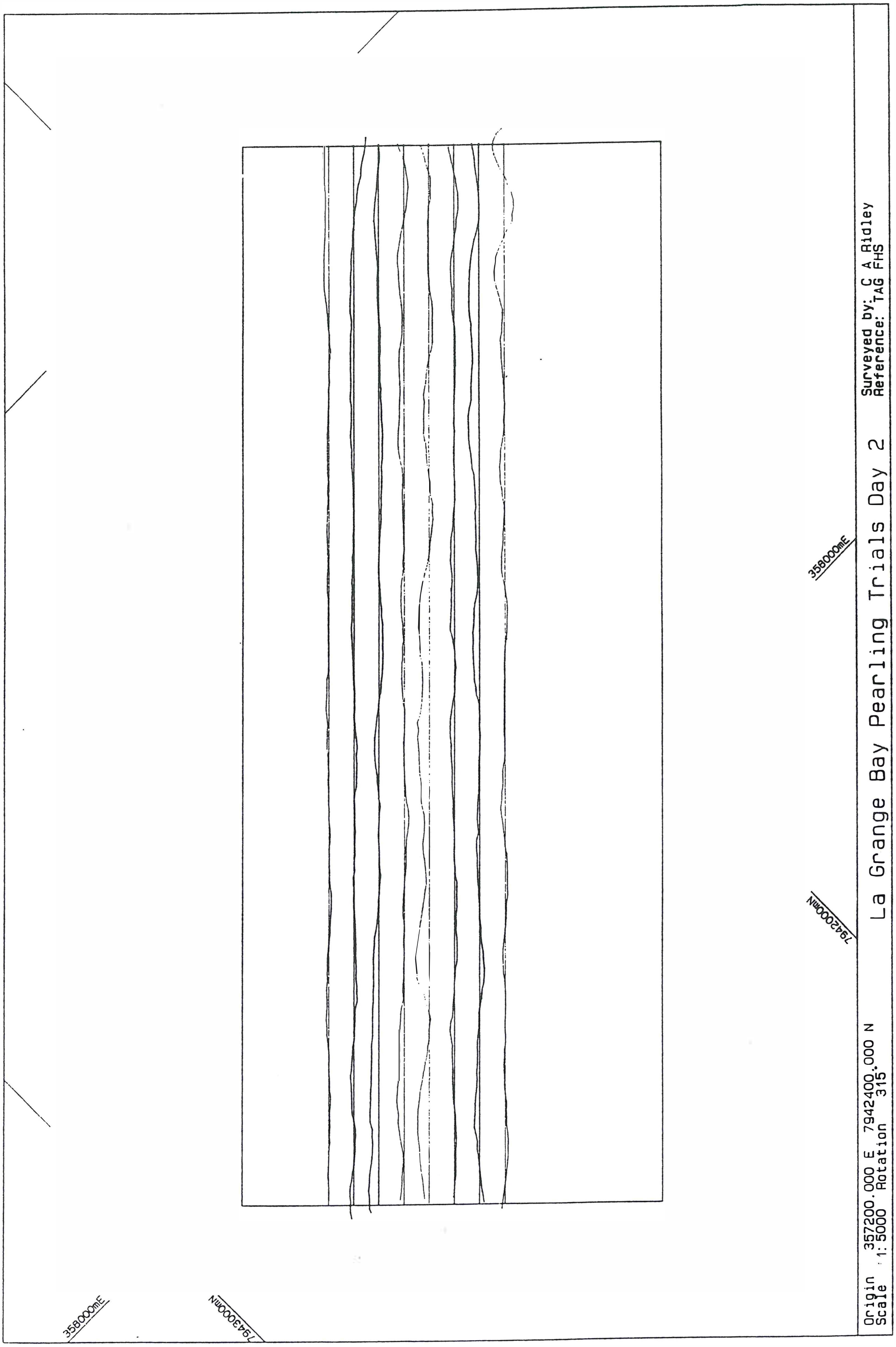


Fig. 4c Vessel tracks for the DeLury experiment on "Garden" bottom. Sweep 3

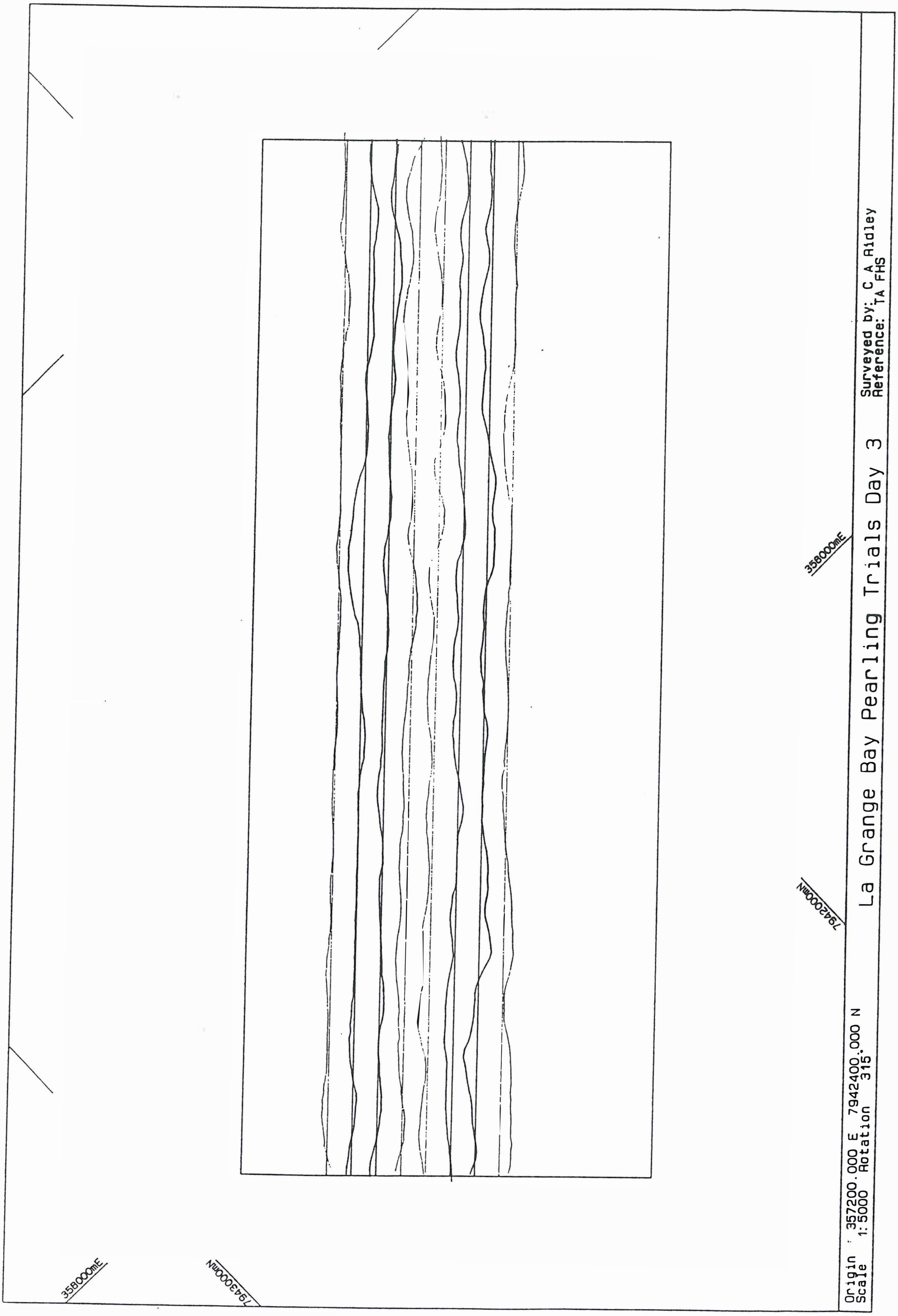


Fig. 4d Vessel tracks for the DeLury experiment on "Garden" bottom. Sweep 4

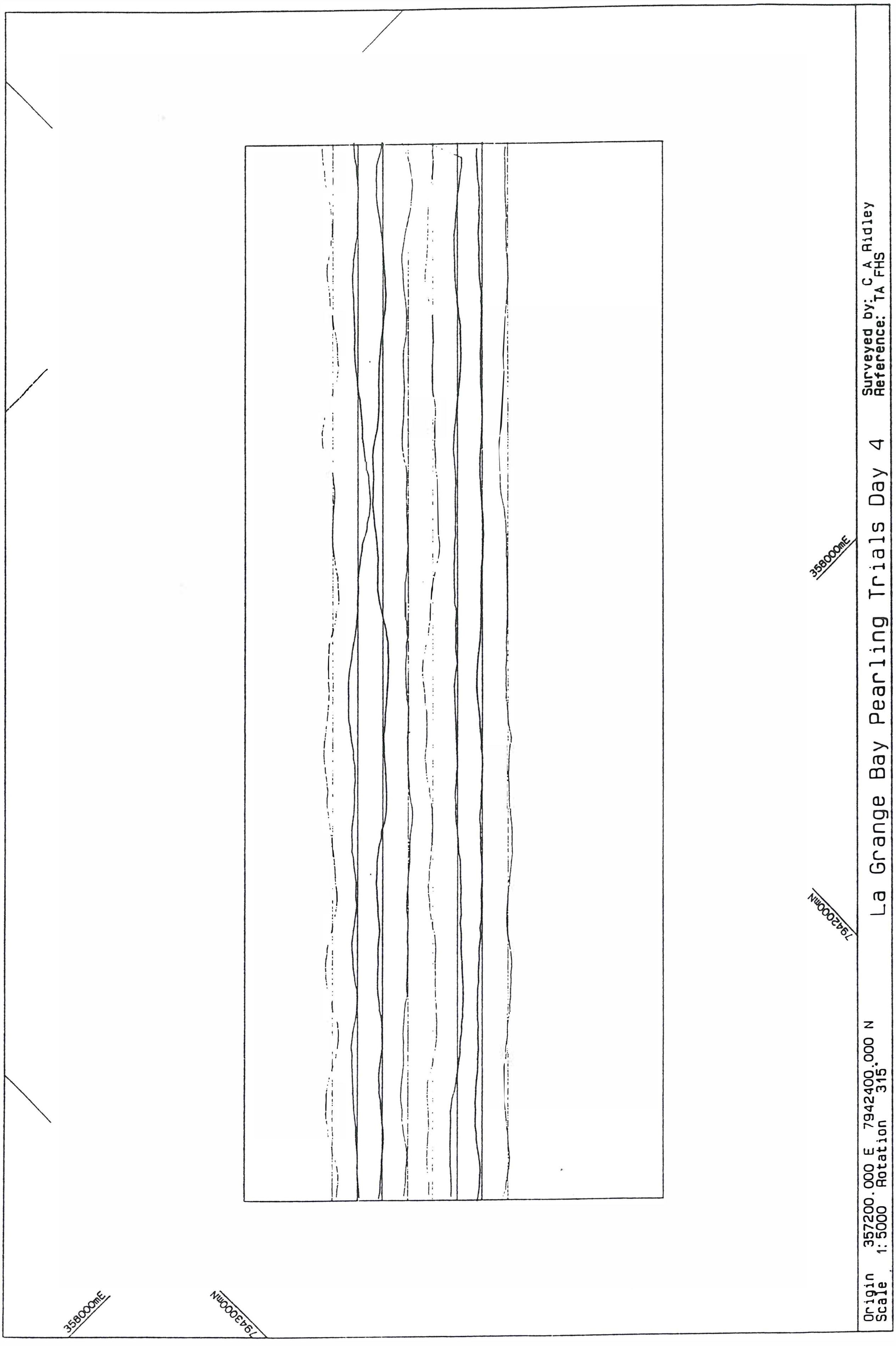


Fig. 5a Vessel tracks for the DeLury experiment on "Potato" bottom. Sweep 1

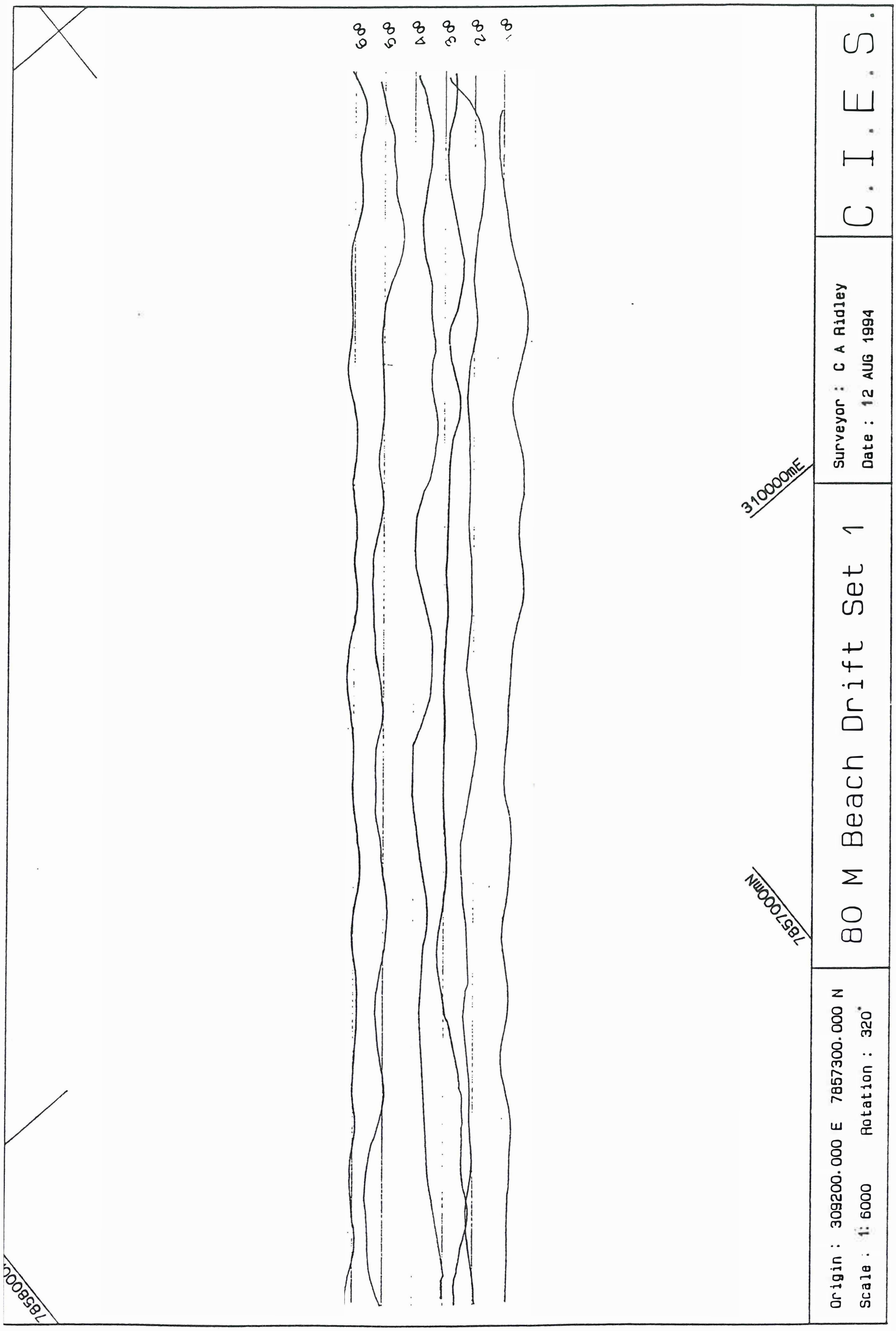


Fig. 5b Vessel tracks for the DeLury experiment on "Potato" bottom. Sweep 2

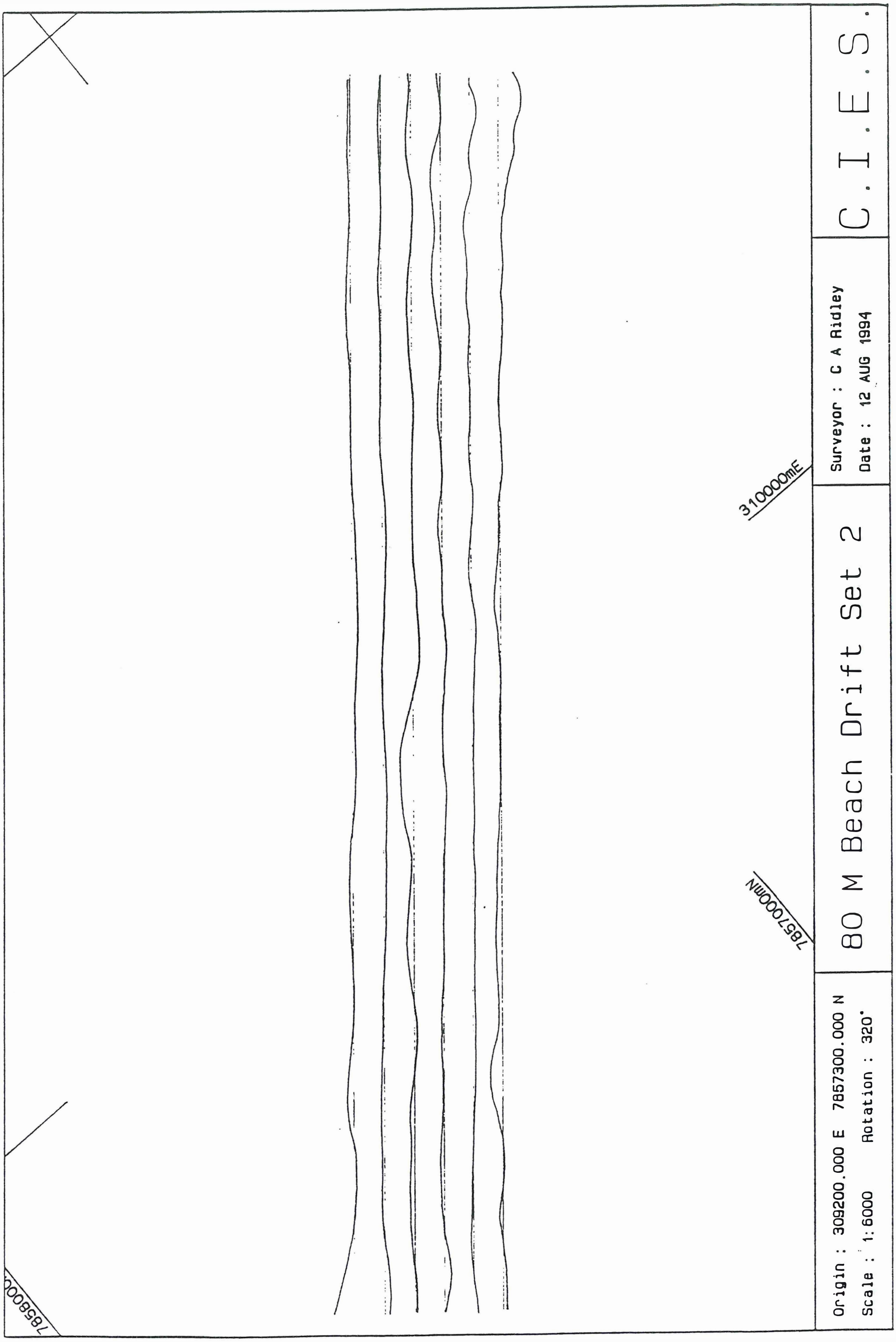


Fig. 5c Vessel tracks for the DeLury experiment on “Potato” bottom. Sweep 3

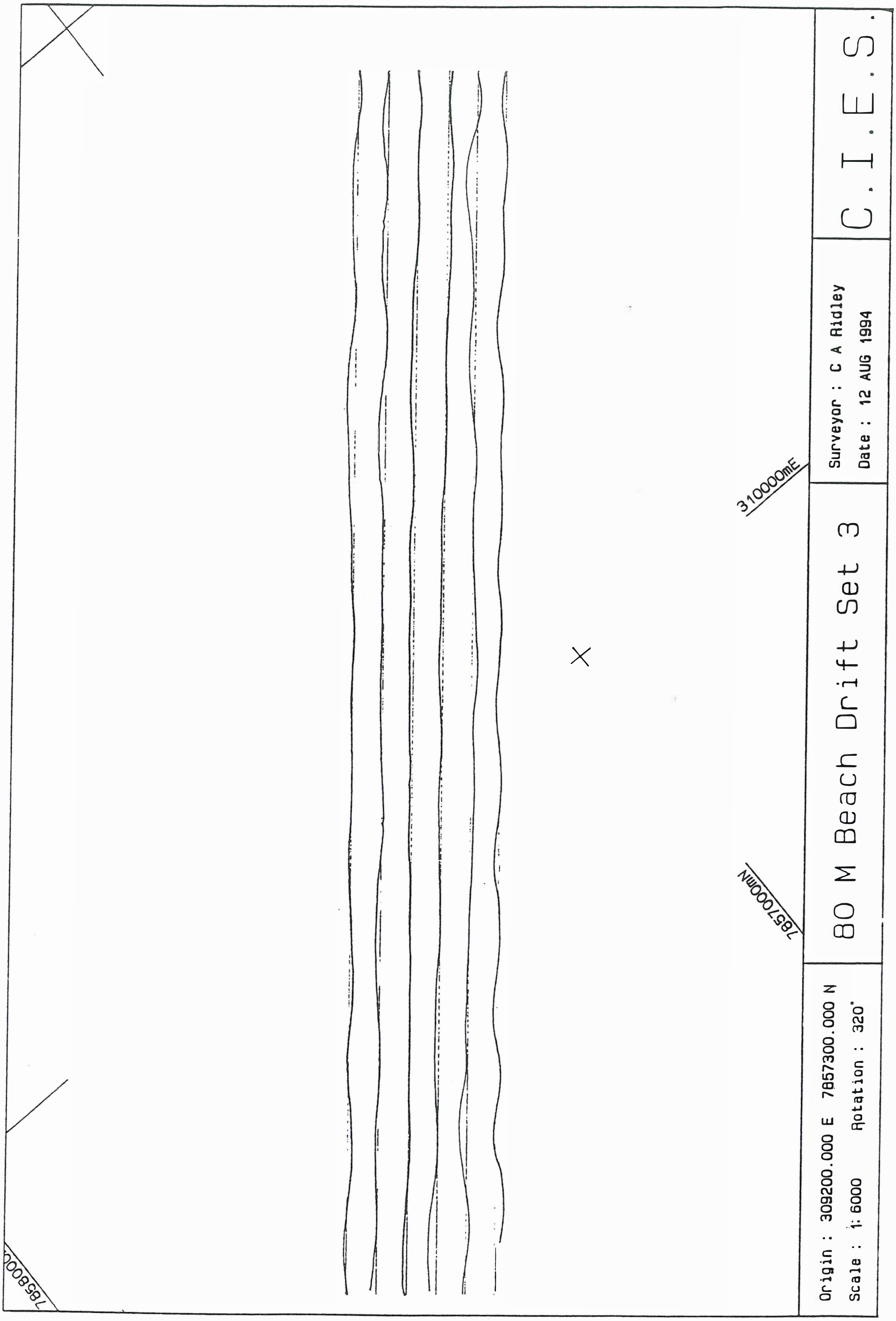


Fig. 5d Vessel tracks for the DeLury experiment on "Potato" bottom. Sweep 4

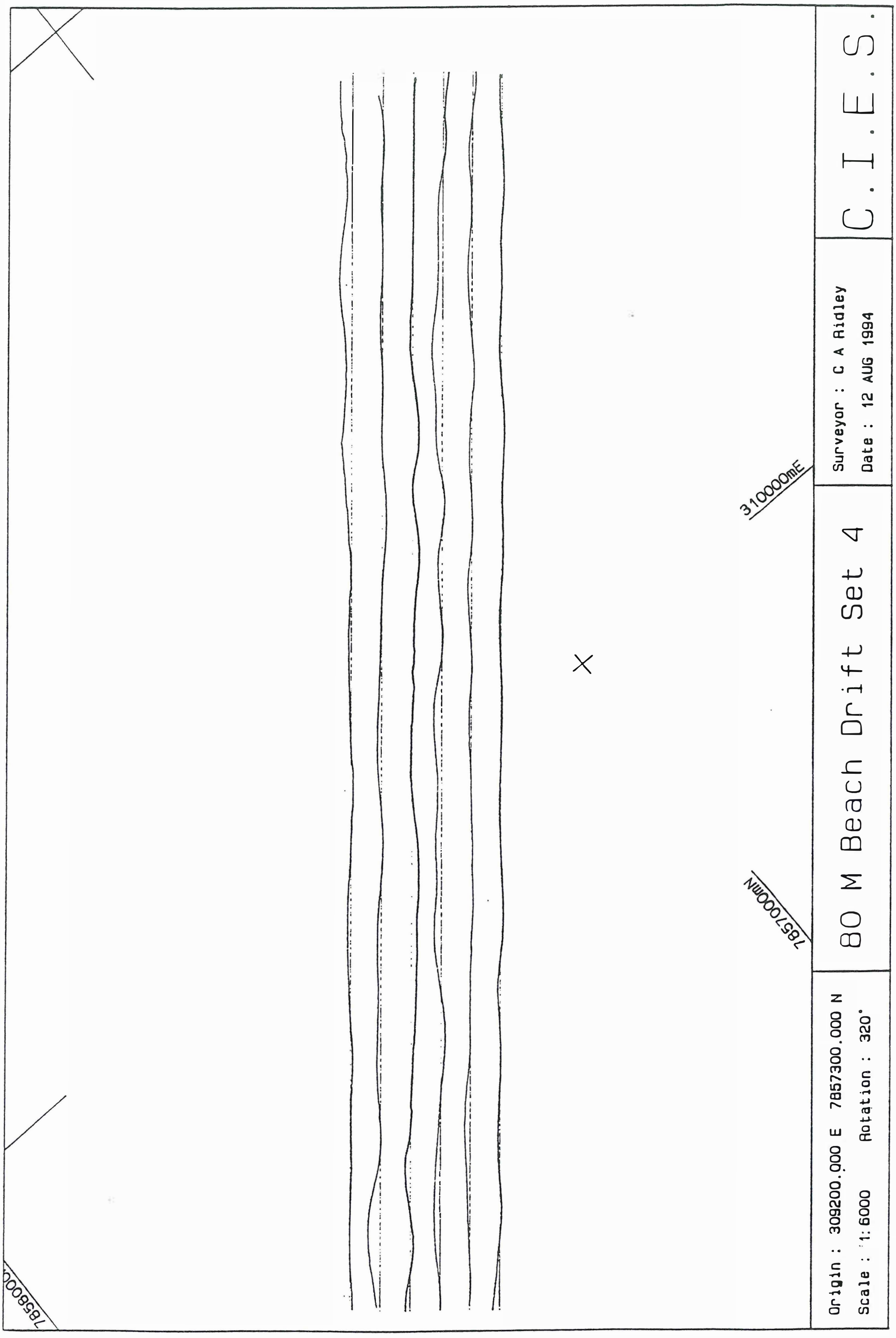


Table 2. Navigational results from the "Potato" bottom DeLury.
Data in the table show mean deviations (in m port or starboard) from the planned steering line. Value shown is the difference between the sum of the deviations to port and to starboard from the fixes recorded at 10 second intervals.

	Strip No.					
Sweep No.	**1**	**2**	**3**	**4**	**5**	**6**
1	8.0 P	1.6 S	14.0 S*	14.3 S*	2.2 P	1.0 P
2	3.6 P	0.8 S	1.6 S	2.9 S	0.4 S	1.2 P
3	0.4 S	2.1 S	0.7 S	2.5 S	3.0 S	1.3 S
4	0.3 P	1.5 S	3.9 S	1.1 S	1.8 S	4.9 S

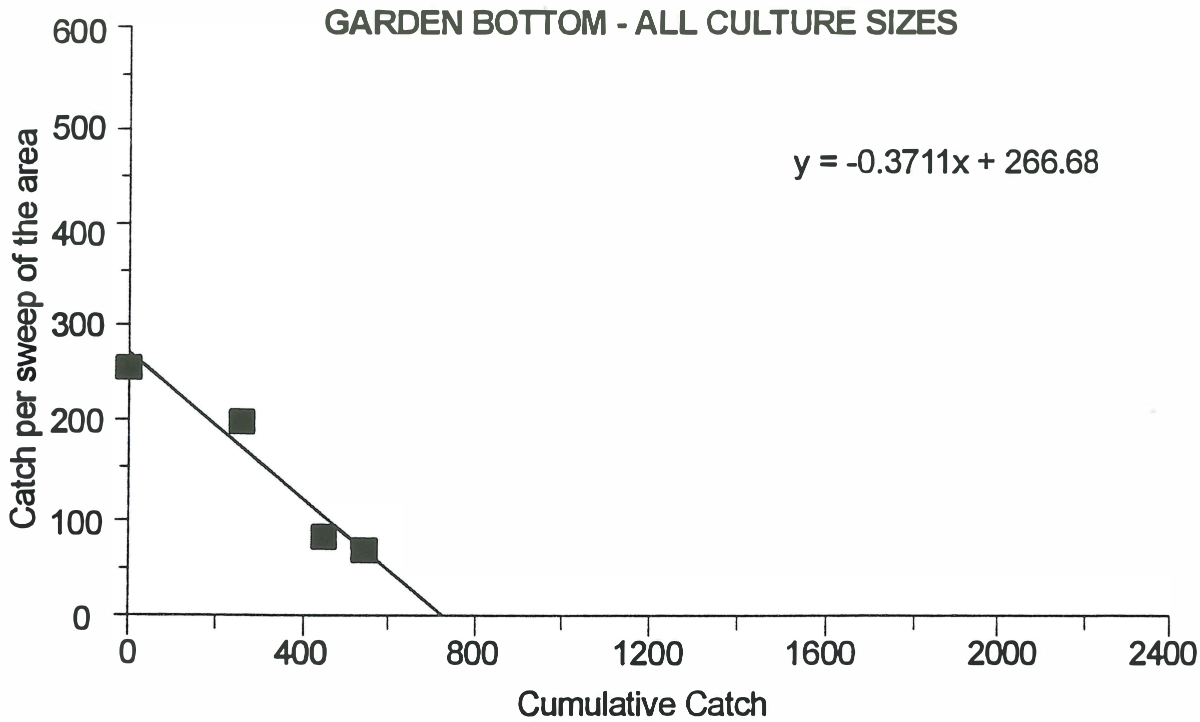
* Drifts where vessel pushed offline by wind or tide.

Because equal units of effort were used to take the successive catches (i.e. one drift of six divers wide over 1500 m), the data can be treated under the Leslie method, a special case of the DeLury method (Ricker, 1975), where the successive catches are plotted against the cumulative catch. Because each drift is of a separate part of the overall area (within the limits of navigation) the total effort has been taken as the sum of the effort of the 6 or 8 drifts required to nominally complete one 'sweep' of the area.

The regressions of catch per sweep against cumulative catch (Fig. 6) show that the catchability/diver efficiency on each of the two bottom types was 37% on "Garden" bottom and 24% on "Potato" bottom. However, with only four data points for the regression, the confidence limits for the slopes of the regressions are fairly wide and further analysis is required to consider whether these results are significantly different. One approach may be to treat each strip as an independent DeLury experiment and use the mean slope for the family of lines as the estimate for the catchability/diver efficiency. Any future use of the technique would need to consider carrying out more sweeps of the area as this would improve the degrees of freedom available in the determination of confidence intervals. However, when catch rates become very low, there is a problem in maintaining diver interest (and efficiency) and this needs to be recognised in planning the number of sweeps.

Data from the two trials were also examined on a size-stratified basis by grouping the catch from each drift into three size categories - small (110-129 mm S.H.), medium (130-149 mm S.H.) and large (150-169 mm S.H.). These indicate that catchability of each of the three size categories on "Garden" bottom was very similar (36 - 38%) (Fig. 7) whereas on "Potato" bottom the catchability declined with increasing size, with small shell having a catchability around 27% and large shell having a catchability around 16% (Fig. 8). However, the statistical significance of the apparent differences in catchability of small and large shell on

Fig. 6 DeLury plots of catch rate per sweep against cumulative catch for "Garden" and "Potato" bottom for all culture sizes.



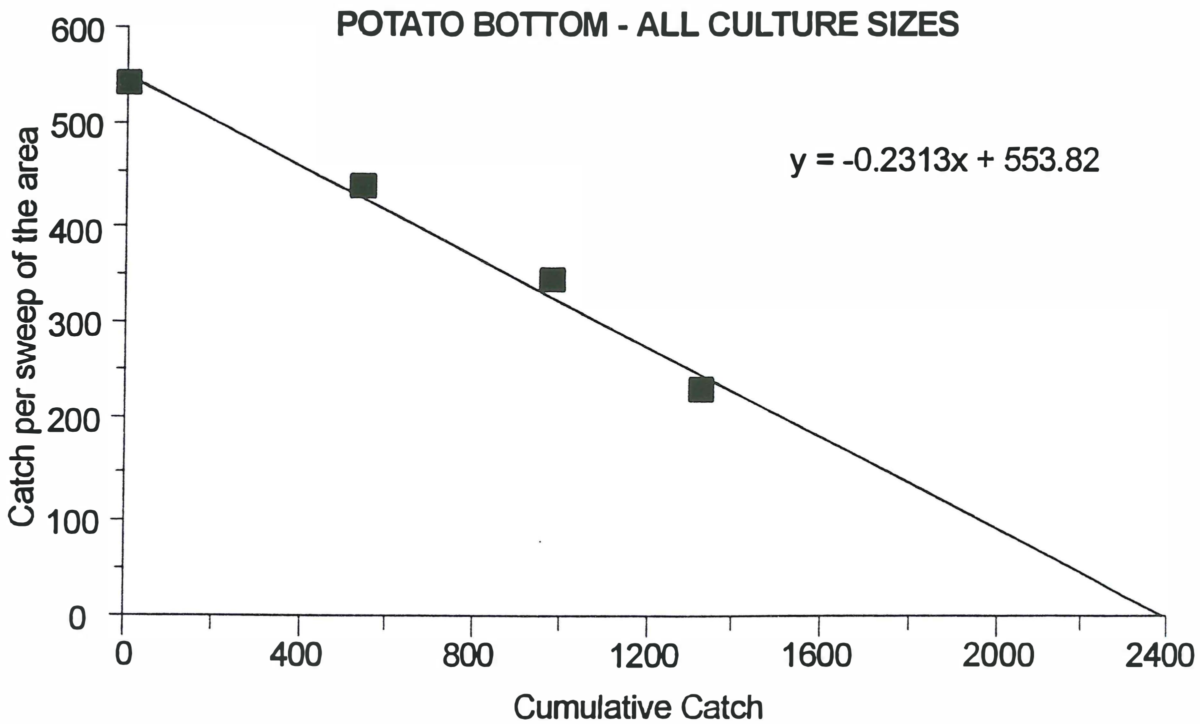


Fig. 7 DeLury plots of catch rate per sweep against cumulative catch for "Garden" bottom for the three size categories of culture shell.

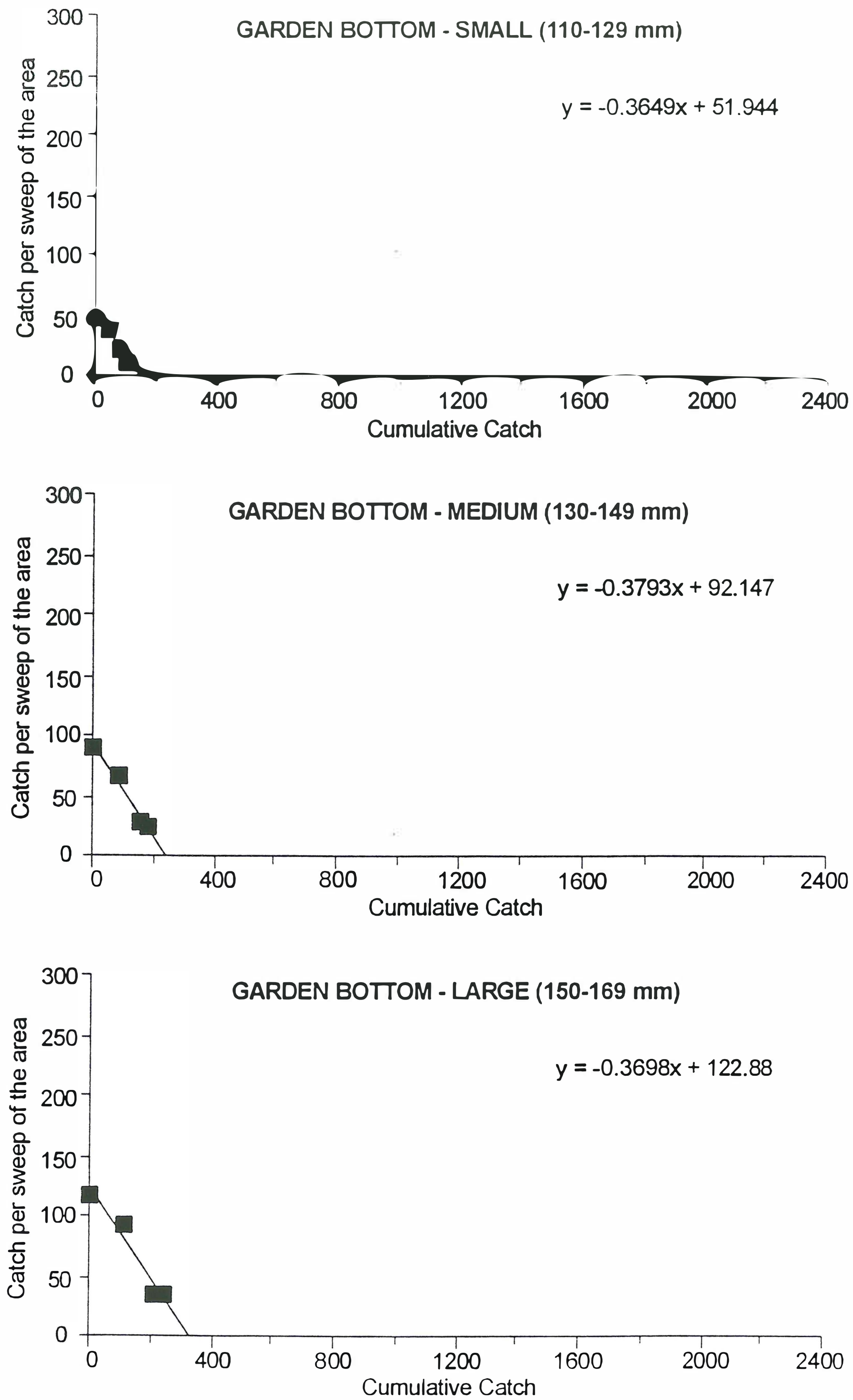
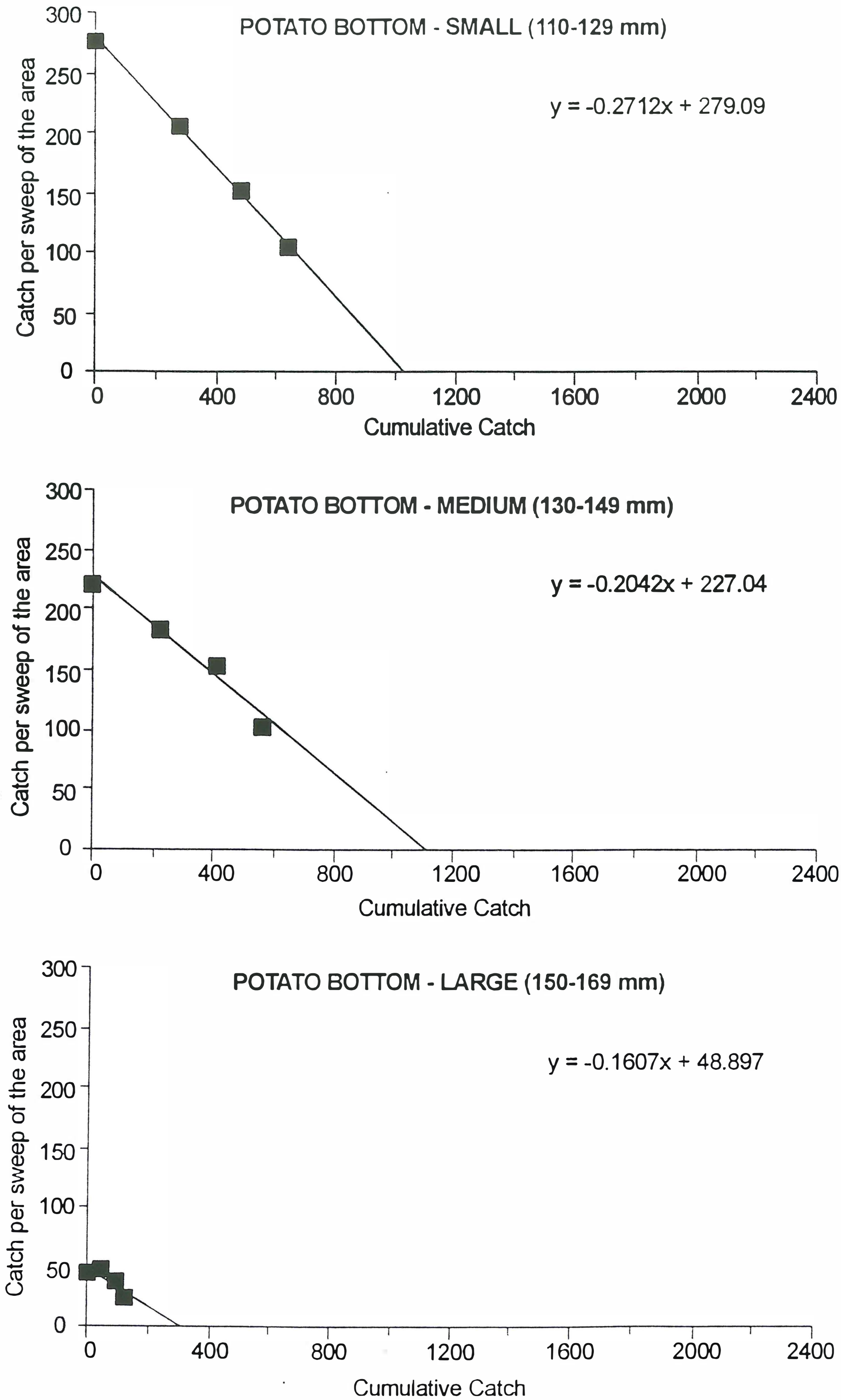


Fig. 8 DeLury plots of catch rate per sweep against cumulative catch for "Potato" bottom for the three size categories of culture shell.



"Potato" bottom is subject to the same problem of wide confidence limits noted above. Nevertheless, the nature of the result is in general agreement with the views of divers, who regard smaller shell as easier to detect because it is both less heavily camouflaged by encrusting ascidians and because the prominent 'fingers' around the shell aperture of smaller shell make a clearly distinguishable feature.

The results also provide a means of estimating the average density of oysters on the bottom. The total population in the fishing area is estimated from the intercept of the regression with the cumulative catch (X axis) while the area is simply the length of the fishing 'strips' times the width of the six or eight 'strips' fished. For the "Garden" bottom trial the estimated total population of commercial-sized oysters was 719 over an area of 432,000 m^2, while for the "Potato" bottom trial the estimated total population of commercial-sized oysters was 2394 over an area of 324,000 m^2, giving a mean density of 1 oyster per 600 m^2 ("Garden" bottom) and 1 oyster per 135 m^2 ("Potato" bottom). These density estimates, which also have fairly wide confidence limits, apply to the total area fished. However, if the experimental areas contained only say, 50% habitable bottom type, then the density figures on the area of habitable bottom would be double those calculated from using the whole area. While no direct measure of the total habitable bottom type in each area was made, comments by the divers and echo sounder observations by the skipper suggested that in both the "Garden" and "Potato" bottom areas the habitable bottom type was around 70 - 80% of the total area fished. Using an average of 75% habitable bottom type for both areas, the mean density estimates on habitable bottom would be 1 oyster per 450 m^2 ("Garden" bottom) and 1 oyster per 101 m^2 ("Potato" bottom). However, personal observations and observations by commercial pearl divers indicate that pearl oysters are often weakly aggregated. The density figures determined are, therefore, minimum values.

The presentation of the results of the analyses using the special case of the Leslie method is not directly referable to catch rate data used in the industry, which is expressed in shell per diver hour. However, the data obtained can be converted to a shell per diver hour basis using the time taken for each drift. On this basis, the catch rate of the first complete sweep of each of the two areas was 9.2 shell per diver hour for the "Garden" bottom and 20.1 shell per diver hour for the "Potato" bottom. Expressing the data in these terms provides, within limits, the ability to make some direct inferences about the abundance and density of pearl oysters on the bottom from commercial catch data.

The limits to the ability to make inferences come about through the comparability of the available habitable substrate in different areas, differences in the length or width of the fishing path of different vessels and differences in visibility. If the area fished commercially was composed of more or less habitable bottom type than that of the experimental fishing areas, then the density estimates would need to be adjusted accordingly. Similarly, if the area fished differed (e.g. through the use of larger booms or longer drifts), this would also affect the calculation of mean density. Differences in visibility between any commercial fishing dive and the dives undertaken during these trials will also affect comparability of catch rate data and the estimate of total abundance. Visibility in both the "Garden" and "Potato" bottom trials was generally good to very good (7-8 m to 15+ m), but more limited visibility would almost certainly have decreased diver efficiency and reduced the catch rate.

2. Recruitment monitoring

The number of culture shell sampled in 1991 and 1992 as part of FRDC 88/93 was relatively low, around 4 - 8% of the total commercial catch in the main Zone 2/3 area (Table 3). From 1993, with the commencement of FRDC 92/147, sampling intensity was increased from around 10% of the total Zone 2/3 catch in 1993 to around 20% in 1995. This increase in sampling frequency resulted from an attempt to achieve as wide a spread of sampling as possible. Under the arrangements with industry to carry out the sampling, research staff were provided with space and facilities on board commercial vessels. However, vessels fished in various areas at the discretion and direction of the skipper or fleet master and it was not possible to sample either randomly determined or pre-set localities across years, although some areas were fished in several years.

Another consideration in seeking to sample intensively and extensively was the high level of variability in piggyback spat rates within localised areas. Examples of this variability are shown in Figures 9 a - d, which show pigggyback spat rates on individual drifts by commercial vessels in the Lacepede Islands and Eighty Mile Beach areas in a two years, using GPS fixes provided by the vessels. The exact locations of the drifts are not shown in the figures to preserve the confidentiality of fishing areas, but the positions of the drifts are shown to scale. The figures show that piggyback spat rates can vary considerably over a relatively small area and even between adjacent drifts.

Table 3 Number of culture-sized oysters examined for piggyback spat in each of the sampling years.

	Year					
No. oysters sampled	**1990**	**1991**	**1992**	**1993**	**1994**	**1995**
Eighty Mile Beach	6,178	17,867	20,950	31,252	72,284	83,134
Lacepede Islands	-	-	15,536	13,238	15,008	21,576

One of the observations made after sampling was commenced in the Lacepede Islands area was that the size frequency distribution of piggyback spat was different to that of piggyback

Fig. 9 Vessel tracks (based on start and end GPS positions), coded for piggyback spat rate, showing small scale variability in spat rates in the Lacepede Islands and Eighty Mile Beach areas in various years.

a. Eighty Mile Beach 1992

b. Lacepede Islands 1992

c. Eighty Mile Beach 1994

d. Lacepede Islands 1994

Fig. 9a

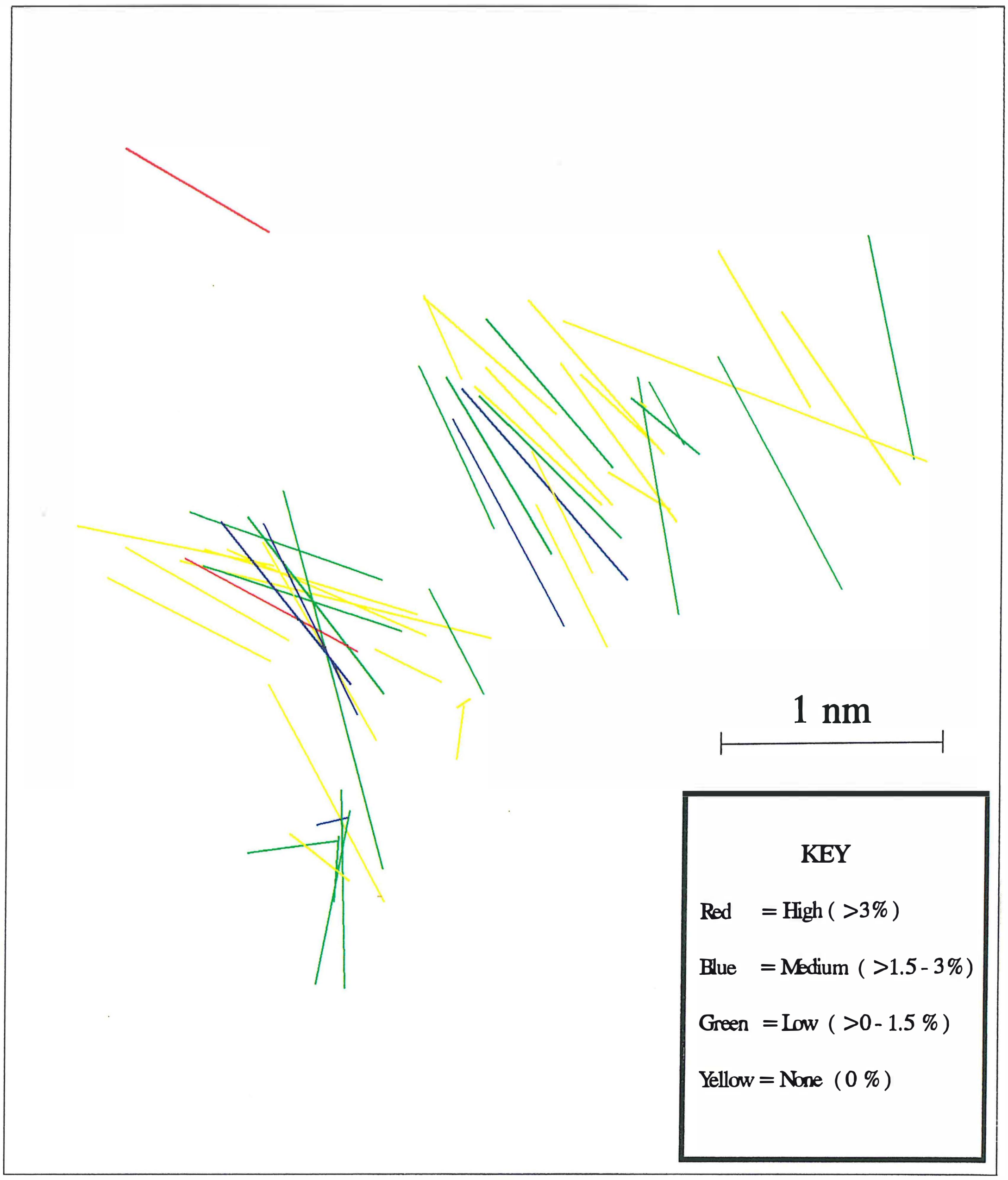


Fig. 9b

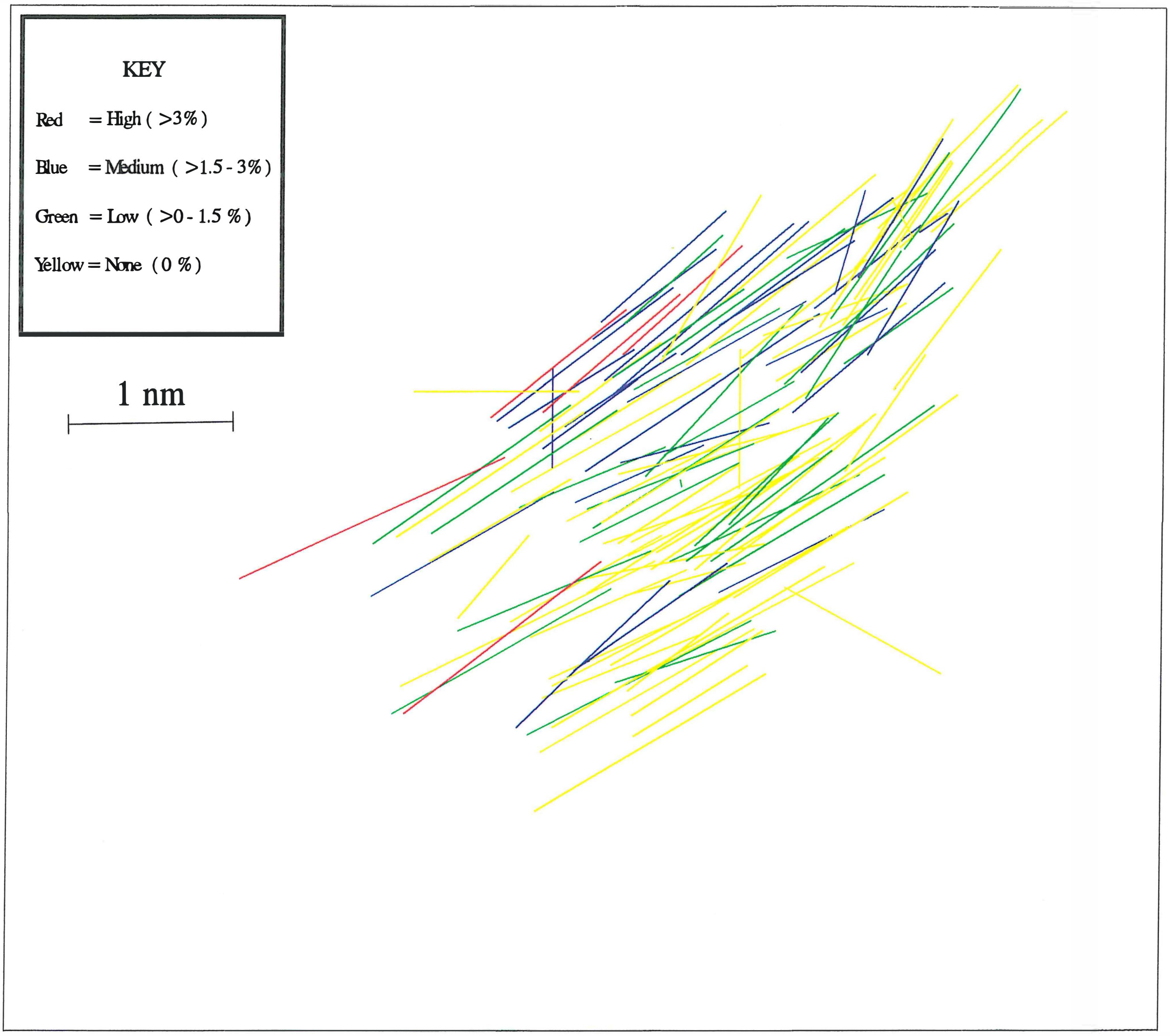


Fig. 9c

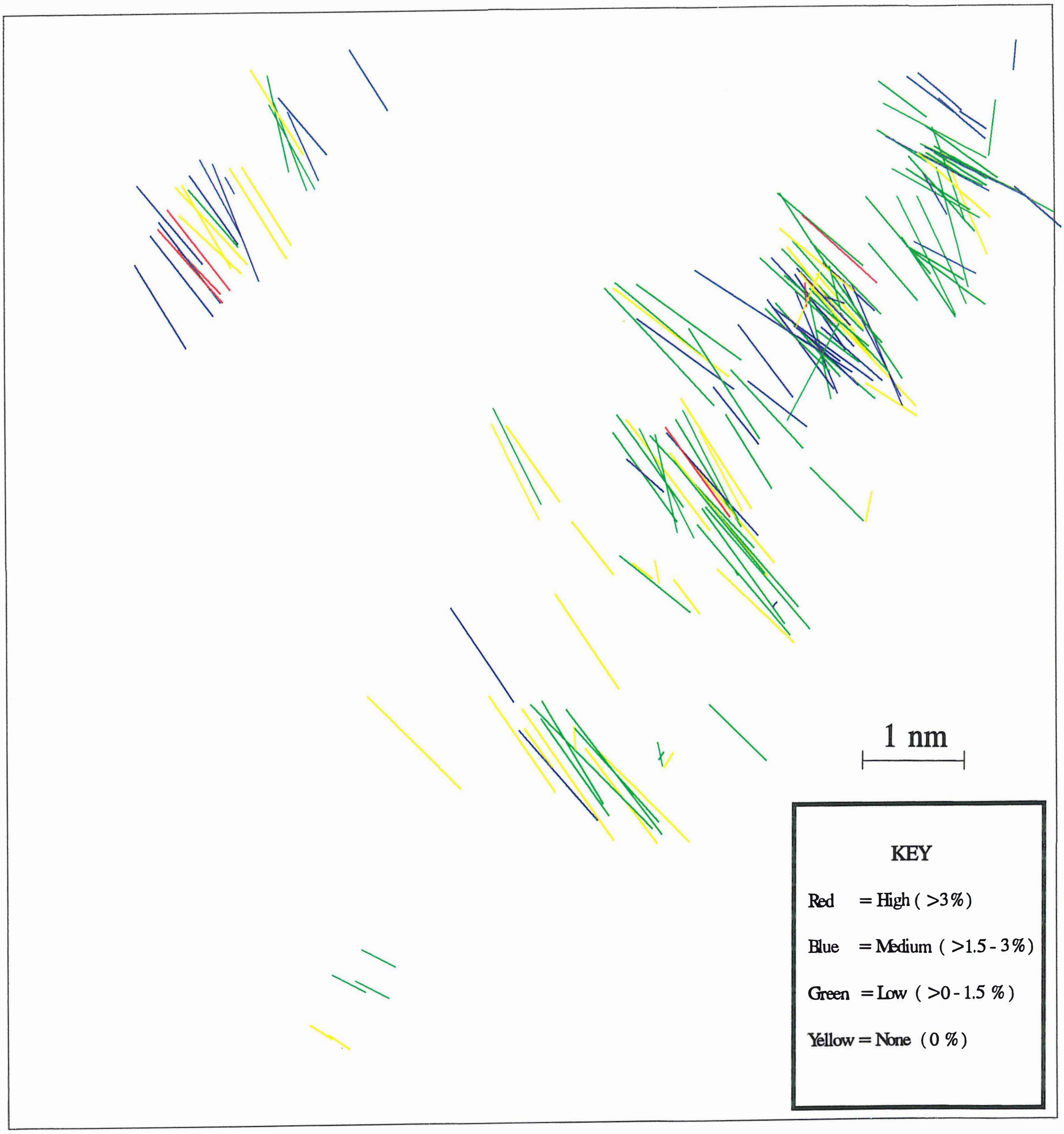
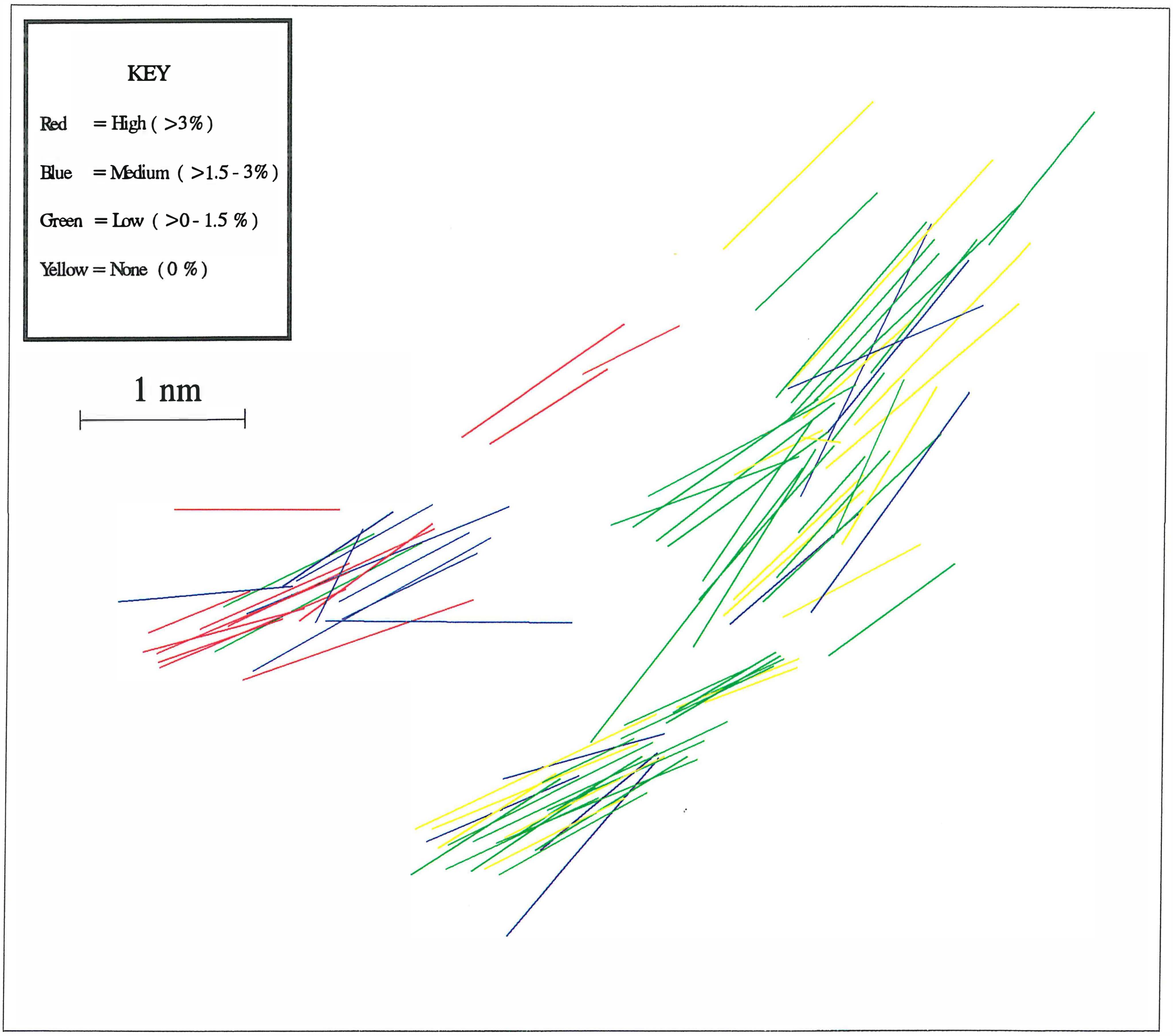


Fig. 9d



spat from the Eighty Mile Beach area (Figs. 10, 11). This difference in size frequency distribution was consistent over the four years in which sampling was conducted in the Lacepede Islands area. Most sampling in the Lacepede Islands area took place in February, at which point the size frequency distribution of piggyback spat was unimodal, with a modal value around 35 - 45 mm S.H. and an upper limit to the mode around 70 mm. The size frequency distribution for the Eighty Mile Beach area, which was sampled over the period March to May in most years, was bimodal, with modal peaks around 20 and 60 mm S.H. The differences in the size frequency distributions and modal sizes between these two localities are considered to be most likely the result of different rates of growth and this is the subject of a current FRDC project. Based on the tag-recapture studies carried out under the present project, and the spat collector studies carried out under FRDC 88/93, it is possible to assign age classes to the two modes detected in the Eighty Mile Beach piggyback spat sampling. These indicate that the modal group around the peak at 20 mm S.H. (0 - 34 mm S.H.) in March - May would be the 0+ age class (i.e. derived from the spawning of late [October-November] the previous year[Year 0]), while the modal group around the peak at 60 mm S.H. (35 - 70 mm S.H.) would be the 1+ age-class (i.e. derived from the spawning two years previously).

Given the understanding obtained from the growth/ageing studies (see below) on the age at recruitment to the fishery it is possible that, for the Eighty Mile Beach area, a relationship between recruitment and catch rate in the fishery, or particular areas of the fishery, could be determined. However, at this stage the analysis did not seek to correlate the recruitment data with the subsequent catch rate in the fishery because a full sequence of fishing years corresponding to the recruitment years is not yet available. Also, because the fishery catch rate is affected by the abundance of the standing stock of at least three year classes (see Growth and Ageing - below), matching data on the catch rate of the various size (age) classes will be required. Data collected by one of the fishing companies on the size distribution of the catch for the 1994 and 1995 seasons should provide some of this information, but the 0+ year class of 1995 will not enter the fishery until 1998, at which point there will be a full sequence of fishing years corresponding to the piggyback spat sampling years.

Because of the apparent differences in size frequency and ages of piggyback spat from the Lacepede Islands and Eighty Mile Beach areas, the data from these locations was treated separately. The data for the Lacepede Islands area for spat in the size range 0 - 70 mm was treated together while the Eighty Mile Beach data were examined both overall for the two size classes (0 - 70 mm) as well as separately for each size (age) class (0 - 34 and 35 - 70 mm). The analysis showed that, for the Eighty Mile Beach area, all the factors examined (year, month, grid and depth) were significant, both overall and for the two size-classes (Table 4a(i)), although a large amount of the variance was unexplained by the factors examined.

Fig. 10

PIGGYBACK SPAT SIZE FREQUENCY
80 MILE BEACH (1991 - 95)

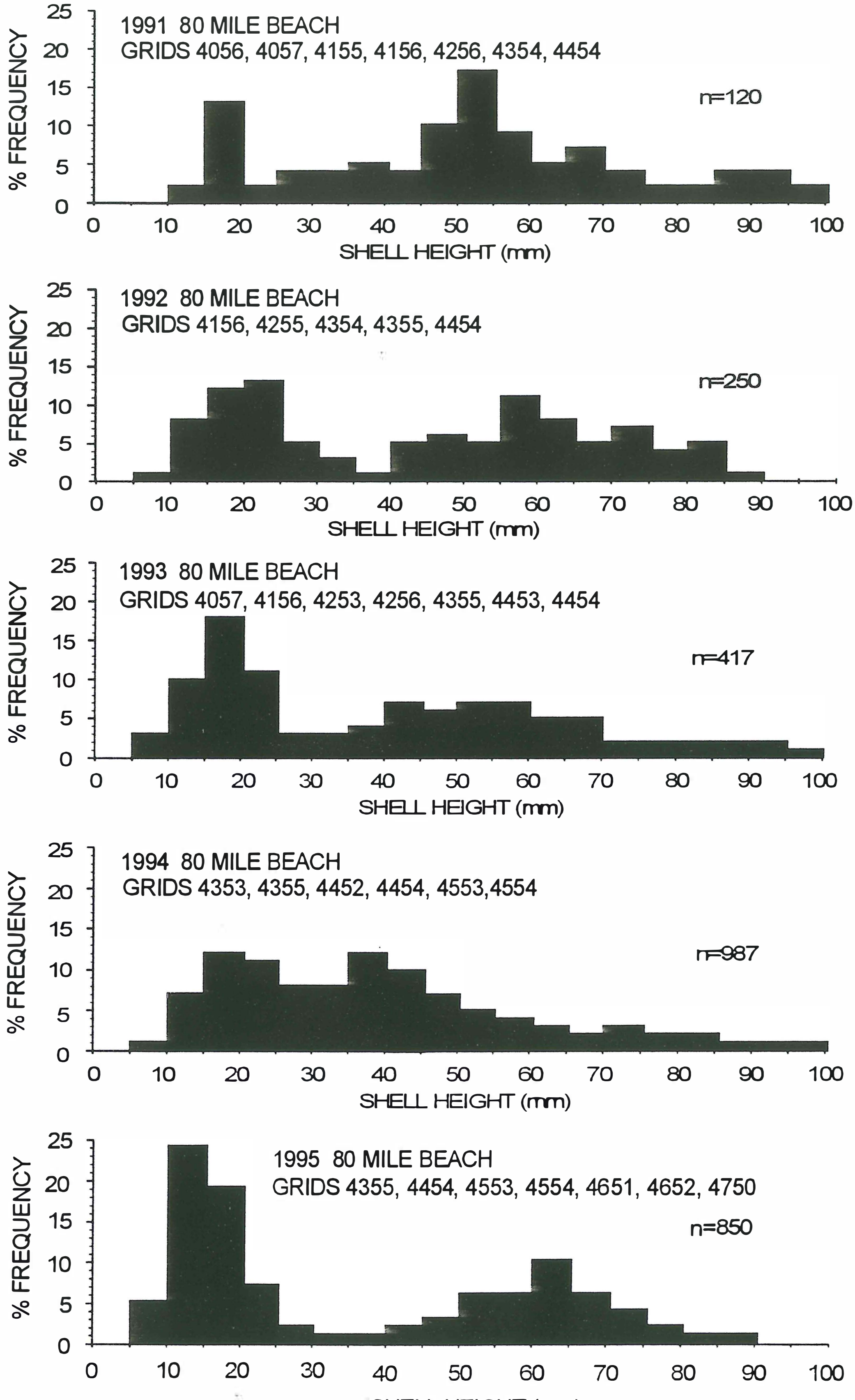
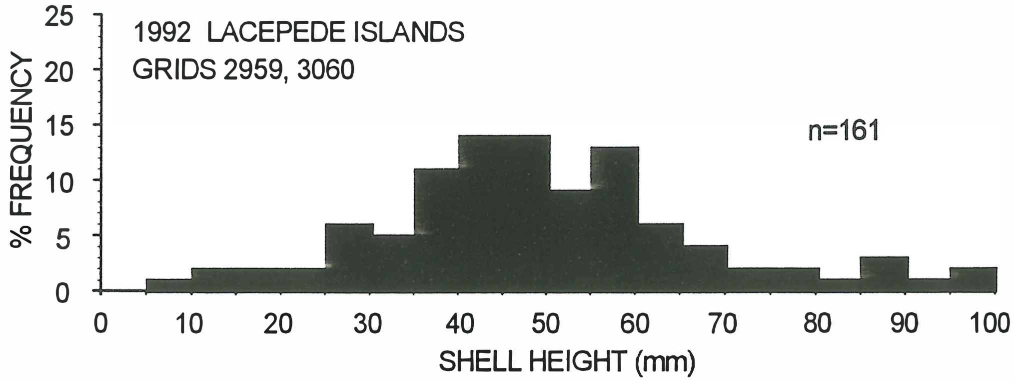
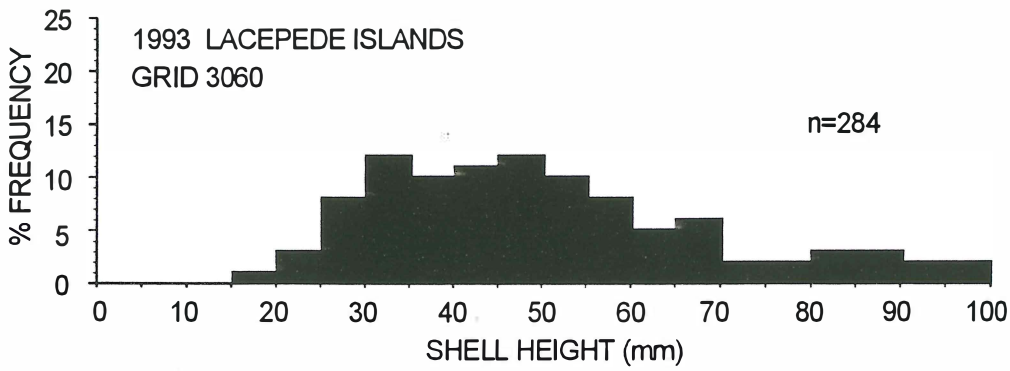
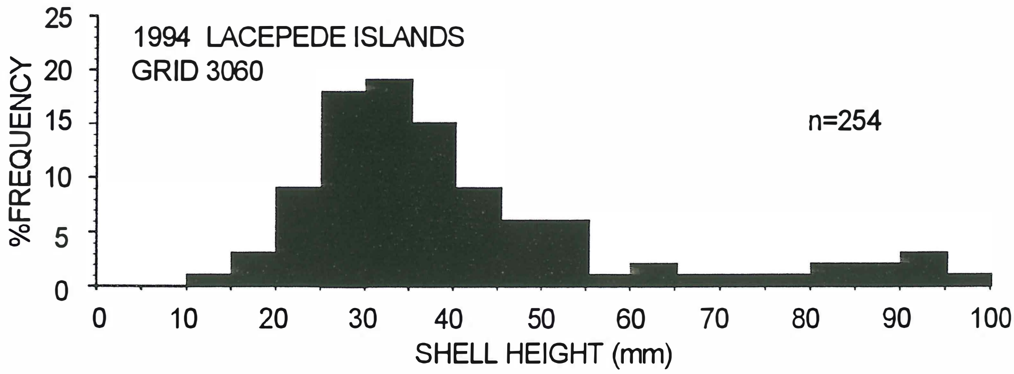


Fig. 11 PIGGYBACK SPAT SIZE FREQUENCY
LACEPEDE ISLANDS (1992 - 95)







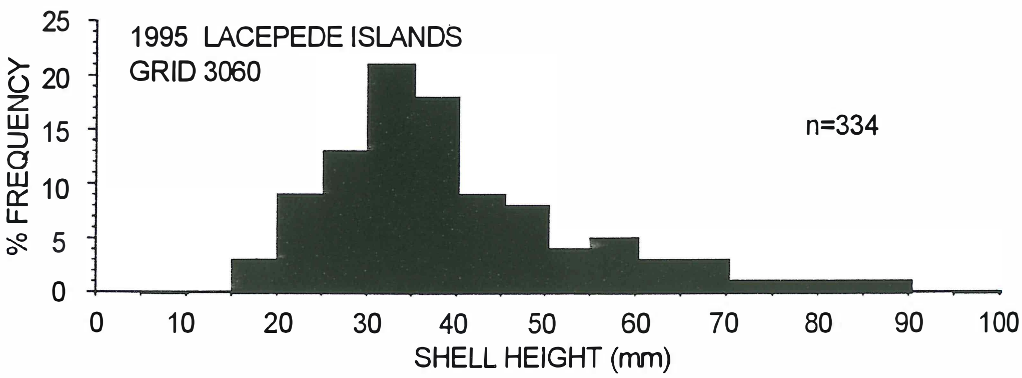


Table 4. Sum of squares related to factors affecting piggyback spat rates, the percentage of the sum of squares explained and the significance.

a. Eighty Mile Beach (Overall [0 - 70 mm] and for the two size classes 0 - 34 and 35 - 70 mm)

i. Main Effects

		0 - 70mm			0 - 34 mm			35 - 70 mm		
FACTOR	**DF**	**SS**	**%SS**	**PROB**	**SS**	**%SS**	**PROB**	**SS**	**%SS**	**PROB**
Year	5	51.1	2.7	<0.001	59.8	3.9	<0.001	27.7	1.8	<0.001
Month	3	9.7	0.5	<0.05	9.8	0.6	<0.01	4.5	0.3	N.S.
Grid	17	177.3	9.3	<0.001	147.1	9.5	<0.001	94.7	6.1	<0.001
Depth	8	28.6	1.5	<0.001	13.5	0.9	<0.05	16.7	1.1	<0.01
ERROR		1428.5			1226.7			1150.6		
R^2		0.249			0.204			0.172		

ii. Main Effects (with Sub-Grid Nested within Grid) and Interactions

		0 - 70 mm			0 - 34 mm			35 - 70 mm		
FACTOR	**DF**	**SS**	**%SS**	**PROB**	**SS**	**%SS**	**PROB**	**SS**	**%SS**	**PROB**
Year	5	42.5	2.2	<0.001	54.7	3.6	<0.001	20.4	1.5	<0.001
Month	2	14.0	0.7	<0.001	11.6	0.8	<0.001	4.0	0.3	N.S
Grid	16	74.7	3.9	<0.001	65.0	4.2	<0.001	36.4	2.6	<0.001
S/grid (grid)	19	38.9	2.0	<0.001	26.0	1.7	<0.05	22.5	1.6	<0.05
Depth	7	22.9	1.2	<0.001	15.7	1.0	<0.01	10.7	0.8	<0.05
Yr * grid	7	32.6	1.7	<0.001	24.7	1.6	<0.001	13.7	1.0	<0.01
Yr*s/g (gr)	4	6.8	0.4	N.S.	8.5	0.6	<0.05	2.5	0.2	N.S.
Yr*Depth	19	31.2	1.6	<0.01	31.2	2.0	<0.01	15.6	1.1	N.S.
ERROR		1253.4			1093.4			1071.1		
R^2		0.341			0.290			0.230		

(gr = grid; s/g = sub-grid)

b. Lacepede Islands (Single size-class, 0 - 70 mm)
(Main effects only, interactions not significant)

	DF	**SS**	**%SS**	**PROB**
Year	3	12.8	2.1	<0.01
Month	2	10.5	1.7	<0.01
Depth	5	35.2	5.7	<0.001
ERROR		507.9		
R^2		0.182		

Of the factors examined, the highest percentage of the sum of squares was explained by the variations between grids, with grid explaining a higher percentage of the sum of sqaures than the other three factors combined. There were also a number of significant one-way interactions (Table 4a(ii)), although their contribution to the sum of squares was only small. Nesting of sub-grid within grid in the analysis including interactions showed that sub-grid (i.e. the 2.5 x 2.5 n.mile areas within the 10 x 10 n.mile grids) was also significant. The combination of the factors grid and sub-grid nested within grid, together with the interactions involving grid and sub-grid, accounted for a higher percentage of the sum of squares than all the other factors and interactions combined.

In the Lacepedes Islands area, where the data all came from one grid, there were no interactions and the factor accounting for the highest percentage of the sum of squares was depth, although again a large amount of variance not explained by the factors examined. However, although depth was the most important of the three factors examined, the contrast in depth in the Lacepedes Islands area (after removing the effects of tidal variation) was small - less than 4 m in all years except 1992, when the maximum range was around 8 m. Given the much lower contribution of year to the sum of squares, the significance of the differences in depth is most likely the result of the variations in the spat rate which occur over small distances, with depth being a proxy for small differences in location.

A major problem identified in the analysis of the data was the high level of confounding between the explanatory factors. This came about through the sampling strategy, which utilised commercial pearling vessels for the sampling platform during the course of normal fishing operations. Because commercial pearling vessels make decisions about their area of operation on the likely catch rate in an area, and will repeatedly fish an area until the catch rate falls to a level where they believe they can achieve a better catch rate elsewhere, the sampling regime was not random or representative of the area as a whole. In the Eighty Mile Beach area in particular, there were differences between years in the areas (grids) sampled as well differences between years in the areas sampled within the same grid or sub-grid.

The results produced from this method of sampling were exacerbated by the high level of patchiness in pearl oyster distribution, presumably as a result of patchiness in the habitat and in the settlement of larvae. This patchiness operates at a number of levels. The very fine-scale clustering which affects piggyback spat rates between individual drifts has been noted above (Fig. 9), and is probably the cause of the significance of the depth (small distance) factor in the ANOVA for the Lacepede Island data. On top of this is a mid-scale mosaic related to fishable areas of one to two square miles where pearl oysters are generally abundant and which form the core focus of commercial fishing activity. This level of patchiness is reflected to some degree by the significance of sub-grid in the ANOVA for Eighty Mile Beach. Furthermore, at

Eighty Mile Beach where the fishing grounds are much bigger than in the Lacepede Islands area, there is large scale patchiness in the habitat and the abundance of oysters on these areas of habitat, as well as differences in the spat rates in these areas. This level of patchiness is reflected in the significance of grid as a factor in the ANOVA for Eighty Mile Beach.

The inability to follow a random or structured sampling strategy is intrinsic in the use of commercial pearling vessels as sampling platforms. However, the lack of a random or structured sampling strategy (which would minimise the effects of patchiness in the patterns of pearl oyster and piggyback spat distribution) and the confounding of factors examined for their effects on piggyback spat rates, makes the piggyback spat rate data difficult to interpret. However, the least squares mean rate - the rate after accounting for all the main effects - can provide a relatively robust estimate of the year to year variations in the piggyback spat rate (Fig. 12). (The least squares mean rate cannot be used to account for the effects of interactions, but these account for only a minor percentage of the sum of squares). The figure shows the relative levels of recruitment from year to year (as measured by the piggyback spat rate) of the two size (year) classes for Eighty Mile Beach and for the one size (year) class for the Lacepede Islands area.

At Eighty Mile Beach (Fig. 12a) the overall least squares mean rate of piggyback spat (0 - 70 mm), as well as the rate for each of the size (age) classes, decreased from 1990 to 1991 and then increased over the period 1991 - 1994. However, the general applicability of the spat rate recorded for 1990 needs to be interpreted with caution as the areas sampled in 1990 differed from the areas sampled in nearly all the other years. The increase in the rate from 1991 to 1994 shows some superficial correspondence with the increases in catch rate which have occcurred in the fishery over the period 1993 - 1995. However, based on the age-class interpretation for spat and the age at entry to the fishery (see Growth and Ageing - below), any increase in recruitment to the lower end of the fishable sizes from increases in the abundance of 35 - 70 mm (1+ age class) would not be detected in the fishery until 1994, which does not correspond with the approximately 30% increase in catch rate over 1992 levels which occurred in the fishery in 1993. Increases in catch rate in the fishery over the period 1993 - 1995 may, however, not be the result of increases in the abundance of pearl oysters through increased levels of recruitment, but arise from increases in vessel efficiency resulting from technological change in the industry (primarily GPS and plotters). If there are factors other than the abundance of fishable-size oysters which affect the catch rate in the fishery, the determination of a causal relationship between piggyback spat rate data and the fishery catch rate will be difficult unless the other factors can be identified

One of the interesting features of the piggyback spat rate data for Eighty Mile Beach is the lack of any significant correlation between the the least squares mean rate for 0+ piggyback spat in one year and the rate for 1+ piggyback spat a year later ($r = 0.131$, $DF = 4$, $P > 0.05$).

Fig. 12a Annual recruitment trends as indicated by the least squares mean piggyback spat rate.

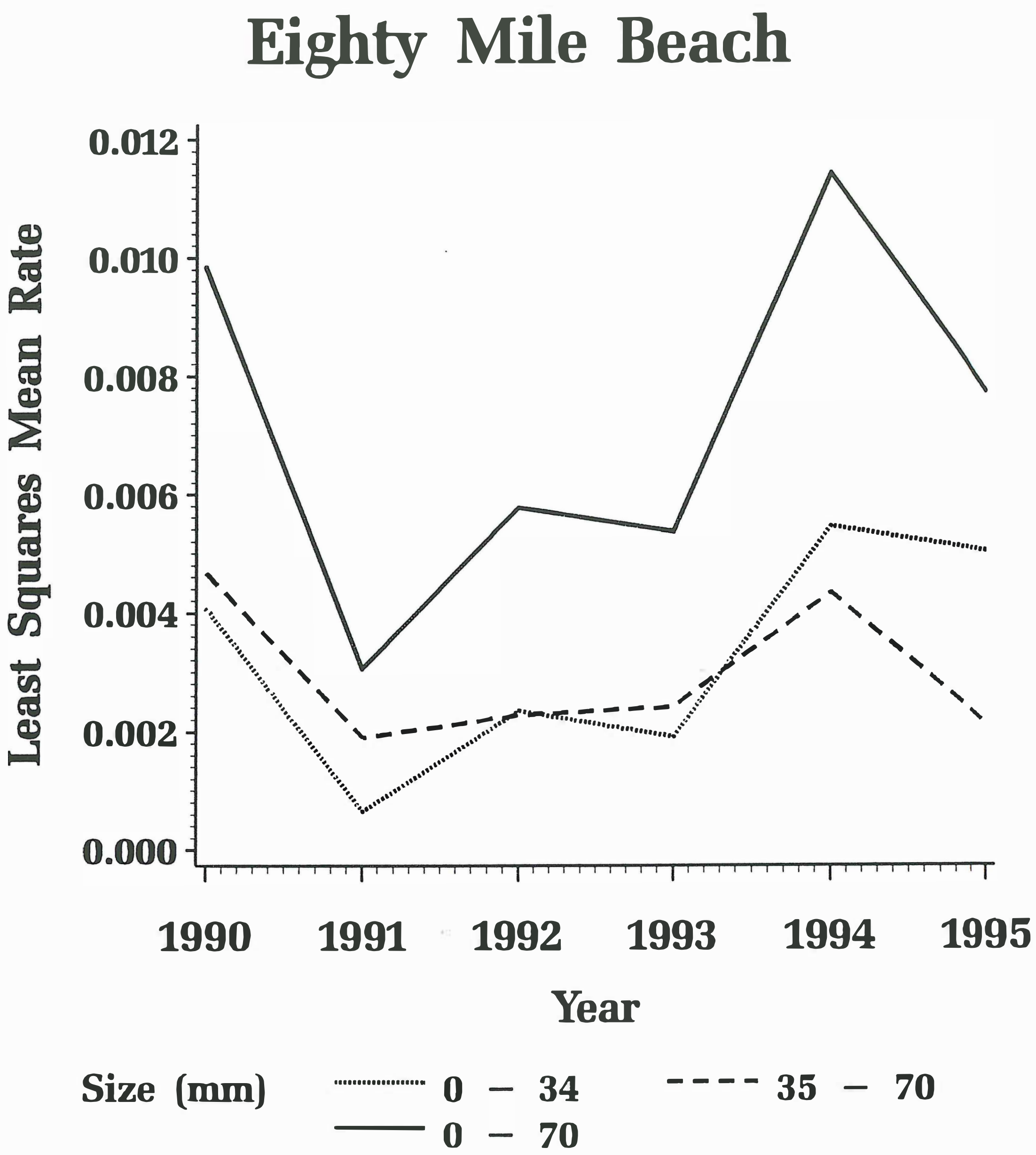
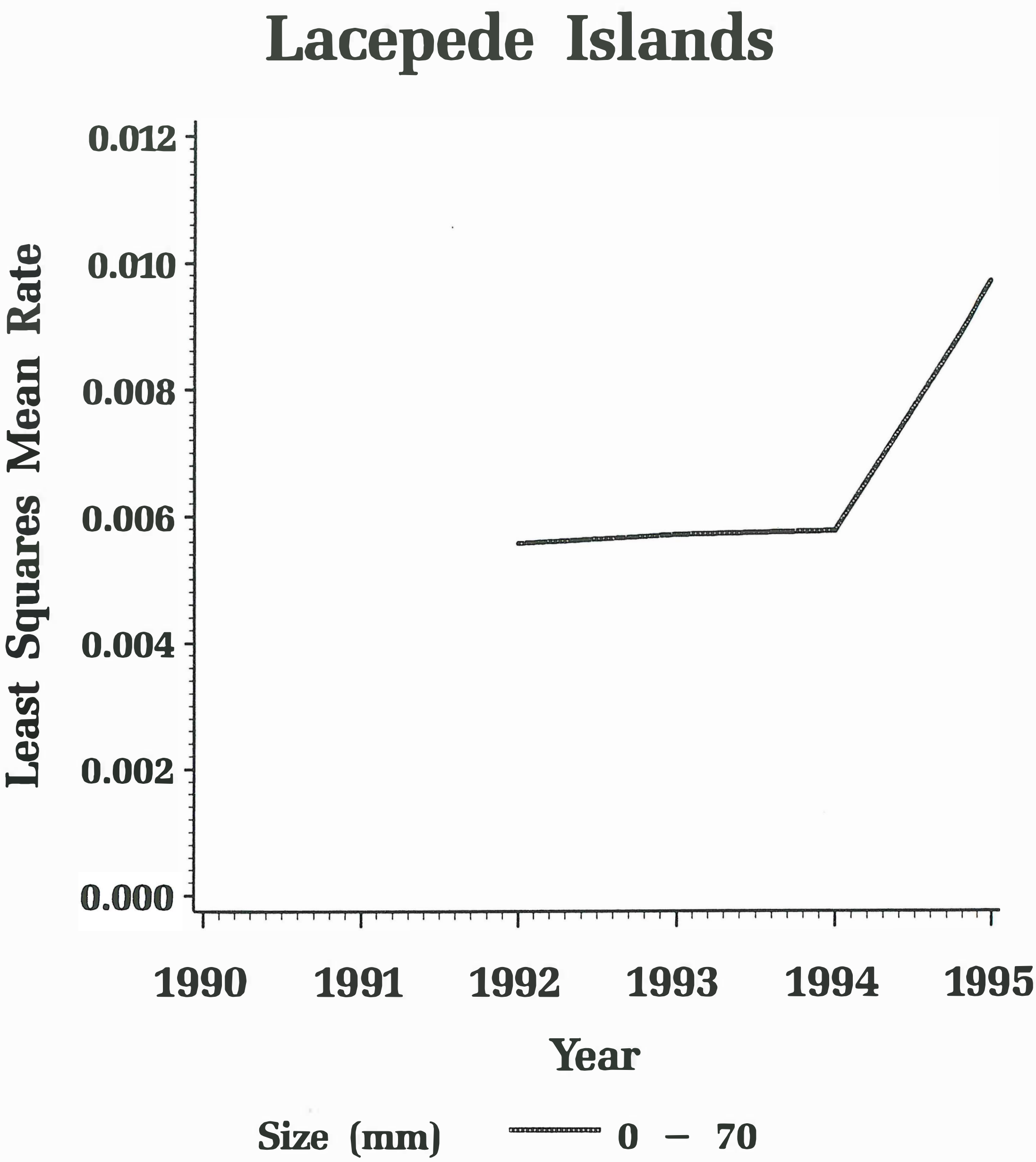


Fig. 12b Annual recruitment trends as indicated by the least squares mean piggyback spat rate.



This may be partly the result of the lack of standardised sampling localities, with the sampling in different years in different areas measuring patches of recruitment of either one or the other of the two year classes. An alternative explanation may be that the piggyback spat rate does not provide a robust measure of recruitment.

The usefulness of piggyback spat data, and its ability to be correlated with subsequent catch rate data in the fishery, will be reviewed again when more years of fishery data corresponding to the years of piggyback spat collection become available and there is growth data available for the Lacepedes Islands area. However, at this stage, the high level of variablity in piggyback spat rates and the inability to operate a sampling programme which can account for this variability, results in data which do not appear to provide a particularly useful index of recruitment to the pearl oyster stock.

3. Growth and ageing

i. Growth of tagged shell in the Eighty Mile Beach area.

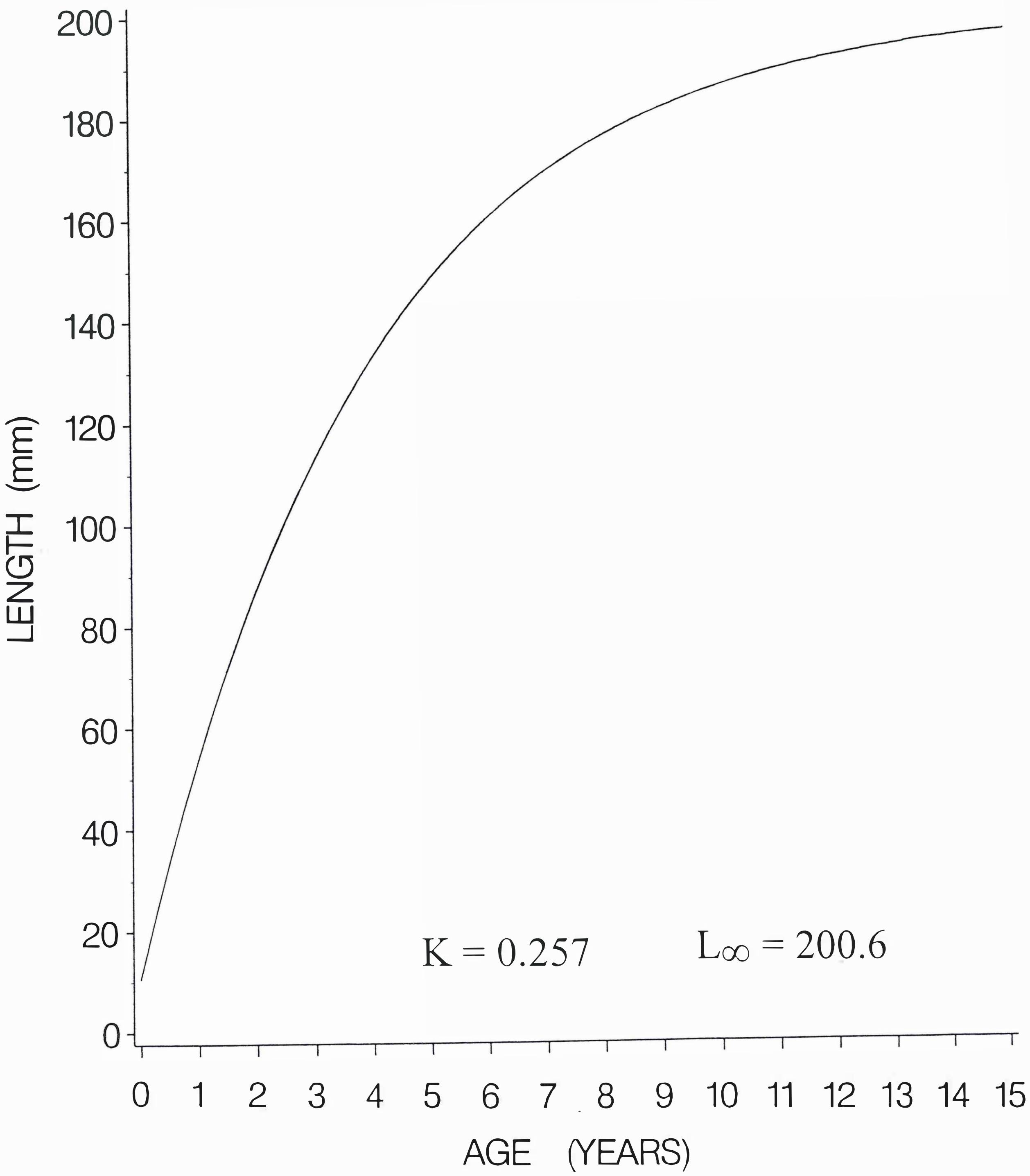
A total of 1853 single year at liberty recaptures covering oysters in the size range 20 - 210 mm were made over the period of the programme (Table 5). Analysis of the data (ANOVA) indicated that, except for the 1989-90 period, between year variations in growth (increase in shell height) were not significant. Growth of oysters in the 1989-90 period was significantly less than in the other four years ($P<0.01$). This difference may reflect normal yearly variations in growth or may possibly be due to less than optimum conditions of storage of some held on some commercial vessels prior to tagging. Further analysis of the data (by source vessel) should determine whether the effects on growth are attributable to handling.

Table 5. Number of single year at liberty recaptures of tagged pearl oysters for the various recapture years.

Period at liberty	89/90	90/91	91/92	92/93	93/94
No. recaptures	204	362	553	343	391

As a preliminary analysis, however, the data for the five years of single year at liberty growth in shell height data were used to generate a growth curve, using the von Bertalanffy growth equation. Because there was a reliable estimate of T_o derived from the earlier spat collector work, this preliminary growth curve was able to be expressed as a curve of size at age (Fig. 13). It should be noted that the measurement of S.H. to the base of fingers gives a measurement which is some 5 - 8 mm shorter than the measurement to the tip of the fingers, so that entry to the fishery at a size of 120 mm to the tip of the fingers would relate to a size around 112 -115 mm on the size at age curve. This equates to an age of 3+ years at entry to

Fig. 13 P. MAXIMA 80 MILE BEACH
PRELIMINARY AGE–LENGTH CURVE
FROM TAGGING DATA 1989–94)
(based on 30 mm = 5 month old)



the fishery. At the maximum useful size for round pearls of 160 - 170 mm, pearl oysters would be 6+ to 7 + years of age. This would mean that a year class entering the fishery would be present in the fishery for 3 - 4 years. The L_∞ value for the growth equation was 200.6 mm, which approximates the average size of the largest pearl oysters in the medium to shallow depths of Eighty Mile Beach. The size at age relationship indicates an age of around 15 - 20+ years for oysters at this size.

ii. Ageing

a). Marking of pearl oyster shells with chemical markers.

Of the 29 pearl oysters released, most were recaptured after one year at liberty (Table 6) and the results of ICPMS analyses conducted on four of the one-year at liberty recaptures are presented. The data show that regions of elevated strontium-calcium ratios were present at points across the thickness of the valves of a number of the specimens examined (Fig. 14). Specimens L882 and L905 both showed clear, single peaks of elevated strontium levels, which presumably reflect the period of exposure to elevated strontium levels. Specimen L925 did not show such an obvious region of elevated strontium levels, although the region of elevated strontium levels in the shell about 3.5 mm from the origin may reflect the period of exposure. Specimen L934 showed two regions of elevated strontium levels at about 0.8 and 1.6 mm from the origin. The cause of these two peaks is not understood, although a similar problem of multiple banding was noted in the tetracycline-exposure trials and will be further discussed in that section.

Table 6. Releases and recaptures of pearl oysters marked with elevated strontium levels by soaking in strontium chloride solution.

Released	Recaptured		
1992	1993	1994	1995
29	6	1	1

Observation under a dissecting microscope of the surface of the cut face of the shell and the small 'craters' left by the laser beam allowed the points related to a particular strontium-calcium ratio reading to be determined. In some instances, (e.g. L905) the highly elevated strontium band was associated with a thin band of calcite prisms, while in other instances (e.g. L905, L882, L925) some points with mildly elevated strontium-calcium ratios were also related to thin bands of calcite prisms. The presence of a thin band of calcite prisms associated with the elevated strontium area of L905 may relate to stress associated with the capture, handling and marking of this animal, although not all marked specimens showed such a 'stress'

Fig. 14 Strontium-calcium ratios at points across the thickness of strontium chloride-treated pearl oysters at liberty for one year.

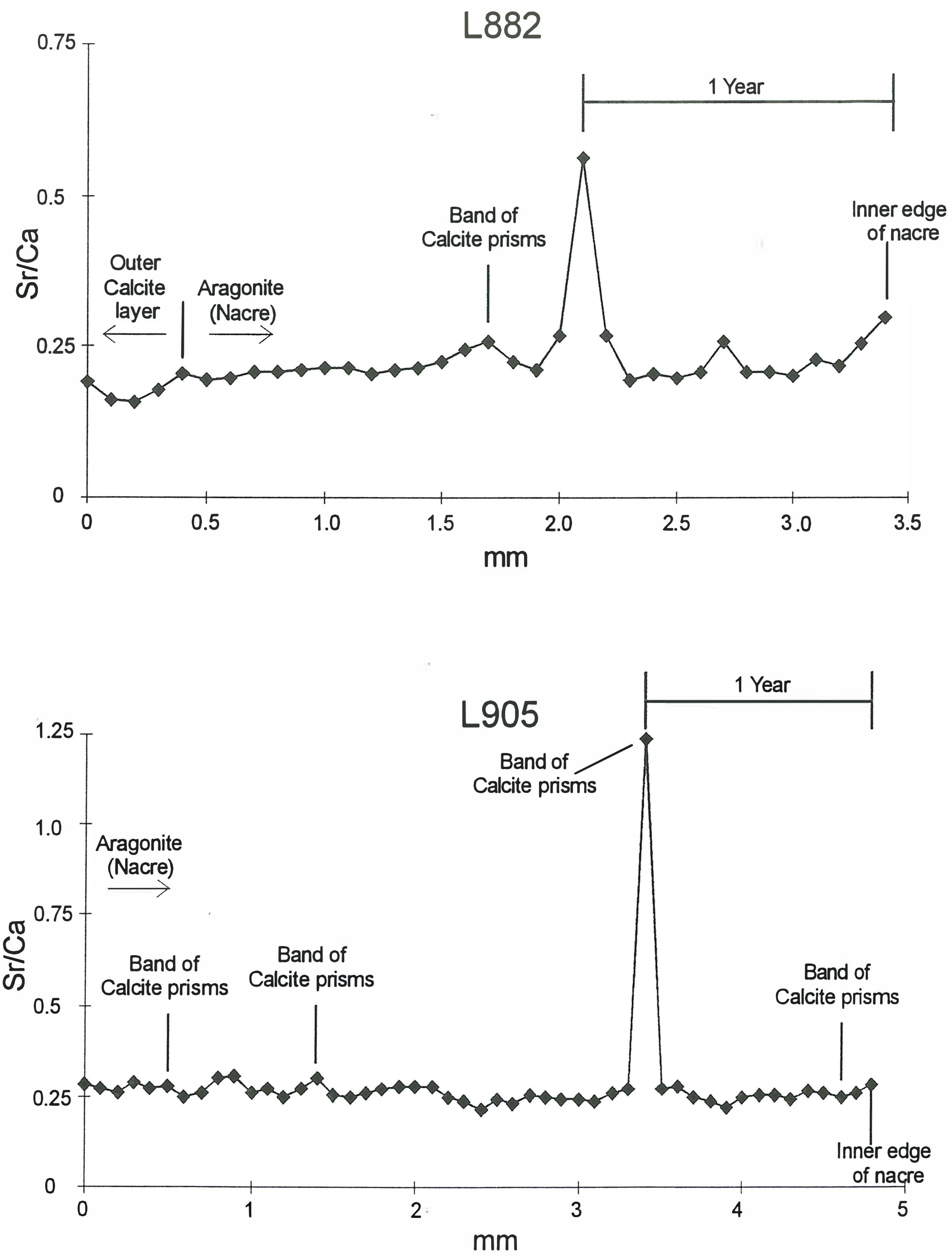
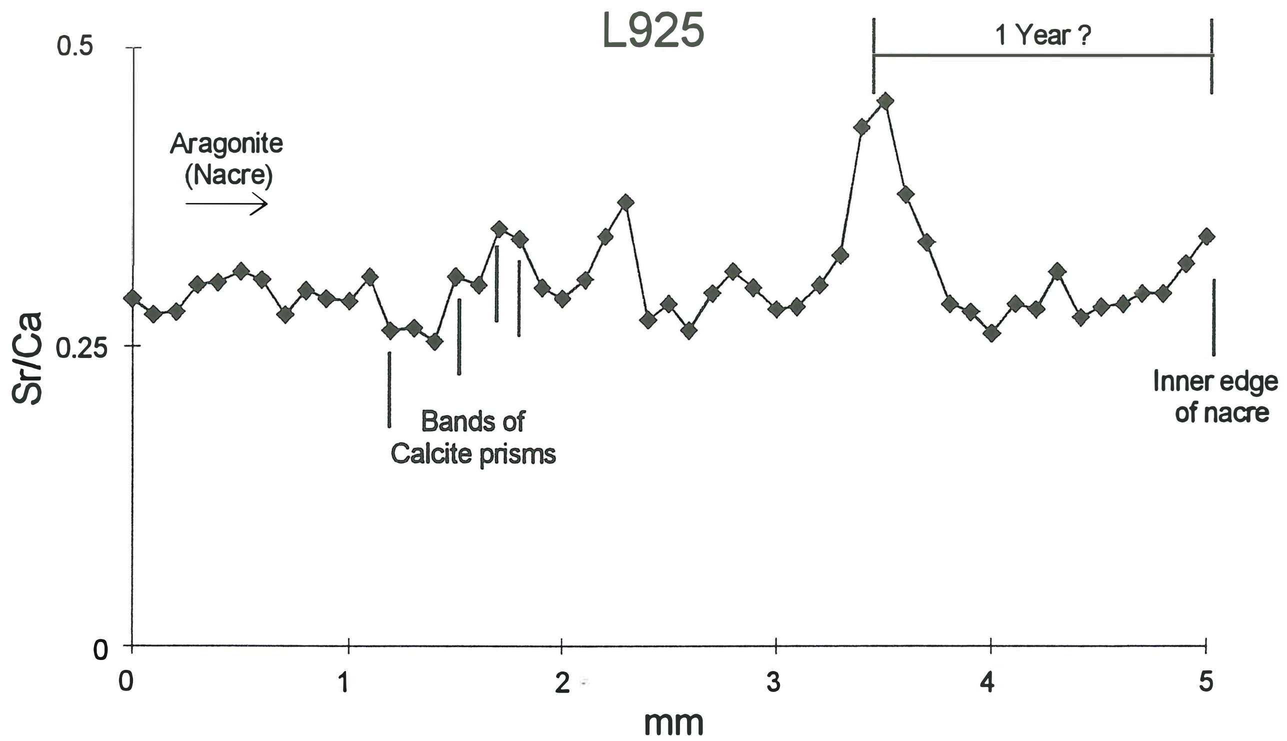
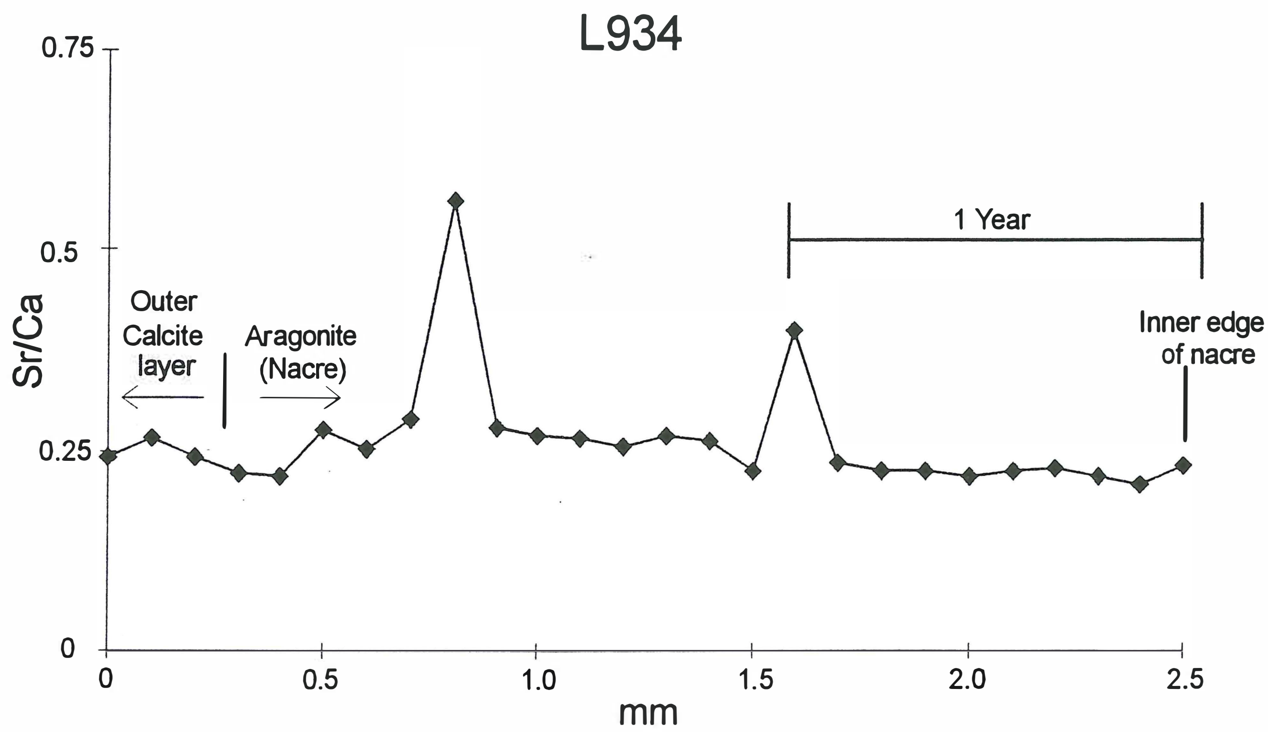


Fig. 14 (Cont'd) Strontium-calcium ratios at points across the thickness of strontium chloride-treated pearl oysters at liberty for one year.





point associated with marking. However, the presence of thin bands of calcite at other points in the shell thickness indicates that a change in the nature of the crystal morph deposited, from aragonite to calcite can occur naturally. The association of mildly elevated strontium-calcium ratios with some of the calcite bands may indicate a greater ability of strontium to be incorporated in the crystal lattice of calcite. However, for those samples where part of the calcitic outer layer of the shells was incorporated in the path of the laser ablation sampling (L882, L934), there was no indication of generally elevated strontium-calcium ratios in the calcitic layer.

The other outcome from the ICPMS analysis was that the strontium-calcium ratios over the period from the time of strontium exposure to the time of recapture 12 months later did not appear to show any clear cycle. Also, although all individuals figured were between 97 and 118 mm S.H. at the time of exposure (which, based on the tagging data, would make them 2+ to 3+ years of age) there were no obvious cycles in the Sr/Ca ratio in the pre-exposure shell which corresponded with the estimated age of the shells. Consequently, while the method of putting a chemical 'time-stamp' into the shells was successful, the hoped-for cycle in the strontium-calcium ratio, which could be used to infer age, was not apparent.

b.) Marking of pearl oysters shells with visual markers.

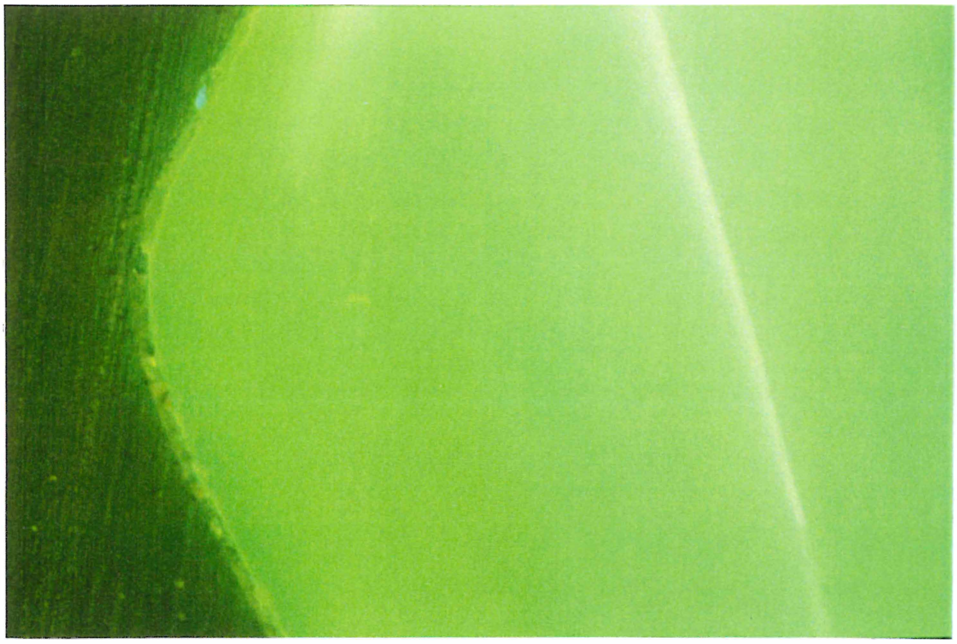
Recaptures of oxytetracycline-injected pearl oysters were made in 1994 and 1995 (Table 7). Examination of injected oysters showed a mark which was visible as a fluorescent band at magnifications over 10x under ultra-violet illumination (Fig. 15a). However, some specimens (e.g. Fig. 16a) showed multiple fluorescent bands, although one band was usually much stronger and probably resulted from the oxytetracycline injection. Examination of the same sections under illumination in the visual spectrum (Figs.15b, 16b) showed that the fluorescent band was usually associated with some obvious discontinuity in the shell and that this discontinuity often included a change in crystal morph from aragonite to calcite. The causes of multiple fluorescent bands in the tetracycline-treated pearl oysters is unknown, but may be related to the same circumstances which give rise to the additional elevated strontium bands detected in the strontium-calcium ratio analysis, as these are also often associated with discontinuities.

Table 7. Releases and recaptures of oxytetracycline-injected pearl oysters.

Released	Recaptured	
1993	1994	1995
298	38	61

Fig. 15 Single oxytetracycline flourescent band in the shell of pearl oyster K187 one year after marking by injection.

a. Viewed under ultraviolet illumination.



b. Viewed under visible light illumination. Note the discontinuity in the shell associated with the flourescent tetracycline band.

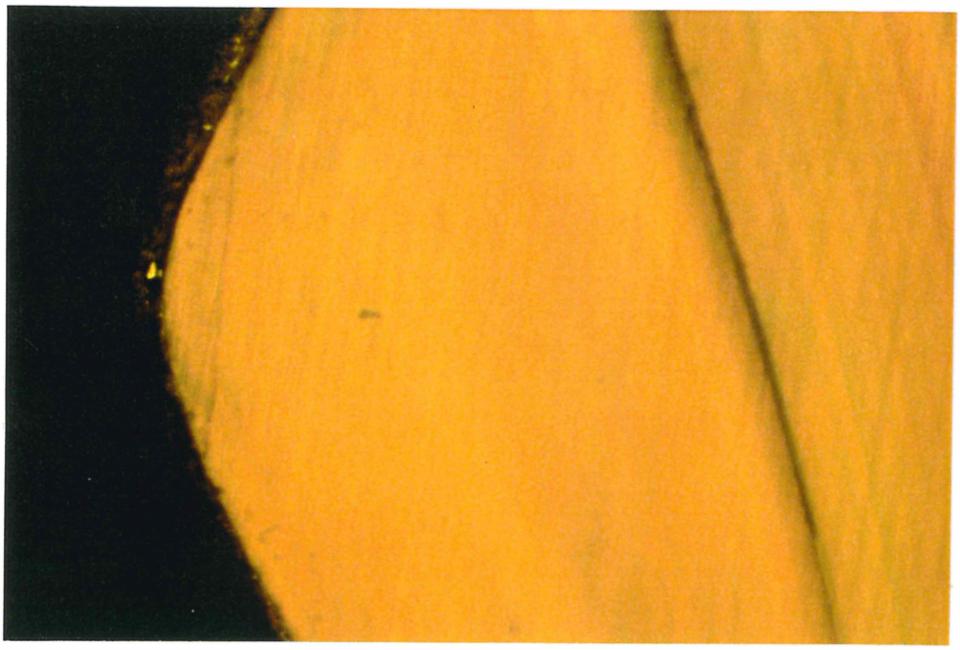
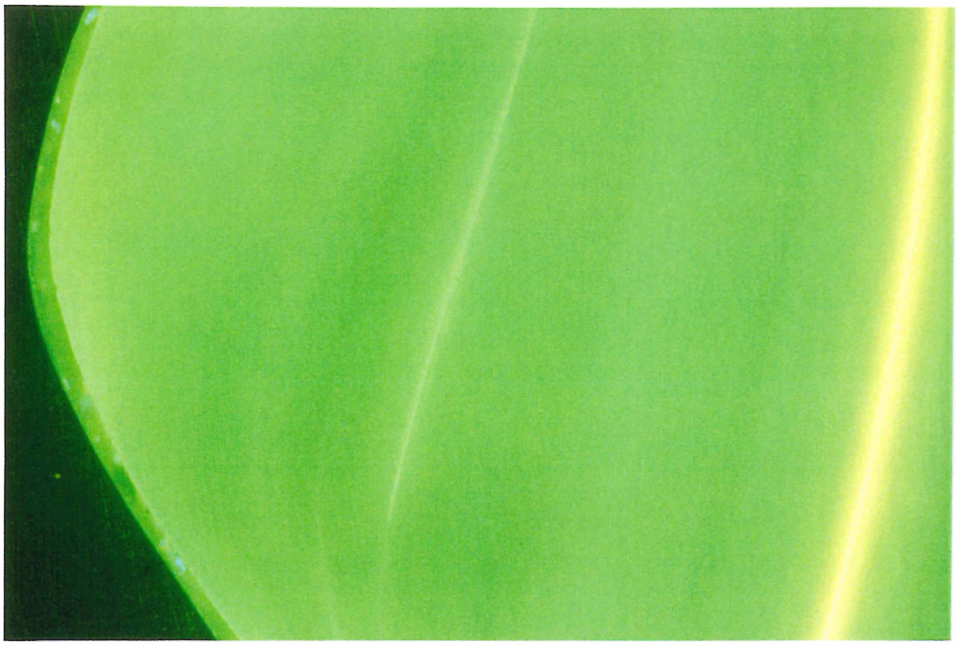


Fig. 16 Multiple flourescent banding in the shell of pearl oyster K314 one year after marking by injection.

a. Viewed under ultraviolet illumination. The thicker, brighter band is believed to be the oxytetracycline mark and the thinner, weaker flourescent band from some other cause.



b. Viewed under visible illumination. The bright (oxytetracycline) band is associated with the slightly darker band in the shell near the edge of the photograph, while the thinner, weaker flourescent band is associated with the the very dark line in the centre of the photograph.

However, although it was possible to put a 'time-stamp' into pearl oysters using oxytetracycline, there was a problem in visualising this 'time-stamp' on the same scale and under the same illumination as that under which the macroscopically visible banding can be seen. The necessity to view the oxytetracycline band at a fairly high magnification caused the macroscopically visible banding to be subsumed in the detail of the surface structure of the polished face of the cut shell. Although it might be thought that the causes and nature of the macroscopically visible banding in pearl oyster shells would be more apparent at these higher magnifications, this was not the case and they were effectively invisible at the magnification required to locate the fluorescent oxytetracycline band. This begs the question as to the cause of the macroscopically visible banding in pearl oyster shells. It may be that this dark and light banding is the result of marginally different thicknesses in the protein matrix in which the nacre crystals grow and, while this produces a macroscopically visible difference in shading, it is not amenable to viewing at the relatively low magnification of a light microscope. At the time of preparation of this report a method of marking the position of the fluorescent band in a visible form on the shell surface, which could then be viewed with the macroscopically visible bands had not been achieved. However, it is hoped that a small amount of further laboratory work will develop this technique to the point where it can be applied to the calibration of the macroscopically visible banding.

Sub-programme B

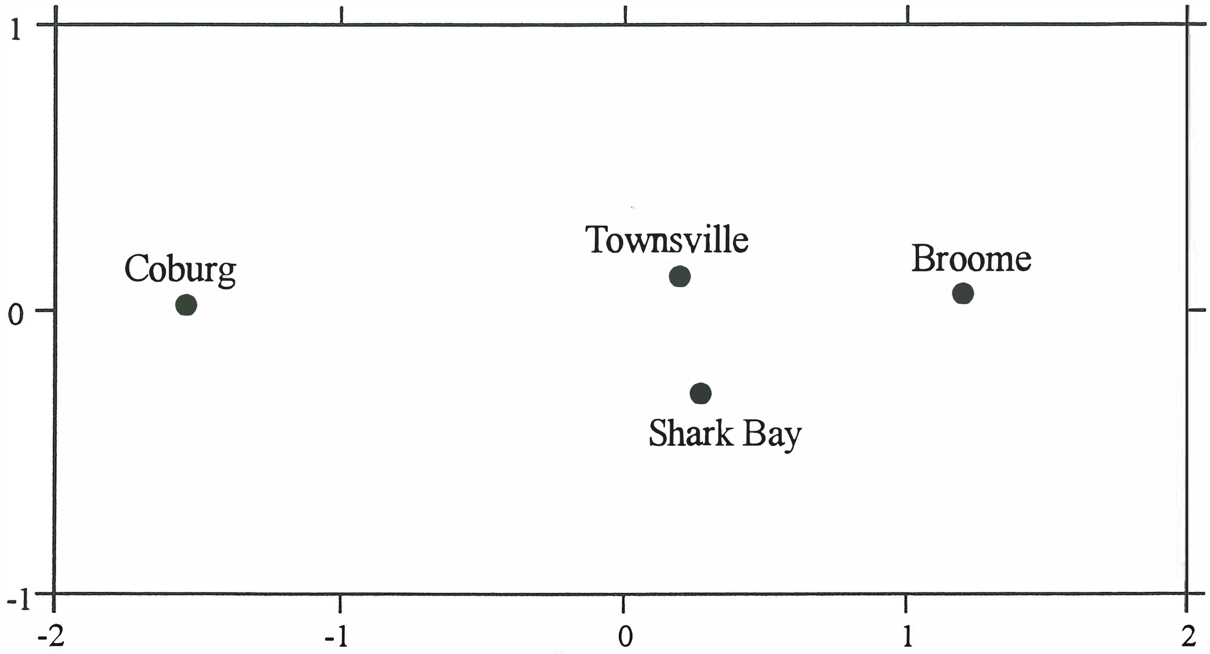
1. Genetic status of populations of *Pinctada albina*

Each of the samples was polymorphic for all eight loci examined. However, because of the small sample size from the Lacepede Channel area, the results were not included in the statistical analysis, although in general they were consistent with the overall results. Genetic divergence among the four other sites (Shark Bay, Broome, Coburg Peninsula and Townsville) was low, with only two of the eight loci showing significant variation. Despite this lack of variation, the combined tests across all loci indicate that there was an overall heterogeneity, with a mean F_{ST} of 0.017. The two sites showing the greatest divergence were Broome and Coburg Peninsula, with a pairwise F_{ST} of 0.039. However the two Western Australian sites showed a much lower level of genetic sub-division, with a mean F_{ST} of only 0.002.

Nevertheless, the apparent absence of genetic sub-division between Shark Bay and Broome does not represent a simple geographic pattern. The multi-dimensional scaling (Fig. 17) shows that the smallest genetic separation was between Shark Bay and Townsville, with these two sites being genetically intermediate between Coburg and Broome. These contrasts highlight the need for caution in interpreting these results. The finding of significant genetic differences among populations gives strong evidence for subdivision and the differences between Broome, Coburg Peninsula and Townsville indicate that these populations are substantially isolated from each other. However, the evidence for extensive connections between Shark Bay and Broome is more tenuous. While genetic differences between areas provides strong evidence for subdivision, failure to demonstrate genetic differences is weak evidence for mixing. The comparison between Shark Bay and Townsville demonstrates this point. Although separated by 5000 km, with intervening populations which are genetically divergent, these two sites were genetically the most similar. This similarity clearly does not indicate extensive mixing, but indicates the limitations of genetic data when divergence is slight.

The mean F_{ST} for *P. albina* from Shark Bay and Broome of 0.002 compares with a mean F_{ST} of 0.007 for *P. maxima* from Exmouth and Cape Bossut (Broome) (Johnson and Joll, 1993). Although the mean F_{ST} is lower for *P. albina* than for *P. maxima* over a similar distance, the lack of a consistent geographic cline for *P. albina* may indicate that, unlike *P. maxima*, the small genetic differences in the loci examined are not the result of genetic mixing. This would indicate that translocation of *P. albina* from Shark Bay to Broome could lead to some genetic impacts on the stocks of *P. albina* in the Broome area.

Fig. 17 Ordination of four geographically separated genetic samples of *P. albina*, based on non-metric multidimensional scaling of pairwise values of F_{ST}.



2. Disease Status of Shark Bay and Broome *Pinctada albina.*

Neither the macroscopic and histological examinations, nor the thioglycolate incubation revealed the presence of any pathological organisms in the samples from either Broome, the Lacepede Islands area or Shark Bay. The only organisms encountered in the Shark Bay sample were the larval tapeworm *Tylocephalum*, while the sample from the Lacepede Islands area contained one specimen with a rickettsia-like infection on the gills and another with possible *Vibrio* lesions in the digestive diverticulae. Samples of *P. albina* previously sampled by the Fish Health Section from a number of sites between Shark Bay and the Kimberley region found only rickettsial infections, the larval tapeworm *Tylocephalum*, and trematode sporocysts. Rickettsial infections are generally regarded as non-pathogenic and are widespread in bivalves. Trematode sporocysts are also widespread in bivalves and are usually non host-specific. They are also non-pathogenic, although they may cause castration by destruction of the gonad tissues. *Tylocephalum* larvae use many species of bivalves as intermediate hosts and they are ubiquitous in bivalves throughout the tropics. The adult stage infects the spiral valves of elasmobranchs which eat infected molluscs.

The parasitic organisms present in *P. albina*, both from Shark Bay as well as other sites in northern Western Australia, are non-pathogenic and common to a wide variety of bivalves - including *P. maxima*. On disease grounds, there would not appear to be a problem with the translocation of *P. albina* from Shark Bay to Broome.

BENEFITS

The benefits of this research programme will be felt in a number of areas. In the area of management of the pearl oyster stock the results provide a first estimate of the catchability of pearl oysters, which provides a means of interpreting the impact of the fishery on the abundance and density of the wild stock. The programme has also developed a workable methodology which could be used to repeat or enhance this first estimate of catchability. The growth and ageing studies have provided the first scientifically-based estimates of growth and age in pearl oysters for the Eighty Mile Beach area, the first estimate of the time lags between recruitment and entry to the fishery and the first estimate of the period of vulnerability of a year class to fishing mortality. The techniques aimed at the independent assessment of age provided a means of putting "time-stamps" in pearl oyster shells. Although the technique of calibrating possible time-related features in the shell subsequent to the "time-stamp" was not initially successful, the oxytetracycline marking method may become useable with further development. In the interim, the size-age relationship developed from the growth studies provides a means of estimating the age of pearl oysters from Eighty Mile Beach.

The benefits which will be derived from the recruitment studies will be more limited. At this stage the value of piggyback spat as a broad-scale indicator of levels of recruitment appears limited. This appeared to result from localised patchiness in the recruitment of pearl oysters and an inability within the available method to sample in a way which would properly reflect recruitment to the area as a whole. Because the method involved the use of commercial fishing vessels, most sampling in any year was focussed on areas which had had a strong recruitment three to four years previously, as these provide the best catch rate. In the Eighty Mile Beach area in particular, this produced an inconsistency in sampling areas from year-to-year and an inability to obtain a broad view of recruitment. However, the growth studies provide a means of understanding the timing of the entry of recruit groups to the fishery and the period of exposure of the recruits to fishing. This does provide some ability to respond to major changes in the level of recruitment at the time of entry into the fishery of a strong or weak year class. For this method to be of use to managers, however, there would need to be a more widespread collection of size-frequency data from the fishery to allow the catch rates of the various size (age) classes to be considered.

The small programme on the potential for translocation of *Pinctada albina* to Broome for use as a training species pointed to translocation being a possibility, although the results from the genetic studies were somewhat inconclusive. Further consideration of the genetic issues would need to take place before any formal decision on translocation from Shark Bay to Broome. However, the requirement for any translocation of *P. albina* will be dependent on the arrangements which industry is able to achieve for the supply of seeding technicians.

INTELLECTUAL PROPERTY

No intellectual property arose from this research.

FURTHER DEVELOPMENT

Further development of a research base for the pearl oyster fishery requires work in several areas. Firstly there is a need to develop an understanding of the growth rates of pearl oysters in the other areas of the fishery. The need for this is highlighted by the apparent differences in growth between the Lacepede Islands and Eighty Mile Beach areas, but it is likely that growth in the southern areas of the fishery is also different to that in the Eighty Mile Beach area. A knowledge of growth rates over the full range of the fishery is of vital importance in understanding the impacts of the fishery on the stock.

Given the difficulties experienced with recruitment monitoring using piggyback spat, a better understanding of the levels of recruitment - at least in the sectors of the stock being exploited - may be obtained through increased levels of monitoring of the size classes taken in the fishery. The growth data for Eighty Mile Beach provide a basis for interpreting the size-frequency data for the Eighty Mile Beach area in terms of age, but size-frequency monitoring for other areas of the fishery would need to be matched to growth information relevant to the area. Further development of the independent ageing methodology could provide a faster tool for determining the time periods from settlement to recruitment to the fishery and the period of exposure to fishing mortality for other areas, although the method may need to be independently calibrated for different areas.

The work on pearl diver efficiency/catchablity provided an objective means of estimating pearl oyster abundance and mean density. However, diver observations indicate that pearl oysters tend to be weakly aggregated, while the occurrence of piggyback spat in itself indicates that pearl oyster larvae may be attracted to settle on or around adult pearl oysters. These observations may indicate that minimising the degree of separation between oysters has some value, and the most obvious is that it increases the potential for successful fertilisation. Distance between spawning adults has been shown to have a major impact on fertilisation success in other sedentary invertebrates and the indications of a tendency to aggregate suggests it may be important in pearl oysters. The importance of the degree of separation as a factor in fertilisation success needs to be examined experimentally, along with the development of means to measure the densities, separations and sex ratios of adult oysters under natural conditions. An understanding of the importance of separation on fertilisation success will be critical if industry moves to fish large mother-of-pearl shell for use as seeding nuclei.

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FINAL COST

Fisheries Research and Development Corporation

Statement of Receipts and Expenditure for the period ending 31 December 1995

Name of Research Organisation	FRDC Project Number	Title of Project
Fisheries Department Western Australia	92/147	Stock evaluation and recruitment measurement in the WA pearl oyster fishery.

Budget Summary	1992-93	1993-94	1994-95	1992-95(1)
Original Budget	$ 141,042.00	$ 188,672.00	$ 214,162.00	$ 543,876.00
Current Budget(2)	$ 141,042.00	$ 188,672.00	$ 214,162.00	$ 543,876.00

Summary Receipts and Expenditure for the Project since commencement

	1992-93	1993-94	1994-95	1995-96
B/F	$ 0.00	$ 24,221.56	$ (48,088.15)	$ 55,941.05
FRDC Funds (Plus)	$ 141,042.00	$ 94,336.00	$ 254,958.00	$ 0.00
Expenditure (Minus)	$ 116,820.44	$ 166,645.71	$ 150,928.80	$ 2,661.15
Refunds(3)	$ 0.00	$ 0.00	$ 0.00	$ 0.00
Balance C/F	$ 24,221.56	$ (48,088.15)	$ 55,941.05	$ 53,279.90

Details Financial Year to 30 June 1996

Funds Available	Balance bought forward from previous year		$ 55,941.05
	Total funds received from FRDC during Financial Year 1995-96		$ 0.00
	Funds Available for Financial Year 1995-96(4)		$ 55,941.05
Allocation FY(5) 1995-96	**Less Expenditure**		
$ 0.00	Salaries	$ 0.00	
$ 0.00	Travel	$ 1,541.40	
$ 0.00	Operating	$ 404.75	
$ 0.00	Capital	$ 715.00	$ 2,661.15
$ 0.00	**Balance as at 31 December 1995**		$ 53,279.90

Notes:

(1) Use this column for the final ONLY regardless of the length of the project
(2) Total current budget shall not exceed Total original budget without approval in writing from the FRDC
(3) Refunds should only be paid at completion of the project together with the final audited statement.
(4) ACTUAL EXPENDITURE (whether cash or accrual) ONLY. Commitment shall not be included..
(5) Show allocation for the current financial year. Transfers between budget heads allowed under 9(f) of the Project Agreement, or approved in writing by the FRDC shall be listed in the comments.

Comments:

Project concluded at 30 June 1995. Statement includes expenses incurred prior to 30 June 1995 but paid in 1995-96 financial year.

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