ENHANCEMENT AND FARMING OF SCALLOPS IN NSW USING HATCHERY PRODUCED SEEDSTOCK.

by

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Enhancement and Farming of Scallops in NSW Using Hatchery Produced Seedstock.

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Project Title: Enhancement and Farming of Scallops in NSW Using Hatchery Produced Seedstock.
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OBJECTIVES:

1. To extend hatchery and nursery rearing techniques and equipment developed in an earlier grant (FRDC 91/53) to the production of triploid P. fumatus.
2. To compare the relative quality and production of diploid and triploid scallops in hanging culture and for reseeding enhancement of the Jervis Bay fishery.
3. To evaluate alternative methods of rearing scallops to a harvestable size using:
   I) Direct adhesion to tapes etc.
   II) Intermediate rearing in pearl cages and lantern cages and thence to bottom seeding, ear-hanging or second stage lantern cages.
   III) Intermediate bottom rearing of seeded scallops in predator proof cages.
4. To assess seeding and harvesting strategies for Jervis Bay using hatchery reared scallops.

NON TECHNICAL SUMMARY:

This is a report on the second stage of a two part program investigating hatchery production, farming and seeding of the commercial scallop, Pecten fumatus. A total of more than four million scallop spat were produced for farming trials and over 250 000 scallops ranging in size from 20-60 mm were released into Jervis Bay.

Hatchery techniques were broadened to include procedures for the production of triploid P. fumatus. In general, the survival of triploid scallops was considerably lower than that of normal (diploid) scallops, particularly, immediately after treatment and during early larval rearing. No differences were observed between triploid and diploid spat, however, juvenile triploid scallops tended to be larger and heavier with significantly larger muscle tissues. The significant reduction in embryo development percentages (>80%), the relatively low percentage triploidy achieved initially (41%) followed by consistent reductions in percentage triploidy, mean that further research is required before it would be applied to the commercial sector.

Although our previous research had, for the first time, demonstrated reliable large scale rearing of P. fumatus larvae was possible, several major constraints to hatchery production were noted. Previously, larvae were either retained in the hatchery until they were large enough to be transferred to land based upweller systems, or larvae were settled on mesh in collector bags and transferred directly to the field. A new technique was developed in which larvae were settled on mesh screens held in the hatchery for about a fortnight before being placed in mesh bags at known densities and moved to the field for culture. This technique allows control of spat stocking density, and has produced threefold increases in spat yields over previous bag settlement techniques while significantly reducing maintenance costs.

The bulk of hatchery produced scallop spat were reared in Jervis Bay using pearl and lantern cage techniques similar to those used overseas. While excellent growth has ensued, extensive trials were undertaken to evaluate alternative techniques for culture. In one of the most promising modifications, scallops were glued to plastic mesh disks which produced growth and survival equal to or greater than that of similar sized scallops cultured using the alternative techniques. Disk spacing was found to affect levels of predation, while the valve by which the scallops were glued was found to affect survival and meat weights, especially in the gonad and adductor muscle. Disk culture was also found to affect mudworm infestation of the shell when scallops were glued in particular orientations.

Preliminary seeding trials were undertaken to establish protocols for subsequent seeding attempts. Notably, several aspects of diver survey accuracy were assessed. The ability of P. fumatus to avoid detection was greater than had been expected, with the best of the trialed survey techniques (10 m long, 1 m wide transects) underestimating scallop densities by approximately 17.5%. Diver experience in scallop collection was not a factor in survey accuracy, however, experienced divers did perform surveys significantly faster. Subsamples of the seeded scallops were placed in cages and deployed at the time of seeding, which proved useful in estimating handling mortality and subsequent predation of the seeded scallops.

Attempts to seed scallops in Jervis Bay using thousands to tens of thousands of hatchery produced juvenile scallops were largely unsuccessful due mainly to predation. On one occasion, major apparent losses were attributable to dispersion from the seeded area over a period of 11 weeks, although more commonly all scallops were depleted within 6 weeks. There were no consistent affects from bottom type on scallop survival and seeding areas which had existing populations of wild scallops offered no apparent advantage. In these cases, populations of larger wild scallops remained unaffected while

naive seeded scallops were depleted rapidly. Size dependent losses were evident in most seedings with larger scallops (35-65 mm) often showing longer survival times.

This report describes the various predators and parasites encountered at several sites within three estuaries in NSW. While crabs, rays starfish and octopus were the among the most important predatory species, the impacts of other species are discussed. An ancillary finding of this study is that deployment of small naive scallops under protective plastic mesh developed by the horticulture industry limited losses to less than 1% per week. Research could, therefore, be extended using equivalent biodegradable mesh canopies to protect seed scallops until they lose their naivety and attain a size of about 65 mm, beyond which they are vulnerable to few predators.

The potential ecological impact of aquaculture and the paucity of information with respect to farming practices in NSW prompted an attempt to measure the accumulation of organic material beneath experimental longlines in Jervis Bay. Although the line increased the amount of organic material in the area, this increase appeared to remain within the assimilative capacity of the environment as no significant increases in organic material could be found in sediment samples taken in or around the site. Subsequent sampling, six months after the removal of the longline, found significant increases in organic loads in the sediment compared to earlier work. While this increase was a product natural processes within the bay, it gives some perspective to the scale of disturbance induced by experimental farming procedures.

Natural settlement of P. fumatus spat in Jervis Bay was monitored, which supported previous work (Fuentes et al., 1989) that despite several spawning events, significant recruitment occurred only in late winter to early spring. The intensity of this recruitment was also similar to that reported earlier and remained far too low for commercial farming purposes. This confirmed the need for a commercial hatchery if P. fumatus farming is to occur in NSW.

The research presented in this report has contributed to a continual process of improvement to the hatchery techniques developed in a previous FRDC grant (91/053). Specifically, triploidy induction, chemical induction of settlement, settlement substrates, spat transport and handling techniques have all been improved. These production techniques have been documented in a separate manual to be published in conjunction with the FRDC concurrent with this report (Project 94/084, Vol II).

KEYWORDS: Commercial scallop, hatchery production, farming, reseeding.

2.0 BACKGROUND AND NEED

In January 1990, NSW Fisheries submitted a research proposal to the FRDC (then FIRDC) entitled "A plan for reseeding and subsequent management of the commercial scallop (Pecten fumatus) in inshore waters of NSW (90/53)". FRDC did not endorse this proposal opting instead to fund a study tour to evaluate existing scallop fisheries enhancement and suspended culture operations in New Zealand and Tasmania before reconsidering the application. The major conclusions reached by this study tour (Williams, 1991) were that:

1) Scallop fisheries enhancement through reseeding is generally viable provided that the optimal seed sizes, depths, bottom substrates and depth etc, which are extremely site and species dependent, are predetermined ie. there is no standard recipe for successful enhancement programs.

2) Regardless of species and location, enhancement programs need to be of a scale involving the reseeding of millions or tens of millions of scallop per annum to be commercially viable.

On the basis of these generally optimistic findings an amended proposal "Enhancement and management of the commercial scallop Pecten fumatus fishery in Jervis Bay" was resubmitted to the FRDC in December 1990.

This modified proposal like its predecessor comprised two parallel R + D components ie.1 Field collection and spawning of ripe scallops from a wild population in Jervis Bay and subsequent fine tuning of hatchery and nursery production techniques, successfully trialed at the Brackish Water Fish Culture Research Station in May 1989 (Frankish et al., 1989).

Large scale pilot seeding and stock management trials in Jervis Bay to identify optimum combinations of time seeding; size and density of seed, depth, substrate type and location together with optimum size/age of harvesting.

In reconsidering this amended proposal, the FRDC recommended that the projects two components be split and run in series rather than in parallel, ie as two separate projects with the 2nd component (stock enhancement and management trials) being funding and implemented subject to completion of the hatchery and nursery development stage. The rationale for this recommendation was on the basis of two important reservations held by FRDC ie.

a) Uncertainty as to whether wild stocks in Jervis Bay did in fact constitute a predictable and reliable source of ripe ready to spawn stock and hence the possible need for research to develop reliable broodstock ripening protocols.

and

b) That the spectacular success achieved by the inaugural hatchery production trial with P. fumatus at PSRC in May 1989 Frankish et al. 1989 ran contrary to results of many previous attempts to hatchery rear this species by a number of competent commercial oyster hatcheries in Tasmania employing essentially identical equipment and rearing protocols.

In acknowledgment of, and response to, these reservations and recommendations, NSW Fisheries successfully resubmitted the application as a first stage hatchery and nursery development proposal Evaluation of hatchery production of scallops (Pecten fumatus) (91/53) in January 1991.

Results of the initial proposal have proven that FRDC reservations were well founded. Contrary to expectation, wild P. fumatus from Jervis Bay were found to be a chronically poor and unreliable source of ready to spawn broodstock (Heasman et al., 1994, 1995). This prompted extensive broodstock conditioning research that is now enabling rapid ripening and reliable spawning of broodstock throughout the year (Heasman et al., 1995). Standardised oyster hatchery rearing equipment so successfully applied in May 1989, also proved unreliable with yields of post settlement larvae generally falling within the range of < 1 to 5%. This in turn has prompted intensive and protracted research that has now exposed a range of fertilisation, incubation, larval rearing and settlement and nursery rearing constraints.

These constraints were systematically addressed and overcome paving the way for the second phase of the project.

The value and necessity of these specialist hatchery and nursery rearing protocols has been strengthened by findings of Fuentes et al. (1992) that capture rates of wild spat in Jervis Bay, averaging less than 36 per collector bag, fell far short of minimum commercially viable rates of 500 per bag (Cropp and Frankish, 1989).

It was proposed that the second (fisheries enhancement and management) phase of this project, as elaborated below, be expanded to include production of triploid scallops and to appraise alternative secondary nursery and suspended hanging culture farming techniques.

As stressed in the original application, scallop fisheries are notorious for large fluctuations and catches of P. fumatus in NSW are no exception. In recent times there have been two main booms in NSW scallop catches (1970-71 and 1981-82) separated by years of negligible catch. The most recent 1981-82 boom was solely the result of large catches in Jervis Bay where 2 800 tonnes (currently valued at $8 million) were harvested.

Research in Jervis Bay by NSW Fisheries between 1989 and 1993 (Fuentes et al., 1991; Worthington, 1992, 1993) reinforced the view that Jervis Bay constitutes a near perfect location for seeding trials and ultimately full blown enhancement of scallop stocks. Censuses of total stocks within the Bay in 1991 and 1992 by Worthington et al. indicated that scallops having reached a size of 40-60 mm as 1 + year olds (ie a size range commonly found optimal for reseeding) sustained negligible mortality during their subsequent growth to harvestable size of 70-90 mm as 2+ year olds.

This encouraging finding was in keeping with relatively high (@50%) survival rates previously reported for P. fumatus from Jervis Bay (Hamer and Jacobs, 1987) and Port Phillip Bay (Gwyther and McShane, 1988) and for strategically seeded scallops in Golden Bay New Zealand (Bull, 1989) and in Japan (Ito and Byakuno, 1989). In the latter case more than 150,000 tonnes of seeded scallops are produced annually. High survival of scallops in Jervis Bay is however in stark contrast with survival rates of < 1 to 1 0% for 40-60 mm P. fumatus seeded in Great Oyster Bay and the D'Entrecasteaux Channel in South Eastern Tasmania (J. Thomson and W. Zacharin Tasmanian Fisheries, pers. comm. 1993).

The proposed inclusion of triploid as well as diploid scallops in enhancement and farming trials in this study was based on consideration of a number of factors.

1) Triploidy in other scallop species such as Argopecten irradians has been shown to increase adductor weight by up to 73% over that of diploids (Tabarini, 1984). A secondary advantage of triploidy is that the gonad, which unlike muscle tissue is subject to accumulation of biotoxins from toxic algal blooms (Shumway, 1993), is small or insignificant in triploid scallops.

2) During the course of the first phase of the project, hatchery reared scallops held in lantern cages over summer and autumn grew at a mean rate of 2.9 mm per week. This was almost twice the fastest rates of about 1.5 mm per week recorded in summer for lantern cage reared P. fumatus in Tasmania (Cropp, 1985). Winter growth rates of scallops held in lantern cages in the extreme south of NSW have been found to be a

mean of 0.9 mm/week. Again this was much faster growth than rates of 0.33 mm/week reported for equivalent winter growth in southern Tasmania (Cropp, 1985) and a rate of 0.5 mm/week recorded in Port Phillip Bay (Sause et al., 1987).

3) Earhanging culture of scallops in Tasmania has been found to increase growth rate by 20-30% over that of bottom seeded scallops and to yield up to twice the saleable flesh (adductor muscle and gonad) yield of bottom seeded scallops of the same shell size and age (B. Horian, Tasmanian Scallop Co Pty Ltd Triabunna, pers. comm. 1993). Indeed live scallops grown by ear hanging command a premium price and position on export markets by virtue of their superior quality to either bottom seeded or lantern cage reared counterparts and are returning survival rates of 80-90%.

Preliminary trials involving the attachment of scallops to ropes and tapes using adhesives rather than conventional ear-drilling techniques were initiated in Twofold Bay in June 1993. This technique was originally tested with P. fumatus with encouraging results by Cropp (1985).

Hanging culture was further refined to enable the attachment of juvenile scallops as small as 20 or even 10 mm and to retain such scallops until they attain a harvest size of 70-85 mm. If successful, such a technique will eliminate the need of intermediate lantern cage nursery rearing of 10 to 20 mm spat to a size of 40 to 60 mm suitable for either conventional earhanging culture or for reseeding. It has been estimated that this could in turn reduce capital and operating costs of farming and stock enhancement by as much as 50%.

3.0 OBJECTIVES

a) To extend hatchery and nursery rearing techniques and equipment developed in an earlier grant (FRDC 91/53) to the production of triploid P. fumatus.

b) To compare the relative quality and production of diploid and triploid scallops in hanging culture and for reseeding enhancement of the Jervis Bay fishery.

c) To evaluate alternative methods of rearing scallops to a harvestable size using:
   I) Direct adhesion to tapes etc.
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d) To assess seeding and harvesting strategies for Jervis Bay using hatchery reared scallops.

4.0 RESEARCH METHODOLOGY, RESULTS AND DISCUSSION

4.1.1 INDUCTION OF TRIPLOIDY IN *PECTEN FUMATUS*

Introduction

Molluscs are diploid, each cell having two sets of chromosomes which may range in number from 10 sets for oysters and 14 sets in mussels to 19 sets in scallops of the genus Pecten (Beaumont and Fairbrother, 1991; Beaumont and Zouros, 1991). Through the use of chemical and physical stressors, it is possible to produce an organism with its genome number increased to three (Triploid) or four (Tetraploid).

In scallops, ploidy manipulation has been achieved using heat and pressure shocks, although the most commonly used techniques are chemical, generally involving cytochalasin B (CB)(Table 4.1.1). In most cases, zygotes are exposed to a dose of between 0.1 to 1 mg l^{-1} CB for 10 to 20 min, commencing 10 to 20 min after fertilisation. Commonly, CB exposure is followed by rinsing eggs with DMSO in seawater to remove residual CB (Tabarini, 1984; Beaumont, 1986; Baron et al., 1989; Komaru and Wada, 1990), a procedure that has been found to significantly improve survival of oyster zygotes (Allen et al., 1989).

Using chemical induction techniques, triploid P. fumatus were produced as part of this research to establish appropriate techniques and to allow an evaluation of the performance of triploid larvae. In particular the timing of CB administration, dose response and the value of DMSO rinses were assessed.

TABLE 4.1.1 Triploid induction in scallops.

Scallop species	Induction method	Treatment time and (duration)	Triploidy (%)	Determination method / ontogeny	Authors
Argopecten irradians	CB (0.1 mg l^{-1})	10 min (20 min)	94.0%	FC / 1-year-olds	Tabarini (1984)
Argopecten purpuratus	CB (0.5 mg l^{-1})	24 min (20 min)	17.0%	DC / embryos	Winkler *et al.* (1993)
	Heat shock (31°C)	10 min (10 min)	66.7%	DC / embryos	Toro *et al.* (1995)
Chlamys farreri	CB (0.5 mg l^{-1})	10 min (20 min)	50.0%	DC	Lu and Wang (1992)
	Cold shock (1°C)	17 min (20 min)	30.4%	DC / embryos	Wang *et al.* (1990)
Chlamys nobilis	CB (0.5 mg l^{-1})	15 min (15 min)	71.4%	DC / embryos	Komaru and Wada
	Hydropressure (200 kg cm^{-2})	20 min (10 min)	19.8 & 23.3%	" "	(1989)
Chlamys varia	CB (1.0 mg l^{-1})	20 min (15 min)	78.5%	DC / embryos	Baron *et al.* (1989)
Mimachlamys asperrima	CB (1.0 mg l^{-1})	16 min (10 min)	59.0%	FC / 5-day-old larvae	Unpub. data
Patinopecten yessoensis	Heat shock(29°C)	46 min (11 min)	26.7%	DC / embryos	Wang *et al.* (1990)
Placopecten magellanicus	6 DMAP (300-600 µM)	70 min (15 min)	95.0%	DC, IA / embryos	Desrosiers *et al.* (1993)
Pecten maximus	CB (0.5 mg l^{-1})	10 min (20 min)	30.0%	DC / embryos	Beaumont (1986)

FC = flow cytometry, DC = direct chromosome count, IA = image analysis of nucleus volume
CB = cytochalasin B, 6DMAP = 6-dimethylaminopurine

Materials and methods

Gamete production and larval rearing

Sexually mature *P. fumatus* were collected from Murrays Beach, Jervis Bay and spawning and fertilisation conducted at 18±0.5°C using the methods described by Heasman *et al.* 1995. To avoid problems associated with self-fertilisation in this hermaphroditic species (Heasman *et al.*, 1995), each batch of eggs was inspected upon release to ensure significant numbers of sperm were not present and then each batch was fertilised immediately. For these experiments, fertilisation is assumed to be instantaneous and the reported times post-fertilisation refer to the time at which sperm was added to egg suspension.

Seawater (35±1 g kg^{-1} salinity) for all triploidy induction and larval rearing was filtered (1 µm nominal) and treated with 1 mg l^{-1} EDTA (Utting and Helm, 1985) as a precaution against metal contamination. For each experiment, each replicate was stocked at 10 embryos ml^{-1} in individual 8 L aerated aquaria maintained at 18±0.5°C. Sea water in each aquaria was exchanged every 48 h, with the larvae retained on 20 µm nylon mesh sieves.

Initially, ploidy of *P. fumatus* was determined after 16 h by chromosome counts on trochophores, but for experimental purposes ploidy was assessed at day 5 by flow cytometry on D-veligers. In all experiments, survival was determined at day 2 on the basis of the number of embryos developing to D-veliger stage. This was determined from four replicate 10 ml samples taken immediately after the contents of each 8 L aquaria had been thoroughly mixed using a perforated plastic plunger. The samples were placed on Petri dishes and the numbers of D-veligers counted with the aid of a dissecting microscope.

In each experiment, fertilised eggs were treated with a stock solution of CB (Sigma-Aldrich, Castle Hill, NSW, Australia) dissolved dimethylsulfoxide (1mg CB ml^{-1} DMSO) (Downing and Allen, 1987). Following exposure, eggs in all experiments were rinsed in filtered seawater.

Ploidy examination

Ploidy in *P. fumatus* trochophores was determined by direct counts of metaphase chromosomes from 50 randomly chosen cells from trochophores stained with Giemsa's stain (Gurrs improved R66, BDH chemicals, Kilsyth, Australia). Using techniques modified from those of Nell *et al.* (1996), approximately five thousand 16-hour-old trochophores were placed in 10 ml of 0.01% colchicine in seawater for 7 h. The embryos were then centrifuged and 9 ml of colchicine solution removed and replaced with 0.7% sodium citrate solution. After 10 min, 1 ml of Carnoy's solution (3:1 absolute methanol: glacial acetic acid) was added for 2 min, before larvae were fixed in full strength Carnoy's. Fixed samples were then refrigerated.

Trochophore samples were pippetted up and dropped onto clean microscope slides and allowed to air dry. Each slide was stained for 10 min in a 10% Geimsa in phosphate buffered saline solution, then rinsed with distilled water and air dried. Chromosomes were counted using a light microscope (400x).The ploidy classification used was as follows: <36 haploid, 36-40 diploid, 41-53 aneuploid, 54-60 triploid. The range of chromosome numbers for each class were broadened to account for artificial chromosome losses and cell overlap (after Guo *et al.*, 1992; Shen *et al.*, 1993; Nell *et al.*, 1996) (Fig. 4.1.1).

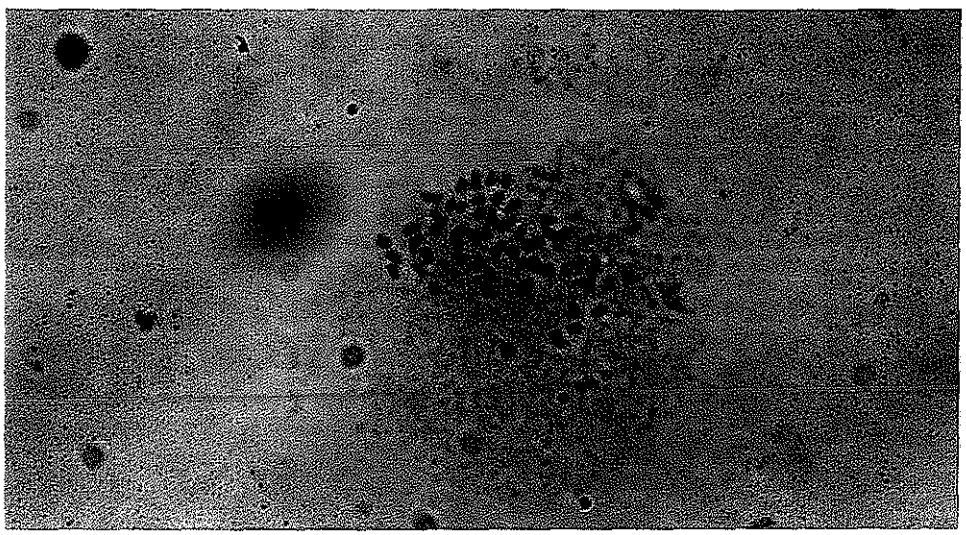

Figure 4.11 Photomicrograph of Giemsa stained chromosomes from a *Pecten fumatus* trochophore (3n)

Percentage triploidy in 5-day-old D-veligers was determined by crushing approximately 10 000 larvae in a clean glass Petri dish using a 5 ml rubber tipped syringe plunger. The crushed larvae were resuspended in 2 ml of phosphate buffered saline solution (pH 7.0) and any debris allowed to settle. A small volume of the supernatant (1-2 ml) was then allowed to drip through 5 µm nylon mesh into a 15 ml Falcon tube. Ten min before cytometric analysis, 0.5 ml of a solution of 250 mg l^{-1} propidium iodide in 1% Triton X 100 was added to the sample. The samples were then gently mixed and kept refrigerated until analysis. On each occasion a diploid sample was included to locate the position of the diploid peak (Fig. 4.1.2).

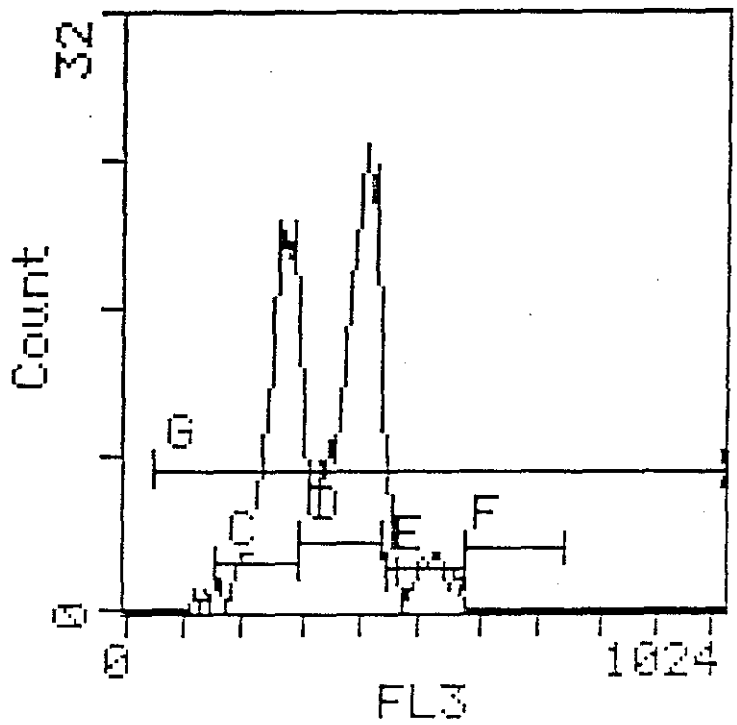

Figure 4.1.2 Example of plots of *Pecten fumatus* larval ploidy generated by flow cytometry illustrating diploid and triploid peaks.

Experiment 1: Effect of timing of commencement of exposure to cytochalasin B

The effect of timing of commencement of a 10 min exposure to CB (1mg l^{-1}) on triploid induction was tested at seven different times, 10, 20, 25, 30, 35, 40 and 45 min post-fertilisation, however replicated trials were only conducted at 20, 25, 30 and 35 min post-fertilisation. One sample was taken from each of the four treatments for ploidy determination to allow comparison of percentage triploidy in trochophores with those

of 5-day-old larvae.

Experiment 2: Effect of exposure to differing concentrations of cytochalasin B

The effect of CB concentration on percentage triploidy and larval survival was tested using the three concentrations suggested in the literature for other pectinid species (0.1, 0.5, and 1.0 mg l^{-1} CB; Table 4.1.1). Three replicate batches of *P. fumatus* zygotes were exposed at each concentration for 10 min, commencing 30 min post-fertilisation. A control treatment (n=3) of fertilised eggs from the same parents were treated in the same fashion without CB exposure.

Experiment 3: Efficacy of DMSO rinses following cytochalasin B exposure

From a mass spawning of 35 scallops, a single batch of 100, 000 eggs was taken from each of six individual scallops, The eggs in each batch were immediately fertilised by the addition of small quantities sperm suspension and then inspected using a microscope to ensure sufficient sperm were present and that eggs had no obvious deformities. These batches of eggs were then pooled and the eggs thoroughly mixed using a perforated plastic plunger. Eight 200 ml aliquots of pooled egg suspension (200 eggs ml^{-1}) were separately treated with 1mg l^{-1} CB for 10 min, commencing 30 min after fertilisation. Four aliquots were then rinsed in filtered seawater and resuspended in the 8 L aquaria. The remaining four aliquots were resuspended in 0.1% DMSO for 15 min to remove residual CB, before rinsing and resuspension in separate aquaria. After 48 h, the number of D-veligers in each aquaria was estimated from counts of D-veligers in four replicate 10 ml samples. Ploidy of larvae each aquaria was determined on day five using flow cytometry.

Statistical analysis

When necessary, percentage triploidy and development data was arcsine $x^{0.5}$ transformed to satisfy requirements for homogeneity of variance before analysis and in all cases homogeneity was confirmed using Cochran's test (Winer, 1971). Data was analysed by ANOVA (Sokal and Rohlf, 1981) and where significant differences were detected, means were compared using an SNK test (Winer *et al.*, 1991).

Results

Induction of triploidy by CB caused abnormal development in some larvae which consistently resulted in greater mortality of treated larval batches compared to untreated diploid controls. Affected trochophore frequently displayed gross morphological changes, while D-veliger larvae often displayed an erratic spiralling motion. In addition, veliger larvae from high concentration treatment groups frequently had bent hinges, notched valves and/or velum deformities.

Experiment 1 - Effect of timing of commencement of exposure to cytochalasin B

The timing of the CB treatments significantly effected percentage triploidy when assessed in 5-day-old larvae (F= 7.237, df 4/10, P<0.005). Among the treatment times tested (Fig. 4.1.3), the highest triploidy percentages for larvae (41%) were achieved by the addition of CB at 30 min post-fertilisation.

Limited comparisons of trochophore triploidy percentages based on direct observation of chromosomes with those derived flow cytometric analysis of 5-day-old larvae (Figure 4.1.3) showed a consistent trend for reductions in triploidy percentages in 5-day-old larvae. However, flow cytometric analysis indicated a triploid percentage of approximately 4% in 5-day old batches of diploid control larvae when chromosome counts of diploid batches had failed to find any triploid larvae. Never the less, triploidy percentages from the two ploidy determination methods were significantly correlated (r= 0.95, P<0.05).

In comparison to controls, the percentage yield of D-veligers from eggs was significantly reduced in all CB treated replicates (F= 64.998, df 4/10, P<0.001), however, no significant differences could be detected between CB treatments (SNK, Table 4.1.2). *A posteriori* power analysis of the percentage of eggs developing to D-veliger stage following exposure to CB, indicated insufficient replication for ANOVA to adequately test for treatment effects (β = 0.26 interpolated from Searcy-Bernal (1994), α= 0.05, k=4, n=3). D-veliger yield was not correlated with triploid percentage (r= 0.41, P=0.17).

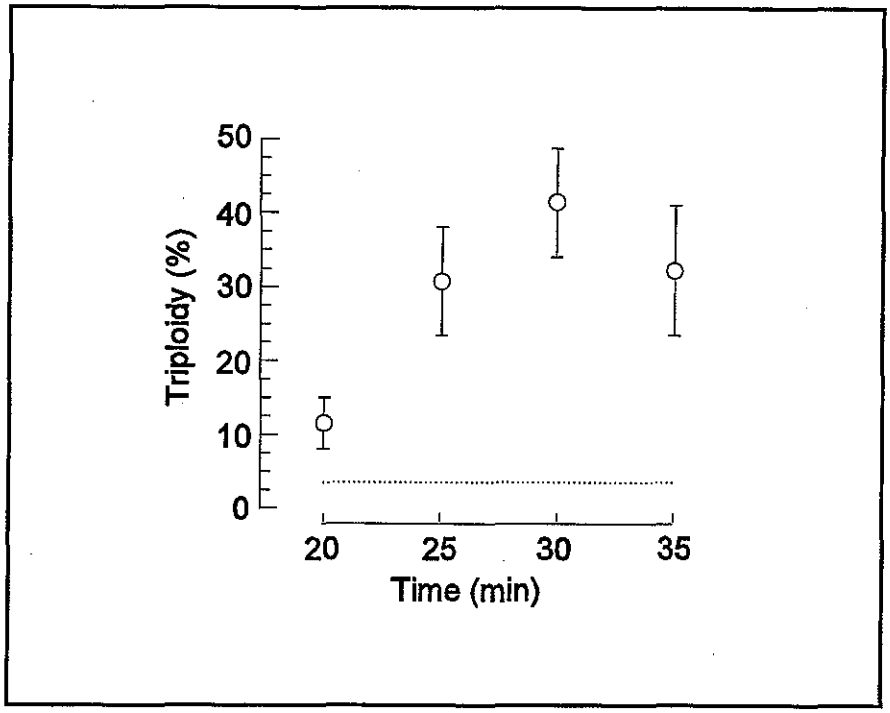

Figure 4.1.3 The effect of the timing of cytochalasin B administration on percentage triploidy in trochophores and 5-day-old larvae. n=3 for values with SD bars

Experiment 2: Effect of exposure to differing concentrations of cytochalasin B

CB concentration significantly effected the percentage triploidy observed in 5-day-old larvae (F= 12.72, df 2/6, P<0.01). Of the concentrations tested (Table 4.1.2), the highest triploidy percentages in 5-day-old (45%) larvae were achieved by the addition of CB at 0.5 mg l^{-1}, although mean percentage triploidy in this treatment was not significantly higher than observed in either embryos or larvae treated with 1.0 mg l^{-1} CB (41%). The mean percentage triploidy observed in both 1.0 and 0.5 mg l^{-1} treatments, significantly exceeded the percentage triploidy in larval batches treated with 0.1 mg l^{-1} CB, although the percentage of eggs developing to D-veliger stage was unaffected by CB dose (F= 0.842, df 2/6, P=0.48).

TABLE 4.1.2 Effect of cytochalasin B concentration on triploidy induction in *Pecten fumatus*.

Cytochalasin B ($mg\ l^{-1}$)	Day 5 triploidy (%)	D-veliger yield* (%)
0.1	20 ± 2^{b}	12 ± 2^{a}
0.5	45 ± 2^{a}	10 ± 1^{a}
1.0	40 ± 11^{a}	10 ± 2^{a}

[1] Values are means ± sd (n=3). Within columns, means with common superscript letters do not differ significantly ($P<0.05$).

* D-veliger yield is the percentage of eggs developing to D-veliger stage after 48 h.

The percentage yield of D-veligers from eggs was significantly reduced in all CB treated replicates in comparison to controls ($F= 20.977$, df 3/8, $P<0.001$), however, no significant differences could be detected between CB treatments (SNK, Table 4.1.2).

Experiment 3: Efficacy of DMSO rinses following cytochalasin B exposure

No significant differences were detected between DMSO rinsed and non-rinsed treatments in the number of eggs developing to D-veliger stage (8.0 and 8.1% respectively). Power analysis however confirmed that in both cases the power of these experiments were particularly low ($\beta >0.05$) and that impractically large numbers of replicates (>100) would be required to detect any differences that may exist.

Discussion

The chromosome number in *P. fumatus* was determined to be 2N=38 and as such was the same as has been determined for other members of this genus such as *P. maximus*, *P. albicans* and *P. jacobeus* (Beaumont and Fairbrother, 1991; Beaumont and Zouros, 1991). This consistency in chromosome number for the genus pecten has previously been noted (Beaumont and Zouros, 1991) however, it is not necessarily typical of all pectinids. 2N= 38 has been the most commonly reported number, but members of the genera *Chlamys* and *Mimachlamys* vary greatly.

Triploidy percentages (a mean of 41% in Expt. 1) were generally lower than those reported for chemical induction techniques with other species, although they were greater than had been reported with *Pecten maximus* using very similar techniques (Beaumont 1986). It should however be noted that ploidy determinations were made on veliger larvae and not embryos. Previous experience, that embryo ploidy determinations gave rise to significantly greater triploidy percentages than assessments made on 5-day-old larvae (Nell *et al.*, 1996; O'Connor and Heasman unpublished data, 1997), was confirmed. Embryo ploidy levels in *P. fumatus* were between 5 and 15% greater than those detected by flow cytometry. It has been suggested that the explanation for this reduced survival may be high mortality in triploid larvae *per sé*. There appears to be little doubt that CB treatment is stressful with this and other studies on molluscs (Utting and Doyou, 1992), including pectinids (Beaumont, 1986; Baron and Diter, 1989; Beaumont and Fairbrother, 1991), showing CB treatment reduces larval survival. Utting and Doyou (1992) found that in comparison with untreated Manila clam (*Tapes philippinarum*) embryos, CB treated embryos (diploids and triploids) used approximately twice the amount of lipid reserve to reach D-veliger stage.

Alternatively, the variation in percentage ploidy with ontogeny could arise wholly or partly from the fact that direct chromosome counts on shelled larvae are difficult (Gérard *et al.*, 1991; R. E. Hand, pers. comm., 1996), and as a result, different ploidy determination techniques were used for each ontogenetic stage. Both methods of ploidy determination used here have potential problems which may lead to inaccuracies. Overlapping of ruptured cells during chromosome staining may elevate estimates of triploidy and might explain the aneuploidy levels observed in Experiment 1, although aneuploids have often been associated with triploids (Yamamoto *et al.*, 1988; Guo *et al.*, 1992; Nell *et al.*, 1996). On the other hand, flow cytometry requires arbitrary decisions to be made on the proportions of peaks attributed to the various ploidy levels and throughout this study, flow cytometry was seen to be sensitive to sample contamination. Recently, differences in the results obtained by the two methods have

been found when used concurrently with *S. commercialis* spat (G. McMahon, pers comm., 1997), fostering a reticence to attribute the difference in survival at day 5 solely to triploid mortality.

Regardless, there remains scope for improvement in triploidy percentages. Alternative techniques involving chemicals such as 6 dimethyl amino purine (6 DMAP) were beyond the scope of this research, and the variables associated with the use of CB have not been exhausted. In particular, the duration of CB exposure was selected on the basis of literature reported values and the efficacy of alternative (particularly lengthened) exposure times were not evaluated.

The optimum among the tested CB concentrations (0.5 and 1.0 mg CB l^{-1}) for triploidy induction in *P. fumatus* are similar to used for most pectinids (Table 4.1.1) and are generally within the range of CB concentrations suggested for triploidy induction in other bivalves, such as the pacific oyster (0.5-1.0 mg CB l^{-1}, Yamamoto *et al.*, 1988; Allen *et al.*, 1989).

Although this study used more replication than other studies (Quillet and Panelay, 1986; Yamamoto *et al.*, 1988, 1990; Desrosiers *et al.*, 1993; Gérard *et al.*, 1994), it still lacked statistical power. Typical minimum significant difference ($P<0.05$) values ranged from 10-30%. Variation among replicates was also a major problem for Downing and Allen (1987). More replication may be needed if better resolution of optimum concentration is to be achieved. Similarly, it would be difficult to establish the efficacy DMSO rinses.

Despite the commonality of DMSO rinses following exposure to CB and the significant increase in survival of oyster embryos treated in this fashion (Allen *et al.*, 1989), percentage development of *P. fumatus* embryos was not apparently increased by the additional rinse. Similar results have been observed with *Mimachlamys asperrima* and as was suspected then, there remains the possibility that the additional handling may negate any positive effects of DMSO rinse. In particular, *P. fumatus* embryos have responded poorly to excessive handling, especially to dry screening (where embryos held on screens and are not suspended in water) which occur during the standard rinsing procedure.

4.1.2 PERFORMANCE EVALUATION OF TRIPLOID *PECTEN FUMATUS*

Introduction

With the advent of simple triploid induction techniques for a variety of bivalves, numerous studies have been conducted to evaluate the performance of triploid stock. This has been particularly so for oysters, where triploids have been shown to differ in various growth and reproductive characteristics (Nell and Maguire, 1994). However, despite the number studies demonstrating the capacity to induce triploidy in pectinids (Table 4.1.1), there has been a dearth of information with respect to the subsequent performance pectinid larvae, spat, juveniles and adults. The most notable exception has been the study of Tabarini (1984).

The induction of triploidy has been purported to have several potential advantages. The inability of homologous chromosomes in cells of adult triploids to synapse during cell division can produce sterility (Tabarini, 1984). In scallops, including *P. fumatus*, meat yields vary seasonally in accordance with the reproductive cycle as energy is transferred from the muscle to promote gonadogenesis (Barber and Blake, 1981). As a result, sterile adults should grow faster as less energy is diverted to reproduction (Tabarini, 1984). This was particularly evident in *Argopecten irradians*, where triploid scallops had 36% heavier total body tissue weights and 73% heavier muscle weights than diploid controls (Tabarini, 1984). Other advantages of triploidy may also include increased heterozygosity, the ability to introduce sterile organisms to sensitive areas and a reduced potential for accidental introductions (Beaumont and Fairbrother, 1991).

Following the development of techniques for producing triploid *P. fumatus*, a batch of triploid scallops was produced and ongrown to 50 mm. In part this batch was produced to evaluate the comparative performance of diploid and triploid *P. fumatus* during culture, while also making triploid scallops available for reseeding trials if appropriate techniques could be developed.

Materials and methods

General

Approximately 40 *P. fumatus* broodstock collected from Jervis Bay were reproductively conditioned for a period four weeks using the techniques described by Heasman *et al.* (1995). These scallops were then removed from the conditioning system and left

emersed for 30 min at ambient temperature (approx. 22°C) before being placed on a spawning table in 10 cm of water (16°C). Four of the 40 scallops were injected with 0.05 ml of 10-3 N serotonin solution (creatinine sulfate, Merck, Darmstadt, Germany) to induce sperm release before all the scallops were subject to a water temperature increase of 3°C. The four injected scallops commenced sperm release within 25 min and an additional nine scallops subsequently released eggs. Within 10 min of egg release, the eggs from a minimum of four scallops were pooled and then fertilised using sperm suspension pooled from each of the four serotonin treated scallops. Four batches of approximately 2×10^6 eggs were collected and treated with cytochalasin B as described previously (Section 5.1.1, ie 1 mg/L CB for 10 min commencing 30 min after fertilisation, DMSO rinsed). The remainder of the fertilised eggs from the spawning, that were not treated with CB, were pooled and placed in a separate 1000 L tank.

Each batch of CB treated eggs was placed in an individual 1000 L tank and cultured for five days. Ploidy was then determined individually for each batch of treated larvae using flow cytometry (Section 5.1.1). The four batches of treated larvae were then combined and transferred to a 20 000 L tank for culture through to settlement. Due to equipment limitations, diploid larvae were retained in 1000 L tanks through to settlement.

Before metamorphosis (Day 17), pediveligers were removed from both the 1000 and 20 000 L tanks and transferred to separate groups of 160 µm nylon mesh screens at a density of approximately 1.0×10^5 screen^{-1} and held in 1700 L downwelling systems. The number of spat surviving in both treatments was estimated one week later and again approximately one month later. On these occasions, spat attached to the wall of the screen were gently brushed from on to the mesh surface before the screen was placed on a 1 cm grid drawn on clear plastic. The number of spat in each of 20 randomly chosen squares was counted and used to estimate total spat numbers per screen (total screen area = 1590 cm^2).

N.B. The specific techniques for larval culture, settlement and nursery culture are described in detail in the hatchery manual produced in conjunction with this report.

Growout

Five weeks post settlement, both diploid and triploid spat were transferred on 1000 µm mesh screens to an outdoor upweller system at Wanda Head and maintained using ambient seawater. After six weeks the scallops were then placed in pearl cages and placed in oxygenated fish transport tanks for translocation to Jervis Bay. Growout of both diploid and triploid scallops was completed on a subsurface longline at Murrays Beach.

Ploidy determination

In this study, initial attempts to determine scallop ploidy were made using direct counts of metaphase chromosomes in colchicine treated gill tissue. This technique had previously been used successfully with both Pacific oysters, *Crassostrea gigas*, (Allen *et al.*, 1989) and Sydney rock oysters, *Saccostrea commercialis* (Roz Hand pers. comm., 1997) and offered the advantage of the detection of mosaicism of ploidy. Regrettably, despite considerable effort, we were unable to routinely achieve acceptable chromosome slides using either gill or mantle tissue from *P. fumatus*.

Direct counts were replaced with flow cytometry for ploidy determination. For each scallop, a piece of gill tissue approximately 10 mm^2 was excised and placed in 1 ml 10% DMSO in marine PBS and then frozen. The tissue and PBS were then thawed before the sample was centrifuged for 1 min at 2000 rpm. The supernatant was discarded and replaced with 0.75 ml of propidium iodide (PI) solution made as follows. Combine 2 parts CTX-100 (0.1% $Na_3C_6H_5O_7.2H_2O$ and 0.1% Triton X-100 {Sigma} in distilled water), 1 part PI stock (1 mg PI per 1 ml CTX-100), 1 part RNAse (1 mg RNAse {Type 1AS, Sigma} and 10% of the total volume as DMSO. The samples were then re-frozen. Immediately before flow cytometry the samples were again thawed before 0.5 ml or more of the suspension was filtered through 15 µm nylon mesh.

Tissue weights

For comparison of diploid with triploid *P. fumatus*, the shell height (hinge ligament to the anterior edge of the left valve) of each scallop was measured to the nearest mm and recorded. The soft body was then removed from the shell and the total wet tissue weight determined to the nearest 0.01 g. The adductor muscle and gonad were then excised and placed in pre-weighed Petri dishes. Any fluid lost following excision was retained

within the dish and included in the weight of the tissue. In the case of all scallops from the CB treated group a sample of gill tissue was excised for ploidy determination. For untreated scallops, gill samples were taken from a minimum of four scallops on each sampling occasion for use in establishing diploid peaks for flow cytometry.

Results

Development of cytochalasin B treated embryos was low (8.3± 1.1%) but typical of the levels observed in earlier trials (Section 5.1.1). Development of untreated larvae was also lower than expected (38%) and was attributed to the delays and additional handling associated with experimental manipulations. None the less, the percentage development of CB treated embryos was less than 25% of the percentage development observed in untreated larvae.

Comparisons of the growth of CB treated larvae and untreated larvae have not been made as facilities did not permit the use of similar rearing vessels, a variable previously found to impact greatly upon the performance of larval batches (Heasman *et al.*, 1995). Rather, the growth of treated larvae has been plotted over the range of growth rates previously observed for diploid *P. fumatus* cultured in 20 000 L tanks to show that growth was within the range that would normally be expected (Fig. 4.1.2.1). Survival however exhibited a different pattern. The percentage of treated larvae reaching pediveliger stage was well below that of untreated larvae (48% and 61% respectively) and was significantly lower than had been expected on the basis of previous 20 000 L culture attempts (Fig. 4.1.2.1). With the exception of one culture in which larvae failed to consume food and stoped growing by day six, survival had previously always exceeded 69% from D-veliger to pediveliger, considerably higher than the 48% observed with CB treated larvae. Further, survival of *P. fumatus* larvae had, with the exception of the one previously mentioned batch, always been greater than ever recorded in the 1000 L tanks used for untreated larvae in this study.

Settlement and nursery rearing of control and CB treated scallops were run concurrently using common equipment and management techniques. Of the pediveligers, 41% of untreated larvae and 26% of treated larvae metamorphosed and survived until Day 25 (approx. 1 week post-settlement), however, spat survival from that time onward in the nursery system remained high (>90% at Day 48). Overall survival of *P. fumatus* from zygote to eleven-week-old spat was estimated to be 8.6 % for untreated scallops compared with an 0.9 % for CB treated scallops.

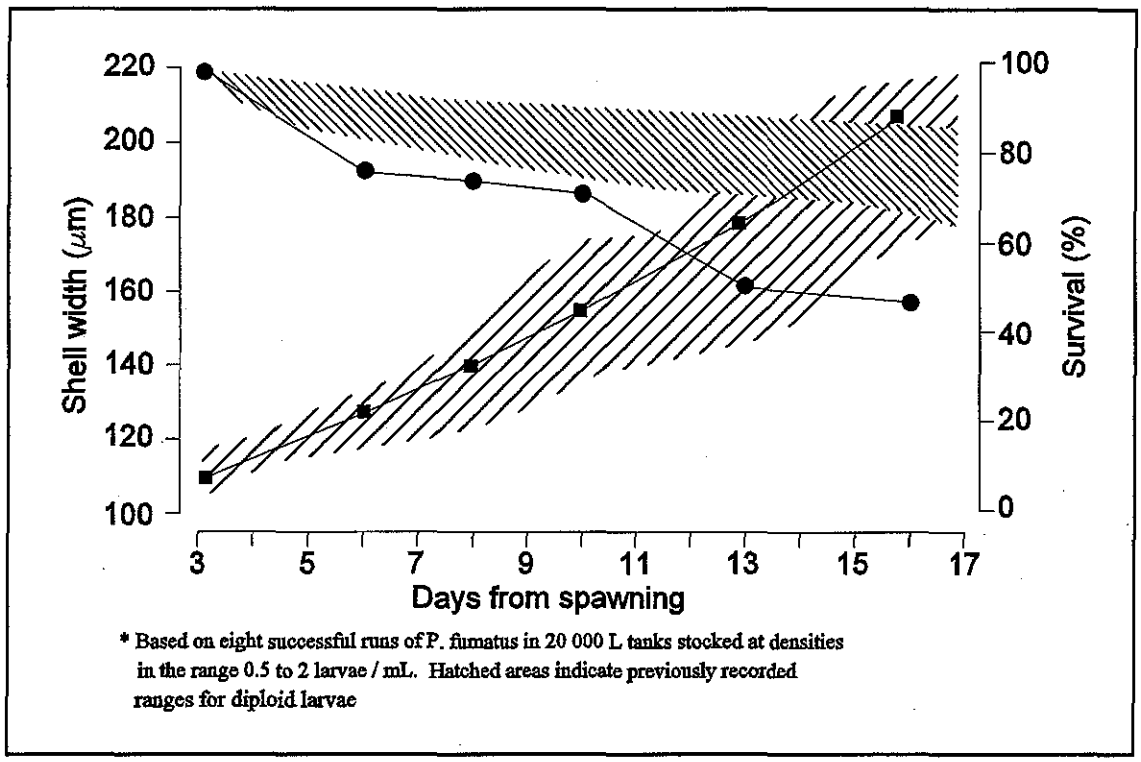

Figure 4.1.2.1 Growth of CB treated *Pecten fumatus* larvae in comparison to previous recorded growth rates. Lines indicate growth and survival of cytochalasin B treated larvae.

Tissue comparisons

Collections of scallops for tissue comparisons commenced when the CB treated group reached approximately 40 mm in shell height. At this time flow cytometry of individual tissue samples indicated that the percentage triploidy had fallen to less than 15%. Among these scallops, it was macroscopically apparent that maturation had commenced in all scallops examined with distinct development of male and female gametogenic tissue. Of the scallops examined, there was no evidence of spawning having taken place. With the exception of the gonad abnormalities discussed later, no spent gonads or gonads typical of those having undergone partial spawning were observed. Excluding the small number of abnormal gonads, there was also no apparent basis on which to macroscopically discern triploid from diploid scallops beyond the tendency for triploid scallops to be slightly larger.

TABLE 4.2.1 A comparison of mean (± se) shell height and mean (± se) tissue weights between diploid and triploid scallops.

	Shell height (mm)	Visceral weight (g)	Muscle weight (g)	Gonad weight (g)
Diploids	42.7 ± 0.6	4.28 ± 0.29	0.99 ± 0.10	0.43 ± 0.03
Triploids (n=8)	44.9 ± 0.7	4.62 ± 0.63	1.44 ± 0.50	0.39 ± 0.07

From Table 4.2.1, it can be seen that triploid scallops were on average larger than their diploid siblings with heavier total visceral weights, considerably heavier muscles and lighter gonads.

Having reached 50-60 mm in shell height, 30 scallops were collected for flow cytometric analysis (4 diploids, 26 CB treated scallops). One of these scallops exhibited the gonad abnormality described later that had been previously been associated with only with triploidy, however, neither this nor any of the treated scallops were found to be triploid. This indicated a further significant reduction in the number of triploids present, possibly to the point of total extinction within the treated batch.

Notable in comparisons of tissues of CB treated and untreated scallops was a clear morphological changes in the gonads of some scallops (Figure 4.1.2.1). This abnormality had never previously been observed in *P. fumatus* and was only present in CB treated scallops. Macroscopically, the proximal portion of the gonad contained spermatogenic tissue and the mid region held oogenic tissue (*P. fumatus* is normally hermaphroditic), however the distal third of the gonad remained clear. In most cases, this abnormality was restricted to scallops that were later determined to be triploid, however, this was not exclusively the case.

Figure 4.1.2.2 Gonad with clear distal portion, typical of those found among CB treated scallops

Discussion

Despite the promising early results observed in growth and tissue weights, the overwhelming feature of our attempts to produce triploid scallops has been the reduction in the percentage triploidy of batches with time. Although the reduction in percent triploidy in field populations is by no means a new phenomenon, or one restricted to scallops (See Allen, 1994), it appears to have been particularly severe in this case. In successive larval batches, percentage triploidy has regularly fallen 5-15% between day 1 and day 5 of larval development. In this study this trend has alarmingly continued with each successive ploidy assessment. In maturing scallops (ca. 40 mm), the percentage triploidy detected using flow cytometry had fallen to approximately 15% and was further reduced by the time the scallops had reached 50-60 mm in shell height, creating severe constraints for assessing the effects triploidy in adult *P. fumatus*. The cause for this reduction is not clear and may arise from one or a combination of the following factors:

1) an inherent reduction in the viability of triploid *P. fumatus*
2) mosaicism
3) variations as a product of the method used to determine ploidy

Triploid vulnerability

As indicated in the triploid induction trials, CB treatment is clearly stressful (Beaumont, 1986; Baron and Diter, 1989; Beaumont and Fairbrother, 1991; Utting and Doyou, 1992) and reduces both embryo development and larval survival. However it is possible that the negative impact of CB treatment extends well beyond larval growth. Utting and Doyou (1992) demonstrated that CB treated *Tapes philippinarum* embryos (diploids and triploids) used approximately twice the amount of lipid reserve to reach D-veliger stage. The inability to accumulate sufficient lipid prior to metamorphosis in molluscs has been suggested to influence settlement success. In this study, the percentage triploidy was not evaluated between the larval cycle and early maturity and thus the major reduction in percentage triploidy may well have occurred during metamorphosis and settlement.

Significant losses of CB treated scallops were experienced later in the growout period, although we have no evidence to suggest that a disease outbreak was responsible for the selective reduction of triploid numbers. Indeed, the available reports of differential effects of disease events on diploid and triploid molluscs (Matthiessen and Davis, 1992;

Hand *et al.*, in press); have suggested the potential for increased survival of triploids. Rather, the losses of scallops have been attributed to dramatic increases in the numbers of the predatory tritons, *Cymatium parthenopium*.

Mosaics and methodological difficulties

In oysters, polyploidy induction techniques have been found to give rise to what have been called mosaics, oysters that have both diploid and triploid cells in some form of mosaic throughout their bodies. Allen (1994) noted two hypotheses for the origin of mosaics. First, that mosaics arise during triploid induction when the polar body is incorporated into only one of two or four blastomeres. This gives rise to two stem cell populations that in accordance with the fate of the blastomeres during development, subsequently results in different tissues of varying compositions of diploid and triploid cells. A second hypothesis involves the reversion of triploid cells to diploid status through some sort of "mitotic miscue". As in the first hypothesis these cells could form a stem cell line that resulted in varying ploidy within and between tissues.

Irrespective of the cause of mosaicism, the fact that reversion of cells can give rise to tissues or parts of tissues that are diploid raises the possibility of incorrect identification of ploidy which would only result a reduction in the triploid percentage. Early in ontogeny, the flow cytometric technique used involves pooling several thousand larvae for each sample, however in assessing adult ploidy, a small tissue sample (10 mm^2) was taken from an individual. A sample that taken if from a mosaic could indicate either triploid, mosaic or diploid status depending on the tissue and the site within tissue from which the sample was taken.

The likelihood of misidentification will in part depend on the numbers of mosaics present. In Allen's (1994) study, mosaic *Crassostrea gigas* were evident in dual peaked flow cytometric curves and were not particularly common (>7% of triploids). However in a recent evaluation of ploidy in *C. gigas*, McMahon *et al.* (pers. comm. 1998) found flow cytometry was not an accurate technique for detecting mosaics and that using direct chromosome counts, 98% of spat with predominantly triploid cells also contained haploid, diploid and aneuploid cells. That is to say vast majority of triploid *C. gigas* were in deed mosaic to some extent.

A second factor, affecting the impact of mosaicism on the determination of ploidy is the distribution of cells of various ploidies throughout the mollusc. If cells are distributed at random with respect to ploidy, even relatively small samples of tissue should show the

dual peaked flow cytometric curves observed by Allen (1994). This however seems unlikely, rather, as stem cells proliferate one might expect to see patches or discrete areas of cells of a particular ploidy. Considerable work could be done in this area however, it is worth noting that despite the high percentage of mosaics in the work of McMahon *et al.*, dual peaked flow cytometric curves were not frequently observed (G McMahon pers. comm., 1998).

In our work with *P. fumatus*, direct counts were the preferred method of ploidy determination, however, we were unable to modify the existing techniques for consistent success with adult tissues. Instead flow cytometry was used exclusively which could partially explain the dramatic reduction in the percentage triploidy between larval and adult assessments and could confuse the interpretation of results. While it is likely that mosaicism occurs in *P. fumatus*, without having quantified its occurrence it is difficult to speculate upon the influence it may have in ploidy percentages, although it could possibly explain a large proportion of the observed reductions.

Mosaicism may also assist in explaining infrequently occurring phenomena such as the occurrence of the earlier described gonad abnormality. While it is thought unlikely that the clear portion of the gonad reflects an area of different ploidy it is possible that it is a characteristic unique to polyploid scallops (not diploid). It is also possible that the only occasion on which a scallop with the abnormality was found to be diploid reflects misidentification of ploidy due to mosaicism.

Tissue weights

Attempts to fully evaluate the effect of triploidy on *P. fumatus* have to some extent been hampered by the reductions in percentage triploidy, however, the results available are both encouraging and consistent with previous research on other scallop species.

Immediately following maturation, triploid scallops were already larger and heavier than their diploid siblings, but perhaps more important, had began to show markedly greater increases in adductor muscle weights. During maturation, the adductor muscle is as a store of energy to drive gametogenesis (Barber and Blake, 1991), thus in *P. fumatus* in the wild, muscle weights tend to reduce as the gonad ripens. Thus the reduced gametogenic activity in triploids allows the maintenance larger muscle tissue weights. In this case, the increase in weight (45%) in 40-45 mm recently matured scallops was not yet as great as the 75% reported by Tabarini (1984) for *A. irradians*.

however, as the effects of triploidy are cumulative with age and as *P. fumatus* in Jervis Bay undergo up to four annual spawning peaks (Heasman *et al.*, 1995) it is possible that triploid *P. fumatus* will eventually exhibit weight advantages as high as those recorded for *A. irradians*.

General

A key question posed by the above results is whether or not the potential benefits of higher tissue yields of triploid *P. fumatus* outweigh the costs of the elevated mortality rates sustained during the earlier developmental stages.

Regardless of the cause, increased mortality of embryos and early larvae is not necessarily a major impediment to commercial hatchery production. The cost of triploid induction is relatively low (AUD$16.72 for 1 L of egg suspension at 1.0 mg CB l^{-1}, Nell *et al.*, 1996) and the poor yields of D-veligers following CB exposure can easily be compensated by increasing the number of zygotes treated. More important are the increased mortalities that have persisted throughout larval development (similar to that found with *Chlamys varia* (Baron *et al.*, 1989) and *Mimachlamys asperrima* (W.A. O'Connor and M. Heasman, unpub. data 1996), and post metamorphosis reductions in percentage triploidy. There are few reports of postlarval performance of triploid pectinids and no mention of poor post-metamorphic survival. It is possible that this was an unusual result in what was an unreplicated growth trial, but, larval growth, survival, and metamorphosis are related to larval quality. If larval "quality" continues to be affected by CB treatment its ultimate cost may be well in excess of AUD$16.72 per treatment.

4.2 INVESTIGATIONS OF LARVAL SETTLEMENT

Introduction

While hatchery production of the commercial scallop *Pecten fumatus* Reeve has been deemed to be economically viable (Cropp and Frankish, 1990), several technical constraints to high efficiency production still exist. In particular, reliable techniques for controlled settlement and nursery rearing. Commonly, ready-to-set scallop larvae are placed in tanks with a collector substrate, allowed to settle and once bysally attached are translocated to a nursery site (Bourne *et al.*, 1989; Frankish *et al.*, 1990; Heasman *et al.*, 1997; Fig.4.2.1). Direct settlement to collectors is thought to be the most economic approach (Bourne *et al.*, 1989; Heasman *et al.* 1994); however, *P. fumatus* spat retention can be relatively low (5 to 10% of ready-to-set larvae); it is difficult accurately predict the density of set upon collectors and there is great variability in spat numbers between collectors (Frankish *et al.*, 1990).

Our initial response to the problems encountered with direct settlement was to adopt a screen settlement system similar to that used by Delaunay *et al.* (1993). Ready-to-set *P. fumatus* larvae (220 to 230 µm shell length) were transferred to downweller screens fitted with 160 µm mesh (Fig. 4.2.2). Following settlement and metamorphosis, spat were retained in the hatchery until they were approximately 750 µm in size, when they were then transferred on screens to field upwelling systems. Spat remained on the screens in upwellers for eight to ten weeks before being transferred to pearl cages for culture on longlines. This screen settlement system avoided the problems associated with control of spat densities and provided higher survival to a size at which spat were transferred to pearl cages, typically 25-35%. However, during the time they are held in field upwellers, spat reach a size at which they no longer attach by byssal threads. Overcrowding of detached spat can lead to inadvertent "biting" where swimming scallops lock valves damaging the mantle and other soft tissues. This soft tissue damage results in reduced growth, shell deformity and increased mortality (Palmer, 1980; Ventilla, 1982; Bourne *et al.*, 1989; Hardy, 1991). As a result, spat densities in upweller screens must be continually monitored and periodically reduced, requiring frequent attention, many screens and considerable nursery space. These demands initiated a reappraisal of nursery techniques from which two divergent approaches emerged.

First, in attempting to increase the efficiency of nursery systems for clams, Malinowski and Siddal (1989) found that passive water reuse in upwellers could double the production per unit of water pumped without compromising growth or survival. This was achieved by connecting upwellers in series such that ambient water cascaded

through four successive upweller units. The demand for nursery space for *P. fumatus* spat prompted and adaption of this principle involving the use of multi tiered (stacking) upweller screens. This approach allowed passive water reuse as well as far more efficient use of available upweller space.

Second, an alternative to direct settlement on collectors was derived by combining the better features of the direct settlement and screen settlement techniques (Fig. 4.2.1). Spat were settled on inexpensive mesh and maintained in the hatchery until they reached a predetermined size. Next, the screen mesh was removed, cut into sections and finally placed in mesh collector bags for deployment in the field. This approach permited controlled stocking densities and allowed the removal of spat from the hatchery/nursery before excessive space was required.

This study was undertaken to assess and compare the practical utility of the two approaches to nursery rearing *P. fumatus*. While the recovery of spat from upwellers had already been found to be significantly greater than that of direct settlement, the impact of the factors associated with tiered upwellers, such as screen position and rotation and stocking density, were unknown. In the case of deployment from screens to spat bags, percentage recovery had not been determined, nor was there any understanding of the effect of spat size at the time of deployment upon later recovery.

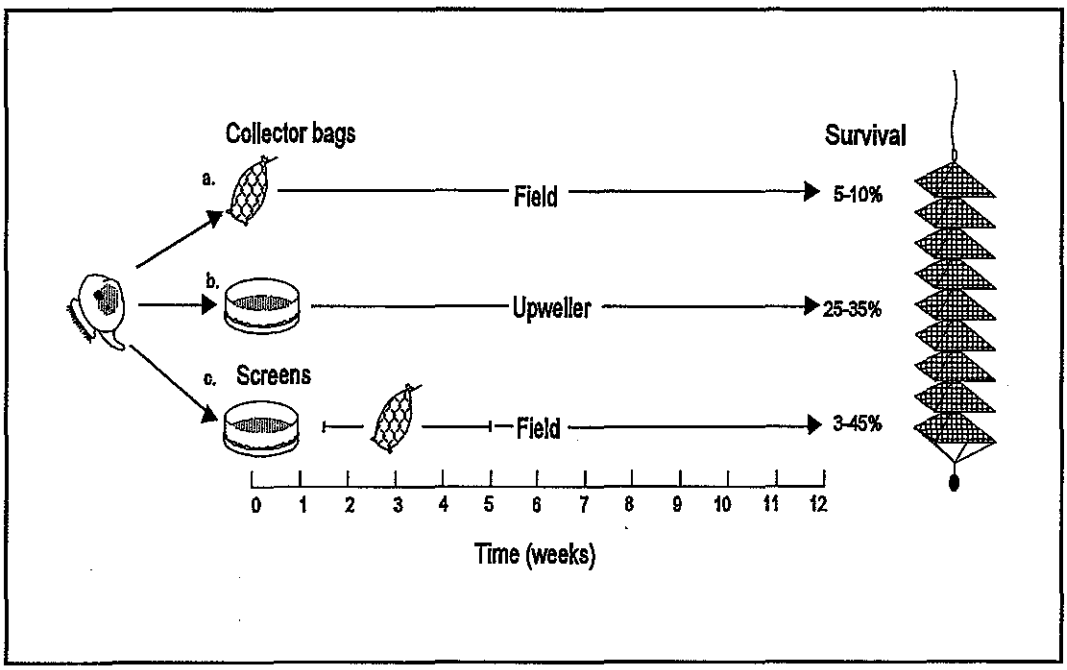

Figure 4.2.1 Techniques used for settlement and culture of *Pecten fumatus* spat and the survival of spat with each technique.

Methods

General

All *P. fumatus* spat used in these trials were produced in the hatchery at Port Stephens Research Centre by methods described in the hatchery manual produced in conjunction with this report and settled onto 160 µm nylon mesh screens. These screens were held in downwelling systems (Fig. 4.2.2) in the hatchery and fed a combination of *Pavlova lutheri*, Tahitian *Isochrysis* aff. *galbana*, *Chaetoceros calcitrans* and *Skeletonema costatum* until the spat had reached the size required for the respective experiments.

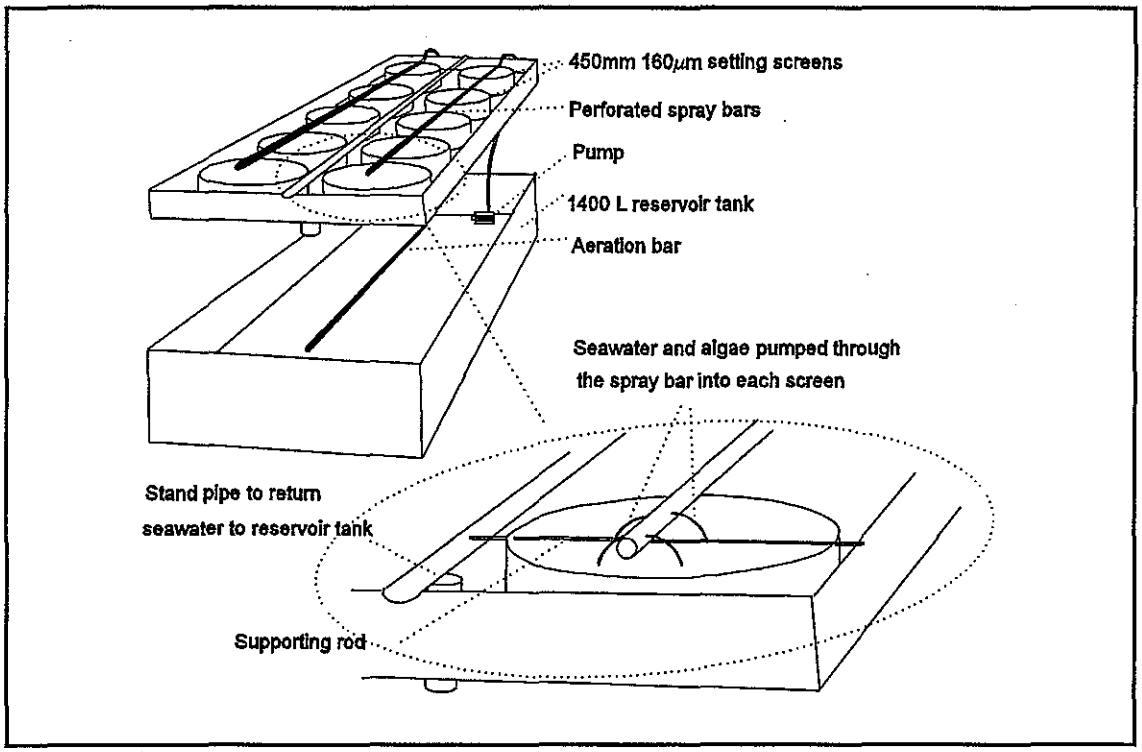

Figure 4.2.2 Downwelling system used for larval settlement of *Pecten fumatus*.

Tiered upweller nursery culture

An experimental upweller system was installed at Tomaree headland adjacent to the entrance to Port Stephens (32° 43'S; 152° 11'E). Five mini-upweller modules (Fig. 4.2.3) were installed and fed raw seawater drawn from a depth of 1-3 m by a 1 Kw pump located at the end of a 30 m wharf. Each module accommodated eight sets of miniature screens constructed from PVC plumbing components. Each of the 40 sets of screens comprised a 90 mm PVC pipe outlet manifold to which stacks of up to nine

interlocking screens, made from 90 mm PVC end caps fitted with 1.8 mm reinforced plastic fly-wire mesh (surface area 63.6 cm^2), could be attached. Seawater entered the stack via the bottom screen, flowed out through the top screen into the outlet manifold and then to the sump via an overflow port. Spat could thus be housed on as many as eight levels (screens) in each stack with the top (ninth) screen preventing scallop spat on the eighth screen from escaping through the overflow port.

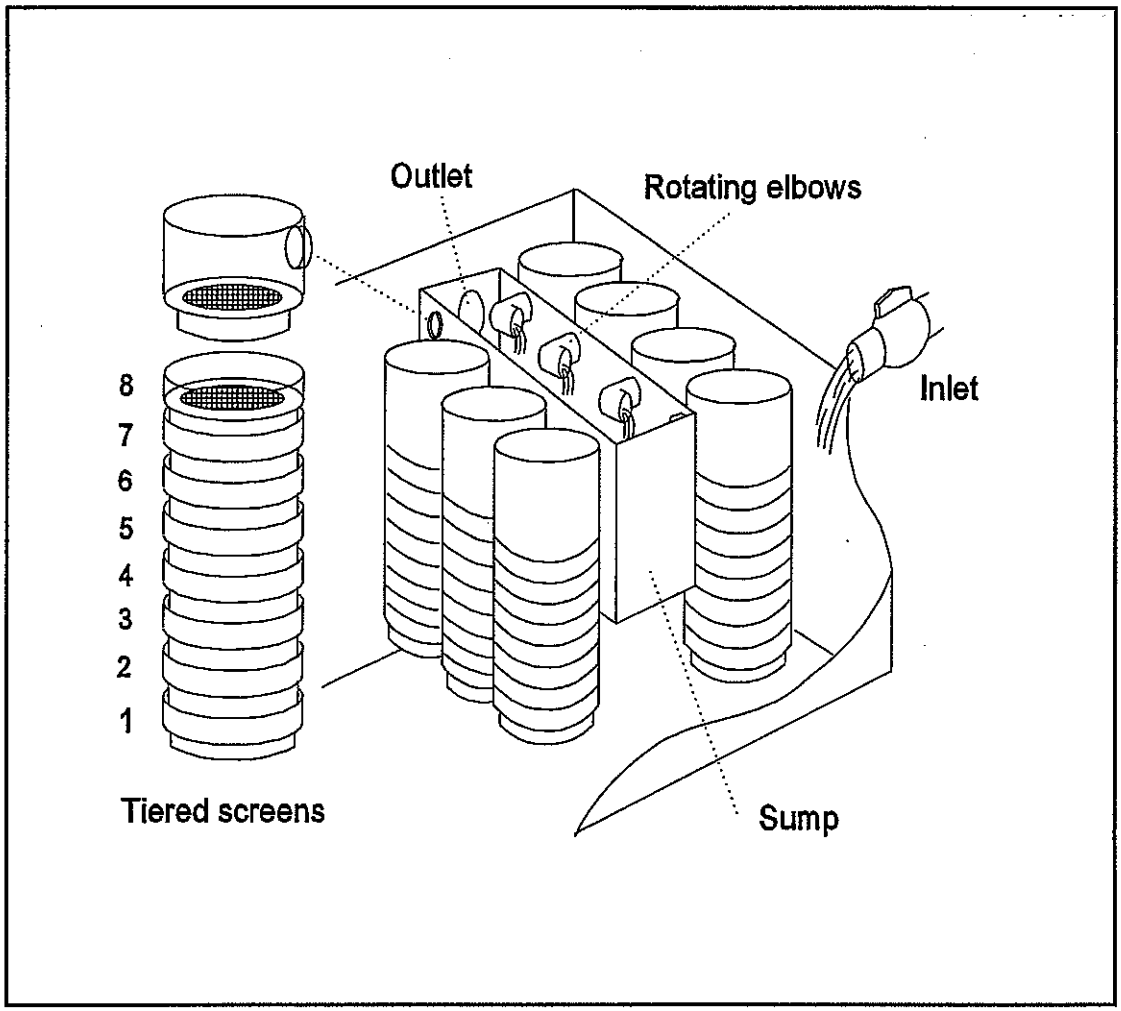

Figure 4.2.3 Experimental upweller unit.

The flow rate of seawater upwelling through each stack of miniature screens was regulated in two stages. The first stage was achieved by adjusting flow into the main chamber of the upweller unit with a 50 mm PVC ball valve. The second stage enabled regulation of flow rates through individual mini upweller stacks. This involved the use of a simple adjustable weir comprising a 20 mm PVC 90 elbow (bend) attached to the outlet port of each mini-upweller stack. Each outlet elbow could be rotated through 90°

to adjust the static head between the incoming seawater (set by the relative height of water within the main chamber of the upweller unit) and exhalant (waste) water discharging from the over the weir serving each stack. Flow rates through each stack could thus be individually adjusted to a common rate, the sum of which (obviously) still matched the seawater inlet flow rate. Measurement of flow rates through each mini-upweller stack was achieved by recording the time required to fill a 100 mL measuring cylinder with seawater discharging from their individual standpipes. All stacks were removed from sumps daily and rinsed clean of accumulated silt, detritus, faeces and pseudofaeces by back-flushing (from top to bottom) with running sea water.

All spat used in Experiments 1 to 3 had been maintained on upweller screens at Tomaree Headland for at least one month. These spat were also graded through mesh sieves to restrict size variability between individuals. Use of the term "size" in relation to spat refers to shell height. This is the distance from the centre of the hinge to the central anterior margin of the cupped right (bottom) valve. All values are mean ± s.d. unless otherwise stated.

Screen to collector bag nursery rearing

For Experiment 4 in which spat were initially reared in the hatchery then transferred at varying sizes and ages to a field longline, *P. fumatus* spat were settled on mesh screens made using inexpensive nylon material obtained from a haberdashery. No particular criteria were set for the selection of mesh material beyond the fact that it had a pore size of approximately 160 µm, and that it did not disintegrate in seawater within four weeks. When spat reached a predetermined size, the mesh was cut from the screen frame using a scalpel, and then cut into sections with the approximate number of spat desired on each section. The exact number of spat was determined by placing the section of screen on a 1 cm grid drawn on clear plastic sheeting and counting the number of spat cm^{-2}, when necessary, with the aid of a magnifying glass (10x magnification). The mesh section was then folded into the middle a of 4 m^2 sheet of pliable 10 mm square grid, black polythene netting ("Vine mesh", Kinnears, Sydney, NSW, Australia) which was then placed inside a spat collector bag (2 mm mesh, Hoyo Corp., Toyohashi, Japan).

Experiment 1 the effects of spat density and screen position upon growth in tiered upwellers

Scallop spat (5.8 ± 0.2 mm, 26 ± 1 mg) were placed on screens at one of eight densities 0.3, 0.45, 0.6, 0.9, 1.2, 1.8, 3.6 or 7.2 g screen^{-1}. Eight screens were stocked at the same density and were combined in fixed positions to form each mini-upweller stack. Five replicate stacks at each density were randomly placed among the five mini-upweller modules within the total upweller unit. Flow rate through each stack was set at 1±0.2 L min^{-1}, checked and if necessary, re-adjusted at low, mid and high tide on the first day and daily there-after. The experiment was terminated after two weeks when the scallops from each screen were drained on absorbent paper before being weighed to the nearest 0.1 g.

Experiment 2 the effect of rotation of screen order within tiered upwellers

During Experiment 1, spat growth was found to be significantly affected by position (level) of screens within stacks. To determine if this growth variability could be overcome by regular screen rotation, eight stacks of eight screens were stocked at 1.8 g screen^{-1} (14.4 g stack^{-1}). Two stacks were positioned in each of four separate mini upweller modules. Each day, the bottom screen (position 1) on one of the stacks within each mini upweller module was rotated to the top of the stack (position 8, Fig. 4.2.3). Growth of scallops on the four rotated screen stack replicates was then compared with those on similarly stocked unrotated stacks serving as controls. " Control stacks" were removed from the sump manifold, emersed and rinsed daily to simulate handling of rotated screens. The experiment continued for eight days to permit one complete rotation of stocked screen positions. The biomass of scallops on each screen was then determined as for Experiment 1.

Experiment 3 the effects of spat crowding in tiered upweller screens

To separate the effects of crowding from varying food availability and rate of waste removal at different levels within stacks, scallop biomass and water flow per stack were fixed and only the number of stocked screens within each stack was varied. In each stack, 19.2 g of scallop spat were distributed over 1 to 8 screens to achieve a surface area stocking rates equivalent of 50, 100, 150, 200, 250, 300 & 400% of available screen area. For example, if spat were distributed equally across all eight screens their surface area stocking rate was equivalent to 50% of the mesh surface area in each

screen. At the other extreme, if spat were all placed within one screen within the stack of 8, the surface area of the spat in the stocked screen was equivalent to 400% of available surface area.

Due to a shortage of suitable sized spat, only one replicate of each stocking density was used. A single replicate per stocking density was adopted in preference to alternative protocols such as the use of 4 replicates in each of a high and a low stocking density. The reason for this was a perceived need to determine the approximate threshold of stocking density beyond which growth rate diminishes. Based upon the results of Experiment 1, flow rate was set and maintained at 4 ± 0.5 L min^{-1} $cylinder^{-1}$, which was not expected (at least initially) to be limiting to growth even at the highest stocking density. Each week for three weeks, the scallops in each treatment were removed, weighed and the screens cleaned. The scallops were returned to their original screen which in turn was returned to its original position within the same stack.

At the completion of each experiment, the spat were removed, weighed and the number of mortalities recorded. The formula used for estimating equivalent surface area increase was

$$S_p=(\sqrt[3]{W_p})^2$$

where: S_p= proportional increase in horizontal plane (dorsal) surface area
and W_p= " " " weight (biomass)

Experiment 4 the effects of spat size and transport method used for mesh transfer

To determine the effect of initial spat size on retention and survival, batches of spat were deployed using the mesh transfer system when they had reached approximately 350, 500, 750, 1000 and 1400 µm in shell height. In each case, eight replicates batches of spat in individual bags were transported to the field in an air conditioned vehicle in either 10-l plastic containers of seawater (four batches) or dry in buckets (four batches). Air emersion time during spat counting and bagging was less than 20 min with an additional 40 min for those bags transported to the field dry. Following transport, the collector bags were taken by divers and attached to moorings at a depth of approximately 4 m, 50 m offshore of Tomaree Headland at a sheltered site that lies within and about 300 m south of the southern side of the entrance to Port Stephens. At the time of deployment of each treatment, four similar "control" bags without spat were deployed to determine if natural catch contributed to the numbers of spat recovered. Seawater temperatures within the hatchery were adjusted to be within 1°C of those expected in the field at the time of deployment. This same temperature was maintained

during wet transport so as to reduce the possibility of temperature shock that is known to cause spat to detach from the collectors.

The collector bags were left at Tomaree for a minimum of approximately one month, which, based upon previous observations, was expected to be sufficient for spat to grow to a size at which they could be removed and placed in pearl cages. After this time, each collector bag was returned to the hatchery where the contents were carefully harvested by rinsing with seawater onto a submerged 300 μm mesh screen. The number of spat in each bag was counted and a subsample of 30 scallops from each bag were measured to the nearest 0.1 mm using a binocular microscope and an eyepiece micrometer.

Statistical analysis

Homogeneity of variance in each case was confirmed using Cochrans test (Winer, 1971) and data were analysed using ANOVA. Where significant differences were detected, means were compared using SNK tests (Winer, 1971). Spat weight gain data was log transformed when necessary to satisfy the assumption of homogeneity of variance. All survival data was arcsine $x^{0.5}$ transformed before ANOVA.

Results

Seawater temperatures during Experiment 1 ranged from 18.5 - 22°C and salinity remained within the range 34.0 to 36.0 g kg^{-1}. For Experiments 2, 3 and 4, seawater temperature ranged from 17 - 22°C and salinity was in the range 33.0 to 35.0 g kg^{-1}.

Experiment 1 the effects of spat density and screen position upon growth in tiered upwellers

Both the initial stocking density and the screen position within the stack significantly effected spat biomass increase and there was a significant interaction between these factors (F=2.016, P<0.001). Biomass increase was greatest in the first screen in each stack and exhibited a progressive decline over successive screens at all densities tested (Fig. 4.2.4). Declining biomass increase with increasing screen position were most pronounced for initial stocking densities in the range 0.6 to 7.2 g $screen^{-1}$. Slopes of lines linking data of spat stocked at the two lowest initial densities of 0.3 and 0.45 g

screen^{-1} were less pronounced, although, least squares linear regressions found all but the lowest socking rate of 0.3 g per screen^{-1} to have significant negative slopes (P<0.05).

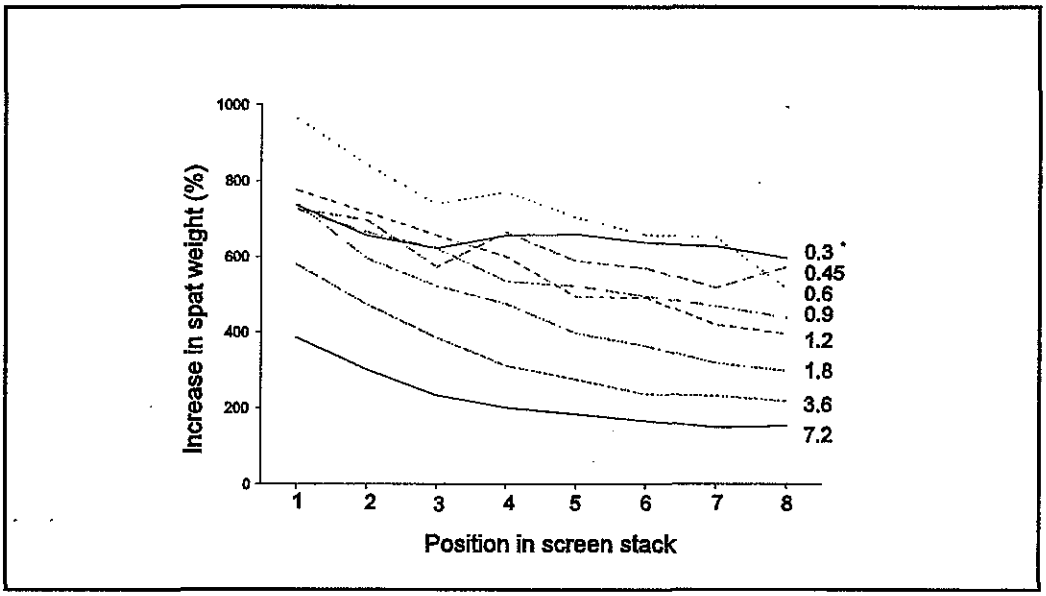

Figure 4.2.4 Biomass increase in commercial scallop spat held at different levels within stacks of upweller screens. Positions 1-8 indicate the order in which screens received seawater. * Initial biomass (g screen^{-1})

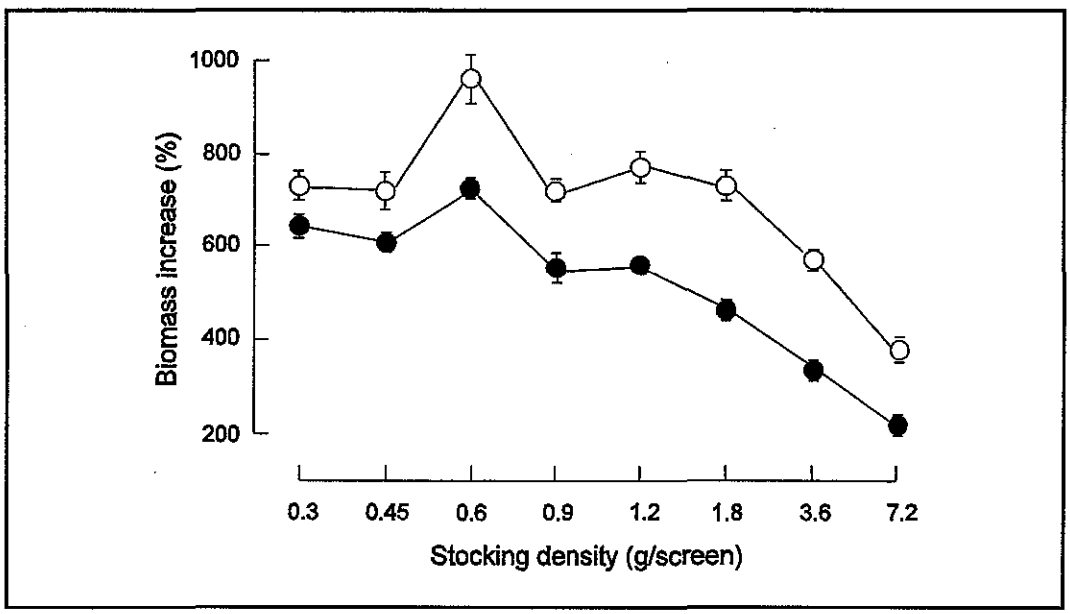

Figure 4.2.5 Biomass increase of *Pecten fumatus* held at one of eight densities in stacks of eight upweller screens. Initial biomass 26±1 mg. Values are means ± SE for a) spat held in the first screen to receive water in each stack and b) for the total stack.

Experiment 2 the effect of rotation of screen order within tiered upwellers

When stocked at a density of 1.8 g screen^{-1} (at which screen position effects could be expected) mean survival of 77% for spat on screens subjected to daily rotation did differ significantly (F= 1.452, df=5, P>0.05) from that of 72% for spat on screens in fixed positions. The mean biomass gain of 66% for spat on rotated screens was however significantly greater (F=9.434, df=5, P<0.05) than the 39% increase exhibited by spat held on screens in fixed positions thereby demonstrating a major advantage of screen rotation.

Experiment 3 the effects of spat crowding in tiered upwellers

In Experiment 3, a constant initial biomass of graded scallop spat was spread through a varying number of from 1 to 8 screens within stacks. As indicated in Fig. 4.2.6, the effects of surface area stocking density *per sé* were clear. After one week, surface area occupied by spat initially stocked at 50% screen coverage had increased to 88% and for spat initially stocked at 100%, to 175 % of screen area. The corresponding figures for spat stocked at higher initial stocking densities of 150 - 400% screen coverage had increased to the range 250 -630%.

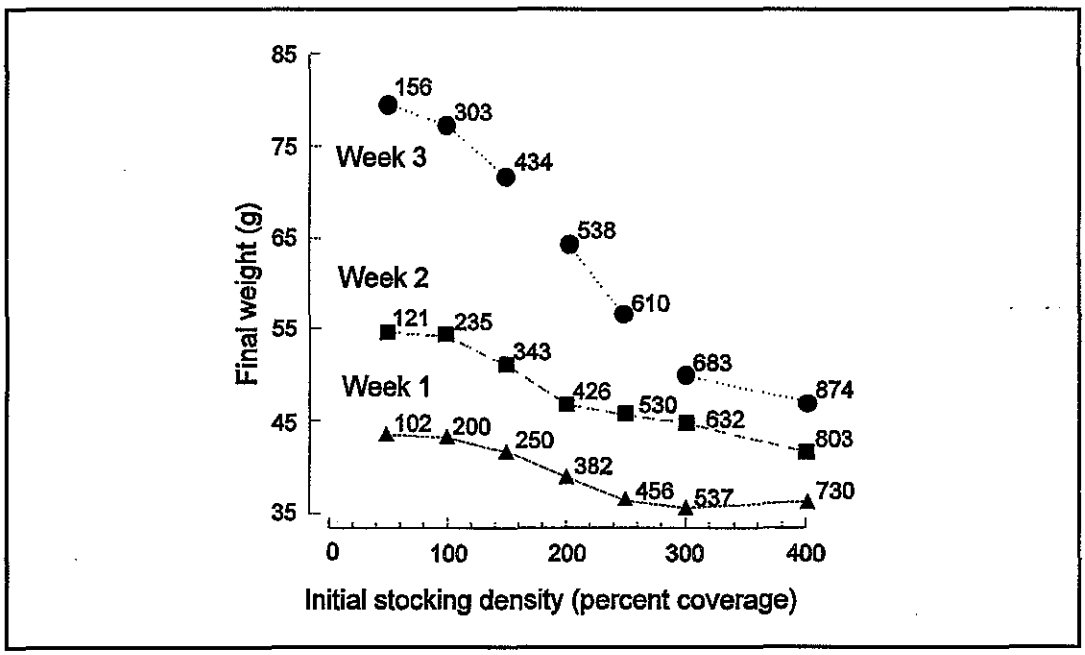

Figure 4.2.6 Biomass increase over time for 14.4 g of *Pecten fumatus* spat stocked at various densities within stacks of upweller screens. * Values are the estimated percentage of available screen occupied by spat at the end of each week at each biomass/surface area stocking density.

After 2 weeks, spat stocked initially at 50 and 100% screen coverage had continued to grow at equally fast rates, attaining mean surface area density of 102% and 202% of screen area respectively. However, spat initially stocked at 150% screen area and above, which had now reached at least 295% screen coverage, exhibited a pronounced density dependent reduction in growth .

After 3 weeks, spat initially stocked at 50% coverage had made the greatest increase in biomass and attained a surface area density of 156% screen coverage. Spat stocked initially at 100% screen coverage, had now reached a surface area coverage of 300% and had fallen only marginally behind in biomass gain.

It is clear from the results of Experiment 3 (Fig. 4.2.6) that surface area density per sé is a critical factor determining growth rate of *P. fumatus* spat on field upweller screens. It would also appear from these results that, in the absence of other growth limiting factors such as food, the upper limit of stocking rate at which maximum growth rate is maintained lies at about 200% screen coverage. However, only marginal reduction in growth occurs at stocking rates of up to 300% screen coverage and only moderate reduction at socking rates up to 400%.

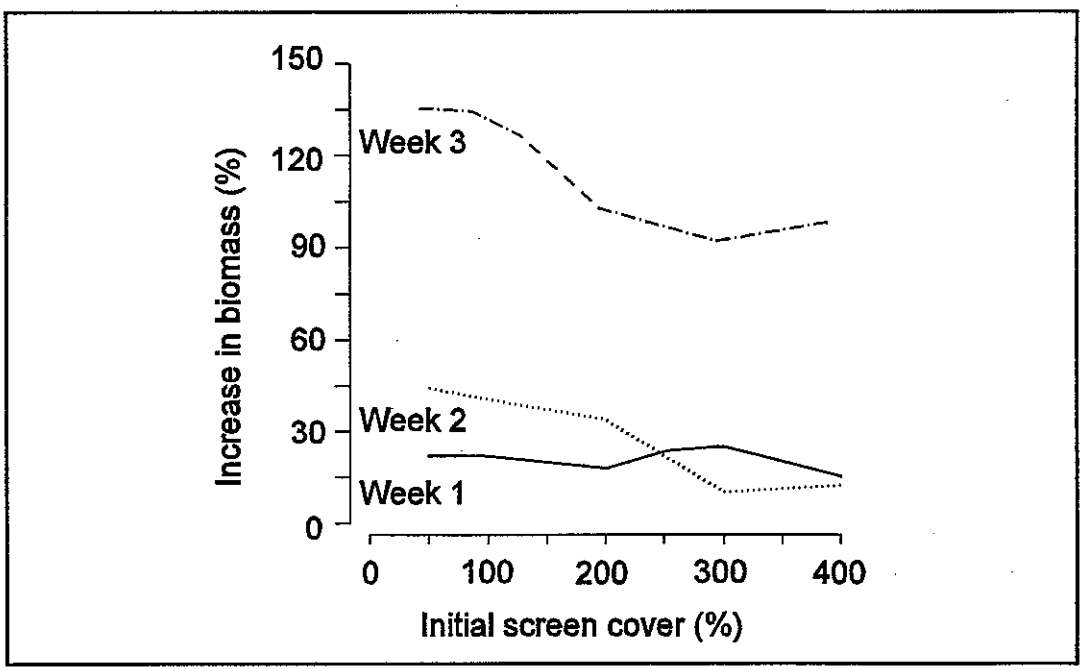

Figure 4.2.7 Weekly biomass increase of *Pecten fumatus* spat held in two or more screens held within an eight screen stack. Initial stocking density 14.4 g stack^{-1}.

The relative (proportional) increase in biomass from week to week varied greatly in this experiment (Fig. 4.2.7). As expected, relative biomass increase was greatest during the first week when absolute biomass per stack was relatively low and the spat at their smallest. During week 2 however, the proportional increase in biomass was unexpectedly much lower and almost uniform across all stocking densities. In week 3, the expected pattern of biomass increase returned. Salinity varied little over the three week period and temperature, although variable, showed a similar range for each week. Thus salinity and temperature are not thought to have caused the reduced growth in week 2.

Experiment 4 the effects of spat size and transport method used for mesh transfer

By the end of this experiment, spat originally attached to the screen mesh had detached and moved out into the surrounding black polythene mesh. No *P. fumatus* spat were found in the control bags thereby confirming that no wild spat recruitment had occurred. In all but the 350 µm treatment, spat had grown to a size at which they could no longer escape through the surrounding 2 mm mesh collector bag (Table 4.2.1).

The number of spat recovered from each collector bag was expressed as a percentage of the original number deployed into that bag and those percentages were arcsine $x^{0.5}$ transformed to satisfy requirements for homogeneity of variance. A two-way ANOVA found the number of spat recovered from collectors was effected by both spat size at deployment to the field and the transport method used, with a significant interaction occurring between these factors (Table 4.2.1). Within the range tested, the percentage of spat recovered increased as the size of spat at the time of deployment increased and percentage recovery was greater for those treatments transported to the field site in seawater (wet) than out of water (dry). A comparison of spat numbers recovered after wet and dry transport within each size class showed a general trend for spat recovery after dry transport to improve with spat size. For example, the numbers of 350 µm (initial shell height) deployed spat recovered after dry transport were less than 10% of those in wet transported collectors, but, by the time spat deployment size reached 1 000 µm, recovery following dry transport had increased to approximately 50% of the numbers in wet transported collectors. The method of transport had no significant effect upon the mean size of spat recovered after one month in the field ($P>0.05$).

Table 4.2.1 a) The effect of spat size at deployment on percentage recovery *Pecten fumatus* spat settled on nylon screen and transferred to mesh filled spat bags. Spat were transported to the field either wet (in seawater) or dry. b) Results of two-way-ANOVA of the effect of spat size and transport method (wet or dry) on percentage of *Pecten fumatus* spat retained after 30 days.

a)

Shell height Approx. (actual µm)	Days post-set	Percentage recovered Wet	Dry	Mean spat size at recovery (mm)
350 (358 ± 114)	9	5.5 ± 1.6	0.5 ± 0.2	2.1 ± 0.3
500 (494 ± 176)	15	25.4 ± 1.5	9.5 ± 0.9	6.8 ± 0.7
750 (764 ± 162)	23	30.3 ± 0.7	2.5 ± 0.3	5.9 ± 0.6
1000 (1004 ± 110)	27	41.8 ± 6.9	20.2 ± 0.7	6.3 ± 1.3
1400 (1409 ± 255)	35	75.3 ± 5.7	30.5 ± 5.1	4.6 ± 0.7

Values are means ± SE.

b)

Source*	SS	df	MS	*F*	*P*
Size	2.03	4	0.59	80.1	<0.001
Transport	0.92	1	0.92	144.9	<0.001
Interaction	0.15	4	0.04	6.1	<0.01
Residual	0.19	30	0.01		
Total	3.30	39			

* Data arcsine $x^{0.5}$ transformed prior to analysis.

Discussion

Two benchmarks were adopted to gauge whether or not the modified nursery rearing technology being evaluated would provide significant advantages over existing technology. The first benchmark was a relatively high spat retention rate of 25-35% acheived with nursery upwellers, but high costs are incurred by setting pediveligers onto hatchery based downweller screens and then transferring these to field upwellers once the resident spat can be retained on larger (>500µm) mesh screens. The second benchmark was that of the relatively low spat retention rates of 5-10%, but very low maintenance costs of direct settlement of pediveligers on mesh within spat collector

bags and then almost immediate transfer to field longlines.

Tiered upwellers

Spat survival in tiered upwellers throughout this trial was consistently high irrespective of stocking density and equivalent to that experienced in the past with single layered upweller systems (25-35%). Thus, the percentage of ready-to-set larvae reaching a size at which they can be deployed to longlines was expected to be of the order of 25-35%, an assumption that has since been corroborated in commercial scale tiered upwellers trials (Heasman *et al.*, unpublished data). The advantage of tiered upwellers over single layered systems arises from a more the efficient use of pumping operations and capital infrastructure.

Studies with several bivalves have indicated that maximum feeding and growth rates are coincident with flow rates that produced about 20% filtration of suspended material passing through upwelling screens (Manzi *et al.*, 1986; Spencer, 1988). Such rates of removal generally coincided with seawater flow (L min^{-1}) to biomass (Kg) ratios in the order 15:1 to 30:1. Comparable flow to biomass ratios of 25:1 can be derived from data provided by Rhodes and Widman (1980) for raceway reared scallop (*Argopecten irradians*) spat and in the present study for the first (lowest) screen within non rotated stacks, 20:1 to 30:1(Experiments 1 and 2). An important inference of the above is that any attempt to maximise growth rate by adjusting seawater flow to a rate that at which spat filter out only 20% of available food is inherently inefficient.

Clearly available food is important and flow rates are a determining factor in food availability, however determining stocking density based on food concentration is problematic and commonly impractical. Feeding mechanisms and an understanding of what components within natural seawater constitute a suitable food source for scallops, particularly juvenile scallops held in field nurseries, are not clear, let alone measurable. Further, food availability changes temporally and often rapidly. The location of the present study on Tomaree Headland lies only 300 m from the entrance to mouth of Port Stephens, and is subject to large and rapid changes in physiochemical characteristics of seawater. In the East Australian current, phtyoplankton biomass may change by as much as an order of magnitude (Hallegraeff, 1981) and could help to explain very high week to week variability in growth detected in Experiment 3, in particular the uniformly poor biomass increases recorded during week 2 across all stocking densities.

The question of food availability in natural seawater is further complicated by the

potential for selective feeding. Despite apparent contradictions between qualitative observational and anatomical evidence, and indirect quantitative studies of scallop feeding (Beninger, 1991), several studies indicate scallops are capable of selective particle rejection (Shumway *et al.*, 1985; Shumway and Cucci, 1987; Newell *et al.*, 1989; Lesser *et al.*, 1991). Within stacked upweller nursery systems, the potential exists for those scallops first exposed to incoming seawater to selectively deplete food. This may be of particular significance in relation to *P. fumatus* spat and could account for profound effects of fixed screen position within upweller stacks on the growth observed in Experiments 1 and 2. Spat in the first (bottom) screen not only had greater food availability but also a greater scope to differentially select and retain preferred food items from the complex array present in raw incident seawater.

As demonstrated in Experiment 2, stocking density, independent of flow rate, has significant effects upon growth of *P. fumatus* spat. This highlights the importance of providing adequate surface area to enhance production efficiency (both in relation to fixed (capital) and variable (labour, power etc.) costs. Having maximised available surface area with the use of stacked screens, achievement of acceptable mean growth rates in combination with high carrying capacity (and hence high production efficiency), is still critically dependent on regular rotation of screens.

Rotation of screens can overcome some of the adverse effects of screen position within stacks and can significantly increase net biomass gains per unit of volume within upwellers and per unit of seawater flow. The reason for this is not clear, however, it may arise from the fact that pectinids, being typical suspension feeding bivalves, exhibit decreasing efficiency with which ingested particles are absorbed with increasing food concentration (Bricelj and Shumway, 1991). The regular alternation of screens through positions of differing food availability may enhance the net absorption of food already filtered and ingested.

With the provision of adequate water flow, stocking rates of *P. fumatus* spat in tiered upweller units can be maintained at up to 200% the available screen surface area whilst maintaining high mean growth rates. Rhodes and Widman (1980) reported that *A. irradians* could be grown from a mean shell height of 5 mm to 10 mm at stocking rates up to 170 000 scallops per 12 m^2 raceway whilst maintaining optimum growth rate. This represents equivalent initial and final stocking rates of 27 and 110% respectively of available horizontal surface area. Results of Experiments 2 and 3 are also comparable to the Malinowski and Siddall (1989) finding that passive reuse of seawater through a succession of four upwelling units more than doubled nursery production of clams *(M. mercenaria)* per unit of pumped seawater even in the absence of screen

rotation.

Results of the present study also indicate that very high net biomass gains, and hence overall operational efficiency of stacked screen upwellers, can be attained at surface area stocking rates up to about 400 % of available screen area without seriously compromising mean growth rate. However, as possible long- term detrimental effects, such as irreversible shell deformity, were not evaluated, densities beyond 200% of available screen area are not advised.

Use of tiered nursery systems does nevertheless have a number of practical disadvantages. Unlike monolayer systems, stock within stacked screens cannot be inspected at a glance and are more prone to water flow restriction caused by multiple rather than single layers of mesh and associated biofouling. They are also prone to " channelling" whereby seawater, taking the course of least resistance, may enter stacks via imperfect seals between successive screens, thereby by-passing upstream screens. Moreover, in the event of power or equipment failure, associated risks of major stock loss are greatly increased. Regular rotation of screens must therefore be coupled to increased vigilance and use of other safeguards such as alarm systems plus increased cleaning, predator removal and other maintenance operations. Otherwise, cost advantages of tiered systems over monolayer systems may be more than offset by occasional catastrophic losses.

Screen to collector bag transfer

The mesh transfer system for *P. fumatus* spat was found to be useful compromise between direct settlement on collectors and the maintenance of spat on screens for nursery rearing. Retaining spat until they reached a shell height of 500 µm before transfer to collectors, provides greater average spat recovery than would be expected from direct settlement on collectors (approximately 8-15% and 5-10% of ready-to-set larvae, respectively). To do this implies the maintenance of spat within the hatchery for an additional 1-1.5 weeks, however this cost may be offset by the ability of the intermediate screen method to permit control of spat densities in bags. Direct settlement results in huge variations in stocking density. Spat numbers can range from a few hundred to 25 000 per bag (Frankish *et al.*, 1990; Heasman and O'Connor, 1996) with significant impacts on subsequent spat growth at higher densities.

Retention of larvae on screens until they reach 1 000 µm further improves spat recovery percentages to approach those observed with screen systems (24% and 25-35%,

respectively), albeit at considerably less cost. In this case spat are retained in the hatchery on screens for up to four weeks, in contrast to the three or more months of maintenance required for the retention of spat on field upweller screens until they reach a size suitable for deployment in pearl cages.

Air exposure is a key factor in transport of spat to the field as increasing emersion times cause progressive increases in detachment of *P. fumatus* spat (Heasman *et al.*, 1994). Following settlement directly onto mesh within spat collector bags, spat are taken to the field in fish transport tanks so that emersion time is limited. In this study, the process of accurate counting of spat inadvertently increased emersion times for all treatments. This extended emersion was thought to explain the relatively poor recovery (3%) of wet transported 350 µm spat in comparison to that (5 - 10%) of similar sized spat settled directly on mesh collector bags and deployed directly to the field. Similarly, the consistent reduction in spat numbers following dry transport to the field were largely attributed to emersion. In practice, spat numbers are estimated while the screen remains immersed and transport occurs in tanks. Emersion is then limited to the minute or two taken to cut the screen into pieces and place each piece in a collector bag (although this could also be carried out in water if necessary) and the time taken to move collectors from the tank to sea.

With respect to the limitations of the screen to collector bag transfer system, it permits control of initial stocking density in collector bags, but, the increased retention time in the hatchery increases the possibility of mortality due to equipment failure and unlike upwellers, the exact number of spat remains largely unknown until the time of harvest.

Conclusions

Both alternative nursery techniques investigated in this study overcome some of the drawbacks of direct settlement of larvae on collector bags. Most notably, the percentage of newly settled spat reaching a size at which they can be transferred to pearl cages can be significantly increased. However, each new technique implies increase in the cost of production. Despite the improvements in efficiency provided by tiered upwellers, intermediate screen to spat bag transfer has been adopted for recent *P. fumatus* spat production and is being trialed in commercial hatcheries elsewhere in Australia.

4.3 DEVELOPMENT OF GROWOUT TECHNIQUES

4.3.1 DISC CULTURE.

Introduction

The commercial scallop, *Pecten fumatus* Reeve, is found along Australia's eastern coast south from central Queensland (Lamprell and Whitehead, 1992), and is farmed commercially in Tasmania and experimentally in New South Wales (Heasman *et al.*, 1995). The techniques employed to culture *P. fumatus* have included meshed enclosures such as pearl cages, lantern cages, and plastic trays, earhanging and adhesion to tapes (Cropp, 1985; Cropp 1989; O'Connor *et al.*, 1994).

A major problem with meshed enclosures, apart from high capital costs, has been biofouling, which can reduce growth rates in scallop culture (Cropp, 1985; Paul and Davies, 1986; Bourne *et al.*, 1989; Côté *et al.*,1993; Claereboudt *et al.*, 1994a; Lodeiros and Himmelman, 1996), either by direct competition for food (Mook, 1981; Lesser *et al.*, 1992) or by reducing water flow (Cropp, 1989). A second problem, inadvertent clamping " biting", can occur when scallops are forced together by swimming (due to overcrowding) or by cage motion. Biting results in damage to the shell secreting cells of the mantle which in turn can give rise to deformities and stunting (Palmer 1980; Ventilla, 1982; Bourne *et al.*, 1989; Hardy, 1991).

Earhanging can reduce the negative effects of the surrounding mesh on growth rates (Ventilla, 1982; Roman and Fernandez, 1991; Dadswell and Parsons, 1991; Heasman *et al.*, unpublished data) and prevents "biting". However, handling mortalities due to the ear drilling process and predation have limited the initial size at which scallops can be safely earhung. Generally scallops are not earhung until they have reached 55 mm or more in shell height (Ventilla, 1982; Roman and Fernandez, 1991; Dadswell and Parsons, 1991).

Alternatives to mesh enclosed culture include fixing scallops to a variety of substrates, such as rope (Hardy, 1991), polypropylene tapes (Cuthbertson, 1979; Cropp, 1985; Day, 1995; Cropp, 1989) or "Bondo" (Ventilla, 1982) a technique using modified lantern cages . Adhesive culture techniques alleviate some fouling and biting problems and have the additional advantage of being able to accommodate smaller scallops than used in earhanging techniques.

Disk culture, an adaption of the "Bondo" technique (Ventilla, 1982), involves the adhesion of scallops to a vertical array of rigid plastic disks spaced down a central

supporting rope. This technique utilises the advantages of non-enclosed culture while providing greater flexibility in predator protection and maximising growth. It also increases the surface area available for stocking, although the previously demonstrated effects of flow velocity, seston concentration (Cahalan *et al.*, 1989; Wildish *et al.*, 1992; Eckman and Duggins, 1993) and orientation to flow (Wildish *et al.*, 1987; Claereboudt *et al.*, 1994a) on growth rate needs further evaluation. This study was undertaken to investigate the utility of disk culture and to assess the impact of disk spacing and scallop orientation on growth and survival. Due to the impact of spionid polychaete infestation in molluscs, their putative effects on scallop population regulation (Hamer and Jacobs, 1987) and their previous impact upon trial farming operations in Twofold Bay (O'Connor *et al.*, 1994), cultured scallops were examined for spionid polychaete prevalence.

Materials and methods

General

Disk culture involved gluing five outward facing scallops, at regular intervals, set 40 mm in from the perimeter of 250 mm diameter, 10 mm mesh, rigid plastic disks (Fig. 4.3.1). A combination of two adhesives, a polyurethane marine adhesive (Sikaflex-241; Sika, Auckland, New Zealand) and cyanoacrylate adhesive (Loctite 454 instant adhesive; Loctite, Caringbah, NSW, Australia), were used to attach the scallops to disks. The adhesives were allowed to cure for 5 min before the scallops were returned to water. Seven disks of scallops were then threaded onto 5 mm rope and separated using spacers made of 20 mm diameter PVC conduit. An additional disk without scallops was placed at the top and bottom to complete each stack (Fig. 4.3.1). Two experiments were conducted in which growth and survival of scallops glued to discs were compared to those of scallops earhung on loop line (Hoyo Corp., Toyohashi, Japan) and/or scallops held in plastic lantern cages (eight 250 mm diameter, 10 mm mesh plastic disks, enclosed in 13 mm mesh size polyethylene netting; Fig. 4.3.1). Three replicate stacks of disks, loop lines and lantern cages were used for each treatment, with each replicate containing a total of 35 scallops.

Both experiments were preceded by two preliminary phases of one and two weeks duration, respectively. During the first preliminary phase (week 1), each replicate not already protected with mesh (all except lantern cages) was enclosed in a stocking of 13 mm mesh size, plastic netting. All replicates were then suspended subtidally at a breakwater enclosed pontoon wharf at HMAS Cresswell, Jervis Bay for one week.

During this first week, adhesion to disks, detachment from loopline and handling mortality were recorded. In the second preliminary phase (weeks 2 and 3), the protective mesh was removed from all but the lantern cages and predation (scallops with visible signs of damage, such as cracked valves or scallops lost from disks) and further mortality (moribund scallops, not damaged and with soft tissue intact) were recorded daily.

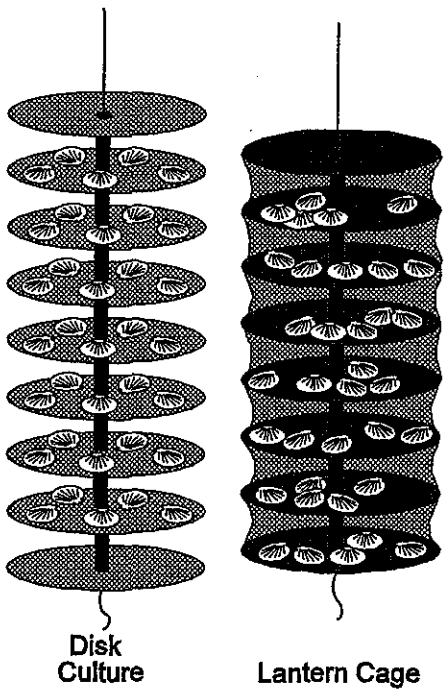

Figure 4.3.1 Disk and meshed cage culture techniques used for *Pecten fumatus*.

In the final growout phase of each experiment, all replicates were restored to their original stocking numbers (n=35) using spare scallops or disks of scallops that had been held concurrently at the pontoon. Each replicate was then transferred to a mid-water longline system located in 17 m of water at a sheltered location near Murrays Beach just inside the entrance of Jervis Bay, NSW (35°04' S, 150°44' E).

At the commencement of each experiment, 20 spare scallops were sampled at random and shell height recorded to the nearest 0.1 mm. Soft body tissue was removed from the shell, blotted dry and weighed to the nearest 0.1 g, then dissected and the muscle and

gonad tissues were weighed separately to the nearest 0.1 g. A stereo microscope was used to inspect each valve for the presence or absence of mud blisters and other evidence of "shell boring" indicative of spionid polychaete worms. Upon retrieval from the longline, the number of scallops remaining in each replicate stack was recorded. Shell height and tissue weights of 20 scallops from each replicate were determined and both their left and right valves inspected for evidence of polychaete worm infestation as previously described.

Experiment 1: The effect of culture method and orientation in disk culture on scallop growth and survival

The performance of scallops, initial mean shell height of 35.0 ± 2.5 mm, glued to disks in one of four orientations, glued by the left (flat) or right (cupped) valve in either normal (left valve uppermost) or inverted orientation (Fig. 4.3.2), was compared to that of similar sized scallops held in lantern cages. For all treatments including lantern cages, disks were spaced 80 mm apart so that stocking densities for each treatment were the same. As previously described, each treatment, following three weeks at the pontoon, was transferred to the longline for 13 weeks ongrowing from mid summer to mid autumn (January to April 1996).

Experiment 2: The effect of disk spacing on scallop growth and survival

For disk culture, scallops of initial mean shell height 40.2 ± 2.8 mm were glued by the left valve in normal orientation at each of four disk spacings of either 40, 60, 80 or 100 mm. Three additional treatments were included: scallops in lantern cages (100 mm spacing), mesh enclosed disk culture (glued by the left valve in normal orientation, 100 mm spacing) and earhung scallops. After the preliminary three weeks observation at the pontoon, all treatments were relocated to the longline for a further 25 weeks from mid autumn to mid spring (April to September 1996).

Statistical analysis

Percentage data was arcsine $x^{0.5}$ transformed and shell height, muscle, soft body and gonad weight where log transformed where necessary, to satisfy requirements for homoskedasticity before ANOVA (Sokal and Rholf, 1981). Where significant differences ($P<0.05$) were detected, treatment means were compared using Student-

Newman-Kuels procedure (Winer *et al.*, 1991).

Results

Experiment 1: The effect of culture method and orientaion in disk culture on scallop growth and survival

Scallop mortality over the first week (attributed to handling) was generally low (<3%) and did not differ significantly between treatments (F=1.13; df 4/10). However, detachment from disks, attributed to poor adhesion, did differ with treatment (Table 4.3.1), being significantly greater among scallops glued by the right valve (F=10.70; df 3/8). Scallop survival after the two weeks exposure at the pontoon also differed significantly with treatment (F=4.97; df 4 /10). Scallops glued by the left valve had the highest survival, being significantly greater than that of scallops held in lantern cages (Table 4.3.1).

After 13 weeks under warm water conditions on the longline (Table 4.3.2), significant differences had developed between treatments in survival, shell height and muscle, gonad and soft body weights (F= 4.11, df 4/10; F= 1.08, df 4/10; F=28.44, df 4/10; F= 16.06, df 4/10; F= 22.41, df 4/10, respectively). Scallops glued by the left valve had the greatest survival, mean shell height and tissue weights. With the exception of survival, scallops held in lantern cages consistently exhibited the lowest performance of all treatments trialed.

Within disk treatments, normal or inverted orientation of glued scallops had no significant effect upon any of the variables measured. In contrast, the choice of valve by which the scallops were glued to disks effected performance. Gluing scallops by the left valve provided significant advantages in survival and all growth criteria except gonad weight (Tables 4.3.1 and 4.3.2).

Table 4.3.1. The effects of culture method and scallop orientation in disk culture on handling mortalities, escapement, predation and overall retention of *Pecten fumatus* suspended from a pontoon in Jervis Bay, NSW, for three weeks (Experiment 1, preliminary phase).

Treatment		Handling mortalities*	Escapement†	Predation	Survival (%)
Lantern cage		2 ± 1^{a}	-	0^{a}	78 ± 4^{b}
Disk:	Glued by left valve in normal orientation†	1 ± 1^{a}	1 ± 1^{a}	0^{a}	84 ± 2^{a}
	Glued by left valve in inverted orientation	1 ± 1^{a}	1 ± 1^{a}	0^{a}	86 ± 5^{a}
	Glued by right valve in normal orientation	2 ± 1^{a}	7 ± 4^{b}	5 ± 3^{b}	66 ± 10^{b}
	Glued by right valve in inverted orientation	1 ± 1^{a}	4 ± 2^{b}	3 ± 2^{b}	76 ± 9^{ab}

Values are means ± SD. Values within columns with a common superscript do not differ significantly ($P>0.05$).

† Escapement losses attributed to poor adhesion of the scallop to the disk.

* Percentage mortality of scallops occurring during the first 7 days of suspension at the pontoon.

† Normal orientation is left (flat) valve upper most.

Table 4.3.2 The effects of culture method and scallop orientation in disk culture on survival, shell height, muscle, gonad and soft body weight of *Pecten fumatus* held on a longline in Jervis Bay, NSW for 13 weeks (Experiment 1, growout phase).

Treatment	Shell height (mm)	Muscle weight (g)	Gonad weight (g)	Soft body weight (g)	Survival (%)
Lantern cage	50.4 ± 0.7 ^{a}	3.4 ± 0.1 ^{d}	2.3 ± 0.2 ^{c}	10.4 ± 0.4 ^{b}	83 ± 5 ^{ab}
Disk: Glued by left valve in normal orientation †	52.1 ± 0.2 ^{b}	4.1 ± 0.0 ^{a}	2.6 ± 0.1 ^{b}	12.2 ± 0.1 ^{a}	91 ± 3 ^{b}
Glued by left valve in inverted orientation	51.7 ± 0.6 ^{b}	4.1 ± 0.1 ^{ab}	2.7 ± 0.1 ^{b}	12.1 ± 0.2 ^{a}	89 ± 2 ^{b}
Glued by right valve in normal orientation	51.3 ± 0.1 ^{b}	3.7 ± 0.1 ^{c}	2.9 ± 0.0 ^{a}	11.8 ± 0.2 ^{a}	84 ± 8 ^{a}
Glued by right valve in inverted orientation	51.6 ± 0.2 ^{b}	3.9 ± 0.1 ^{bc}	2.9 ± 0.0 ^{a}	12.0 ± 0.4 ^{a}	77 ± 5 ^{a}

Values are means ± SD. Values in columns with a common superscript do not differ significantly (P>0.05). † Normal orientation is left (flat) valve upper most. Initial mean shell height, muscle weight, gonad weight and soft body weight were 35.0 mm, 0.9 g, 0.5 g and 3.4 g, respectively (n= 30)

Experiment 2: The effect of disk spacing on scallop growth and survival

In the first week of deployment at the pontoon (Table 4.3.3), handling mortalities differed significantly with culture method (F=5.16; df 6/14). Earhung scallops suffered high handling mortalities, while all other treatments had similar low handling mortalities. Losses during the first week due to poor adhesion or escape from cages and earhung lines were low (≤ 3%) and did not differ significantly between disk treatments (F=0.96; df 4/10). During the second phase at the pontoon, following the removal of protective mesh from the four disc spacing treatments and earhung scallops, significant differences in predation occurred between culture methods and between disc spacings (F=56.15; df 6/14). Predators, notably blue groper (*Archoerodus viridus*, Steindachner), were observed removing scallops from earhung treatments. Other culture methods were less effected (Table 4.3.3). Among the disk treatments, disks spaced at 100 mm had significantly greater losses through predation, while the remaining disks experienced similar low levels of predation. Over-all survival at the pontoon differed significantly with treatment (F=37.31; df 6/14) ranging from total mortality on earhung lines to 100% survival for mesh enclosed disk culture.

After 25 weeks on the longline, survival did not differ significantly among treatments (F=3.29; df 6/14). Earhung scallops again suffered the greatest losses, however, *post hoc* multiple range tests were insufficiently powerful to detect treatment differences. Shell height (Table 4.3.4) increase differed significantly among treatments (F= 35.65; df 6/14), as did soft body, muscle and gonad weights (F=17.37; df 6/14, F=37.64; df 6/14 and F=36.91; df 6/14, respectively). All types of tissue weight gain were much greater in treatments not enclosed in mesh. Indeed, gains in adductor muscle weight in non mesh enclosed scallops were in the order of twice that of their enclosed counterparts, while gonads weights were of the order of four times greater in non enclosed scallops. Although tissue weights were uniformly heaviest in scallops glued to disks spaced at 100 mm intervals (Table 4.3.4), increasing the distance between disks beyond 60 mm did not significantly increase the yield of saleable flesh (muscle plus gonad) nor had any effect on mean survival which was uniformly high (90 to 93%, Table 4.3.4) in all disk grown scallops.

Table 4.3.3 The effects of culture method and disk spacing on handling mortalities, escapement, predation and survival of *Pecten fumatus* held at a pontoon for three weeks (Experiment 2, preliminary phase).

Treatment	Handling mortalities*	Escapement†	Predation	Retained alive (%)
Lantern cage	1 ± 2^{a}	_	16 ± 17^{bc}	83 ± 16^{bc}
Earhung	16 ± 6^{b}	_	100 ± 0^{d}	0^{d}
Disk spacing:				
40 mm	2 ± 2^{a}	1 ± 2^{a}	0^{a}	97 ± 3^{ab}
60 mm	2 ± 2^{a}	5 ± 2^{a}	0^{a}	93 ± 3^{abc}
80 mm	3 ± 3^{a}	5 ± 8^{a}	3 ± 3^{ab}	90 ± 14^{abc}
100 mm	2 ± 3^{a}	0^{a}	23 ± 6^{c}	75 ± 9^{c}
100 mm + mesh	0^{a}	0^{a}	0^{a}	100 ± 0^{a}

Values are means ± SD. Values in columns with a common superscript do not differ significantly ($P>0.05$).

† Escapement losses attributed to poor adhesion of the scallop to the disk.

* Percentage mortality of scallops occurring during the first 7 days of suspension at the pontoon.

Table 4.3.4 The effects of culture method and disk spacing on survival, shell height, muscle, gonad and soft body weight of *Pecten fumatus* held on a longline in Jervis Bay, NSW, for 25 weeks (Experiment 2, growout phase).

	Shell height (mm)	Muscle weight (g)	Gonad weight (g)	Soft body weight (g)	Survival (%)
Lantern cage	52.2 ± 0.4 [c]	3.2 ± 0.3 [c]	0.7 ± 0.3 [b]	9.9 ± 2.1 [b]	90 ± 10 [a]
Earhung	55.2 ± 1.3 [b]	4.9 ± 0.4 [b]	2.9 ± 0.2 [a]	15.3 ± 1.0 [a]	71 ± 10 [b]
Disk attached:					
40 mm spacing	58.2 ± 0.8 [a]	5.1 ± 0.3 [b]	2.1 ± 0.1 [a]	14.5 ± 1.1 [a]	93 ± 2 [a]
60 mm spacing	58.8 ± 1.0 [a]	6.0 ± 0.4 [a]	2.7 ± 0.2 [a]	17.1 ± 1.1 [a]	91 ± 8 [a]
80 mm spacing	57.9 ± 0.6 [a]	5.8 ± 0.4 [a]	2.7 ± 0.2 [a]	16.6 ± 0.5 [a]	91 ± 5 [a]
100 mm spacing	59.3 ± 0.6 [a]	6.1 ± 0.8 [a]	2.9 ± 0.2 [a]	17.8 ± 0.4 [a]	92 ± 4 [a]
100 mm + mesh (control)	54.2 ± 0.4 [b]	3.6 ± 0.2 [c]	0.6 ± 0.2 [b]	10.5 ± 0.3 [b]	90 ± 7 [a]

Values are means ± SD. Values in columns with a common superscript do not differ significantly ($P>0.05$). Initial mean shell height, muscle weight, gonad weight and soft body weight were 40.2 mm, 1.5 g, 1.0 g and 4.2 g, respectively (n= 30)

Prevalence of spionid polychaetes (mudworms) and the incidence of shell deformities

Mudworm prevalence (Table 4.3.5) differed significantly with culture method and orientation (F= 74.62; df 4/10). Scallops contained in lantern cages and scallops glued by the right valve in normal orientation had identical, relatively high levels of mudworm infestation, while scallops in the remaining three treatments had lower incidence of polychaete infestation. The 41% increase in shell deformity in lantern cages was significantly higher (F=25.00; df= 4/10) than disk culture, where it varied from 7 to 16%.

Increasing the distance between disks (Table 4.3.6) had no significant effect on the prevalence of mudworm infestation or number of shell deformities (F=0.73; df 6/14). Scallops from all disk culture and mesh enclosed disk culture treatments had similar percentages (1 to 9%) of mudworm infestation (Table 4.3.6), although infestation in these treatments was significantly lower than the 23 and 34% infestation observed in scallops from lantern cages and earhung scallops, respectively (F=9.65; df=6/14). There were no significant differences in the number of shell deformities between any of the treatments in this experiment.

Table 4.3.5 The effect of orientation in disk culture on mean percentage increase of *Pecten fumatus* displaying evidence of polychaete infestation and shell deformities (Experiment 1).

Treatment	Lantern cage	Disk			
Orientation		Glued left Normal†	Glued left Inverted	Glued right Normal†	Glued right Inverted
Mean prevalence of spionind polychaetes (%)	51 ± 3 ^{a}	19 ± 3 ^{b}	15 ± 2 ^{b}	51 ± 5 ^{a}	21 ± 2 ^{b}
Mean increase in shell deformities (%)	41 ± 6 ^{a}	12 ± 5 ^{bc}	7 ± 3 ^{b}	16 ± 2 ^{c}	11 ± 2 ^{bc}

Values are means ± SD. Values in rows with a common superscript do not differ significantly (P>0.05). † Normal orientation is left (flat) valve upper most.

Table 4.3.6 The effect of spacing between disks on mean percentage increase of *Pecten fumatus* displaying evidence of Spionid polychaete (mudworm) infestation and shell deformities (Experiment 2).

	Lantern cage	Earhung	Disk spacing				
			40 mm	60 mm	80 mm	100 mm	100 mm + mesh
Increased mudworm infestation (%)	34 ± 8 [b]	23 ± 8 [b]	2 ± 0 [a]	2 ± 4 [a]	7 ± 5 [a]	9 ± 3 [a]	1 ± 7 [a]
Mean increase in shell deformities (%)	8 ± 5 [a]	6 ± 6 [a]	2 ± 2 [a]	6 ± 4 [a]	13 ± 5 [a]	3 ± 1 [a]	3 ± 2 [a]

Values are means ± SD. Values in rows with a common superscript do not differ significantly ($P>0.05$).

Discussion

Techniques developed for scallop cultivation frequently face inherent problems in attempting to optimise growth rates without compromising survival. Disk culture of *P. fumatus*, a modification of the Japanese "bondo" technique (Ventilla, 1982), has shown particular promise in comparison to earhanging and mesh enclosed culture in Jervis Bay. Initially the process of gluing scallops to disks was found to be relatively benign with mortality attributed to handling generally below 3%. Although losses due to poor adhesion (escapement, Tables 4.3.1 and 4.3.3) were uniformly low (< 7%), irrespective of orientation, scallops glued by the flat left valve were retained significantly more securely.

Overall, predation was found to be the greatest immediate threat to suspended culture of scallops in Jervis Bay, although, even within this study, site dependent effects were clearly evident. Scallops have a wide variety of predators including fish, crabs, octopus, starfish and predatory molluscs (Olsen, 1955; Medcof and Bourne, 1964; Elner and Jamieson, 1979; Ventilla, 1982; Robinson, 1993). Rates of predation can be exacerbated by the fact that scallops from suspended culture have been shown to have thinner shells than wild scallops (Ventilla, 1982; MacDonald, 1986). When suspended at the sheltered pontoon wharf site in Jervis Bay known to host a wide range of potential predators, particularly large fish, loss of earhung scallops was both rapid and complete. In contrast, alternative suspended culture methods offering some measure of protection against fish, such as mesh protected lantern cages and mesh enclosed disks spaced at 80 mm or less were relatively unaffected. Even so, predation was not consistent at the pontoon. In Experiment 1, lantern cages offered complete protection, however, in Experiment 2, mortality rose to 16% and was attributed to the arrival of several large groper (*A. viridus*). Although these fish had no apparent affect on scallops glued inwards of the margins of mesh protected disks, free moving scallops that strayed to the edges of the lantern cages were vigorously attacked through the mesh.

At the longline site, the type and severity of predation changed markedly. Survival of earhung scallops was the lowest recorded but had increased from no survival at the pontoon to 71% on the longline, while the advantage of mesh protection over exposed disks was reduced. In the latter example, mesh did not confer protection against starfish which had apparently settled as planktonic larvae and metamorphosed in the suspended cages. With respect to disk culture, fish predation still occurred and the advantage of closer disk spacing became apparent. However, in all disk treatments, starfish were far less numerous, possibly because they too were not afforded mesh protection from predators in this situation. At both the pontoon and the longline site, scallop orientation

conferred an advantage against predators (Tables 4.3.1 and 4.3.3). Observations by divers suggested that those scallops glued by the left valve presented a convex surface which prevented fish such as leatherjackets (Monacanthids) chipping away at the edges of the shell to gain access.

Growout

In Jervis Bay, disk culture of scallops has been shown to support growth greater than that observed in mesh enclosed culture and comparable with that of earhung scallops. Differences in shell height of scallops grown by the suspended culture techniques tested in Experiments 1 and 2 were more pronounced than those indicated by soft tissue indices of growth. However, as shell height increase is irreversible, it is seen as a poorer short term indicator of environmental conditions. Fluctuations in somatic tissue weights are a preferred indicator of short term environmental fluctuations (MacDonald and Thompson, 1985), especially for animals such as scallops that are sold largely on the basis of meat weight. Having said this, non-enclosed disk culture and earhanging produced similar soft tissue yields that exceeded those of mesh enclosed scallops. (Tables 4.3.2 and 4.3.4).

The choice of valve by which scallops were glued to disks and the disk spacing significantly effected soft tissue weights (Tables 4.3.2 and 4.3.4). Despite similar total soft body weights, scallops glued by the right valve had heavier gonads, while those glued by the left valve tended to have heavier adductor muscles. This is possibly the result of differential loading of the adductor muscle as illustrated in Figure 4.3.2.

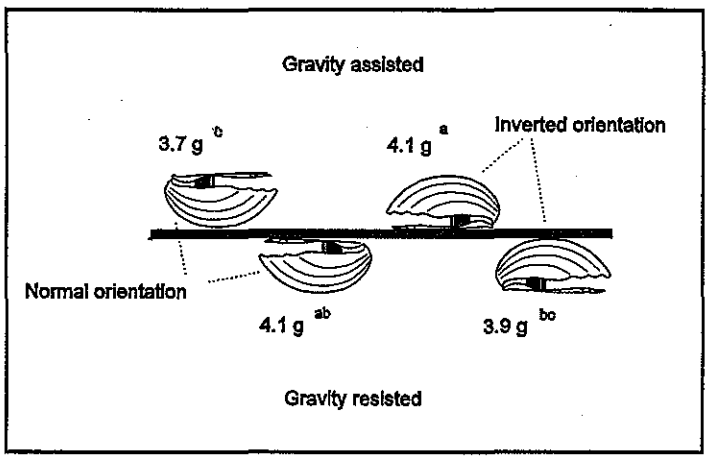

Figure 4.3.2 Resistance to adductor muscle contraction for *Pecten fumatus* glued to disks in differing orientation. Values are mean muscle weights for scallops held in each orientation, those with similar superscripts do not differ significantly ($P>0.05$).

Normally, the smaller and lighter, left valve of *P. fumatus* would be drawn towards the right valve, however if glued by the left valve, muscular contraction occurs against the increased resistance of a larger, heavier, right valve. This may result in increased muscle mass and energy expenditure at the expense of energy that might otherwise be devoted to gonadogenesis.

Growth rates for several bivalves have been well correlated with food availability (MacDonald and Thompson, 1985) and thus the observation that disk spacing effected scallop meat weights was not unexpected. Although mesh enclosure may be beneficial in locations of high current velocity (Claereboudt *et al.*, 1994b), in areas of moderate to low current velocity, the mesh and subsequent fouling reduce water flow past the scallops resulting in decreased growth (Cropp, 1985; Côté *et al.*,1993; Claereboudt *et al.*, 1994a; Lodeiros and Himmelman, 1996). Thus it was expected that a combination of increased stocking density per unit volume, reduced flow rates between disks and biofouling would limit seston availability. In general in this study, there were no appreciable changes in soft tissue weights with increasing disk spacing, except for a small but significant increase in the case of adductor muscle weight of scallops spaced at 60 mm and above (Table 4.3.4). This observation suggested an optimum disk spacing of 60 mm for farming of *P. fumatus* in Jervis Bay.

Polychaete prevalence and shell deformity

Shell boring spionid polychaetes, known colloquially as mudworms, have long been recognised as a major pest to bivalve aquaculture in Australia (Skeel, 1979; Dix, 1981). Mudworms can produce unsightly blisters or shell deformities that prevent scallops being marketed in the shell and in more extreme circumstances have been a cause of mortality in *P. fumatus* in both Tasmania (Dix, 1981) and New South Wales (Hamer and Jacobs, 1987; O'Connor *et al.*, 1994). Thus, the potential economic ramifications of mudworm infestation for scallop farming can be dramatic as illustrated by the fact that in some treatments more than half the scallops were infested.

Typically, mudworm infestation in *P. fumatus* is similar to *Polydora variegata* infestation in *Patinopecten yessoensis* (Mori *et al.*, 1985; Sato-Okoshi, 1994), in that it occurs almost exclusively on the left valve (Hamer and Jacobs, 1987). Whether this results from settlement of polychaete larvae on the flat left valve which is normally uppermost, or from difficulties in boring through the curved right valve is unclear, but, this may help to explain the significant reduction in polychaete prevalence evident in this study. Only 5% of mud blisters found after longline culture were on the right valve

(S.J. O'Connor pers. obs) and those scallops glued by the left valve or glued with the right valve in inverted orientation showed significantly lower polychaete prevalence (Table 4.3.5 and 4.3.6). The adhesives used on the surface of the left valve may have affected any polychaetes present at the time of application and/or inhibited further infestation. In addition, inverted orientation (with the curved right valve uppermost) may reduce the amount of material settling and remaining on the scallop, which may include polychaete larvae. Indeed, Dix (1981) observed reduced mortality in vertically oriented *P. fumatus* and suggested that decreased silt accumulation might have reduced the incidence of Polydora infestation.

The occurrence of shell deformity in Experiment 1(Table 4.3.5) was much lower in disk culture (7 to 16%) than in lantern cages and was attributed to a combination of the prevention of "biting" and a reduced incidence of mudworm. However, in Experiment 2, the number of scallops with deformed shells was relatively low and did not differ among treatments. The lower incidence of shell deformity in Experiment 2 may in part be due to a reduced incidence of mudworm, but may also have been influenced by rough seas in Jervis Bay experienced during Experiment 1. The oscillation of cages caused by large swells can gather loose scallops together, exacerbating the "biting" phenomenon.

Conclusion

Earhanging has previously been shown to promote greater growth rates and meat weights than conventional meshed enclosures (Ventilla, 1982; Mottet, 1979; Hardy, 1991; Roman and Fernandez, 1991), however, the drilling process and predation dictate a minimum size of about 55 mm at which scallops can be safely transferred from nursery culture (Ventilla, 1982; Roman and Fernandez, 1991; Dadswell and Parsons, 199; Heasman *et al.*, unpublished data). Like cage culture, disk culture allows scallops to be removed from nursery culture at a smaller minimum size of about 30 mm without increased predation or handling mortality (Table 4.3.3). Disk culture is capable of maintaining both shell and somatic growth at equivalent rates to those observed in similar sized earhung scallops (Table 4.3.4). To enhance survival and reduce the prevalence of spionind polychaetes, it is recommended that *P. fumatus* be glued by the left valve and that disks be spaced at intervals of 60 to 80 mm to maintain predator protection, promote rapid growth and achieve a high production per lineal metre of longline and hence per unit capital cost. However, it is recommended that alternative, cheaper adhesives be identified and extensively field tested before this method of culture is commercially adopted.

4.4 SCALLOP RESEEDING TRIALS

4.4.1 EVALUATION OF SURVEY METHODS

Introduction

The development of successful fisheries enhancement programs is dependent on the accuracy with which a seeded population can be monitored and the ability to identify the cause of any losses. Therefore, as the initial step in a program to seed scallops in Jervis Bay, a comparative evaluation was made of the efficiency of alternative diver sampling techniques.

Several methods have been used to monitor scallop populations in Australia including catch data from trawls or dredges (Joll, 1994), video monitoring (R. McLoughlin pers. comm., 1992) and dive surveys (Fairbridge 1953, Zacharin *et al.* 1990, Fuentes *et al.* 1994). While each method offers advantages, dive surveys (where practical) offer greater precision in estimating scallop abundance (Zacharin *et al.* 1990, Fuentes 1994) and permit a variety of supplementary *in situ* observations to be made. However, diver surveys can be limited by the availability of competent, experienced divers and are commonly restricted by the short duration divers can work safely at depth.

Handling mortality is considered to be a significant risk when handling juvenile scallops (Salina 1994), particularly if the scallops are held out of water (emersed) for protracted periods. In this study, subsamples of seeded scallops were deployed in "predator proof" cages at the seeding site in order to estimate handling mortality and provide insight into the impact of predators on unprotected seeded scallops.

The aims of this study were firstly to identify an accurate and efficient sampling procedure and to evaluate the influence of diver experience on these procedures. Secondly, to use of caged scallops as a means of quantifying initial handling mortality and predation.

Materials and Methods

All scallops used in this study were produced in the hatchery at the NSW Fisheries, Port Stephens Research Centre and ongrown in pearl and lantern cages at Murrays Beach, Jervis Bay.

Sampling method

At the time of the experimentation (Oct. 1995), the scallop beds in Jervis Bay were in areas varying from sand or silty sand through to more complex substrates of silty sand, shell debris and sparse marcophyte cover. A site representative of the latter, more complex bottom type was selected in approximately 10 m of water off Hole-in-the-Wall on the southern shore of Jervis Bay (Fig. 4.4.1). A rope grid was positioned on the bottom to create a 25 x 25 m square plot comprising 25, 5 x 5 m subplots (quadrats) and any naturally occurring scallops (n=3) were removed. Rope transect lines, 10 m long, were placed randomly within the 25 x 25 m grid. Each transect line was pegged to the bottom at one end and weighted at the other. Next, 100 scallops, ranging in size from 19-50 mm shell height, were placed into each of the 25 quadrats within the grid (ie at a mean density of 4 scallops m^{-2}). Half an hour after seeding the plot, scallop numbers were estimated by each of three methods. In the first, a 1 m long stick was used as an aid to estimate scallop numbers within a corridor of 0.5 m either side of the 10 m transect line. To undertake the second sampling technique the weighted end of the same transect line was rotated through 90° to ensure the transect area remained within the plot. A sampling exercise was then repeated in the same manner as the first, except that the sampled corridor was increased to 1 m either side of the line. In the third procedure, divers were deployed to a designated 5 x 5 m subplot and without handling the scallops or disturbing the plot, counted all scallops sighted during a systematic search of the subplot. Each of the three sampling methods was undertaken three sites chosen at random within the grid by three research divers and experienced in scallop collection.

The following day, six divers, three research and three recreational divers (who had observed scallops in the wild previously), were asked to repeat the sampling exercise from the previous day. In addition, these divers were asked to search a randomly designated 5 x 5 m subplot and remove all the scallops they could find. These scallops were placed in a bag and taken to the surface for measurement. Throughout the exercise all divers were accompanied by a "buddy" to record the number of scallops observed in each sample and the time taken to complete each sample. Divers were instructed that speed was not the objective and that they were to take as long as they considered necessary to satisfactorily complete each sampling task.

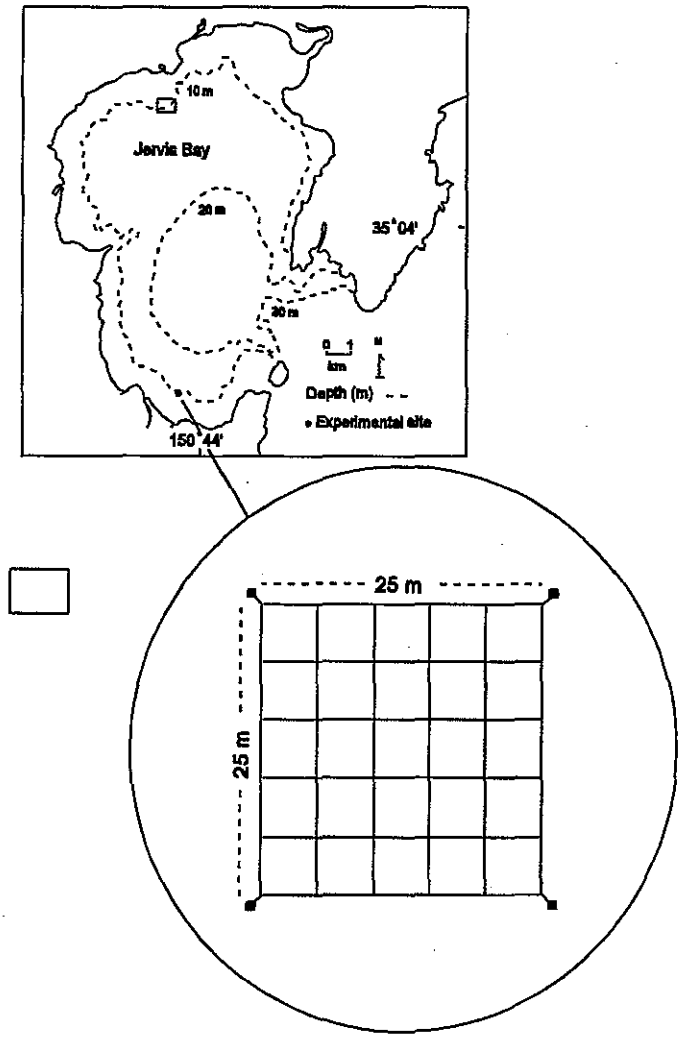

Figure 4.4.1 location of grid site in Jervis Bay seeded with hatchery produced scallops.

Scallop cage trials

Immediately before their deployment, subsamples of scallops were taken, measured and placed in 60 x 35 x 10 cm perforated plastic trays (prawn freezer trays, Nally, Minto, NSW) and enclosed with light-weight polythene netting. These caged subsamples were then deployed by divers on the sea floor within the seeded plot. Growth and survival of scallops (initial shell height 16.5 ± 2.6 mm, mean ± s.d.) were assessed in trays enclosed in either 6 or 10 mm polythene netting (Sarlon, Punchbowl, NSW). Each netting size was used to enclose 16 trays, stocked at a density of 12 scallops/tray. The trays were weighed down by attaching two house bricks to their undersides and deployed on the sea floor in 10 m of water off Hole-in-the-wall. After 24 h and again after 72 h, divers inspected each cage to record any mortality that was ascribed to

handling stress. Every three weeks over the following 16 week period, four replicate trays from each treatment were retrieved by divers and growth (increase in shell height to the nearest 0.1 mm) and survival of scallops were recorded.

In a second experiment, four sets of 16 trays were enclosed in 10 mm light-weight polythene mesh and stocked at a density of either 5, 10, 20 or 40 scallops/tray (shell height 41.2 ± 2.7 mm, mean ± s.d.) before being deployed at the same Hole-in-the-wall site. Four replicate trays of each stocking density were removed at 4, 8, 12 and 16 weeks after deployment and growth and survival recorded as previously described.

Results

Sampling methods

As the area covered by the each of the three sampling methods differed, the scallop densities observed by divers were standardised to scallops m^{-2} and the time spent by each diver conducting each sampling procedure was standardised as time (seconds) m^{-2}. Results of surveys 30 min after scallop deployment and again 24 hrs later are given in Table 4.4.1. Numbers of scallops found after 30 min were always lower than the original stocking density and varied according to sampling method. The 10 x 1 m transects provided highest estimate of scallop density and the estimate most closely approximating the original stocking density, followed by 10 x 2 m transects and 5 x 5 m quadrats, respectively.

After 24 hr, the densities of scallops observed by divers, regardless of survey technique, had fallen dramatically. The numbers of scallops found with each sampling method still differed significantly ($P<0.05$) and were consistent with the order of accuracy found after 30 min (10 x 1 m > 10 x 2 m > 5 x 5 m). The previously untested method of collecting from 5 x 5 m quadrats improved returns over both 10 x 2 m transects and 5 x 5 m observations, but the numbers of scallops found remained significantly lower than in 10 x 1 m transects.

Irrespective of survey method, researchers experienced in scallop collection did not find significantly more scallops than the recreational divers (Table 4.4.2). However, researchers were significantly faster in completing each sampling procedure (Table 4.4.2). Within each of the two groups of divers, the time taken per unit area by divers did not vary significantly between survey methods. Correspondingly, within sampling methods, no significant correlations ($P<0.05$) were found between sampling duration

and the number of scallops found. Estimates of turbidity made by each pair of divers put visibility at approximately 5 m and was not considered by any of the divers to be an impediment to the sampling methods tested.

To assess whether a divers' ability to find scallops was reduced in relation to the smallest size classes likely to be used in seeding trials, a Chi squared comparison was made of the numbers of scallops deployed and those recovered in five size classes (<25 mm, 26-30 mm, 31-35 mm, 36-40 mm and >40 mm). While individual divers differed significantly from one another in the mean size of scallops recovered (F=2.09; df=5/221; P<0.05), all divers were more likely to find larger than smaller scallops (X^2 = 14.83; df 4; P<0.01, Fig. 4.4.2).

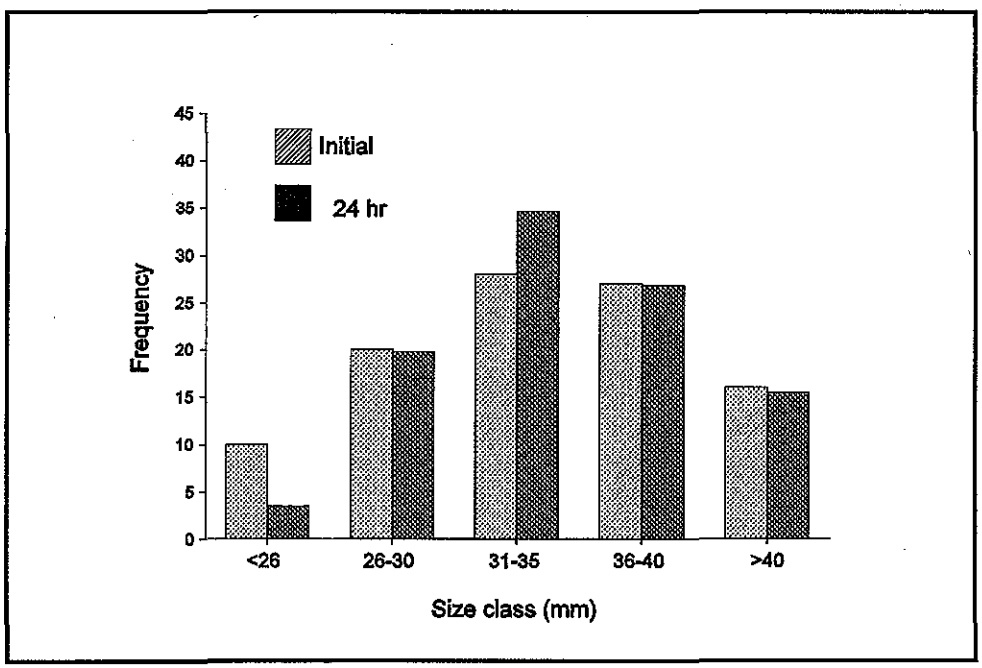

Figure 4.4.2 Comparison of size frequency histograms for scallops deployed and those recovered by divers 24 hr later

No evidence of the presence of predators (Octopus, starfish, crabs, etc) or predation (damaged or empty shells) was seen within the first 24 h of deployment.

Scallop cage trials

Mortality within 72 h of deployment was attributed to handling stress and trauma, and in both cage trials was negligible (<1%). Comparisons of growth and survival of scallops contained in cages under 6 and 10 mm mesh indicated both meshes could serve as suitable controls for seeding trials. Survival after the twelve week period did not differ significantly between meshes, however by the twelfth week scallops housed under 10 mm mesh were significantly larger ($F=6.238$; df 1/10; $P<0.05$, Fig. 4.4.3). Mortality rate remained low and constant averaging about 0.75% per week over the full 12 week duration of the experiment under both sizes of mesh. When stocked at one of four densities under 10 mm mesh, survival did not differ significantly between treatments after 16 weeks ($F=0.412$; df 3/12; $P>0.05$). Nevertheless, there was a general trend of increasing mortality rate that varied from about 0.8% per week at 5 scallops/tray to about 1.5% at 40 scallops/tray. Growth was however density dependent ($F=5.612$; df 3/12; $P<0.05$), decreasing with increasing stocking density from about 0.5 mm a week at the lowest stocking density (5 scallops cage^{-1}) to 0.25 mm a week in scallops at the highest tested density (40 scallops cage^{-1}) (Fig. 4.4.4).

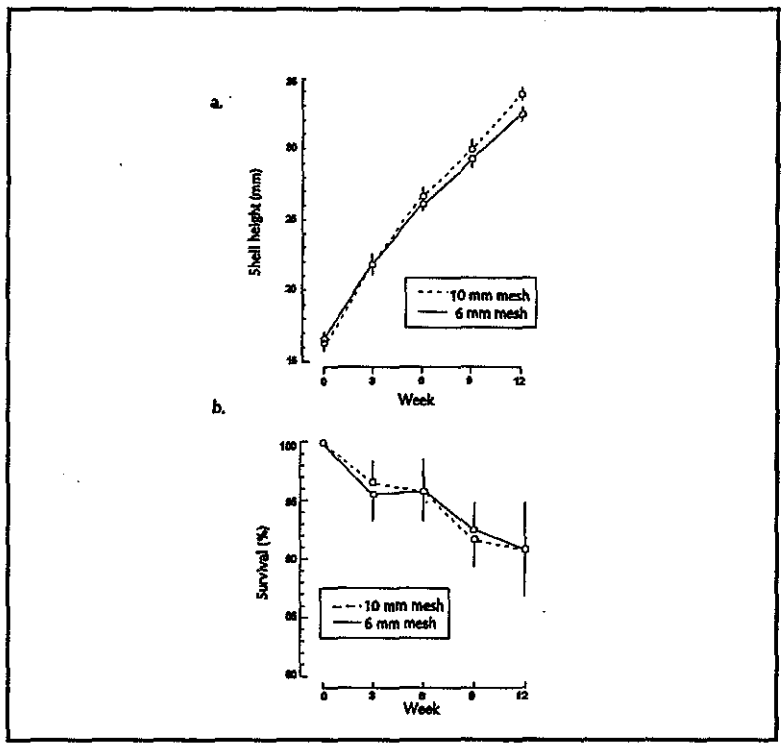

Figure 4.4.3 Growth (a) and survival (b) of scallops held in cages of two mesh sizes.

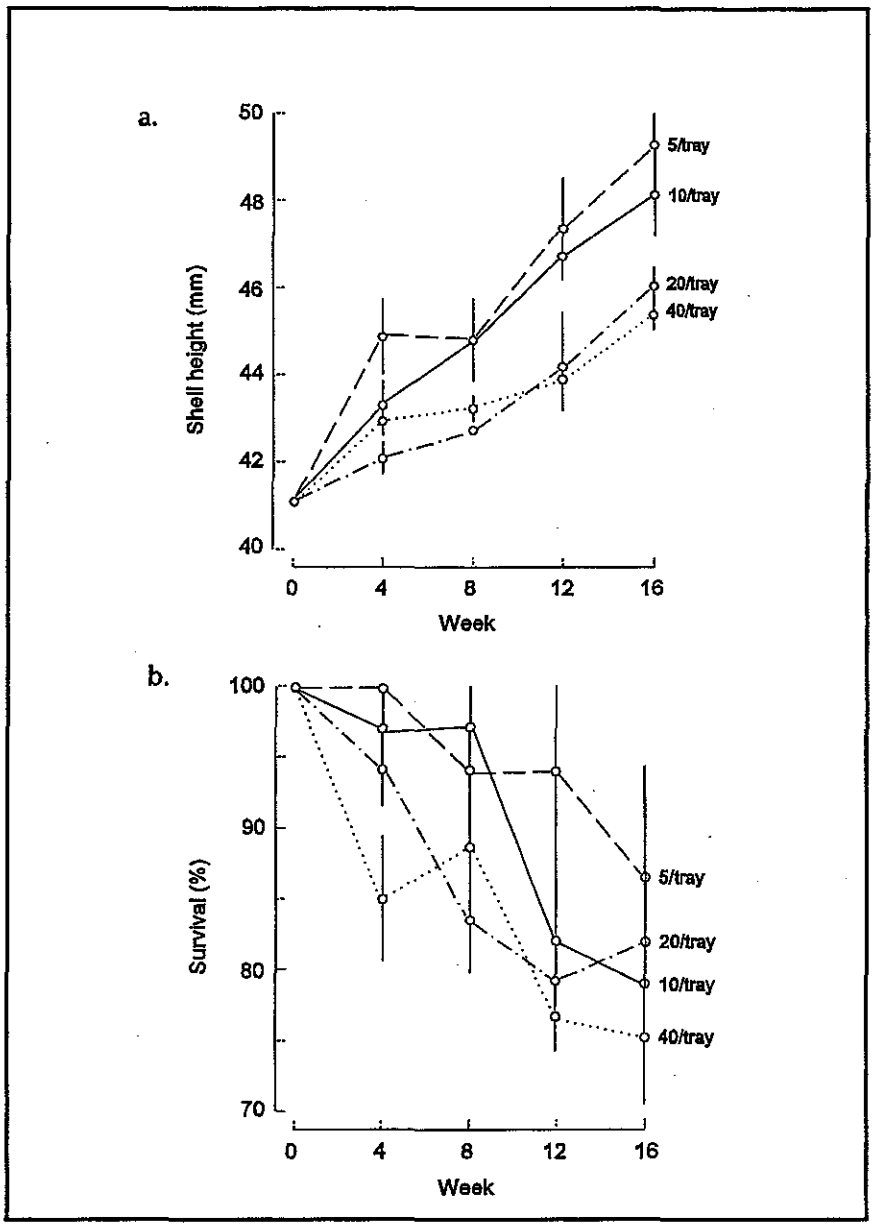

Figure 4.4.4 Growth (a) and survival (b) of scallops held in cages at one of four stocking densities.

Discussion

The success of scallop reseeding programs is variable (Tettelbach *et al.* 1990, Tettelbach and Wenczel 1993, Salina 1994) but will ultimately be a product of a number of variables, primarily, handling induced mortality, natural mortality (disease parasitism age etc), predation, dispersion and fishing pressure. In cases where survival is poor, it may be of some benefit to partition the effects of each factor.

Initially the accuracy of any estimate of scallop survival is to an extent dependant upon the sampling method. Diver surveys are popular as they do not imply disturbing scallops and they allow a variety of observations that may not be available in surveys conducted using other methods (ie dredging). This additional information could include observations of the presence and behaviour of predators, signs of predatory behaviour (crushed or broken shells) and scallop burial. However, diving surveys are not beyond reproach. Although the four methods evaluated in this study sampled only relatively small areas (10-25 m^2), they yield substantially different estimates of scallop density. Despite covering the smallest area and taking the least time to conduct, 1 m wide transects provided the best estimates of scallop density with the lowest levels of inter-diver variation (Table 4.4.1). All methods including 1 m wide transects did however provide a gross underestimate of actual scallop densities (4 m^{-2}). Estimated density only 24 h after deployment ranged from 1.2 m^{-2}, or 30% of initial stocking density in 5 x 5 m quadrats, to 1.9 m^{-2}, or 47.8% of initial stocking density in 1 m wide transects. This under estimate was thought to be indicative of the cryptic habit of scallops rather than any real loss through dispersion or predation.

The reseeding site in this study was chosen for its substrate complexity as it represented the most difficult of the potential reseeding sites to sample. However, it is to be expected that scallops would be recovered with greater efficiency at less complex, sandy sites, and that post-seeding surveys should be conducted after an appropriate interval to quantify the potential for sampling error on a site by site basis.

The interval before sampling needs to be sufficient for scallops to settle into the substrate in preferred areas but, in the case of *P. fumatus* in Jervis Bay, probably should not exceed 24 h. In this and subsequent seedings of scallops, dramatic reductions in numbers occurred rapidly. Although the reductions in this study were the most dramatic observed observed over the ensuing 3 years, and may have been influenced by the relatively small number of scallops deployed and the amount of macrofaunal and -floral shelter at the site, it has not been uncommon to record reductions in the number of seeded scallops observed as great as 10% a day.

Taking a subsample of the seeded stock and containing it in a predator proof enclosure provides a simple reliable estimate of what turned out to be a very low rate of handling mortality. This low rate could not have otherwise been differentiated by follow up sampling due to the high underestimation of the actual scallop density. In the short term, less than 1% of scallops died within 72 h in either cage experiment, and subsequently, scallops stocked at 5/tray (24/m^2) under 10 mm mesh suffered no mortality in the first four weeks (Fig. 4.4.4), allaying concerns of post handling

mortality.

In the longer term, scallops in cages are still presumably subject to diseases, parasites and predators capable entering or feeding through the mesh. The much lower mortality rates (less than 1% per week) sustained by scallops stocked in cages at low to moderate densities than sustained by seeded scallops is indicative of the efficiency of mesh enclosures in the prevention of long term predation. Although most scallops are not well adapted for migration (Brand, 1991), short term dispersion can be extensive and rapid (Cliche *et al.*, 1994). Mesh enclosure of scallops removed the possibility of dispersion and negated the ability of scallops to avoid detection by divers.

Suggested sampling regimen

The results of this study clearly indicate 10 x 1 m transects are the most accurate of the tested sampling methods for either experienced researchers or recreational divers, and that searches should be conducted within 24 hours to assess sampling error with respect to different substrate types characteristic of release areas. In order to assess handling mortality, a subsample of scallops from the seeded population should caged and deployed at the seeding site. Scallop density within cages should not exceed 24/m^2 and for Jervis Bay a mesh size of 10 mm provides adequate predator protection without rapid fouling. Caged scallops can be monitored for several months after deployment to indicate the level of predator protection they afford. Concurrent with continued sampling of the seeded area, sequential surveys should include sampling of the perimeter of the seeded area in order to gauge the rate and direction of dispersion out of seeded areas.

Table 4.4.1 A comparison of scallop recovery and sampling time for 30 minutes post-seeding and 24 hrs postseeding by researchers and recreational divers. Initial scallop density 4 m^{-2}.

Time	Diver	10 x 1m transects		10 x 2m transects		5 x 5m quadrat (search)		5 x 5 quadrat (recovery)	
of sample		Density	Time	Density	Time	Density	Time	Density	Time
30 min	Researchers	3.3±0.1	2.5±0.2	2.8±0.1	2.5±0.2	2.5±0.1	4.8±0.2	--	--
24 hrs	Researchers	1.8±0.2 (14)	7.6±0.6	1.6±0.1 (34)	4.8±0.2	1.2±0.2 (15)	4.8±0.4	1.8±0.3(18)	6.8±0.4
24 hrs	Recreational	1.9±0.1 (6)	12.1±4.1	1.5±0.3 (12)	8.0±3.1	1.2±0.1 (31)	14.6±4.6	1.6±0.2(25)	16.0±2.0

Density data is the mean estimated scallop density m^{-2}, time is sec m^{-2}, all values are means ± SE (n=3). Figures in parentheses are coefficients of variation (Sokal and Rohlf, 1971). -- Data not collected

Table 4.4.2 Summary of results of MANOVA of scallop observations* and duration of samples with respect to sampling method and diver experience.

Data	Source	SS	df	MS	*F*	*P*
Scallop numbers						
	Method	989.0	3	329.7	5.1	<0.05
	Experience	3.4	1	3.5	0.1	0.82
	Interaction	39.7	3	2.8	0.2	0.89
	Residual	1029.2	16	1.0		
	Total	2061.3	23			
Sampling time*						
	Method	79.8	3	26.6	1.4	0.28
	Experience	264.0	1	264.0	13.6	<0.01
	Interaction	48.5	3	16.2	0.8	0.49
	Residual	311.6	16	19.5		
	Total	703.9	23			

4.4.2 ATTEMPTS TO SEED, *PECTEN FUMATUS*, IN JERVIS BAY

Introduction

Annual yields from fisheries for the commercial scallop, *Pecten fumatus* Reeve, fluctuate greatly (Gwyther 1989, Young and Martin 1990), both throughout its range and in particular in Jervis Bay, NSW (Fig. 4.4.1). The Jervis Bay fishery has at times supported up to 35 dredge vessels and numerous divers (Fuentes 1994) and has produced harvests of 2 800 t per annum (Hamer 1987), however, booms in this fishery only occur every ten years or so. In the interim, low and variable recruitment has led to industry decline and fishery closure (Fuentes 1994, Heasman *et al.* 1995). Causes of recruitment success or failure are unclear, but may involve environmental factors associated with El niño Southern Oscillation events, highly variable upwelling events associated with the East Australian current, juvenile predation and parasitic castration (Hamer and Jacobs 1987, Fuentes 1994, Heasman *et al.* 1995 and 1996)

The success of seeding programs in Japan (Ventilla 1982, Lovatelli 1990) and New Zealand (Bull 1989), coupled with recent improvements in hatchery rearing techniques for *P. fumatus* (Frankish *et al.* 1990, Heasman *et al.*, 1995, this report), have provided an impetus and the means to seed beds in Jervis Bay to enhance production from the fishery. Research aimed at seeding scallops in other areas has involved factors including; size specific survival (Salina 1994), predation (Morgan *et al.* 1980) and site specific survival (Tettelbach *et al.* 1990, Salina 1994). Each of these is thought to be important in the development of seeding strategies for Jervis Bay. The interplay of size specific survival and cost of production will determine the optimum seeding size. Estimates of annual scallop mortality have been as high as 50% (Hamer and Jacobs 1987, Worthington 1992), although, having reached approximately 50 mm, scallop survival may be much greater (Worthington 1993). This size (50 mm) is however the size at which scallops begin to recruit to the fishery (50-60 mm Hamer and Jacobs 1987), thus the economics of seeding at this size need further investigation. The potential causes of high mortality are varied, but starfish and octopus predation are likely to have an impact (Hamer and Jacobs 1987). These predators appear to vary in their distribution within Jervis Bay and in combination with varying environmental conditions, such as depth, current flow and substrate type, are likely to give rise to site variability in scallop survival

Materials and methods

Handling and seeding

All scallops used in these seedings were hatchery produced at the PSRC using reproductively conditioned broodstock from Jervis Bay (Heasman *et al.*, 1995). These scallops had then been ongrown in pearl cages and lantern cages on a subtidal longline at Murrays Beach (Fig. 4.4.1). Before seeding, the scallops were collected and stockpiled under a floating pontoon at HMAS Cresswell, an Australian naval training base, where any dead shells and deformed scallops were removed. A minimum of 100 scallops were taken at random from each batch just prior to seeding and the shell height of each scallop was determined to the nearest mm.

In each case, sites for seeding were initially positioned and located by GPS (Meridian, Magellan, San Dimas, CA, USA) and then divers with hand held compasses and measuring lines marked the perimeter of the area at intervals with cement blocks. Each plot was square and arranged so that opposite sides of the plot ran North-South or East-West. Divers then surveyed the area recording observations of substrate type, the presence of potential scallop predators and the number and shell height of any naturally occurring scallops.

In all, six sites were seeded and monitored as described here (Fig. 4.4.1, Table 4.4.3). These sites were all within the natural range of *P. fumatus* during previous boom years in Jervis Bay.

For small seedings of 5000 scallops or less, divers were used to manually deploy the scallops directly on the seabed. In the remaining seedings, the corners of the stocked areas were marked with surface buoys and the scallops were systematically broadcast over the marked area from a boat.

To permit the assessment of mortality associated with handling stress and trauma, a minimum of 160 scallops were taken at random during the seeding procedure and divided equally into one of eight trays (prawn freezer trays, Nally, Minto, NSW) and then enclosed in 10 mm polyethylene mesh (Sarlon, Punchbowl, NSW). Each tray was weighted with baked clay house bricks and were worked into the seafloor within the seeded area by divers such that the scallops had sufficient depth of substrate to bury. Each tray was recovered within a week and any mortalities that had occurred were arbitrarily ascribed to handling mortality.

Sampling method

On each sampling occasion, a minimum of eight 10 m x 1 m transect surveys were conducted by divers at randomly chosen sites within the seeded area. At each site an initial rate of dispersion was estimated within the first fortnight by surveying two additional 20 m x 1 m wide transects radiating from each corner of the plot as indicated schematically in Figure 4.4.5. Each radiating transect was made at 90° to the edge of the site at which it commenced and was divided into proximal and distal 10 m sections. To aid the speed and accuracy of these tasks, each diver carried a 10 m transect rope weighted at each end and a 1 m measuring stick. Underwater slates were also carried by divers to record the results of surveys and other relevant observations such as pressence or evidence of predators and scallop shell fragments.

Where possible, the first thirty scallops found within the seeded area by divers during the survey were collected and taken to the surface where the shell height of each scallop was measured to the nearest mm before being returned to the seeded area. Any dead shells and shell fragments found by divers were collected for later examination.

To investigate the effect of substrate variation on a small spatial scale, a site at Scottish Rocks (Fig. 4.4.1) was chosen as it had three distinct substrate types. Approximately one third of the seeded area was clear sand, one third was weed bed (predominantly *Posidonia australis* and some *Halophila ovalis*), and the remainder a mix of rock and shell rubble (Fig. 4.4.3). The boundaries between each of the substrate types present were sufficiently distinct for divers to confidently confine individual transects to a single substrate type. For the two seedings at Scottish Rocks, a minimum of six transects were conducted at randomly chosen sites within each substrate type on each sampling occasion. When possible, samples of scallops were taken from each substrate type for size comparison.

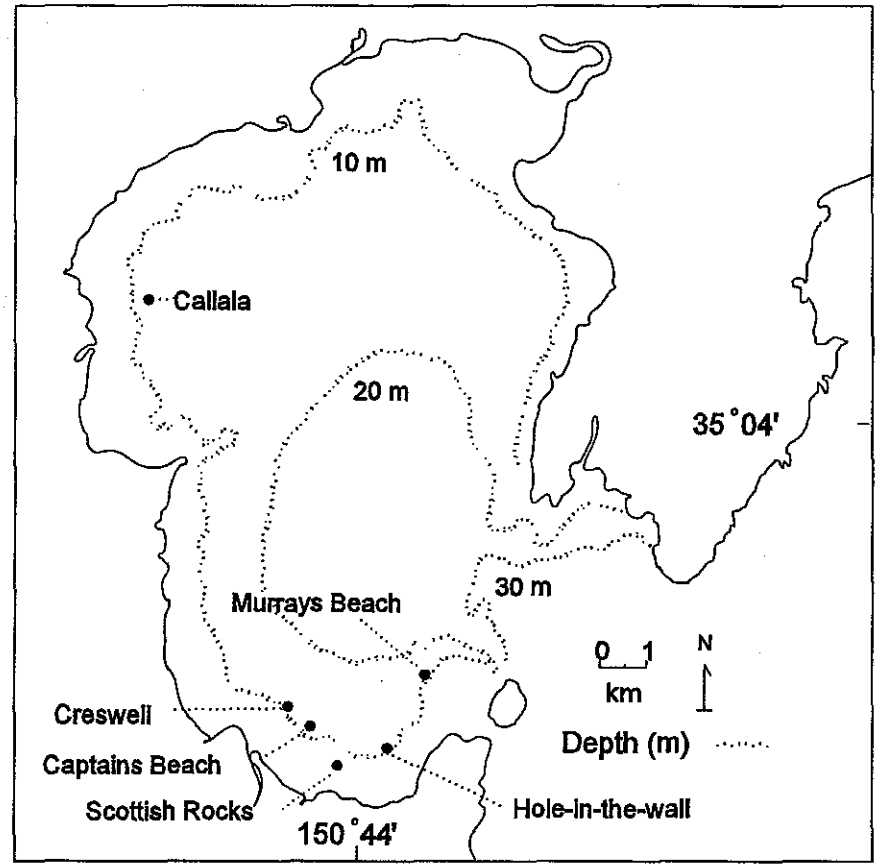

Figure 4.4.1. Map of Jervis Bay indicating the sites seeded with hatchery produced *Pecten fumatus*

Table 4.4.3 Sites seeded with *Pecten fumatus* in Jervis Bay, NSW, Australia.

Site (Seeding date)	No seeded	Size & range (mm)	Area & Depth	Bottom	Handling Mortality	Predators
Hole-in-the-wall* (16 Oct. 1995)	2 500	33.1 (19-38)	625 m^2 10 m	Sand and shell rubble	0	Rays, Crabs, Starfish
Cresswell (16 Jan. 1996)	2 500	47.3 (25-65)	625 m^2 13 m	Sand	-	Starfish and numerous Rays
Captains Beach (I) (16 Jan. 1996)	2 500	47.3 (25-65)	625 m^2 11 m	Sand	-	Rays and Starfish
Captains Beach (II) (8 Feb. 1996)	10 000	42.7 (34-55)	2500 m^2 11 m	Sand	4%	Rays and Starfish
Callala (12 Mar. 1996)	11 000	44.9 (25-60)	2500 m^2 13 m	Silty sand	0	Rays
Murrays Beach (I) (26 Mar. 1996)	5 000	28.1 (20-37)	2500 m^2 17 m	Silty sand	0	Rays, Octopus and Starfish
Murrays Beach (II) (31 May 1996)	20 000	34.7 (21-48)	2500 m^2 17 m	Silty sand	0	Rays, Octopus and Starfish
Scottish Rocks (I) (14 Aug. 1996)	5 000	36.4 (27-47)	2500 m^2 5-7.5 m	Rock, sand and Posidonia Bed	0	Crabs
Scottish Rocks (II) (28 Apr. 1997)	15 000	32.9 (18-50)	2500 m^2 5-7.5 m	"	<1%	

* Initial sampling method validation trial. * The number of scallops (per m^2) present at the site before seeding.

Results

Survival

The survival of caged scallops throughout this research was generally high and scallops contained in cages within seeded areas were no exception. In most seedings, there were no mortalities in caged scallops in the week to fortnight following scallop release and only once did mortality exceed 1%. On that occasion in summer 1996, the caged scallops were the last to be deployed and were thought to have been affected by prolonged emersion during the heat of the day. Without exception, scallop survival in cages was very much greater than that of their concurrently seeded siblings.

The success of seeding attempts was variable with the maximum period during which scallops could be found within the seeded area being recorded in the first seeding at Captains Beach. At this site approximately 10% of the seeded scallops were present after 11 weeks and had reached a mean shell height of 56.1± 3.6 mm (± SD). The recapture rate varied greatly with location (Fig. 4.4.2) and within locations (Fig. 4.4.3). Scallop losses were most rapid at the Callala site which was subject to severe storm action immediately after seeding. The storms prevented sampling for two weeks, at which time no signs of seeded scallops were detected.

On a smaller spatial scale, scallop recovery from the three habitat types at Scottish rocks varied significantly after one week (F=9.49 & F=9.98 {P<0.05} for the first and second seedings respectively). In both seedings, significantly greater numbers of scallops were recovered from the rock substrate than either weed or sand, however no significant differences were found among the sizes of scallops collected from each substrate type (F=1.66 & F=1.22 {P>0.05} for the first and second seedings respectively). Overall, scallops recoveries persisted for a greater period of time from the rock substrate in the first seeding and from rock and weed beds in the second (Fig. 4.4.3).

Comparisons between the size frequency of scallops at the time of deployment and those of scallops recovered by divers, were made using Chi squared analysis in which scallops were arbitrarily assigned to 5 mm shell height classes (15-19, 20-24, 25-29, etc.; Fig. 4.4.1). For most sites, comparisons were made one week after seeding, however, bad weather prevented this for the second seeding at Captains Beach and thus

N.B. Interpretations of scallop survival and the imapct of scallop size on survival should not be made without referal to section 5.4 which describes diver bias in the sampling techniques used. For example initial surveys may have underestimated scallop densities by as much as 17% and this may have been at the expense of smaller scallops.

comparison here was made using collections taken two weeks after deployment. In each case, a factor 0.6 mm/ week was allowed for growth (after Hamer and Jacobs, 1997) which was subtracted from the shell height of each scallop recovered before size frequency comparisons were made. With the exception of the seeding at Cresswell, all seeded scallop populations underwent significant increases in size frequency beyond that expected due to growth alone. In most cases, a reduction in the number of small scallops recovered (or its corollary, increases in the number of large scallops found) was evident (Fig. 4.4.4).

Dispersion

The rate of dispersion over the first week (two weeks for Captains Beach II) was estimated by averaging the numbers of scallops present in the radiating transects (20 m x 1m), adjusting for the total area within 20 m of the seeded plot and expressing the estimated number of scallops to have dispersed beyond the seeded area as a percentage of the original number stocked .

Table 4.4.4 Estimates of dispersion for the first week of deployment for six areas seeded in Jervis bay.

Site	Mean N°/ transect	Area covered	No dispersed	No Stocked	dispersion/ week (%)
Cresswell	0.25	3600 m^2	45	2500	1.8
Captains Beach I	1.38	3600 m^2	248	2500	9.92
Captains Beach II	3.13	5600 m^2	876	10000	4.38*
Callala	0	5600 m^2	0	11000	0
Murrays Beach	1.13	5600 m^2	316	5000	6.32
Scottish Rocks	0.38	5600 m^2	106	5000	2.12

* calculated from fortnightly data.

Dispersion was found to be a significant factor in the reduction of scallop recovery rates at several sites although the distances travelled were generally small. Disregarding the Callala site, where no scallops were recovered, estimates of dispersion varied from 1.8 - 9.92% of the seeded population per week (Table 4.4.4). However, the bulk of these scallops had not moved more than 10 m from the seeded area within the first week (Fig. 4.4.5). Generally, dispersion explained only a small percentage of the reduction in scallop recapture rates, however this was not the case at in the initial seeding at Captains Beach. Here continued dispersion at a rate of 9.92% of the population weekly

would account for over a 75% reduction in scallop numbers within the seeded area over 11 weeks.

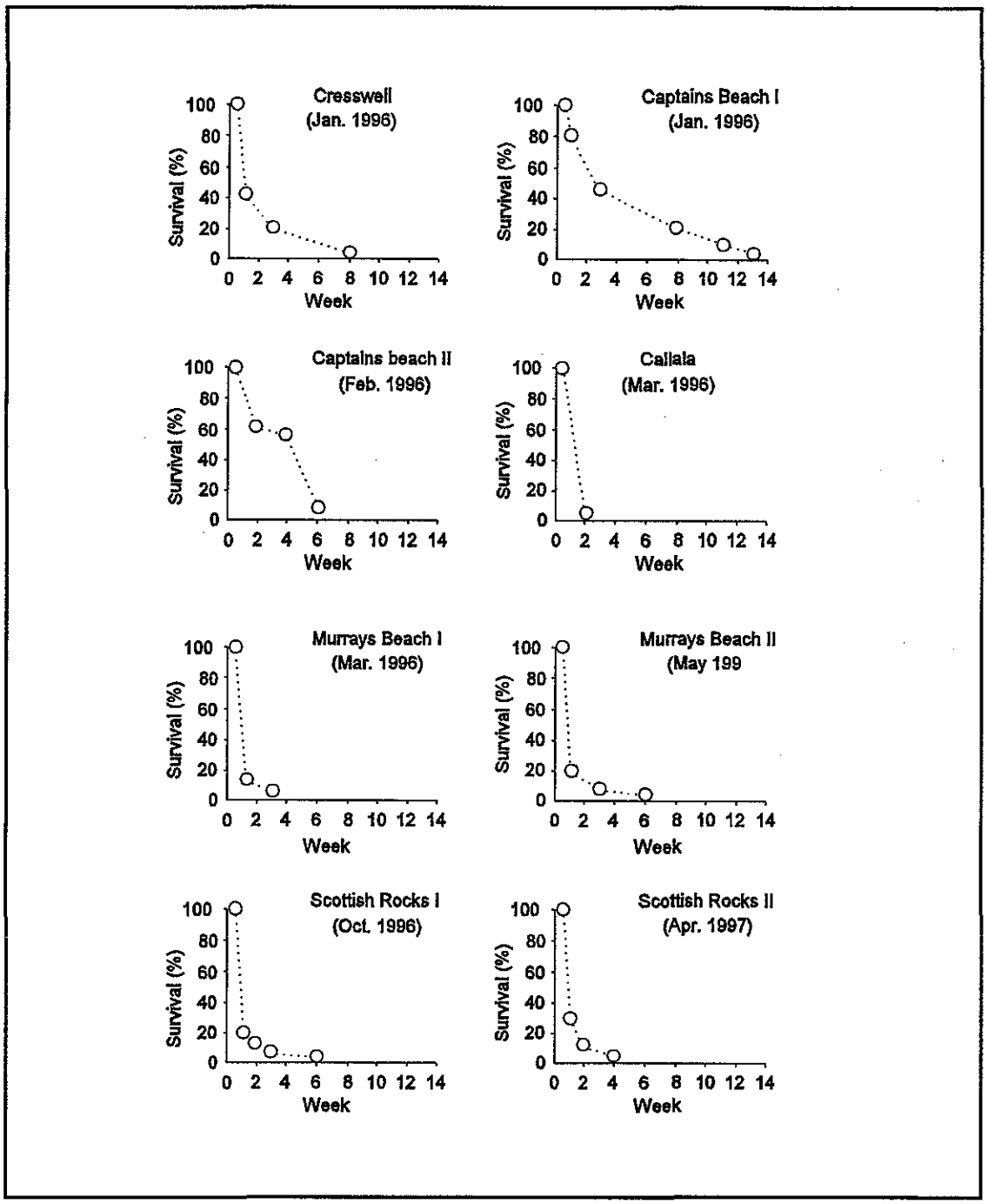

Figure 4.4.2 Survival of *Pecten fumatus* in eight seedings at one of five sites in Jervis Bay. Horizontal scale indicates time after initial seeding, seeding date in parentheses.

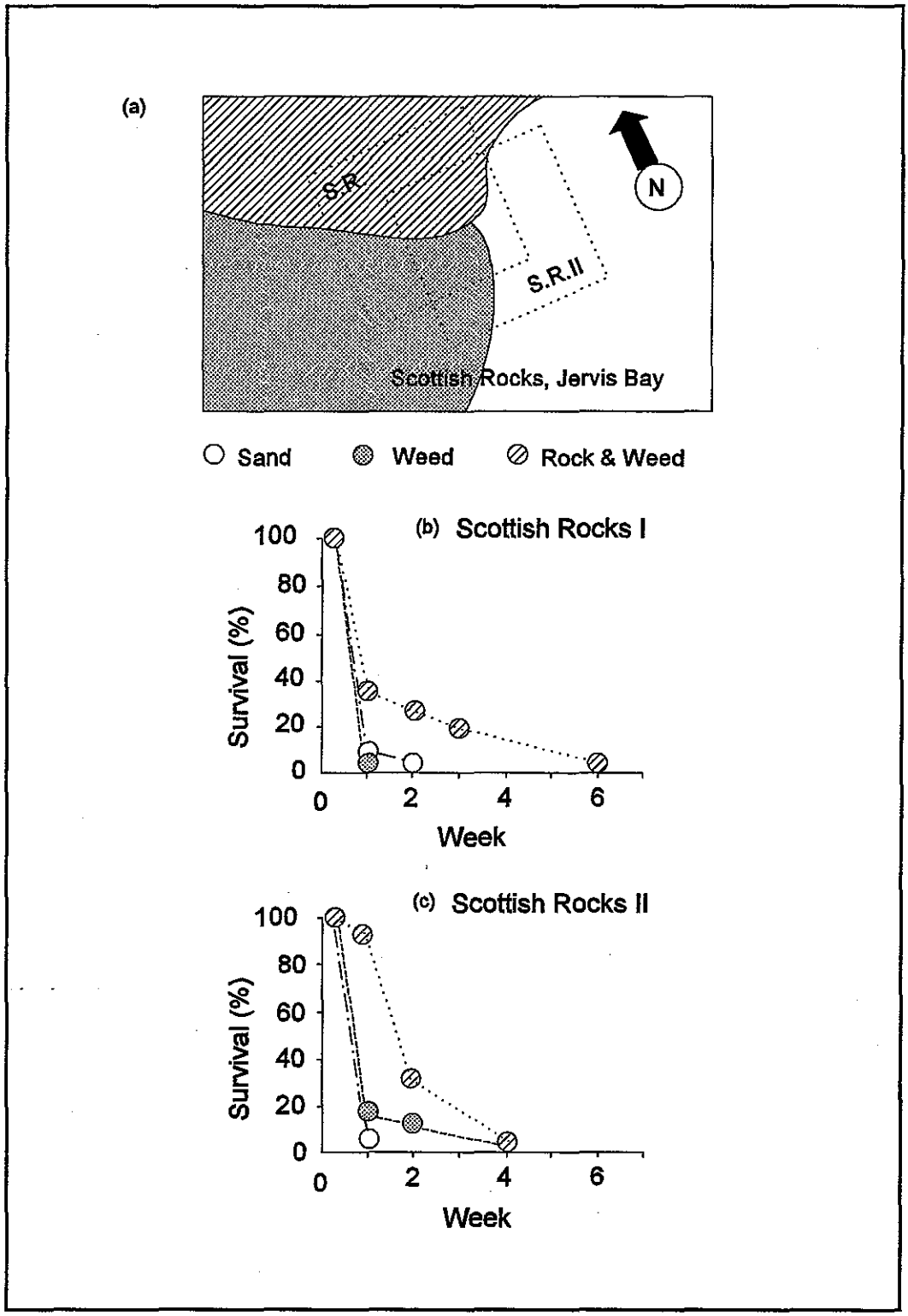

Figure 4.4.3 Survival of *Pecten fumatus* seeded onto one of three substrate types at Scottish Rocks, Jervis Bay.

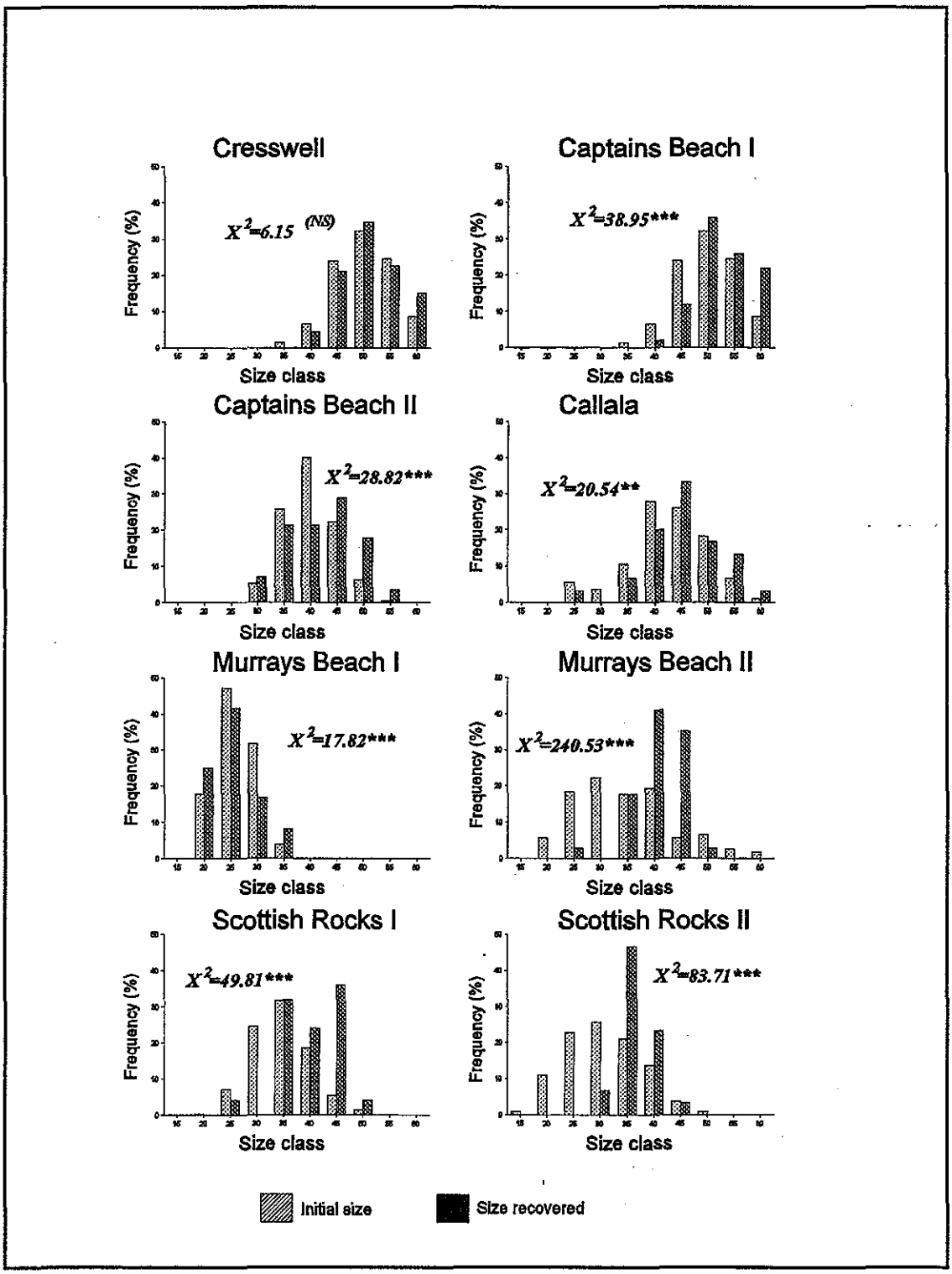

Figure 4.4.4 Frequency histograms of scallops seeded and those collected within one to two weeks after release. X^2 values are shown with * indicating the level of significance, NS P>0.05, * P<0.05, ** P<0.01, *** P<0.001.

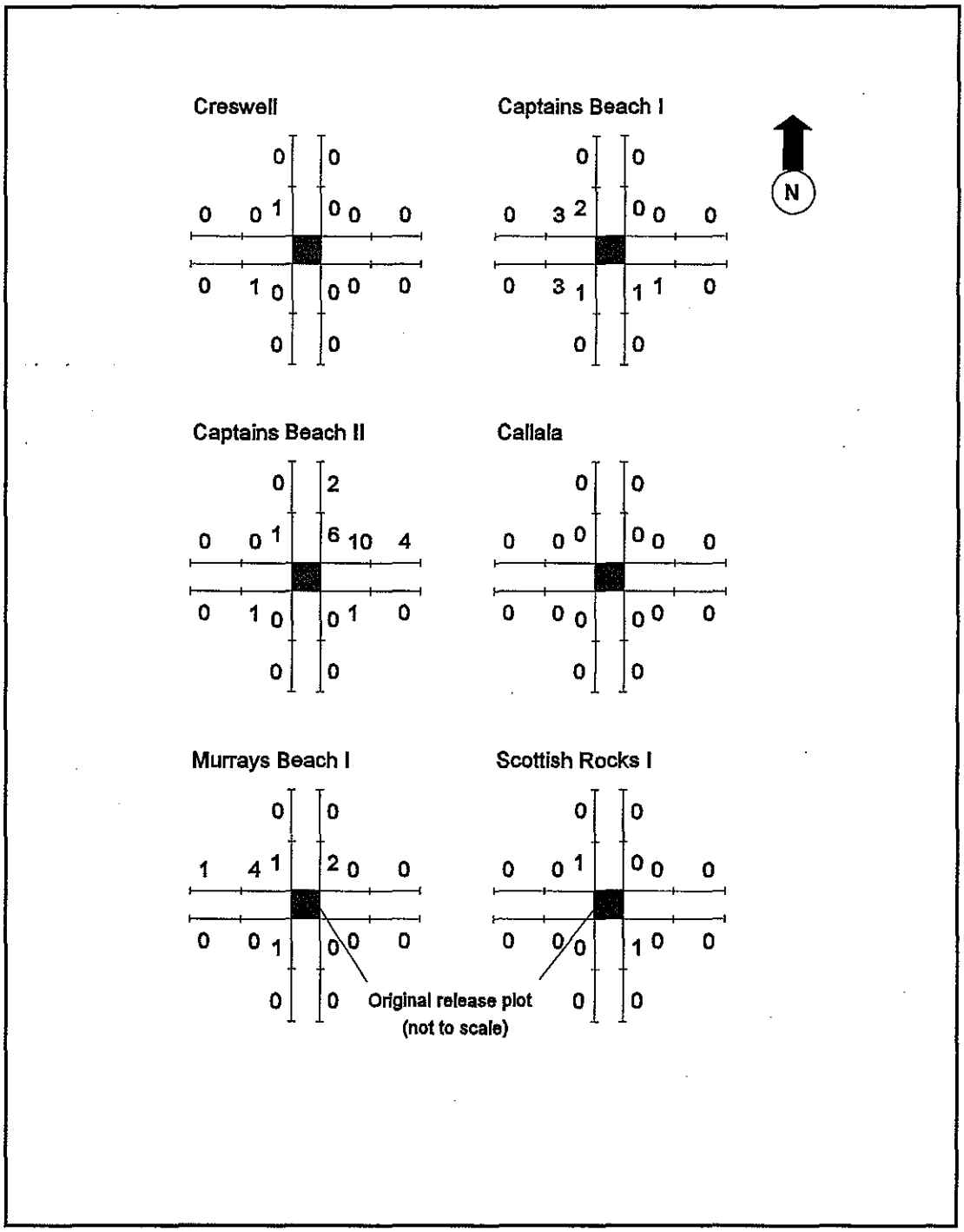

Figure 4.4.5 Schematic representation of results of 20 m x 1 m wide transect surveys radiating from the corners of six seeded areas in Jervis Bay, indicating the direction of travel and the numbers of scallops found within the proximal and distal section (10 x 1 m) of each 20m transect.

Discussion

At the outset of this study there were few estimates of the natural rate of mortality of *P. fumatus* and some evidence to suggest that mortality rates may vary interannually. Estimates from tag and recapture studies in Jervis Bay (Hamer and Jacobs, 1987) and from Port Phillip Bay (Gwyther and McShane, 1988) had found mortality to be approximately 50% annually. However, both populations suffer from a bucephalid parasite that is thought to weaken scallops and increase mortality (Heasman *et al.*, 1995). In Jervis Bay the prevalence of the parasite has been found to vary from 5% to more than 60% of the adult population (Heasman *et al.*, 1996) and therefore may cause significant variations in annual mortality. At about the time of Hamer and Jacobs' (1987) study, they recorded parasite prevalence at a low 8% (see Heasman *et al.*, 1996) and thus their estimated 50% annual mortality in Jervis Bay may be an underestimate for years of higher parasite prevalence. Regardless, the losses experienced in these scallop seeding exercises were well in excess of that expected and were though to be influenced by three factors; diver accuracy, predation and scallop dispersion.

Diver accuracy

Initially, the cryptic habit of juvenile *P. fumatus* had been demonstrated to result in large and rapid apparent reductions in numbers of seeded scallops. Within periods of as little as 30 min after seeding, diver estimates of scallop numbers can be between 17.5% (1 m wide transects) and 37.5 % (5 x 5 m plots) less than the number initially seeded, depending upon the sampling method used (Section 5.4.1). It was thought that this would vary with substrate complexity and would have to be taken into account with changes in site within Jervis Bay. However, the above accuracy estimates are made for relatively complex substrates in which scallops are difficult to find, and yet they are insufficient to explain even the reduction in numbers of scallops observed over the first week of most seedings. While an increased ability for divers to find scallops in open sandy areas may have extended the period over which scallops were recovered from sites such as Captains Beach, scallop recoveries were not consistently greater at sandy sites. This was particularly so at Scottish rocks, where scallops seeded on sandy areas were depleted (disappeared) more rapidly than at other sites.

Observations in natural populations from Jervis Bay had suggested that upon reaching approximately 50 mm in shell height, scallops may be less prone to predation and exhibit higher survival. This was supported to some extent by the finding that after allowing for growth (0.6 mm/week) there were generally small but significant increases

in the mean sizes of most scallop samples recovered in the week to fortnight following deployment. In part, this could again have been a product of diver bias as earlier sampling validation trials (Section 5.4.1) had demonstrated that irrespective of previous experience, divers were less likely to find smaller scallops, particularly those of less than 25 mm shell height. Scallops of this size were present in most seeded batches, however inspection of the size frequency histograms (Fig. 4.4.4) indicates greater differences in larger size classes than would be expected on the basis of our previous work. Earlier, the relative frequencies of scallops deployed and those recovered by divers showed virtually no difference for size classes greater that 35 mm.

The exact size at which a survival advantage was achieved was not clear cut and varied with seedings from 35 to 60 mm. At Captains Beach, where scallops remained for the greatest period of time, any advantage was not apparent until scallops were greater than 60 mm in size and well beyond the size at which cost effective scallop seeding is likely to occur.

Predation

The predominant cause of mortality in seeded scallops was attributed to predation. Caged controls indicated that handling mortality was uniformly low in the week following deployment, averaging less than 1 %, while the longevity and growth of caged scallops in previous studies indicated that parasites and small predators capable of entering the mesh protected cages had little impact. Rather, large predators that have previously been implicated in scallop mortalities were suspected. Of the putative predators of *P. fumatus* in Jervis Bay most have been identified as significant predators of scallops elsewhere in Australia and overseas They include starfish (Olsen, 1955; Dickie and Medcof, 1963; Brun, 1968; Imai, 1971; Chernoff, 1987; Pitcher and Butler, 1987; Ventilla, 1982), octopuses (Oresanz, 1986), crabs and lobsters (Tettelbach, 1985; Hatcher *et al*, 1996), gastropods (Halary *et al*, 1994) and flat worms (Boyer and Meyer, 1995). While the impact of specific predators is addressed in more detail later (Section 5.4.3), it is important to note that the abundance of some predators varied greatly with location in Jervis Bay. Rays and octopuses were ubiquitous (the latter in small numbers), while starfish such as *Luidia australiae* were more commonly reported in sandy areas and the crabs, *Nectocarcinus tuberculosis* were confined largely to weed beds and drift weed aggregations.

Despite the abundance of potential scallop predators (Section 5.4.3), it remains unclear why seeded scallops should be more adversely affected by predators than had been

previously observed in wild populations (Worthington, 1992, 1993). Two possibilities were raised. Initially, the wild population of *P. fumatus* during this study was comparatively small with particularly low numbers of small scallops (<50 mm shell height). Given that the estimates of mortality made by Hamer and Jacobs (1987) were made in the early to mid eighties when scallop populations were much greater than during the present study, it is possible that a relatively stable population of predators would have a greatly increased impact during years of low natural scallop abundance. This is to some extent supported by seeding experiences elsewhere, such as those in Japan, where it has been suggested that survival rate increases with the number of scallops seeded (Ito 1988).

Important predators of *P. fumatus* included stingaress (*Urolopus cruciatus* and *U. testaceus*) capable of consuming many seed size scallops per day, and possibly the seven armed sea star *Luidia australiae*. Both these predators are ubiquitous throughout Jervis Bay being commonly found at densities in the range 0.2 -0.8 /100 m^2 (CSIRO, 1991) and have been seen to increase in density within recently seeded areas. Another significant predator, octopuses, although less prolific, are also capable of consuming large quantities of prey. One species, *Octopus tetricus*, found predominantly in temperate waters on the west coast, but also the east coast of Australia, is capable of growing to a mean weight of 2.2 kg in six months (Joll, 1977). Daily food consumption for this octopus averages 30-40 g tissue weight or 8-10, 40 mm scallops. Like stingarees, large octopus were observed to rapidly infiltrate and construct lairs within scallop release areas. In one instance, the shells of 167 seeded scallops were found at the entrance of a new lair, 10 days after seeding. It was therefore considered probable that collective predation by stingarees, starfish and octopus in Jervis Bay has the potential for seed scallop removal rates in the order of 1-10 scallops/100 m^2/day. Such rates of predation alone could account for for the loss rates observed for most releases in which seed scallops were deployed at densities of 0.7 - 2.0/m^2.

A second reason for higher predation in seeded stocks may arise from the possibility of morphological or behavioural disadvantages (naivety) of artificially propagated seed-stock. Scallops grown in suspended culture (as was the case in this study) have been found to have a thinner shells when compared to wild scallops of comparable size (Ventilla, 1982; Macdonald, 1986) and may be more vulnerable to predators, particularly those that crack or bore through the shell. Additionally, containment during culture may discourage a swimming response to predators both because of its futility within a caged environment and the increased likelihood of damage in collisions with other scallops (biting). Thus seeded scallops may simply opt for valve closure in situations in which swimming may be a more appropriate response.

All the areas seeded in this study had previously supported populations of *P. fumatus*, although, only the area seeded at Murrays beach retained any appreciable numbers of wild scallops, albeit at densities of less than 0.1 m^{-2}. It was suggested that the areas which retain remnant populations between the boom years may well offer some advantage and would be suitable for seeding. This however did not appear to be the case. Both seedings at Murrays Beach were rapidly depleted in numbers by predators, while the remnant population suffered no apparent decrease. This was thought to have arisen from the fact that the wild population were all greater than 65 mm in shell height and were less susceptible to predator attack.

Dispersion

Dispersion was the final factor found to contribute to the reduction in recovery of scallops from seeded areas and importantly was capable of explaining the reduction in numbers of scallops in at least one of the seeded areas. In the first seeding at Captains Beach, an estimated 10% of the seeded population remained within the original deployment area 11 weeks after seeding, despite an estimated initial dispersion rate of 9.92 % wk^{-1}. If this rate of dispersion was maintained and 9.92% of the remaining population left the seeded area each week, this alone would account for a reduction of approximately 75% of the population. However, in most cases dispersion appeared to be largely unidirectional (Fig. 4.4.5) and was consistent with the direction of current movement within that area. Thus it is possible that the entire seeded population at Captains Beach underwent net movement in a direction such that 9.92% of the original population fell outside the seeded area each week. This to say that the population was decreasing arithmetically rather than geometrically as in the previous instance. If this were the case, the entire population would leave the seeded area in approximately 10-11 weeks. This is significant in that it suggests that despite the relative longevity of this seeding, the losses of scallops may have arisen purely due to dispersion rather than predation and that it may be possible to reseed an area in Jervis Bay without decimation from predators.

Dispersion, like diver accuracy, has previously been found to be influenced by substrate type. Stokesbury and Himmelman (1996) found dispersion distances of *P. magellanicus* were usually greater over sand substrates. This trend was also possible in Jervis Bay where the highest dispersion rates were recorded at sandy sites, possibly arising from the reduced resistance to current flow. Again supporting the notion that dispersion is a current driven phenomenon and should therefore be largely unidirectional in Jervis Bay and to some extent predictable. Given that the bulk of substrate types encountered in

Jervis Bay are sand/mud with few macrophytes, dispersion is likely to be a factor in most scallop reseeds.

General discussion

It has been clear throughout our attempts to seed scallops in Jervis Bay that predation is been the major limiting factor and that previous observations for mortality rates and sizes have not held with respect to hatchery produced stocks. However, despite predation, at least one seeding was monitored for a period of two and a half months and appeared to be largely dispersed rather than consumed. Regrettably, there were no features of this seeding that consistently improved scallop survival. Subsequent seeding of that site on a larger scale failed and the use of similar sandy sites produced inconsistent results, particularly at a nearby site at Scottish rocks, where survival over the sandy substrate was the worst of those tested. This is not necessarily atypical of seeding experiments. The variety of factors and the variability in their impact can be so great that at one site successful seedings can be followed by those in which two thirds of small and medium sized scallops (15 & 30 mm) can be missing within three days (Fleury et al., 1996).

The key to successful reseeding of Jervis Bay may well lie with the scale of the seeding program. While the attempts made in this research were of a scale that had been used successfully elsewhere (Golden Bay, NZ), seedings may need to be orders of magnitude greater (100-1000 x) such that predators are overwhelmed. Alternatively, seedings need to be afforded some measure of protection from predators especially during the immediate period of naivity following release. While techniques used elsewhere such as liming and dredging to remove predators are clearly unacceptable in Jervis Bay, meshed enclosures on the bottom similar to those used evaluated in this study and in the United States with clams could be viable.

4.4.3 OBSERVATIONS OF PREDATORS, PESTS AND PARASITES OF THE COMMERCIAL SCALLOP.

Introduction

From the outset of this study it was clear that predators and parasites would pose varying problems in our endeavours, however, despite an average annual mortality in some cases estimated as high 50% (Hamer and Jacobs 1987) there had been only a few, mostly scant descriptions of the potential predators and parasites of *P. fumatus* and their impacts.

During these farming studies, in excess of 4 000 scallops were dissected for the determination of reproductive condition, more than ten million scallops were produced for farming trials and over 250 000 scallops were released into Jervis Bay. As a result, numerous observations were made of species with the ability to impinge upon scallop culture. The following section discusses the major causes of scallop mortality and categorises the various species according to their impact upon our attempts to farm *P. fumatus*.

Materials and methods

Research was confined to three areas on the central and south coasts of NSW; Port Stephens, Jervis Bay and Twofold Bay (Fig. 4.4.6). All broodstock for these studies were collected by divers from Jervis Bay and hatchery production took place at Port Stephens Research Centre (PSRC). Spat were cultured in Port Stephens and Jervis Bay and were held in spat collector bags or on screens in land based upwelling systems. Juvenile scallops were ongrown in trays and cages on the sea floor, in pearl cages, in lantern cages and adhered or earhung on tapes, ropes and plastic discs, at all three locations. All scallop seedings were all restricted to Jervis Bay, but in some cases were protected with a covering layer of mesh.

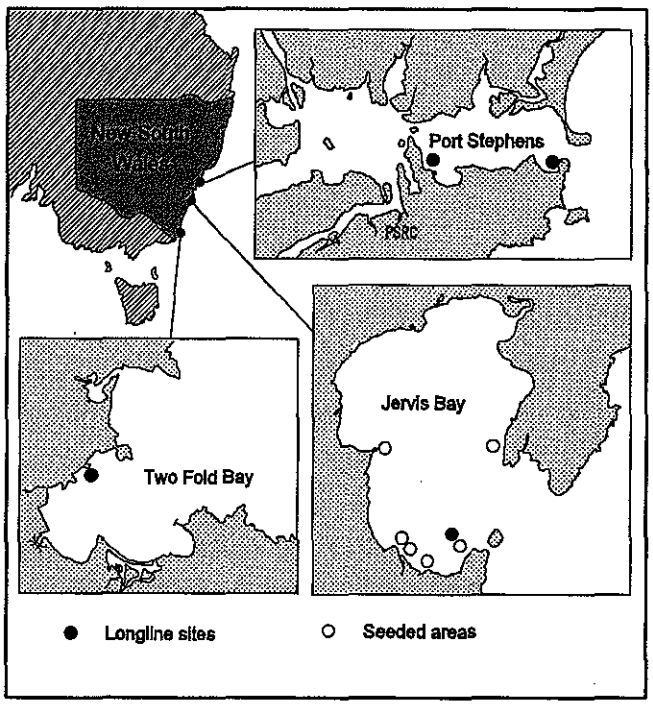

Figure 4.4.6 Maps of NSW, Port Stephens, Jervis Bay and Twofold Bay, showing the location of culture operations.

Results

Fishes

Despite anecdotal reports of fish species predating *P. fumatus*, we have made very few direct observations were made. The three major exceptions were the blue "groper" (wrasse), *Achoerodus viridis*, leatherjackets (Monacanthidae) and stingarees.

Blue groper are omnivores capable of growing to a length of 1.2 m (Grant, 1982) and are generally associated with reefs and rocky outcrops. Although groper have not been observed at either the longline sites or at any of the reseeded areas in Jervis Bay they have been seen at the Tomaree site in Port Stephens and at wharves used to hold scallops in both Jervis Bay and Twofold Bay. Here they are voracious scallop predators capable of stripping earhung scallops from lines in hours and in the case of larger fish also capable of attacking caged scallops through the mesh. In attacks that can be audible to divers, these fish rapidly draw in mouthfuls of water, scallops and mesh, and crush the scallops so that they can be drawn through mesh. In extreme cases, groper

attacks have torn the cage mesh allowing access for other predators such as octopus.

Leather jackets, unlike blue groper, have been almost ubiquitous and are particularly common at wharves, reefs and at the longline site in Jervis Bay. Among the more common species have been the fantail leather jacket (*Monocanthus chinensis*) and the yellow-finned leatherjacket (*Meuschenia trachylepis*). While the feeding of leatherjackets generally assists in the reduction of fouling on lines and cages, they have also been observed attacking scallops. In particular, larger fish can directly ingest spat and, in the case of larger scallops, can chip away the edges of the shells to gain access.

Several elasmobranchs are thought to be among the most significant predators of *P. fumatus*. The stingarees, *Urolophus cruciatus* and *U. testaceus* were ubiquitous in Jervis Bay having been recorded at densities commonly in the range 0.2 -0.8 m^{-2} (CSIRO, 1991) while fiddler rays (*Trygonorhina fasciata*) and shovelnose rays (*Aptychotrema rostratus*) were also common. Each of these species are mollusc predators and frequently reach a size sufficient to prey on seeded scallops, in some cases even the largest of *P. fumatus* (100 mm shell height). In the shallows surrounding HMAS Cresswell Naval Base, stingarees have been seen eating scallops that have escaped from cages or have been lost following groper attacks on mesh cages. However, there have been few other direct observations of rays eating scallops, although there is considerable indirect evidence of their activities. It is often noticeable that in post seeding surveys the number of rays increases dramatically in seeded areas and that the characteristic feeding depressions in the sand left by rays also increase. In some cases, fragments of scallop shell have been found in these depressions suggesting predation by rays.

Several other fish species are suspected scallop predators, however shy, cryptic or nocturnal behaviour has prevented direct observations. Amongst this group are the sparids, *Pagrus auratus* (snapper) and several *Acanthopagrus* spp. (bream). These fish prey upon molluscs and in the case of snapper have been specifically reported to inhabit scallop beds (Coleman, 1980). Fleeting observations of bream and snapper have been made at most of the culture sites however their shy nature has prevented direct observations of their feeding habits.

Crustaceans

Crabs

Among the most commonly reported and wide spread predators of scallops are crabs (Tettelbach 1985, Minchin 1991. Avariety of crabs have been found in the pearl and lantern cages, although, only a small number have been equated with mortality of *P. fumatus*. In particular the powerfully clawed portunid, *Nectocarcinus tuberculosis*, has caused considerable damage and subsequent mortalities to scallops retained in pearl cages.

In Jervis Bay, *N. tuberculosis* regularly appeared in pearl and lantern cages over late winter to early spring (June-September). In the first instance, settling some time after May and growing to reach a carapace width of 10-15 mm by mid September. At this size the crabs did not appear to damage adjacent scallops, however, by November to December the crabs can grow to a carapace width of 25 mm when scallop mortalities began to occur. By February it became clear the crabs were the cause of the increased mortality as newly introduced cages of scallops and those in cleaned crab free cages remained unaffected. By March 1996 the crabs had grown to a size capable of decimating all of the scallops confined in the cages. Crabs as large as 85 mm were recorded in cages later during the year.

Containment trials in pearl cages, with scallops (mean size 35 ± 9.2 mm) and *N. tuberculosis* of various carapace widths found predation was size specific. No significant predation occurred in the presence of *N. tuberculosis* of <25 mm carapace width, however, predation increased with increasing crab size. Crabs 25-30 mm preyed upon scallops of a similar size and by the time crab size had increased to 50 mm or more, all scallops in the experimental treatments were lost.

The impact of *N. tuberculosis* has not been solely confined to cages. The species is particularly common in the seagrass, *Posidonia australis,* beds (Ferrel et al. 1992) and drift algal mats of *Gracilaria verrucosa* (Langtry and Jacoby 1996) in Jervis Bay and thus several reseeding trials have been affected. In particular scallops seeded over an area incorporating seagrass at Scottish Rocks, Jervis Bay in August 1996 encountered predation from large numbers of *N. tuberculosis*. Crabs were found consuming scallops and 5 000 scallops averaging 36 mm shell height were all but lost within 3 weeks. At this time, several female crabs found were in "berry", which was consistent with the timing of settlement of crabs in pearl cages in the previous year and with the later discovery of large numbers of crab juveniles (<10 mm) in the spat catching bags deployed near by at Murrays Beach.

Several other crabs, including the spider crabs (Majidae) *Naxia tumidia* and *Hyastenus elatus*, and members of the genus Dromidae have also been found in cages but do not

appear to cause any problems with the scallops, and may in fact be beneficial by feeding on some of the biofouling organisms.

Crayfish

The crayfish, *Jasus verreauxi*, occurs along the southern and central coasts of NSW and is found on sublittoral rocky reefs throughout Jervis Bay (Bell, 1987) and on rocky headlands within a short distance of the longline sites at both Port Stephens and Twofold bay. At each of these sites, juvenile *J. verreauxi* are occasionally found on mussel ropes (if present) and in scallop cages. While their numbers have always been low, several juveniles of approximately 12.5 mm carapace length were noted in pearl cages in Jervis Bay in August 1995. Continued monitoring of the cages containing crayfish found significantly higher mortality of scallops than in the surrounding cages, however this may have arisen from the fact that the scallops were the only food source available. Regardless, *J. verreauxi* were always removed from the cages as a precaution.

It was considered unlikely that *J. verreauxi* would be a significant natural predator of *P. fumatus* as the habitats for both species show little overlap.

Starfish

Starfish have previously been designated as significant predators of *P. fumatus*. The eleven-armed spiny starfish *Cosinasteria calamaria* (Gray) was linked to the demise of a stock of *Notovola meridionalis* (now recognised as *P. fumatus*) in Tasmanian waters (Olsen, 1955) and Hamer and Jacobs (1987) listed starfish and octopus as the major predators of *P. fumatus* in Jervis Bay. While the latter report did not list a species, it is likely that this was a reference the seven armed starfish, *Luidia australiae*, which has been recorded commonly at densities of 0.2 -0.8 /100m^2 (CSIRO, 1991) in Jervis Bay. Members of this genus are predators of pectinids overseas (McClintock, 1983; Wolff and Alarcon, 1993) and *L. australiae* has a reputation among local scallop divers as a predator, although we have never observed *L. australiae* attacking scallops.

In our experience the seastar, *C. calamaria*, poses a greater threat to *P. fumatus*, particularly in cage culture. Juveniles of this species were found pearl cages in August 1995 which grew to a size of 120 mm within four months. At this size the starfish had the ability to devour even the largest of the scallops (70 mm) held in lantern cages at that time. Subsequent observations of affected cages found two distinct classes of

starfish suggesting a second spawning and recruitment of this species in March 1996.

Molluscs

Predatory Gastropods

In accordance with the variety of techniques and locations used to culture *P. fumatus* there appeared to be a similar number of predatory gastropods capable of producing significant mortalities among cultured stocks. In hanging cages the triton, *Cymatium parthenopium*, posed significant problems in Port Stephens and Jervis Bay.

In Port Stephens, *C. parthenopium*, colloquially known as the hairy oyster borer, presented a significant problem to caged *P. fumatus*. Capable of reaching 100 mm in size and easily recognised by its characteristic periostracum (Fig. 4.4.7), this predator has, as the name suggests, been a pest to commercial oyster production in NSW (Dakin 1952, Bennett 1974). Up to a dozen *C. parthenopium* were found in a single pearl cage which is usually associated with the complete loss of all the scallops present. It is not unknown for *C. parthenopium* to be in cages with 6 mm mesh, nor for the predator to be present in only one cage per string (10 cages). This has led to the suggestion that they have arisen from eggs capsules laid on the cage surface and entered the cage as juveniles. *C. parthenopium* of less than 10 mm shell length have been found in cages.

In Jervis Bay, *C. parthenopium* settled in both pearl and lantern cages and although it appeared to be slow growing, taking 5 to 6 months to attain a size capable of attacking scallops, if allowed to remain could result in 100% scallop mortality. Predation trials subsequently found this mollusc to be capable of killing a scallop as large as 60 mm (when confined to a cage).

For bottom grown scallops the wine-mouthed Lepsiella, *Lepsiella vinosa* and the magnificent volute, *Cymbiola magnifica* pose a greater threat than the previously mentioned species. *L. vinosa* is only small, reaching approximately 3 cm in length but has been found to prey scallop on juveniles in the bottom cage trials. Alternatively, *C. magnifica* can grow to 30 cm in size (Macpherson and Gabrielle 1962) and is capable of encasing an adult scallop (50-80 mm) with its muscular foot and then forcing it into the sand. *C. magnifica* is however relatively scarce in Jervis Bay, and it has more likely been its large and attractive nature that has lead to divers observing its attacks on scallops than the frequency or the impact of such attacks on scallop numbers

Figure 4.4.7 The hairy oyster drill, *Cymatium parthenopium.*

Cephalopods

Octopus are a major predator of scallops in Jervis Bay (Hamer and Jacobs, 1982) and in Port Stephens, particularly of seeded scallops. As observed by Hamer and Jacobs (1982) scallops shells are ever-present in octopus lairs and frequently include the shells of tagged experimental animals. During this study, divers frequently noticed new octopus lairs in seeded areas and on one occasion, 10 days after seeding, divers removed the shells of 167 seeded scallops from one lair ! As discussed previously in Section 5.4.2 these observations corroborate the findings of Joll (1977) that octopus are fast growing and voracious feeders capable on average of consuming the equivalent of eight to ten 40 mm scallops daily over their brief (1 to 2 year) life span.

Although octopus were clearly capable of constructing lairs in sandy areas of Jervis Bay, they showed a tremendous affinity for blocks and anchors. Initially, cheap, readily available, hollow concrete building blocks (390 x 190 x190 mm) were used to stabilise

longlines, weigh cages and mark areas for seeding. However, these proved so popular with octopus that they were removed and only when absolutely necessary, were replaced with smaller solid concrete bricks that did not provide ready shelter.

Worms

Stylochus

A species of unidentified flatworm, a Polyclad of the genus *Stylochus* (commonly 1.5-3 cm long, Fig. 4.4.8), has been observed in spat held field in upweller units and occasionally in pearl and lantern cages. Members of the genus Stylochus are significant predators of oysters (Littlewood and Marsbe, 1990) and mussels (Galleni *et al.*, 1980), but had not previously been associated with mortalities in *P. fumatus*. Commonly, this flatworm does not occur in sufficient numbers (<2 per upweller screen) to cause any immediately discernible impact. However, containment trials with scallop spat indicated that if left to unchecked they could cause significant spat losses. In practice, all flat worms observed during routine maintenance of upweller screens were removed.

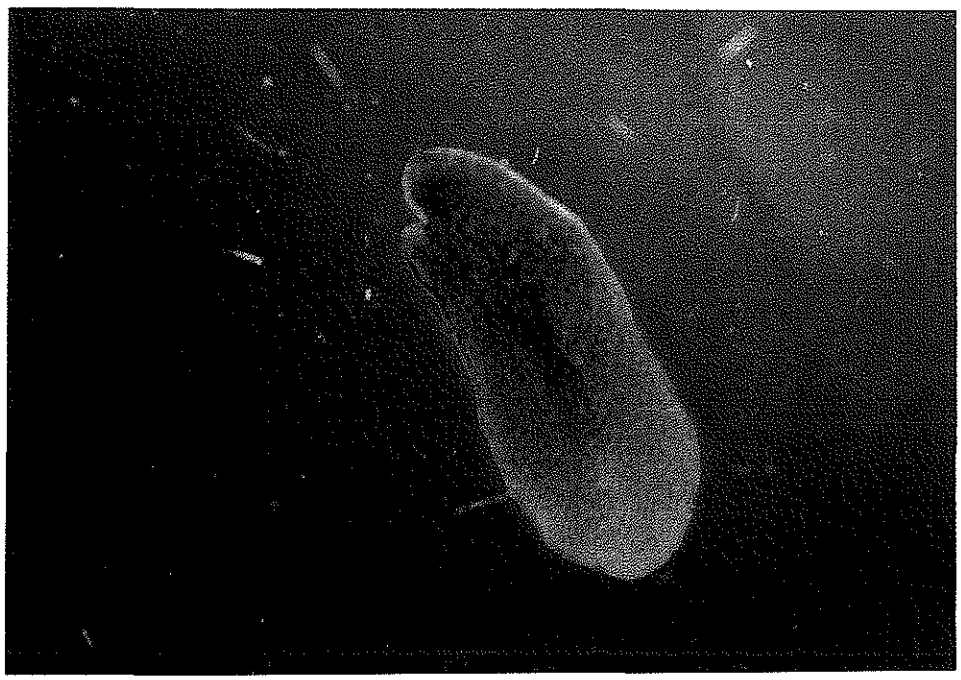

Figure 4.4.8 A turbellarian Polyclad, *Stylochus* spp. found in nursery systems.

Polydora (Mudworms)

Shell boring spionid polychaetes are a major pest to bivalve aquaculture in Australia (Skeel, 1979). In scallops, commensal mudworms can produce unsightly blisters or shell deformities that prevent marketing in the shell and in more extreme circumstances

have been thought to cause of mortality in *P. fumatus* in both Tasmania (Dix, 1981) and New South Wales (Hamer and Jacobs, 1987; O'Connor *et al.*, 1994). Hamer and Jacobs found that the prevalence of polydora increased with age in *P. fumatus* and that when more than 40% of the shell surface was covered with blisters, mortality was likely to occur. During the culture of *P. fumatus* in Twofold Bay and in Jervis Bay up to 100% and 51%, respectively, had been affected by mudworm. Thus, the potential economic ramifications of mudworm infestation for scallop farming can be dramatic. While the spionid responsible for the high level infestation in scallops in Twofold Bay was identified as *Polydora haswelli* and the blisters in scallops from Jervis Bay were similar to those in scallops in Twofold Bay, Skeel (1979) reported two other spionids were found in NSW, *P. websteri* and *Boccardia chilensis.*

Typically, >95% mudworm infestation in *P. fumatus* occurs on the left valve (O'Connor *et al.*, in press and Section 5.3 of this report). Whether this results from settlement of polychaete larvae on the flat left valve which is normally uppermost, or from difficulties in boring through the curved right valve is unclear, however both the early studies of Dix (1981) and those of the authors have confirmed that scallop orientation has a significant impact on polydora prevalence (Fig. 4.4.9). In both cases orientations which reduced the accumulation of suspended material on the scallop, that is having vertical orientation or with the curved right valve uppermost, reduced the incidence of polydora. Additionally, the presence of foreign material such as adhesives or sealants on the uppermost valve also reduced polydora prevalence. These adhesives and sealants may have affected any polychaetes present at the time of application and/or inhibited further infestation.

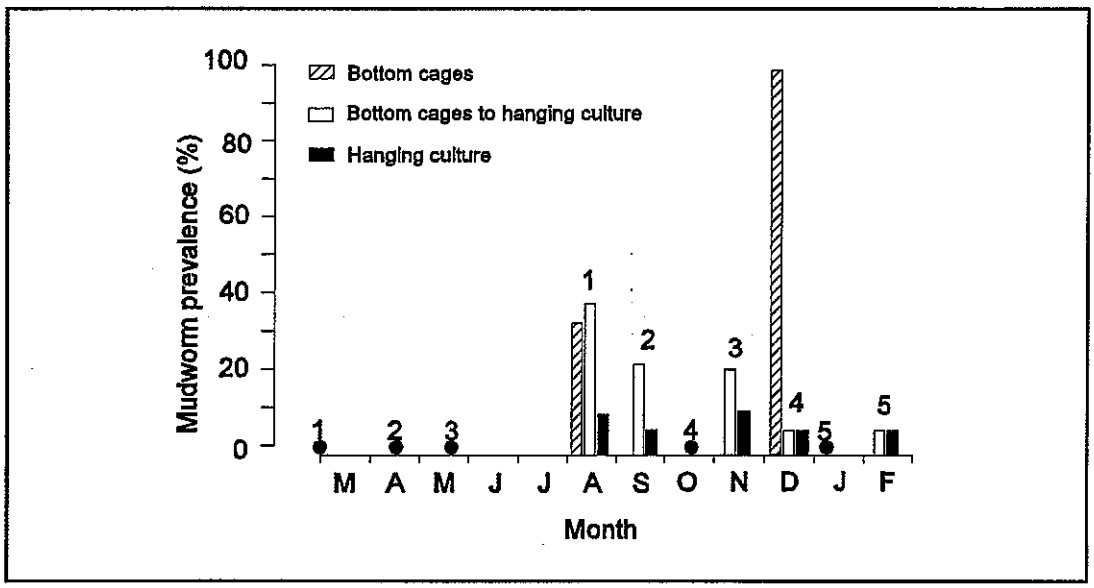

Figure 4.4.9 Prevalence of mudworm, *Polydora haswelli*, infestation in five

shipments of scallops ongrown using one of three rearing methods in Twofold Bay. Circles indicate time of deployment.

Mudworm infestation appeared to be seasonal, site dependent and influenced by the culture method used. This was most evident in shipments of hatchery produced juvenile scallops to Twofold Bay. Here, subsequent infestation was generally more frequent in scallops than was observed in either Jervis Bay or Port Stephens, although, mudworm infestation appeared to vary strongly in accordance with the culture method used and time of deployment (Fig. 4.4.10). Mudworm infestation was consistently lower in scallops maintained in suspended cages than those held on trays in racks on the bottom (O'Connor et al., 1994). While there is some conjecture as to the impact of sedimentation on mudworm infestation (Handley 1997, Handley and Bergquist 1997), noticeably less sedimentation took place upon suspended scallops. However, the differences in prevalence may simply reflect the proximity of mature mudworm populations which were restricted to the benthos.

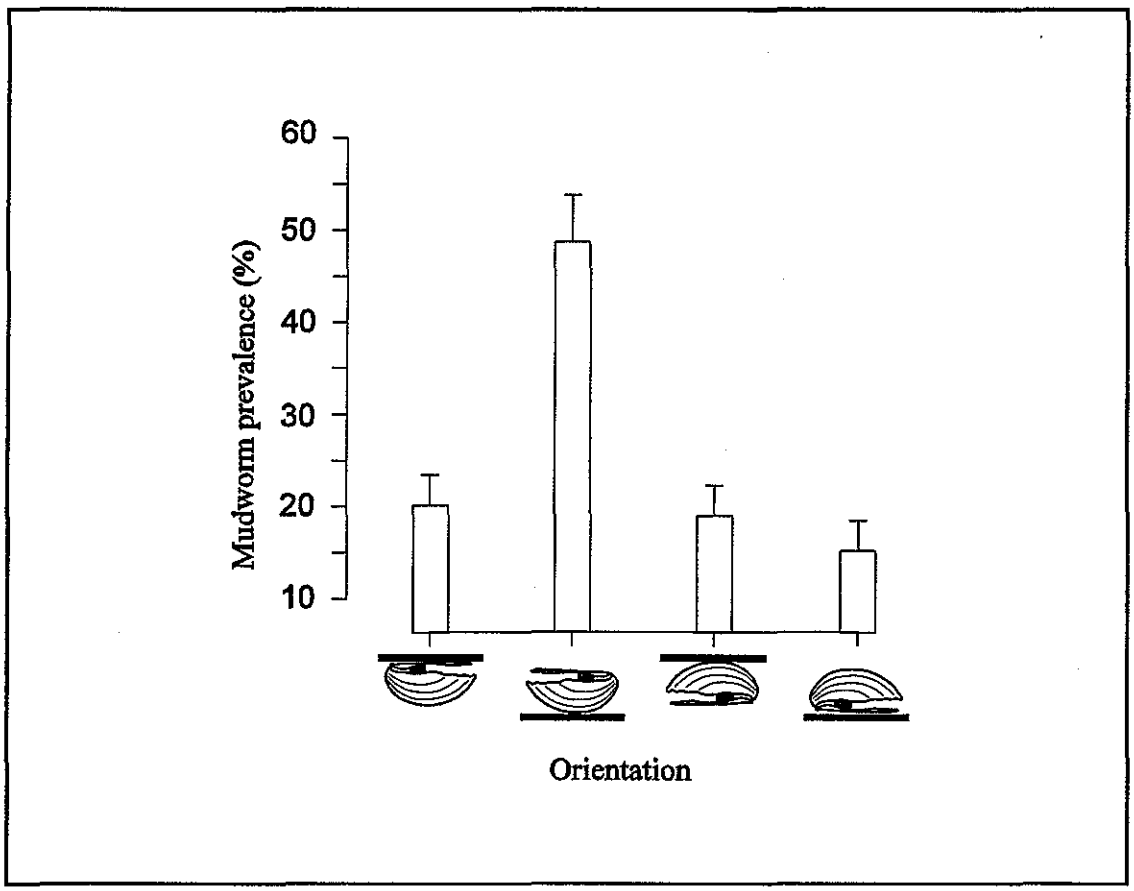

Figure 4.4.10 Prevalence of mudworm infestation in scallops glued to disks in different orientations.

Digenetic trematodes of the family bucephalidae are a common parasite of *P. fumatus* in southern Australian waters and have been well known fisherman and researchers for many years (Sanders 1966). The scallop is thought to be an intermediate host in which the sporocysts proliferate, progressively replacing the gonad and causing castration. The results of this process are clearly visible in *P. fumatus*. Our observations (Heasman *et al.*, 1996), have indicated that the male portion of the gonad becomes transparent before the entire gonad turns orange and later red. In addition there has also been some evidence of muscle weakening and increased mortality in parasitised scallops which has been most evident following periods of emersion during transport to the hatchery. In some cases, over 90% of the scallops to die during transport have been parasitised while the underlying percentage of parasitism within the shipment has been less than 15%.

The prevalence of Bucephalids in *P. fumatus* collected from Jervis Bay has shown strong seasonal and interannual variations with the numbers of infected scallops having ranged from 5% to more than 70% in individual collections. In those years in which the prevalence has been particularly high, increases have been observed in spring, culminating in peaks in percentage parasitised in late autumn and early winter . Although, regardless of the time of year, the prevalence of infection has increased with scallop size.

Despite the distinctive change in appearance, bucephalid parasitised scallops pose no risk to consumers and to date have not been discriminated against in the marketplace. Thus the greatest discernible impact of the bucephalid has been on the collection of broodstock, although with the ability to reduce the effective reproductive population by as much as 70% may well have major implications.

Echinocephalus

A nematode, *Echinocephalus* sp. has been reported to produce orange cysts in the adductor muscle of *P. fumatus* (McShane and Lester, 1984; Hamer and Jacobs, 1987) affecting marketability. Similar orange-brown cysts were observed once early in this study (Aug. 1993) but has not been encountered since.

Discussion

It is difficult to identify the most significant causes of mortality of *P. fumatus* in Jervis Bay as few attempts have been made to quantify the impact of individual pest or

predatory species. However, we can divide pest and predatory species into three groups according to their impact: 1) species of little impact, 2) species of moderate impact or those that are easily controlled and 3) those species with the ability to cause significant losses (Table 4.4.1). This of course assumes that farming techniques and environmental conditions remain much as they have for the past six years.

Low impact predators

Several of the species observed as predators are unlikely to have a major impact. In the case of the blue groper, predation can be rapid and, in earhanging trials total. However, this impact has been limited to experimental work conducted in the vicinity of rocky outcrops. Blue groper have never been observed at either seeding or longline culture sites. In addition, the blue groper is a protected species due to its low numbers, and like the magnificent volute, probably occurs too rarely to have an impact on a large scale. Similarly, the occurrence of crayfish is too infrequent for any significant impact and they have not been observed to occur naturally on the scallop beds of Jervis Bay.

Moderate impact predators

Despite its potential to decimate the reproductive population of *P. fumatus* in Jervis Bay, the bucephalid parasite was also classified as having low impact on farming activities. As long as the market is unaffected by the presence of the parasite, it is likely to be little more than a nuisance during broodstock collection. Several species were found with a greater potential to impact upon *P. fumatus* culture and were classified as a moderate risk, although in many instances their impact was limited to a particular type or phase of culture. The sparids and leatherjackets, which have caused considerable predation problems for New Zealand mussel farmers (Meredyth-Young, 1985), generally posed a threat to scallop spat and juveniles, mostly in seeding exercises or in unprotected culture such as earhanging. Several species such as hairy oyster drills occur in sufficient numbers to have an impact on survival of caged scallops, however, they were easily observed an generally removed during routine cage changes.

High impact predators (excluding man)

The species thought to impose the greatest risk to scallop fisheries enhancement and culture include crabs, rays, octopus, and spionid polychaetes. Of these, octopus were omnipresent and effected both farming and seeding operations. Crabs (*N. tuberculosis*) were responsible for some of the greatest losses of spat, juveniles and adult scallops in farming and reseeding exercises, but were a problem confined mostly to Jervis Bay.

Similarly, the elasmobranchs were thought to be collectively responsible for major losses of seeded scallops but had no observed affect upon farming operations, while the starfish, *C. calamaria*, affected only caged scallops.

In summary, seeded scallops suffered far greater mortality than those farmed in cages which was thought to be mostly the product of crabs, octopus and rays. Caged scallops appeared to provide an opportunity for a greater variety of predators and pests, however careful management limited losses. In many cases the impact of pest species was seasonal and frequently site dependent.

Table 4.4.1 Predators, pests and parasites of the commercial scallop, *Pecten fumatus*, cultured in Port Stephens (PS), Jervis Bay (JB) and Twofold Bay (TB), NSW

Species	Location	Impact
Fishes		
Labridae		
Achoerodus viridis	PS, JB, TB	Low
Monocanthidae		
Monocanthus chinensis	PS, JB, TB	Low-Mod.
Meuschenia trachylepis)	PS, JB, TB	Low-Mod.
Sparidae		
Pagrus auratus	PS, JB, TB	Low-Mod. seeded scallops mostly
Acanthropagrus spp.		
Elasmobranchs	JB	High for seeded scallops only
Urolophus cruciatus		
U. testaceus		
Trygonorhina fasciata		
Aptychotrema rostratus		
Molluscs		
Gastropods		
Cymatium parthenopium	PS, JB	Mod
lepsiella vinosa	JB	Mod. seeded and bottom caged scallops
Cymbiola magnifica	JB	Low, seeded scallops only
Cephalopods		
Octopus	PS, JB, TB	High, mostly for seeded and bottom caged scallops
Crustaceans		
Nectocarcinus tuberculosis	JB	High
Jasus verreauxi	JB, TB	Low
Echinoderms		
Coscinasterias calamaria	JB	Mod-high
Luidia australiae	JB	Low, possibly significant for seeded scallops
Worms		
Stylochus sp.	PS, JB	Low
Bucephalus sp.	JB	Low, but of considerable ecological significance
Polydora haswelli	PS, JB, TB	High
Echinocephalus sp.	JB	Low

Impacts are Low: infrequent, of little impact or easily managed
Moderate: relatively common but manageable
High: Capable of significantly affecting viability

5.0 BENEFITS

Successful commercial application of improved hatchery and nursery technology developed during this project will greatly reduce costs of seed scallops used for both farming and fisheries enhancement. Direct deployment of recently settled scallops in traditional spat collector bags at a mean yield of 10 000 per bag (yields of up to 25 000 spat/bag have already been achieved) will be greatly reduce costs of 10 to 15 mm seed below current levels.

Tascallops Pty Ltd's present target capture of 30 to 50 million of wild spat at Triabunna requires the deployment of 200 000 to 300 000 spat collectors returning an average of only 100 to 200 spat per bag. This massive operation requires up to 100 additional seasonal staff and the chartering of up to 6 additional fishing vessels for the deployment retrieval and processing of collectors and spat. The total estimated cost of these operations is several hundred thousand dollars. With the use of hatchery produced spat settled at an average rate of 10 000 per spat bag, this operation could be scaled down by a factor of at least 90% with commensurate cost savings. Additional benefits of hatchery production is the controlled timing and reliability of production and scope for the developing genetically improved stock.

Mean catch figures for wild *P. fumatus* spat in Tasmania collected in a total of 209 375 spat collector bags deployed in 1994/95,1995/96, 1996/97 and 1997/98 were≈90, 35, 25 and 60 per bag respectively (Scott Crawford,Tascallops P/L, pers. com., June 1998.). These all fell far short of minimum commercially viable rates of 200 to 500 per bag cited by Cropp and Frankish (1989) but were in line with spat capture rates for Jervis Bay. As a consequence, total annual collections of wild spat by Tascallops P/L for their farming operations in S.E. Tasmania have been well short of the minimum targeted level of 30 million p.a..

With respect to NSW, enhancement of the scallop fishery beyond the current 1 in 10 to 1 in 15 year Jervis Bay fishery to a fishery of approximately 300 tonnes per annum (10% of peak natural production) would return direct revenue of at least $1 million. Flow on benefits to the local recreational diving and tourist/catering industries are likely to multiply these benefits by a factor of at least 2 to 3 fold (Greg Pullen, Commercial and Industrial Development Manager, Shoalhaven Shire Council, pers. comm. April 1995).

Ongoing development of farming and fisheries enhancement technology for NSW (see Section 9) and other southern mainland states using hatchery and nursery generated

seed stock will ultimately lead to additional gross export revenue in the order of $3 to $4 million per thousand tonnes of production. Relevant technology developed during this study has already been passed on to interested commercial hatcheries in Tasmania, South Australia and Western Australia. It is estimated that such benefits will begin to be realised within the next 5 years and possibly much sooner in the case of the existing Tasmanian scallop farming and stock enhancement industry.

6.0 FURTHER DEVELOPMENTS

In 1997, *Shellfish Culture P/L* (a commercial oyster hatchery based at Bicheno Tasmania) in collaboration with and *Tasscallops P/L* (a commercial scallop farming company based at Triabunna), were awarded a two year R&D grant by the Tasmanian government to commercialise hatchery and nursery technology developed during the course of the present study and its precursor, FRDC Project 91/ 053.

The aim of the project was to overcome constraints to scallop fanning generated by high costs, associated with highly variable and generally low yields of wild collected spat as previously discussed in Section 6.0.

Over the past two years, *Shellfish Culture P/L* manager Martin John and scallop project leader John Mercer, have made several visits to the PSRC and have remained in regular contact with us to ensure the smooth transfer of hatchery technology and to keep abreast of new developments at the PSRC . Especially significant are improved nursery production and farming techniques, the latter including very promising results with the use of furanone based antifoulant coatings on scallops in suspended culture.

Considerable and continuing interest in transfer of these scallop aquaculture technologies is also emanating from South Australia (Dr Patrick Hone, SARDI - pers comm.), Victoria (Steve Dunn and Dr Neil Hickman, Vic Dept Fish. & Wildl. - pers.comm.) and Queensland (Mike Potter, BIARC - pers. comm.).

7.0 CONCLUSIONS

* Hatchery and nursery techniques initially developed for *P. fumatus* during the precursor project (FRDC 91/53) have been improved during the current project, can now be regarded as both reliable and efficient. A total of more than four million scallop spat being produced for farming trials and over 250 000 scallops ranging in size from 20-60 mm were released into Jervis Bay.

* A threefold increase in spat yield can be achieved by first settling larvae on cheap curtain material equivalent of 160 µm mesh downweller screening, retaining them in the hatchery for approximately a fortnight and then transferring them to field longlines The last step is achieved by interspersing strips of curtain material within folds of plastic netting stuffed into traditional wild spat collector bags. This technique allows control of spat stocking density, significantly reducing maintenance costs.

* There are no apparent differences between the growth and survival of normal (diploid) compared to triploid *P. fumatus* spat. Juvenile triploid scallops however, tend to be larger and heavier than their diploid siblings with significantly greater adductor muscle mass.

* Despite using the optimised dosage, exposure time and duration, use of cytochalasin B to induce triploidy reduced the yield of embryo by >75%. Moreover, cytochalasin B yielded low initial rates of triploidy (41%) followed by further reductions to negligible levels by the onset of sexual maturity. Thus further research, including development of alternative methods of triploid induction, is required before the full benefits of triploidy can be properly evaluated in *P. fumatus*.

* Gluing of scallops to plastic mesh disks (disk culture) yielded rates of growth and survival equal to or greater than that of similar sized scallops cultured by alternative techniques. Disk spacing of 40 to 60 mm minimised predation, while the valve by which the scallops are glued can be manipulated to maximise survival and soft tissue weights, especially gonad and adductor muscle. Gluing scallops by the upper flat left valve in both orientations or by the lower curved right valve in inverted orientation, significantly lowered mudworm prevalence below that of scallops grown in cages, earhung or glued by the right valve in normal orientation.

* The ability of seeded *P. fumatus* to avoid detection was greater than had been expected, with the best of four trialed survey techniques underestimating scallop densities by approximately 17.5%. This "best" technique consisted of sampling 1 m wide transacts that can be simply designated by using a 10 m length of rope stretched between two weights.

* Diver experience did not improve survey accuracy but did improve survey speed and hence efficiency.

* Deployment of scallops in predator proof cages was found to be useful in estimating handling mortality and of subsequent predation of the seeded scallops.

* Seeding of scallops using batches of thousands or tens of thousands of hatchery produced juvenile scallops in Jervis Bay was unsuccessful due mainly to predation. Commonly, all seeded scallops were depleted within 6 weeks regardless of substrate type or presence of existing populations of wild scallops Further research and development of biodegradable mesh canopies to protect naive seeded scallops from decimation by a range of predators is both warranted and recommended .

* Loss rates of seeded scallops in Jervis Bay are size dependent, larger scallops (35-65 mm) often showed longer survival times but triploidy confers no apparent advantage. Crabs, rays starfish and octopus were among the most important predatory species of seeded scallops in Jervis Bay.

* The impact of a scallop long-line in Jervis Bay appeared to remain within the assimilative capacity of the environment in that no significant increases in organic material could be found in sediment samples from in and around the site.

* Despite several spawning events annually, significant natural recruitment of *P. fumatus* in Jervis Bay was limited to late winter and early spring and was too intermittent and sparse to serve as a source of seed for commercial farming purposes. This finding confirms the need for a commercial hatchery if *P. fumatus* farming is to occur in NSW.

* A new generation of antifoulant coatings that include non toxic biodegradable furanones, originally isolated from macroalgae, showed great promise for combating heavy and diverse biofouling of scallops under suspended culture in Jervis Bay.

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9.0 APPENDICES

Appendix 9.1

INTELLECTUAL PROPERTY

Findings arising from the research conducted in this program make a number of valuable contributions towards the development of a scallop industry. The improvements to hatchery rearing techniques made during this and the previous FRDC grants have been compiled in a manual format and forwarded to FRDC for publication. The additional improvements to settlement and farming techniques are either in press or being prepared for publication and do not represent intellectual property opportunities.

Appendix 9.2

STAFF

Project Leader/ Biologist.....Dr. Mike Heasman

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Senior Technical Officer.....Mr. Wayne O'Connor
Technical Officer.....Mr. Stephan O'Connor

Support Staff (Jervis Bay)
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Appendix 9.3

SPATFALL MONITORING

Introduction

Spat fall monitoring is frequently undertaken in scallop research programs to provide information relevant to hatchery production, farming and seeding programs. Initially the relationship between indices of reproductive condition and recruitment can be highly variable, particularly in Jervis Bay, where 3-4 annual peaks in reproductive condition are common (Jacobs, 1983, Fuentes, 1994; Heasman *et al.*, 1996). Spatfall provides a better indication of potential recruitment to the fishery and this information was vital if size matched comparisons of survival in seeded and wild scallops were to be made. Additionally monitoring spatfall in Jervis Bay provided data for comparison with results obtained by Fuentes *et al.* (1993) when resident scallop populations within the bay were considerably larger than those encountered during the period of this research. Finally, if spat fall had increased since the studies of Fuentes *et al.* (1989) it may be possible to use spat from the collectors to supplement hatchery produced seed for experimental seedings.

Materials and Methods

Strings of spat collectors were deployed monthly for twelve months at two sites off Murrays Beach, Jervis Bay. Each string held five 1 mm mesh spat collecting bags (Hoyo Corporation, Toyohashi, Japan) stuffed with 4 m^2 of black plastic mesh (20 mm oyster protection mesh, Kinnears, Sydney). Each bag was spaced 2 m apart, starting 6 m above the sea bed. Each monthly deployment of spat bags was made on the same day at both sites and the bags remained in the water for two months. The two sites chosen were approximately 0.5 km apart, but both in 17 m of water over the Murrays Beach scallop bed.

Upon collection, the site and depth from which each bag had been taken was recorded, before the number of scallops inside each bag were counted. Each bag was carefully inverted and the surface inspected with the aid of a binocular microscope. The mesh material from each bag was placed over a 500 μm nylon screen and any spat were washed from the mesh using fresh water to encourage rapid spat detachment. Separate counts for both *P. fumatus* and the doughboy scallop, *Mimachlamys asperrima,* were recorded.

Results

Data was analysed by ANCOVA, with month of deployment as a fixed factor and depth as a covariate. Site was considered a random factor. An unbalanced design model was used for analysis, as strings of collectors went missing on two occasions. Differences between means were detected using Student Newman Kuels procedure (Winer, 1971). Significant variation in spatfall occurred temporally ($P<0.05$), however, spatfall did not vary as a product of site or depth, nor were there significant interactions between these factors. Spat fall data is presented in Table 9.3.1.

Table 9.3.1 Spat fall data pooled for two sites of Murrays Beach over a twelve month period commencing September 1995.

Collection Month	Spat numbers *M. asperrima*	*P. fumatus*
September	13.4 ± 6.9^{bc}	59.1 ± 26.7^{a}
October	22.2 ± 3.4^{b}	15.7 ± 8.0^{b}
November	12.8 ± 12.2^{bc}	12.3 ± 9.2^{b}
December	406.8 ± 51.9^{a}	10.3 ± 4.6^{b}
January	0.4 ± 0.5^{c}	1.0 ± 0.7^{b}
February	0.6 ± 0.8^{c}	0.6 ± 0.8^{b}
April	1.0 ± 1.4^{c}	0.5 ± 0.7^{b}
June	3.0 ± 2.1^{c}	0.2 ± 0.4^{b}
August	5.4 ± 1.7^{bc}	1.0 ± 1.0^{b}

Values are means ± s.d.. Values within columns with a common superscript do not differ significantly .

Discussion

One of the primary questions encountered when contemplating scallop farming or seeding is the source of spat, either natural or hatchery produced. While the former is undoubtedly quicker and simpler, this and previous studies (Fuentes *et al.* 1991) have failed to collect sufficient spat for farming purposes. Cropp and Frankish (1989) estimated that for economic viability approximately 500 *P. fumatus* spat per collector bag are required, however, neither this study nor that of Fuentes *et al.*(1991), recorded

catches of this magnitude (maximum 59.1 and 35.4 *P. fumatus* spat per collector, respectively).

In both this and the previous attempt to catch *P. fumatus* spat in Jervis Bay, small numbers of spat were collected throughout the year, reflecting the extended temporal availability of reproductively mature scallops. However, despite the presence of mature scallops and evidence that there may be as many as 3-4 spawning peaks in each year (Jacobs, Fuentes 1994, Heasman *et al.*, 1995) in both cases the only significant recruitment arose in late winter / early spring. While we have previously argued that this recruitment occurs at a time when the most favourable environmental conditions for larval growth and survival occur in Jervis Bay (Heasman *et al.* 1996) this does not discount the possibility that the settled spat are not the progeny of Jervis Bay stocks.

As previously described, water flow in Jervis Bay is clockwise, arising from a northerly current that enters along the southern shore past Bowen Island moving around the bay and out on the northern side of the entrance, past Point Perpendicular (Holloway *et al.* 1992). The spat bags were deployed on the southern shore approximately 1 km inside the entrance from Bowen Island in an area in which they would be exposed to water that had recently entered the bay. Thus ready-to-set larvae (18-20 days old) in the water column could well have arisen from spawnings well to the south of Jervis Bay. There is some circumstantial support for this in the fact while both studies used similar collectors at the same site, this study recorded higher spat catches despite significant reductions in the scallop population in Jervis Bay since the Fuentes *et al.* (1991) study. Regardless spat catch has been and remains too small for commercial scallop production.

With respect to the other common pectinid in Jervis Bay, the doughboy scallop, *Mimachlamys asperrima*, spat catches were significantly greater (approx 400/bag) however, these catches are also likely to be too small for commercial purposes. The timing of peak catches was later than that of *P. fumatus*, but was temporally consistent with peaks in reproductive condition observed by O'Connor and Heasman (1996). The persistence of small numbers of spat is also consistent with the prolonged temporal availability of *M. asperrima* in good reproductive condition in Jervis Bay, however, like *P. fumatus* the possibility of recruitments from outside the bay also exists.

Throughout the entire period of this study (1994 -1997) no significant *P. fumatus* recruitment was observed in Jervis Bay and at no time were sufficient juveniles found to permit comparisons of survival of wild and hatchery reared, seeded stock.

Appendix 9.4

THE IMPACT OF SCALLOP LONGLINES IN JERVIS BAY

Introduction

Of particular topical importance both locally and internationally are the social and environmental impacts of aquaculture. These impacts are frequently divided into three broad areas: the aesthetic influence, the influence upon competing activities and the ecological impact. In farming the scallop, *Pecten fumatus*, in Jervis Bay these issues were considered at some length.

Initially, a public information campaign was undertaken to inform the major user groups of the nature of the research and what could be expected. To minimise visual impact, the longlines were designed and constructed to be wholly submerged with only marker buoys to indicate their location. Indeed during daylight hours the line was invisible to the naked eye from the shoreline. The line was positioned at a depth (approx. 5-7 m) which allowed navigation of both recreational and commercial craft which frequented the area to sight-see, fish and occasionally dive. The site for the lines was chosen in consultation with the Navy so as to minimise impacts on the activities of large ships. The success of this process was such that no complaints were received from any source during three years the lines were in place.

Prior to their removal, one of the lines had been fully stocked for in excess of twelve months with scallops destined for seeding trials. This was seen as an opportune time to make some measure of the ecological impact of the farming activities.

One of the most commonly employed methods for the assessing impact is to look for the accumulation of organic material in the sediment. Beyond the natural accumulation of organic material, material also arises from the settlement of faeces and psuedofaeces from the cultured animals, the faecal materials of the many organisms attracted to the line, fouling falling from the line and from the settlement of material from the water column due to localised disruptions of current flow. To investigate the possibility of localised accumulation of organic material in the sediment, sampling was undertaken that indicated both that the level of organic material in the sediment was relatively low and that there was no apparent pattern of accumulation resulting from the presence of the line.

Materials and Methods

Facilities

The dimensions of the longline and the scallop stocking densities used in this study were consistent with those suggested for commercial culture (Hardy 1991). The main line was approximately 400 m long from anchor to anchor, of which the central 200 m was stocked with a lantern cages or strings of pearl cages (10/string) every 1.5 m. Stocking rates for cages varied between approximately 60 to 100% coverage of the cage area. For cleaning purposes, all cages, floats and lines were removed from the site and taken to a shore based facility.

Sampling

Divers taking benthic cores with transparent perspex tubes which are sealed in situ are considered to be the best method for the collection of sublittoral sediment (Fleeger *et al.* 1988). In accordance, divers in this survey used perspex 10 cm x 3 cm sample tubes with screw caps to take benthic cores. Samples were taken along four 200 m transects which ran at 90° to the longline. Each transect involved taking samples at 100, 50, and 10 m either side of the line and from directly beneath the line (Fig. 9. 1). Two of the four transects passed beneath the section of line that was stocked with scallops, while the remaining transects were conducted 100 m up-current and 100 m down-current of the stocked section of the line. Three replicate cores were taken at the seven designated sites on each transect (ie 28 sites and 84 core samples). All samples were immediately placed in a portable freezer and transported to the laboratory for analysis.

Approximately six months after the complete removal of the line, the above sampling procedure of three replicate cores was repeated for sites 4, 11, 18 & 25 (Fig. 9.1), that is one site each directly upcurrent and down current of the previous line location and two sites directly beneath the previous line location. Sites were located with the aid of GPS, but are subject to the accuracy of the device, estimated to be ± 10 m.

To determine the organic content of the sediment, each sample was thawed, before being placed in a drying oven (100°C) for 24 h. Using a balance accurate to 0.1 mg, 10 g (± 1 mg) of dry sediment sample was transferred to a crucible and then ashed at 425°C for 6 h before being re-weighed. The weight of organic material in each sample was determined from the difference in weight to the nearest mg before and after ashing.

Results

Total organic content levels for sediment samples from the 28 tested locations ranged from 4800 to 23866 mg / kg of sediment and are given in Table 9.4.1. Single factor ANOVA of log transformed organic content data found no significant differences between sites ($P>0.05$) when sampled with the line in position and fully stocked (10.4.2a).

Six months after the line removal, total organic contents reamained similar for the four tested sites, however, overall organic material levels rose significantly being 2 to 4 times greater than when sampled with the line stocked and in position at the site. No significant interactions were detected between sites and sampling occasion (Table 9.4.2b)

Table 9.4.1 Total organic content of sediment samples collected from Murrays Beach, Jervis Bay, NSW.

Site	Organic content ($mg\ kg^{-1}$)		Site	Organic content ($mg\ kg^{-1}$)	
1	10566 ± 4401		15	8000 ± 1777	
2	9493 ± 6567		16	12400 ± 1670	
3	5266 ± 1877		17	5066 ± 896	
4	8533 ± 5253	22043 ± 6159*	18	5000 ± 1997	19173 ± 3140*
5	23866 ± 25716		19	8866 ± 3320	
6	9200 ± 6677		20	22133 ± 28477	
7	4900 ± 1400		21	7766 ± 6011	
8	7133 ± 4354		22	9900 ± 529	
9	6266 ± 2274		23	6600 ± 655	
10	15066 ± 9247		24	5500 ± 1928	
11	7500 ± 4334	21717 ± 5253*	25	9733 ± 1677	19313 ± 1023*
12	14266 ± 2074		26	4800 ± 2433	
13	6033 ± 2411		27	8900 ± 1044	
14	6733 ± 611		28	5366 ± 2853	

Values are means ± s.d. * Values 6 months after line removal.

Table 9.4.2 ANOVA analysis of Total Organic Content (TOC) of a) surface sediments for 28 locations at the longline site in Jervis Bay and for b) four of those sites sampled six months after line removal. Data was log transformed to satisfy requirements for analysis of variance.

a)

Source	SS	df	MS	*F*	*P*
Collection site	10.948	27	0.405	1.138	0.33
Residual	19.945	56	0.356		
Total	30.892	83			

b)

Source	SS	df	MS	*F*	*P*
Collection site	0.4199	3	0.1399	0.919	0.45
Sampling occasion	8.9032	1	8.9032	58.425	<0.001
Interaction	0.3702	3	0.1234	0.810	0.51
Residual	2.4381	16	0.1523		
Total	12.1316	23			

Discussion

Longlines are a source of organic material and any accumulation of organic material beneath the longlines was hypothesised to be a function of the total organic output of the line, the hydrography of Jervis Bay and the assimilative capacity of the environment. As we were unable to detect significant differences in the organic content of substrate beneath and around the longline in Jervis Bay, the question was raised, were the hydrodynamics of the site such that organic material was swept away or was the rate of deposition within the assimilative capacity of the environment?

Complex hydrodynamic modelling of the longline site was beyond the scope of this research, however, water movements in Jervis Bay are dominated by nearly continuous inflows on the southern side of the entrance to the Bay with similar outflows on the northern side (Holloway *et al.* 1992). These flows are almost certainly generate a cyclonic (clockwise) gyre in which both current and tidal flows are weak, typically less than 5 cm s^{-1}, and thus flushing times for the bay can be as great as 74 days with a mean of 24 days (Holloway *et al.* 1992).

Under circumstances in which water flows are relatively stable and predictable the dispersion of waste from an aquaculture facility can be simply estimated from the following equation:

$$d=(D \times Cv)/S$$

where **d** is the distance dispersed, **D** is the depth, **Cv** is the current velocity and **s** is the settling velocity of a given particle (Gowen *et al.*, 1989). This equation expresses what might be derived intuitively, that is that heavier particles have greater settling velocities and fall to the bottom closer to the longline. For large clumps of dislodged fouling this would be almost directly beneath the line and indeed this was frequently observed too be the case, although, there was no apparent accumulation of this material. Smaller particles would be transported with the current and deposited according to their settling velocity. As the longline was oriented parallel to the prevailing current along the southern shore line, the directional consistency of the weak current should give rise to a pattern of deposition similar to that illustrated in Figure 9.1. It would therefore be reasonable to expect that the impacted area (Fig. 9.1) would have elevated levels of organic material, while sites up current would be largely unaffected by the farming activities. As this was not the case and there was generally a low level of organic material at the tested sites the suggestion was that the amounts of material deposited were largely assimilated.

The second set of sediment samples provide further support for the contention that the additional organic load imposed upon the environment by the longline may be small in comparison to normal environmental fluctuations. Although unintentional, the second set of samples was taken following a short period of heavy seas, the magnitude of which were not uncommon to Jervis Bay. These seas resulted in heavy drift algal mats in the Murrays Beach area with up to 80% of the bottom covered. This material in many cases was decomposing and was thought to have produced the significant overall increase in organic material levels in the sediment.

While the above suggests that scallop farming at Murrays Beach had little impact on the environment, several additional points should be considered. The high level of maintenance and handling associated with scallop stocks used for this research saw the equipment regularly cleaned onshore. This practice ensured the removal of large quantities of organic material that may have otherwise contributed to the organic load at the longline site. Additionally, is likely that some material was swept beyond the sampled area, although given the weak prevailing currents, this material is thought unlikely to have had any significant impact upon areas remote to the longline site.

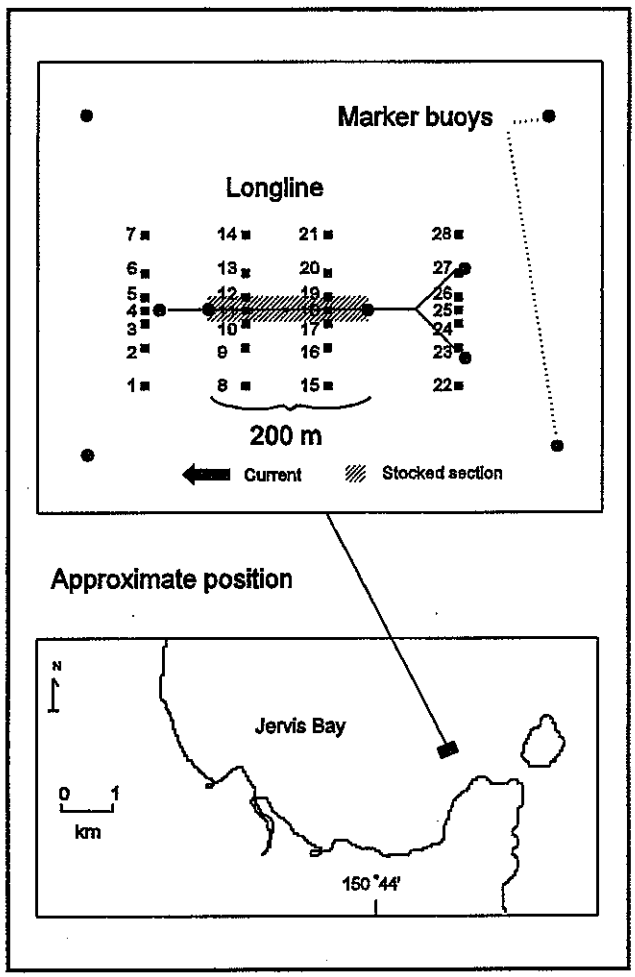

Figure 9.1 Sampling locations and hypothesized area for organic material settlement.

Novel antifouling technologies for shellfish aquaculture

Report I. Field biofouling trials of coatings and active ingredients using live scallops. A summarised report

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I. Live Scallop Coating Experiment

Materials & Methods

Novel antifouling technologies developed at the Centre for Marine Biofouling & Bio-Innovation for the prevention of fouling in shellfish aquaculture were tested for the efficacy in deterring the settlement and growth of fouling organisms on the scallop *Pecten fumatus*. These tests were carried out with the co-operation of Dr Mike Heasman and Mr Steve O'Connor (NSW Fisheries) at the research scallop aquaculture facility, Jervis Bay, NSW. Three treatments were used in the trials. 1) Treatment in which the AI was included in the carrier and coated onto the scallop, 2) Coating controls in which only the carrier for the active ingredient (AI) was coated onto the scallop, and 3)Control scallops which were untreated. Fifteen scallops were used for each treatment. The scallops ranged in size from 1cm to 3 cm.

The coating control and active ingredient treatments were coated onto the scallops using a standard paint brush. The scallops were left to air dry for two minutes before being placed into pearl nets and resuspended in the water. Control scallops were exposed to the same coating and drying conditions as coating controls and the active ingredient treatment.

Field Placement

Cages were hung off the long line in Jervis Bay on April 17, 1997. The cages were hung off one line between a depth of 3 to 6 metres in the following order;

AI Treatment
Coating control
Control

The experiment remained submerged for eight weeks (23/6/97) after which time the scallops were removed and fouling on individual valve of each scallop (cupped & flat) quantified.

Measurement of fouling on scallop shells

Fouling was quantified using the standard point intercept method. A 0.5 x 0.5 cm grid was placed over the shell, and the presence and type of fouling organisms counted. Results are presented as percent cover of the fouling organisms on the shells. The two halves of each scallop were analysed separately as cupped and flat valve. However, as in most cases there was no difference in fouling for the cupped and flat valves the results for individual fouling organisms were combined for ease of presentation. Mortality of scallops was also recorded.

Statistics

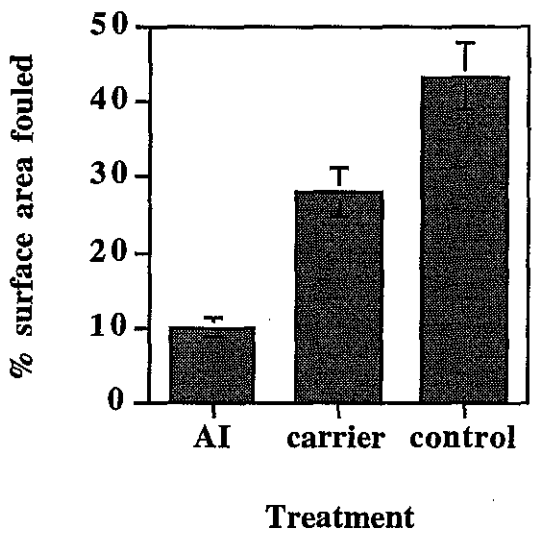
The data were analysed by analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Data were analysed as arcsin $\sqrt{p}$ transformations where applicable.

Results

Total Fouling

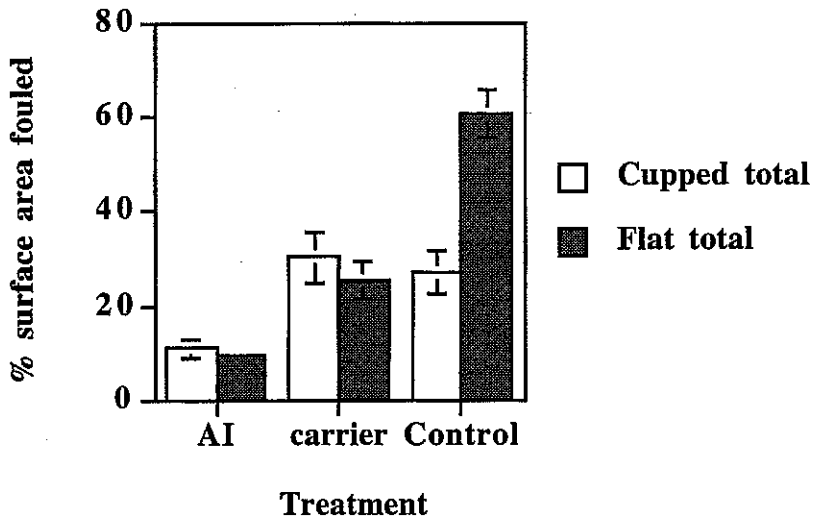
The treatment of scallops strongly deterred the settlement and growth of fouling organisms (single factor ANOVA, $p = 0.0001$) (Fig. 1, Plates 1-3). Mean percentage coverage of whole scallop shells was 10 % for treatment shells compared with 43 % for untreated controls. The coating control (28%) were also significantly less fouled than on treated control shells demonstrating that the carrier alone has significant antifouling activity. (Fig. 1). Mortality only occurred in control scallops where two scallops were dead (13%).

Figure 1. The percent surface area of scallop shells covered by all fouling organisms. Data are means ± SE.



There were significant differences in the fouling community of the cupped and flat valves of control shells but not on scallops which had been treated (Two factor ANOVA; Tukey's test $\alpha = 0.05$). While the distribution of fouling organisms was not a primary goal of this study, the difference in settlement of fouling organisms on different parts of the shell may be of importance in designing appropriate antifouling formulations. Therefore results for total fouling are presented for both cupped and flat valves (Fig. 2), but results for individual species are presented as combined data for both valves of scallops.

Figure 2. The percent surface area of the cupped and flat valves of scallop shells covered by all fouling organisms. Data are means ± SE.

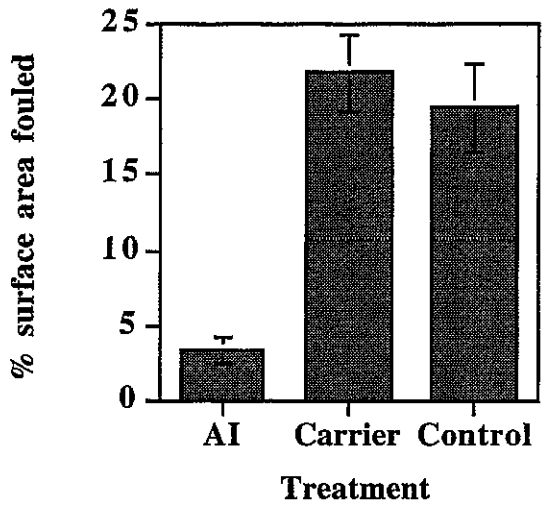


The fouling organisms of whole scallops ranked in order of percent coverage of scallop shell were, the oyster (*Ostrea angasi*), bryozoans (*Membranipora* sp. + others), sponges, tubeworms, the bivalve (*Electroma georgiana*), and barnacles.

Oysters

Oysters were the major fouling organism in terms of % coverage. There was a significant effect of treatment of oysters on % coverage (single factor ANOVA, p = 0.0001). The AI treatment was significantly more deterrent than the carrier treatment or the control, which were not different from each other (Tukey's test $\alpha = 0.05$). Mean percentage coverage for AI treatment shells was 4 % compared with 22 % for the carrier alone, and 19 % for control scallops. (Fig. 3)

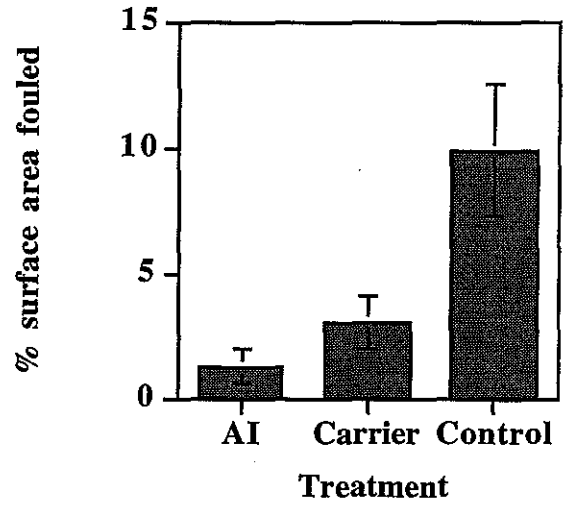
Figure 3. The percent surface area of scallop shells covered by oysters. Data are means ± SE.



Bryozoans

Bryozoans, in particular encrusting species, were the next most common major fouling organism in terms of % coverage. The treatments had a significant effect on % coverage (single factor ANOVA, $p = 0.0001$). However, in contrast to the oyster, both the carrier and the AI treatment significantly deterred fouling and there was no significant difference between the two treatments (Tukey's test $\alpha = 0.05$). Mean percentage coverage for AI treatment shells was 1 % compared with 3 % for the carrier, and 10 % for control scallops. (Fig. 4)

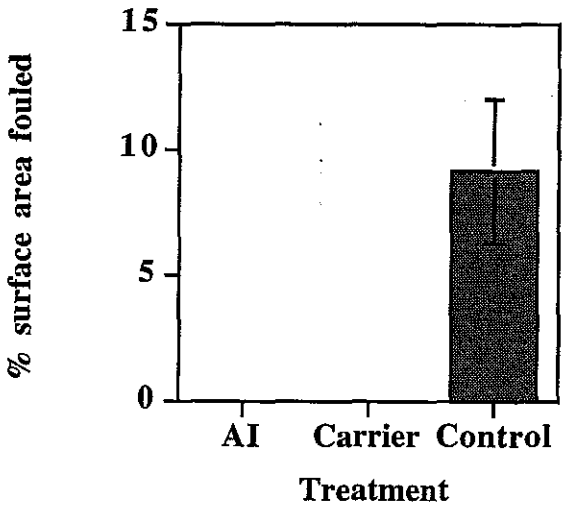
Figure 4. The percent surface area of scallop shells covered by bryozoans. Data are means ± SE.



Sponges

The third most common fouling organism was sponges. Again the AI and Carrier control treatments had a significant effect on % coverage (single factor ANOVA, $p = 0.0001$) and as was the case for bryozoans. There was a significant difference between the two treatments (Tukey's test $\alpha = 0.05$). In fact both treatments completely inhibited the settlement of sponges while the control shells had a mean coverage of 9 % of shell area. (Fig. 5)

Figure 5. The percent surface area of scallop shells covered by sponges. Data are means ± SE.



Tubeworms, Bivalves and Barnacles.

There was a strong decrease in coverage of surface area for the next three groups of foulers; tubeworms, the bivalve *Electroma georgiana* and barnacles. All of these groups had a mean surface area coverage of less than 4% on control scallops. Due to the low values and high variance among replicates there was no significant difference between AI treatment, carrier or control for tubeworms (ANOVA, $p = 0.511$, Fig. 6), *Electroma* (ANOVA, $p = 0.535$, Fig. 7) or barnacles (ANOVA, $p = 0.861$, Fig. 8).

Figure 6. The percent surface area of scallop shells covered by tubeworms. Data are means ± SE.

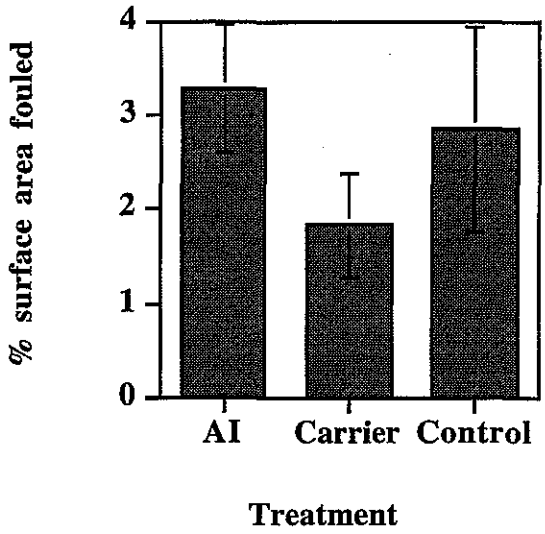


Figure 7. The percent surface area of scallop shells covered by the bivalve*Electroma georgiana*. Data are means ± SE.

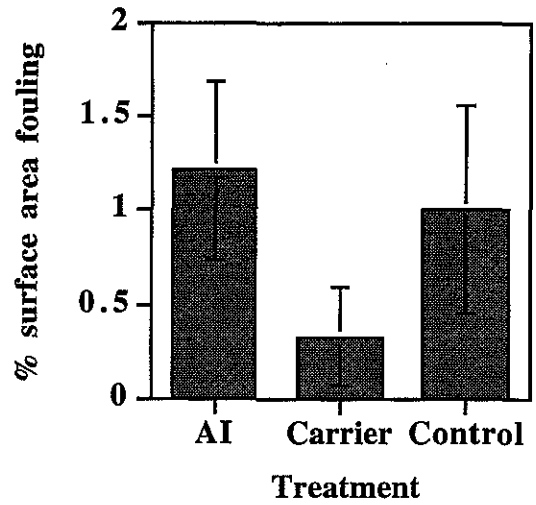
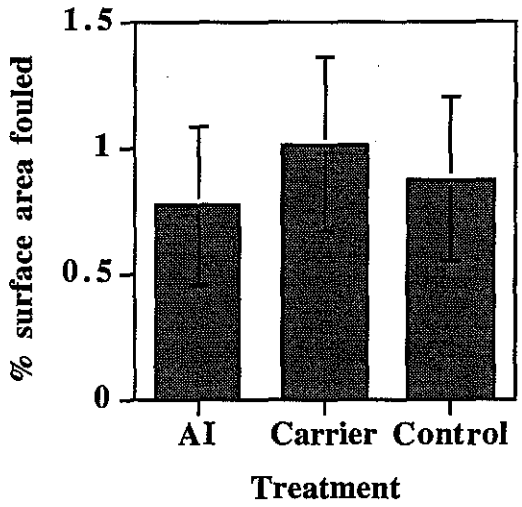


Figure 8. The percent surface area of scallop shells covered by barnacles. Data are means ± SE.



II. Scallop Cage Experiment

Materials & Methods

In a parallel experiment to the scallop coating experiment, novel antifouling technologies were assessed for their efficacy in preventing the settlement and growth of fouling organisms on the lantern cages in which scallops are cultured. These nets are also used for the aquaculture of other shellfish species. Tests were carried out at the research scallop aquaculture facility, Jervis Bay, NSW. Four treatments were used in the trials. 1) Control nets which were untreated, 2) Carrier controls in which only the carrier for the active ingredient (AI) was coated onto the net 3) Active ingredient in which the AI was included in the carrier and coated onto the net and 4) No-Foul in which the commercial antifouling paint No-Foul ™, was coated onto nets. Each lantern cage has four panels. One panel of each cage was coated with a different treatment. Therefore all nets had one panel containing each treatment. Treatments were randomly assigned. Ten cages (replicates) were used for the experiment.

Field Placement

Cages were hung off the long line in Jervis Bay on April 17, 1997. The cages were hung in two groups of five cages. Cages were assigned to positions randomly and the array of cages hung from depth of 3-6 metres.

Measurement of fouling on scallop cages

The experiment remained submerged for six weeks (07/06/97) after which time the cages were photographed *in-situ* using a Nikonus V with 35 mm close-up lens and frame. Slides were viewed using a Telex Caromate 400 AV unit and a 10 cm^2 area of net for each treatment was traced. The trace was scanned and the contrast adjusted (Abode Photoshop 3.0) such that netting and fouling were black and the net spaces white. The imagine was then transferred to NIH image, a black/white threshold

applied, and the image inverted (black to white and *v/v*) and analysed for net space area. Unfouled nets (not immersed) were also analysed in this manner. This allowed the calculation of fouling on the trial nets as occlusion of net space, as a percent of unfouled nets.

After eight weeks the experiment was ended and fouling by invertebrates measured. Individual fouling organisms were removed from each panel of the nets, dried with a paper towel and weighed. The total wet weight of each group of fouling organisms was determined.

Statistics

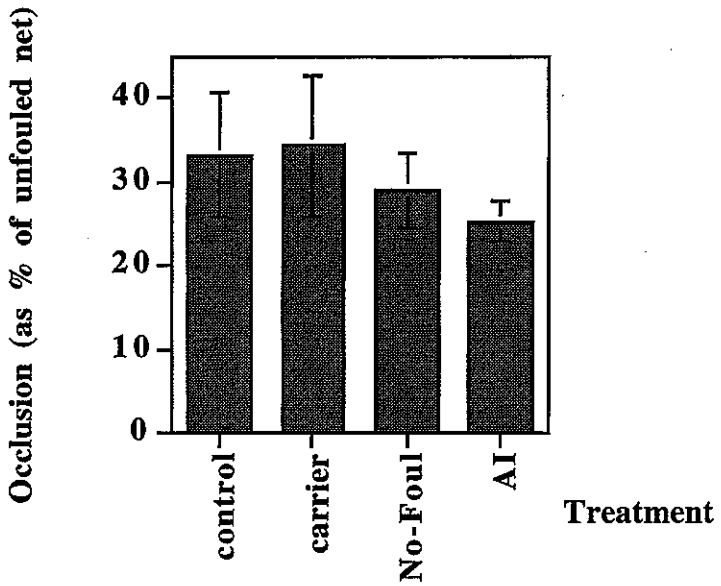
The data were analysed by analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Data were analysed as arcsin $\sqrt{p}$ transformations where applicable.

Results

Net occlusion

The occlusion of net space was due to the growth of an encrusting diatom film. There was no significant difference between occlusion of net space on any treatments (ANOVA, $p = 0.73$, Fig. 9).

Figure 9. The occlusion of net space (as a percent of unfouled nets) by fouling organisms. Data are means ± SE.

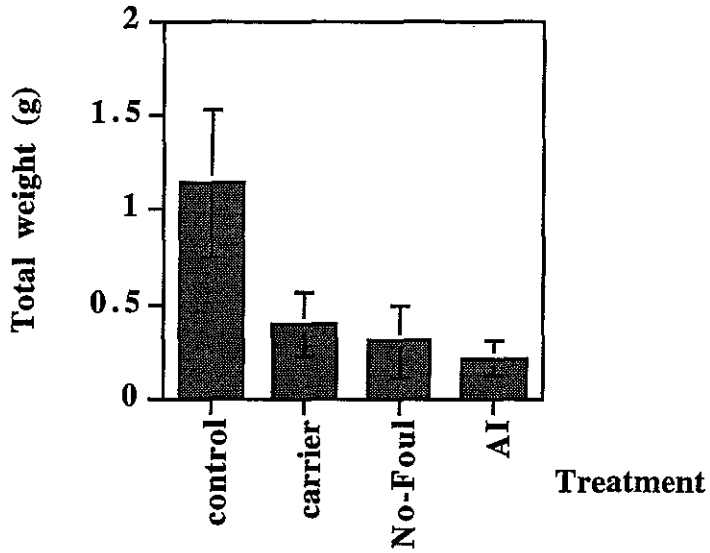


Fouling by invertebrates.

Macrofouling by invertebrates occurred on all control and treatment nets after eight weeks. There was a significant effect of treatment on the total weight of fouling organisms (ANOVA, $p = 0.038$, Fig. 10). The mean weight of all fouling organisms on control nets was 1.2 g compared with means of 0.2 - 0.4 g for the carrier, No-Foul and AI treaments. All three coating treatments were significantly less fouled than the

control but there was no difference between the coating treatments (Tukey's test α = 0.05). The coating control (carrier) was also significantly less fouled than control shells demonstrating that the carrier alone has significant antifouling activity. (Fig. 10).

Figure 10. Weight of all fouling organisms on nets. Data are means ± SE.

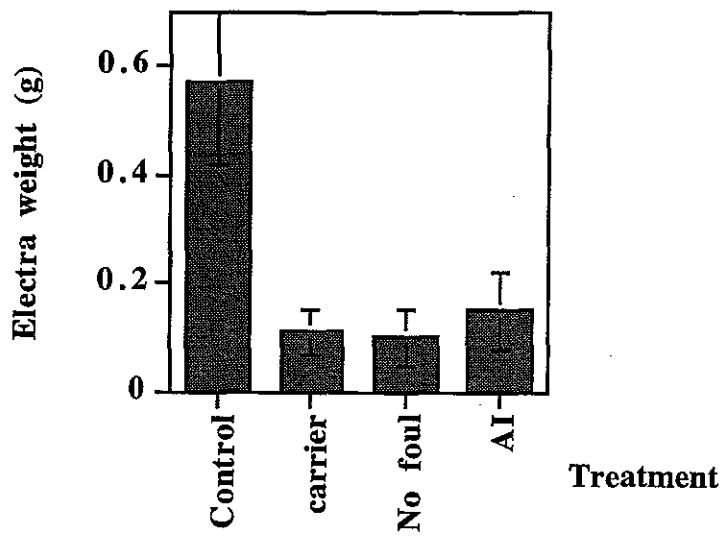


Only three types of fouling were present on the nets. The organisms ranked in order of weight were, the bivalve (*Electroma georgiana*), the bryozoan (*Bugula neretina*) and a tuft forming diatom.

Electroma georgiana

Electroma was very strongly deterred by all three coating treatments (ANOVA, p = 0.002, Fig. 11). There was no significant difference between the three coating treatments (Tukey's test α = 0.05).

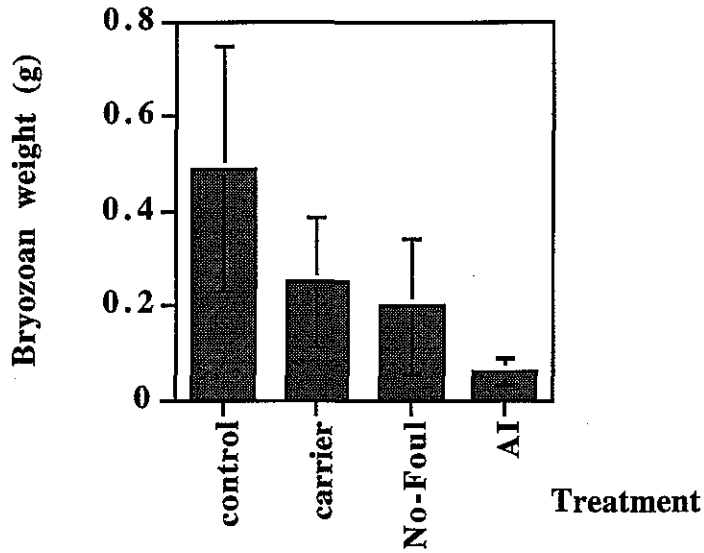
Figure 11. Weight of *Electroma georgiana* on nets. Data are means ± SE.



Bugula neretina

There was no significant difference in the abundance of*B. neretina* between the treatments and the control (ANOVA, $p = 0.322$, Fig. 12). The AI had the lowest mean abundance of *B. neretina* , however, due to the large variances in the control and other treatments this result was not significant.

Figure 12. Weight of *Bugula neretina* on nets. Data are means ± SE.



Diatom Tufts

The weight of diatom tufts on the net panels was not significant between the control and coating treatments (p =0.288, Fig. 13). Although no tufts were found on the net panels coated with the AI, the result was not significant due to the large variance.

Figure 13. Weight of diatoms on nets. Data are means ± SE.

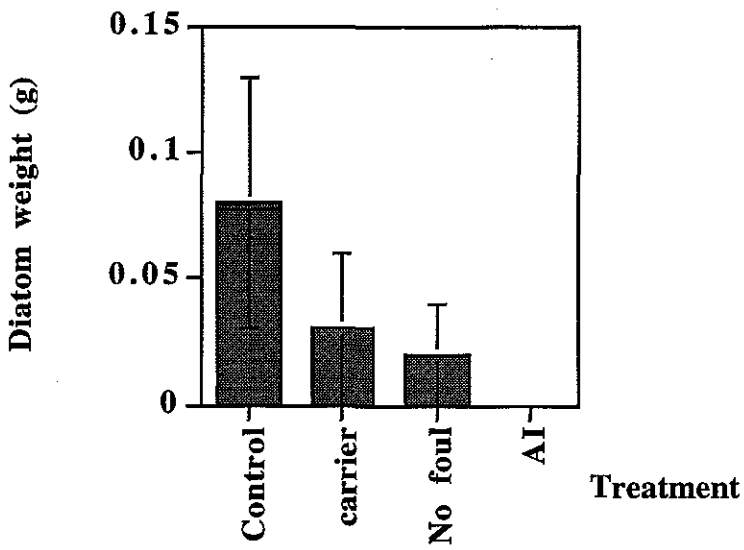


Plate 1. Scallops treated with carrier incorporating the **Active Ingredient**. Photographs were taken following eight weeks of field exposure.

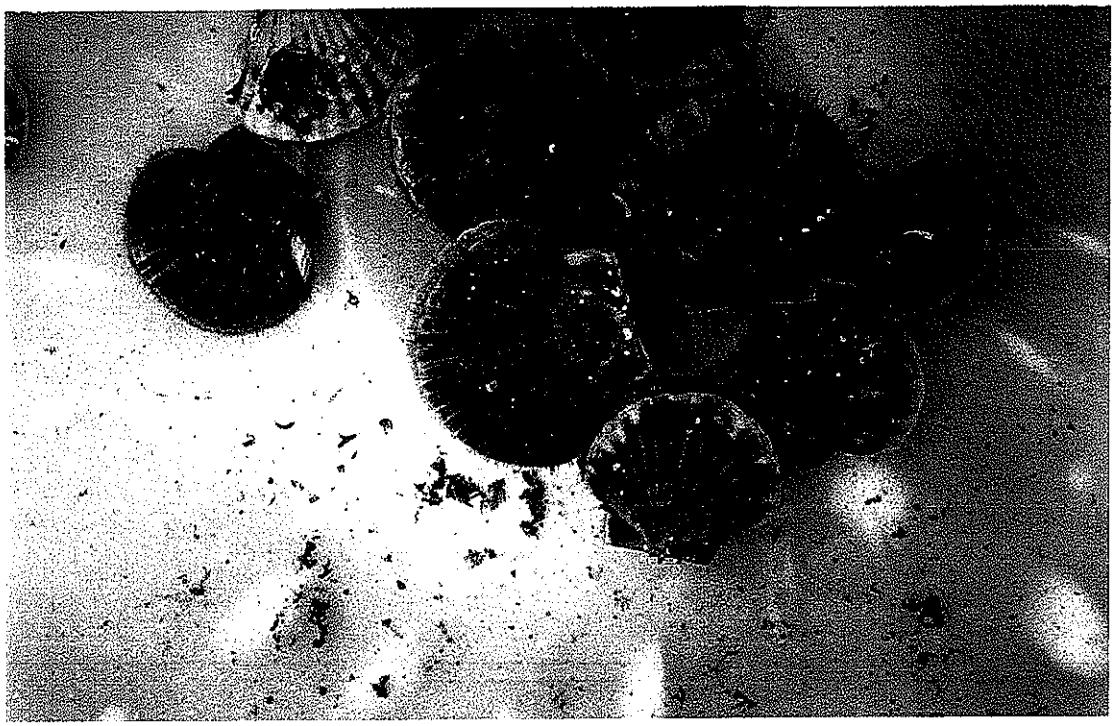


Plate 2. Scallops treated with **Carrier .**
Photographs were taken following eight weeks of field exposure.

Plate 3. Untreated **Control** scallops.
Photographs were taken following eight weeks of field exposure.

Other titles in this series:

No. 1 Andrew, N.L., Graham, K.J., Hodgson, K.E. and Gordon, G.N.G., 1998. Changes after 20 years in relative abundance and size composition of commercial fishes caught during fishery independent surveys on SEF trawl grounds.Final Report to Fisheries Research and Development Corporation. Project no. 96/139

No. 2 Virgona, J.L., Deguara, K.L., Sullings, D.J., Halliday, I. and Kelly, K., 1998. Assessment of the stocks of sea mullet in New South Wales and Queensland waters. Final Report to Fisheries Research and Development Corporation. Project no. 94/024

No. 3 Stewart, J., Ferrell, D.J. and Andrew N.L., 1998. Ageing yellowtail (*Trachurus novazellandiae*) and blue mackerel (*Scomber australasicus*) in New South Wales. Final Report to Fisheries Research and Development Corporation. Project no. 95/151

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No. 5 Kennelly, S.J. and Broadhurst, M.K., 1988. Development of by-catch reducing prawn trawls and fishing practices in NSW'S prawn-trawl fisheries (and incorporating an assessment of the effect of increasing mesh size in fish trawl gear). Final Report to Fisheries Research and Development Corporation.

No. 6 Allan G.L. and Rowland S.J., 1998. Fish meal replacement in aquaculture feeds for silver perch. Final Report to Fisheries Research and Development Corporation. Project no. 93/120-03

No. 7 Allan G.L., 1998. Fish meal replacement in aquaculture feeds: Subprogram administration. Final Report to Fisheries Research and Development Corporation. Project no. 93/120

No. 8 Heasman, M.P., O'Connor, W.A., O'Connor, S.J. and Walker, W.W., 1998. Enhancement and farming of scallops in NSW using hatchery produced seedstock. Final Report to Fisheries Research and Development Corporation. Project no. 94/084

No. 9 Nell, J.A., McMahon, G.A. and Hand, R.E., 1998. Tetraploidy in Sydney rock oysters. Final Report to Cooperative Research Centre for Aquaculture. Project no. D.4.2.