Evaluation of hatchery production of scallops *Pecten fumatus*

Final Report to FRDC

M. P. Heasman, W. A. O'Connor and A. W. Frazer

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to

Fisheries Research and Development Corporation

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Project Title: Evaluation of hatchery production of scallops (*Pecten fumatus).*
(FRDC 91/53)

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Fisheries Research and Development Corporation
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Dear Sir

I hereby submit the Final Report for the project "Evaluation of hatchery production of scallop *Pecten fumatus.*; FRDC No 91/53". All the objectives set out in the original grant application have been met and the diverse array of improved hatchery and nursery technology developed is already being applied in a follow-up project " Enhancement and farming of scallops in NSW using hatchery produced seedstock; FRDC No 94/084 which was commenced in October 1994.

I thank and commend FRDC committee members for their continuing interest and support for research and development of bivalve fisheries enhancement and mariculture in Australia.

Yours sincerely

M P Heasman

Dr Mike Heasman

2.0 SUMMARY

2.1 Non Technical (Lay) Summary

This project was prompted by poor or variable availability of wild commercial scallop spat for fisheries enhancement and farming in southern Australia and by generally poor and variable success of commercial oyster hatcheries in earlier attempts to fill this shortfall.

Scallops from Jervis Bay, routinely sampled for breeding condition over a three year period proved to be a poor and unreliable source of ripe ready-to-spawn scallops for immediate use in the hatchery. This prompted the development of reliable hatchery conditioning techniques that have enabled routine spawning of scallops throughout the year. Improved methods of spawning induction, fertilisation, incubation and larval rearing, have similarly been developed though systematic investigation and experimentation.

Generally low and variable hatchery success experienced with this species of scallop prior to and during the first year of this project was found to be caused by its high susceptibility to vibriosis, a common bacterial disease experienced by bivalve hatcheries throughout the world. .

Altered rearing equipment in conjunction with improved husbandry developed during this project have been identified as the best method of combating this disease. A trial using a 20 000 l hatchery tank instead of standard 1000 l experimental tanks, resulted in 90% (9 million) of larvae reaching the settlement stage in 14 days. These are the highest growth and survival rates reported for this species.

Satisfactory growth and survival rates have also been achieved using a standard 1000 l rearing vessel but with continuous rather than batch feeding and seawater exchange. The validity and reliability of these altered rearing systems are being rigorously evaluated in ongoing research.

A multi-tiered (stacked tray) upwelling nursery system has been developed as an interim cost effective method of producing large (up to 500 000) batches of 10 to 15mm juvenile scallops. These are being used in a follow-up scallop fisheries enhancement project initiated in October 1994.

2.2 Technical Summary

Variability in the reproductive condition of the commercial scallop *Pecten fumatus* in Jervis Bay hampered their immediate use as broodstock for hatchery production of seed and led to continued monitoring of wild stock reproductive condition. Macroscopic observations and gonad weight indices were used to monitor the reproductive condition. Both spatial and temporal variation in reproductive condition were detected with 3 to 4 annual peaks in reproductive activity occurring within the periods March-May and August-October. In comparison to southern stocks of *P. fumatus,* those in Jervis Bay have more frequent reproductive peaks, are less temporally consistent in their reproductive timing but offer a better source of scallops for hatchery conditioning and controlled spawning .

The same *P. fumatus* population, was also sampled between October 1991 and December 1994 to determine rates of infection by a trematode, (*Bucephalus* sp). that causes parasitic castration in this hermaphroditic . scallop . Over the first two years of sampling, an average of only 5.1% of adult *P. fumatus* were infected. A significant increase in the numbers of infected scallops occurred in spring 1993, reaching 63% by May 1994. The frequency of infection increased with scallop size, reaching 66% in *P. fumatus* greater than 80 mm in shell height in 1994. Seasonal and inter annual variations in parasitism for the *P. fumatus* recorded over a continuous period of five years in Jervis Bay are discussed as are the potential impact of the parasitic castration on reproductive potential and marketability.

Difficulty in obtaining ripe unparasitised broodstock prompted the development of hatchery conditioning protocols. Microalgal diets, feeding rates and holding temperatures conducive to rapid gonadal growth and development were identified. Results of microalgal clearance rate experiments indicated that the species *Pavlova lutheri,* Tahitian *Isochrysis* aff. *galbana, Chroomonas salina* and *Chaetoceros gracilis* are ingested by adult scallops at similar rates at 14, 18 and 21° C but more slowly at 11° C.

Maximum (satiation) ingestion rate using mixed diets containing approximately equal

numbers of cells of these four species, was estimated as about 6 x 10^9 cells per day for broodstock in the range 55 to 75 mm shell height. Large numbers of cells of the microalga *Tetraselmis suecica* passed through the digestive tract of scallops undigested. Egg production rate, gonad size and condition factors were all highest when broodstock were held at 15° C, lowest for scallops maintained at 21° C and of intermediate values at temperatures of 12 and 18° C. Gonad condition and egg production increased as feeding rates were raised from 12.5 to 100% of satiation at all test temperatures in the range 12 to 21°C.

Frequent spawning triggered by routine handling of broodstock during these experiments emphasised the need for conditioning methods and equipment that minimise handling and other disturbance factors. No unplanned spawning occurred at feeding rates of 25% or 12.5% of satiation at 12°C. The use low temperature and low feeding rate might therefore enable short term stockpiling of broodstock once they attain prime breeding condition. Findings of these experiments were successfully applied to a subsequent large scale conditioning and induced spawning trial.

Variable quality and yield (percentage development from eggs) of *P. fumatus* D-veligers prompted an assessment of traditional fertilisation and incubation protocols developed originally for hatchery rearing of the Sydney rock oyster. Various sperm to egg ratios were tested on eggs suspended in seawater at different densities. Ratios of 1000:1 led to the highest D-veliger yield when eggs were incubated in suspension at 1/ml. With increasing egg densities, the addition of 1000 sperm/egg led to increasing average numbers of sperm visible at the periphery of each egg, indicating fewer sperm were necessary for fertilisation at higher egg densities.

The time period and temperature over which released gametes were stored before fertilisation were also found to significantly affect D-veliger yield. Decreasing gamete storage temperature 26 to 14°C increased D-veliger yield, as did a reduction in gamete storage period from 6 to 1 h. The incubation of embryos at densities in the range 5 to 50/ml did not affect D-veliger yield. A significant increase in total bacterial counts in the culture water occurred with increasing embryo stocking densities. Presumptive *Vibrionic* counts did

however increase with significantly with increasing embryo stocking densities.

In a comparison of the viability of self and cross-fertilised embryos and larvae, fewer self-fertilised embryos developed to D-veliger stage, although survival did not differ significantly in subsequent larval rearing. Cross-fertilised larvae had attained a significantly larger size by day seven after fertilisation.

Growth and survival of scallop larvae held at two temperatures, 16 and 22°C, and fed one of eight algal species was compared with that of unfed larvae or those fed a reference diet originally developed for rearing Sydney rock oyster larvae. The latter comprised equal amounts of three algal species, *Pavlova lutheri*, Tahitian *Isochrysis* and *Chaetoceros calcitrans*. Growth on all single species diets, including *P. lutheri,* was considerably lower than a rate of 7.3μm/day achieved on the mixed reference diet. Age specific survival was unaffected by temperature. However, because growth rates were generally much higher at 22° C than at 16° C, size specific survival was much higher at 22°C, which is accordingly recommended for routine hatchery operations.

*P. luther*i, was subsequently fed in combination with each of the other eight species and growth and survival at 22°C determined. When fed in combination with *P. lutheri*, the only algal species to produce synergistic increases in growth were T. *Isochrysis* and *C. calcitrans.* With both single and combination diets, a significant negative correlation was found between growth and mortality.

Eight dietary regimes all involving *P. lutheri*, *T. Isochrysis* and *C. calcitrans* but fed in differing combinations and sequences were also tested over a twelve day period. Larvae fed *P. lutheri* for three days before being fed the reference diet for nine days were significantly larger than siblings initially fed T. *Isochrysis* or *C. calcitrans.* Increasing the frequency with which all three species were fed produced greater larval shell length increases and is accordingly recommended for larval rearing of *P. fumatus.*

Embryos, D veliger larvae and early juvenile stages of *P. fumatus*, were held at temperatures ranging from 13 to 27°C. An incubation temperature of 18°C produced the

greatest yield of D veligers. Growth rate of larvae increased with increasing temperature from 2.5μm day^{-1} at 15°C to a peak of 6.5μm day^{-1} at 24°C but decreased with a further increase in temperature to 27°C.

Age specific larval survival rates decreased significantly with increasing temperature in the range 15 to 27°C. However, size specific survival rate, a more meaningful measure of optimum rearing temperature, exhibited a pronounced peak value at an intermediate temperature of 21°C. On the basis of these results, the maintenance of larval rearing temperatures between 18 and 21°C is likely to provide the maximum survival of larvae to the pediveliger stage.

The mean size of early stage spat (3 week post- settlement; mean shell height 1.04± 0.26mm) on-reared in the hatchery, increased exponentially for 4 weeks at all temperatures tested. Growth increased from a negligible rate at 13°C to a maximum rate at 24° C. During the fifth and final week of the trial, a constraint to continued exponential growth became evident at all temperatures tested except 13°C. This result was consistent with previous observations of constrained growth of spat when maintained beyond a period of 6 to 8 weeks post-settlement (mean shell height of 2 to 4 mm.) on a standardised microalgal diet specifically developed for larvae.

Survival and byssus attachment of spat was highest at temperatures supporting the highest growth rates. Possible ecological implications of these results are discussed in relation to the oceanography of south eastern Australia and variability in annual recruitment and catches. The possible use of byssus attachment as an indicator of favourable spat growing conditions is discussed.

Generally low and variable hatchery success with *P. fumatus* was found to be caused by vibriosis, a form of bacterial necrosis. *Vibrio alginolyticus* was consistently identified in diseased larvae while two additional species, *V. harveyi* and *V.parahaemolyticus,* also co-occured with *V. alginolyticus* on occasions.

Chloramphenicol at mg/l totally suppressed vibriosis but also impaired the growth of

larvae. Moreover, cessation of its use at the pediveliger stage resulted in very poor survival through metamorphosis. Oxolinic acid, was found to enhance survival of incubating eggs and of larvae without suppressing growth when used at 1mg/l. Any use of antibiotics in bivalve hatcheries is nevertheless strongly discouraged.

Attempts to identify probiotic microalgae that inhibited vibriosis were unsuccessful. Attempts to evaluate two bacterial strains already shown to enhance growth and survival of *Vibrio* susceptible crab larvae and oyster larvae will be pursued, if and when cultures become available. Very encouraging initial results in circumventing vibriosis in *P. fumatus* were attained with altered rearing protocols when used in conjunction with improved broodstock conditioning, spawning, fertilisation, incubation and larval rearing husbandry.

Use of a 20 000 l hatchery tank rather than 1000 l "standard experimental" tanks whilst retaining standard alternate day batch feeding and seawater exchange, resulted in a 90% survival from D veliger to pediveliger stage in 14 day. These are the highest survival and growth rates yet reported for this species. The use of a 1000 l experimental tank operated with continuous administration of food and seawater exchange also produced rapid growth and an acceptable survival rate of 21%.

These initial results nevertheless await validation in the form of a series of fully replicated trials in which altered hatchery protocols are compared with standard controls (1000 l tanks run with batch feeding and seawater exchange) as used in the past.

Findings of this study are consistent with overseas experience that larval vibriosis is a "management disease" that is best combated with altered hatchery procedures tailored to the particular needs of each species.

A multi tiered (stacking) upweller nursery system, was developed as an interim method of producing large numbers of 10 to 15mm scallop spat. A miniature experimental version of this system was built to determine combinations stocking density and seawater flow rates that would ensure high operating efficiency without seriously compromising growth rate. In the absence of screen rotation, mean growth rates were greatest for spat held on first of

eight screens making up each stack and exhibited a progressive decline in successive screens at all densities tested..

The mean biomass gain for spat on screens within stacks that were rotated daily was significantly greater (F=9.434, df=5, P<0.05) than `that exhibited by spat held on screens retained in fixed positions within stacks. Rotation of screens was shown to overcome some of the adverse effects of screen position within stacks and was able to significantly increase nett biomass gains per unit of upweller space and per unit of seawater flow. However the reason for this was not clear.

Surface area density per sé is a critical factor determining growth rate of *P. fumatus* spat on field upweller screens. 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 100% screen coverage. However, only marginal reduction in growth occurs at stocking rates of up to 200% screen coverage and only minor reduction in growth at up to 300% screen coverage.

Although maximum growth rates recorded for *P. fumatus* spat coincided with flow to biomass ratios of 20 to 30 : 1 (litres/kg/min), such ratios are inherently inefficient. Reuse of seawater via tiered upwellers enables a doubling or trebling of yields per unit of flow. Regular rotation of screens must however 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.

3.0 BACKGROUND AND JUSTIFICATION (NEED)

A " boom or bust" pattern is typical of scallop fisheries the world over including fisheries for *P. fumatus* in throughout southern Australia (Gwyther, 1989; Young and Martin, 1990). For example the fishery in Jervis Bay NSW produces good yields of 1,400 to 2,800 tonnes every 10 years or so (Hamer,1987) which have a current value of $2 to $4 million.

If consistently high scallop fishery yields are to be established, the problem of low and variable recruitment of juvenile scallops must be addressed. Japan has been a world leader in enhancing the yields of wild scallop fisheries with current production more than 200,000 tonnes per annum (Bartrom, 1987). Suitable areas are reseeded with wild caught spat which are subjected to intermediate nursery rearing to a mean size of about 40mm in pearl and lantern cages.

China, with 1986 production levels attaining 230,000 tonnes relies heavily on hatchery produced spat and in contrast to Japan, grow-out to market size takes place in hanging culture rather than by reseeding (Cropp,1989).

The availability of wild *P. fumatus* spat and recruitment to fisheries stocks are often low and variable from year to year especially in NSW (Fuentes et al., 1994) but also in the major producing areas of Port Phillip Bay, Victoria (Sause et al., 1987), the Bass Strait and the East Coast of Tasmania. Development of cheap reliable hatchery and nursery rearing techniques is therefore needed if natural fisheries are to be enhanced to provide consistently high yields or if new aquaculture industries based on this species are to be developed in NSW.

Larval rearing of *P. fumatus* was first achieved in Tasmania by Dix and Sjardin (1975). The Tasmanian government offered contracts for the supply of 4.2 million juvenile *P. fumatus* in the range 10 to 20 mm in 1987 and 1988. In spite of considerable effort local oyster hatcheries were able to supply only 100,000 and 280,000 in 1987 and 1988 respectively (Cropp and Frankish, 1989)

Thus far, the largest artificial spawning of *P. fumatus* had produced 125 million eggs but no

hatchery had yet produced more than 500 000 settled spat from one batch of larvae (Cropp and Frankish, 1989). By contrast, a preliminary hatchery trial conducted in May 1989 (Frankish et al, 1990) at the NSW, Port Stephens Research Centre (PSRC), yielded more than 10 million settled spat. Of these, several hundred thousand were on reared to 10 to 20mm with very little mortality.

These contrasting results may have arisen due to one or more of the following factors:
1) high variability in the quality of eggs or larvae sourced from wild broodstock

2) subtle albeit critically important differences between equipment and techniques used by different hatcheries involved, and

3) disease(s)

4.0 OBJECTIVES OF THE RESEARCH PROJECT

a) To develop reliable year round access to ripe ready-to-spawn broodstock as a source of stock for hatchery and nursery rearing of seed scallops.

b) To identify optimum temperature, salinity, stocking density and feeding regimes for the survival of hatchery reared scallop larvae.

c) To identify stocking densities and seawater flow rates of juvenile settled scallops in nursery systems that promote high growth and survival up to a size (10 to 15mm) suitable for on -growing in pearl nets and lantern cages and thence to a size (50 to 60mm) suitable for farming or fisheries enhancement.

5.0 RESEARCH METHODOLOGY, RESULTS AND DISCUSSION

5.1 ACQUISITION OF RIPE BROODSTOCK

5.11 Evaluation of Jervis Bay *P. fumatus* and as a source of hatchery broodstock

Abstract

Variability in the reproductive condition of *Pecten fumatus* in Jervis Bay hampered the collection of broodstock for hatchery production of seed and led to continued monitoring of wild stock reproductive condition. Macroscopic observations and gonad weight indices were used to monitor the reproductive condition of collections of broodstock from Jervis Bay. Both spatial and temporal variation in reproductive condition were evident in scallop populations within Jervis Bay with peaks in reproductive activity occurring within the periods March-May and August-October. In comparison to southern stocks of *P. fumatus,* those in Jervis Bay have more frequent reproductive peaks and are less temporally consistent in their reproductive timing but offer a better source of scallops for hatchery conditioning and controlled spawning .

Introduction

Pecten fumatus Reeve is a subtidal bivalve found in areas (Fig 5.11.1a) from the southeastern border of Western Australia to central NSW (Young and Martin, 1989). Throughout this range, the level of genetic differentiation detected among populations of *P. fumatus* is low (Woodburn, 1989), however, considerable variability has been reported in the timing of spawning activity at various locations. Spawning in southern Tasmania occurs from August to October (Harrison, 1961) and in Port Phillip Bay, Victoria from June to November (Sause et al., 1987). Further north in Jervis Bay, Jacobs (1983) observed three spawning peaks (late winter, early spring and early summer) and Fuentes (1994) subsequently reported four peaks in the reproductive cycle in both 1989/90 and 1990/91.

Very early in this project high variability in reproductive condition was recognised in wild *P. fumatus* being collected from Jervis Bay as was an apparent chronic scarcity of ripe ready to spawn broodstock This in turn prompted research to identify practical and reliable methods of ripening the gonads of recently captured wild broodstock. However it was also recognised that gonad conditioning protocols are often contingent upon the broodstock being in fair reproductive condition to begin with. For instance attempts to condition spent gonads in the closely related European scallop *P. maxims* have met with mixed results (Cochard and Devauchelle, 1993). An understanding of the reproductive behaviour of *P. fumatus* from Jervis Bay was therefore considered essential to securing a reliable and predictable supply of broodstock.

Routine collections of wild *P. fumatus* were initiated in October 1991 as a source of ready to spawn broodstock for hatchery rearing trials. These collections created an opportunity to continue monitoring the reproductive condition of stocks in Jervis Bay initiated two years earlier (1991) by NSW Fisheries staff as part of a broader Jervis Bay Baseline Study commissioned by the Australian Department of Defence (Fuentes, 1994).

Both macroscopic observations of condition and gonad weight indices were used to monitor changes in *P. fumatus* reproductive condition.

Materials and Methods

Collection Sites

Historically, *P. fumatus* have occurred in most areas in Jervis Bay (35°04' S, 150°44' E), however, only two beds , Murrays Beach and Honeymoon Bay (Fig.5.11.1b), have consistently carried scallops (Fuentes et al., 1992). In September 1991, three sites, two at Murrays Beach and one at Honeymoon Bay, were sampled to choose a site for the routine collection of hatchery broodstock. Scallops were collected from two depths at Murrays Beach, MB1: 15-17m and MB2 8-9m. Both sites have a sandy bottom and are well flushed, although the shallow site had large areas seagrass (*Zostera capricornia*) interspersed with

sand patches from which the scallops were collected. Scallops from Honeymoon Bay (HB) were collected from a sand and silt bottom at a depth of 14-15m.

Collection Methods

From May 1991until October 1993, a minimum of 100 *P. fumatus* were collected fortnightly from Jervis Bay, weather permitting. These were packed over ice, transported to the NSW Fisheries, Port Stephens Research Centre. From October 1991 a minimum representative subsample of 30 scallops selected at random was set aside for assessment of general and reproductive condition within 24 h of arrival. The remainder were retained in aerated seawater before being used to conduct spawning or gonad conditioning trials.

Data collection and analysis

Those scallops used to evaluate general and reproductive condition were subjected to the following procedures. Shell heights (central dorsal to central ventral margin) were recorded, the proportion of the gonad containing ovarian tissue visually estimated and each scallop assigned a stage on an arbitrary scale of gonad maturity (Heasman et al., in prep.a). A numerical ranking (Table 5.11.1) was assigned to each macroscopic stage, ranging from 1 for spent scallops to 8 for ripe, (ready to spawn) scallops. Scallops were then dissected and soft body, adductor muscle and gonad weight for each scallop were determined to the nearest 0.01 g.

GSI was calculated using the method of Latrouite and Claude (1979) , where:

GSI = (Gonad weight / (Soft body weight - Gonad weight))*100

As the average shell height varied with collection, direct comparisons of soft tissue weights between collections were not made. Nutrient reserves used in gonad development of bivalves

are stored in other soft tissues especially the adductor muscle (Barber and Blake, 1991). To enable a meaningful comparison of the general physiological condition of scallops, estimates of the means of total soft tissue weight, adductor muscle and combined adductor muscle and gonad weight corresponding to a standardised shell height, were calculated for each collection using least-squares linear regression analyses (Daniel and Wood 1980; Dredge 1981).

The same procedure was applied to gonad weight as an alternative to GSI in evaluating breeding condition. Rationale for the latter is that GSI is potentially vulnerable to a bias imposed by the general physiological condition of animals. For example, if two scallop populations have the same size structure and mean gonad weight but differ significantly in mean soft tissue weight, the population with the lower soft tissue weight (poorer general condition) will exhibit a significantly higher GSI. In other words, poorer general physiological condition imposes a positive bias on GSI (thereby over estimating breeding condition) and vice versa.

A standardised shell height of 67 mm was chosen as it approximated both the overall mean and median shell height of scallops collected during the study. For adductor muscle the equation, $y = a + bx$ (where: y is adductor muscle weight (g) and x is shell height (mm)) was fitted to data of each fortnightly sample and a predicted gonad weight for a "standard" 67mm scallop calculated. The same procedure was repeated for adductor muscle and total soft tissue weights.

Results

Characteristics of Scallops at three initially sampled sites

Of the three sites initially sampled (Fig 5.11.1b), MB1 contained the greatest densities of scallops. Time to collect 111 scallops at this site was less than a quarter that required to collect smaller numbers (Table 5.11.2) at the other two sites. Scallops collected from MB1 and MB2 did not differ significantly ($P>0.05$) in size or reproductive condition . Scallops

collected from HB were however significantly ($P<0.05$) larger (67±8mm) than those from Murrays Beach (55± 6mm), but in poorer reproductive condition and had a significantly higher ($\chi^2 = 258.3$; $P<0.001$) frequency of parasitised gonads (25%) than scallops from the two Murrays Beach sites (2.3 and 1.8% respectively).

The parasite, a bucephalid trematode, invades the gonad causing functional sterility (parasitic castration). Accordingly, parasitised scallops, which have since become the subject of a separate investigation (section 5.12 and Heasman et al., in prep. a) 1995/Appendix 3) are of no use as broodstock. Because of these results and the protection afforded divers in most weather conditions, MB1 was chosen for all subsequent collections.

"Least squares" linear regression analyses (Table 5.11.3) were conducted and best fit regression lines (Sokal and Rohlf, 1981) fitted to pooled data of non parasitised scallops collected from the three sites in September 1991. These scallops fell within the collective size range 50 to 85mm. The analyses were used to define relationships between shell height (independent variable), soft tissue weight, gonad weight and GSI (dependent variables). GSI was independent of shell height (slope of regression not significantly different from zero). To further reduce the possibility size dependant variation in GSI (Gonor, 1972; Grant and Tyler, 1983; West, 1990) and to ensure all collected scallops were sexually mature, the minimum scallop size for all subsequent collections was set at 50mm.

Routine collections

During the two years, October 1991 to October 1993, 1528 scallops were examined of which approximately 7% were parasitised (also see -Section 5.12). A comparison of shell height data for each scallop collection indicated that variances were highly heterogeneous (Cochrans Test, $C=0.06822$; $P>0.001$). Mean shell height of this bed of scallops, which has since been shown to comprise single year class (Worthington, 1993), increased significantly (Kruskall Wallace test, $X^2 = 845.9$; $df = 50$: $P<0.001$) and progressively from about 57mm in October 1991 to about 72mm two years later in October 1993 (Fig 5.11.2).

Bottom temperatures (Fig 5.11.3) followed a distinct seasonal trend, highest temperatures occuring in late Summer (February) and lowest in mid to late Winter (July and August).

Macroscopic observations

The changes in mean ranking for macroscopic stages for each collection are provided in Fig 5.11. 4.. These were significantly correlated with the changes in GSI ($r = 0.61$; $p<0.001$; Table 5.11.2). Most scallops had developing gonads with " Developing 2" and "Developing 3" stage gonads comprising 25.9 and 26.0% respectively of all non parasitised scallops examined. Only 2.0% of the scallops (Fig 5.11.5) were spent. This was significantly less ($\chi^2 = 44.16$, $P<0.001$) even than the small proportion (4.5%) of scallops classed as ripe. The relative incidence of spawning scallops although also low (9.8%), was significantly higher ($\chi^2 = 80.53$, $P<0.001$) than that of ripe scallops.

No clear recurrent seasonal trends could be discerned from gonad stage data presented in Fig 5.11.6. Most gonad stages were represented in all months including the Summer months of December, January and March when mean GSI values were chronically low.

Gonado-somatic index (GSI)

A comparison of GSI data for each collection found variances to be highly heterogeneous (Cochrans Test, $C=0.0640$; $P<0.001$) precluding the use of ancova to determine the proportion of variance due to changes in shell height (Grant and Tyler, 1983). In lieu, GSI was regressed with shell height for each collection. As only one collection (13/8/92) was found to have a significant relationship between GSI and shell height, ($F= 6.01$: df 29; $P=0.02$), we considered GSI to be independent of shell height during this study. Significant variation in GSI was evident between collections (Kruskall Wallace test, $X^2 = 601.8$; $df = 50$: $P<0.001$). In two years, 7 or 8 apparent peaks in GSI could be discerned (Fig.5.11.7), all falling between March (early Autumn) and October (mid to late Spring). The summer months

of December to February were characterised by consistently lower mean GSI values indicative of minimum breeding activity.

Within the generalised 8 to 9 month season encompassing peaks in mean GSI, the exact timing and amplitude of individual peaks was inconsistent from year to year. The minimum period between successive peaks in mean GSI was in the order of 6 to 8 weeks.

Discussion

All apparent peaks in breeding activity (as indicated by GSI values) of *P. fumatus* in Jervis Bay were confined to Autumn, Winter and Spring in the present 2 year study. These observations were consistent with those of Fuentes(1994) over the preceding two years (August 1989 to July 1991) and with those of Jacobs (1983) except for an early Summer peak in GSI reported by the latter. Considerable year to year variation in the exact timing and degree of spawning synchronisation (as indicated by the variations in timing and amplitude of GSI peaks) was evident in all three studies. The number (3 or 4) of annual peaks in mean GSI recorded over each of these successive studies was however consistent.

Low GSI values recorded through all or most of Summer in Jervis Bay coincide with rising sea temperatures in the approximate range 18 to 24°C .(May et al. 1978, CSIRO, 1994). This observation is consistent with the experimentally determined upper temperature limit of 18°C for gonad development in *P. fumatus* from Jervis Bay (also see Section 5.13).

Reported spawning seasons for other populations of *P. fumatus* vary considerably with geographical location. Winter/Spring spawning was reported for *P. fumatus* in Southern Tasmania (Harrison, 1963) and Port Phillip Bay (Sause et al., 1987). Young et al. 1991, found that major spawning activity occurred in Spring and Summer at both King Island and Banks Strait, but at King Island there was also an Autumn spawning between March and May.

Gametogenic cycles are controlled by a combination of exogenous and endogenous factors including genetically determined neural and hormonal responses to environmental cues such as temperature, food and light (Barber and Blake, 1991). Whether the geographical differences observed in *P. fumatus* reproductive behaviour are due to genetic variation or altered environmental cues is difficult to assess. Woodburn (1989) found that all populations of *P. fumatus* in south eastern Australia exhibit a low degree of genetic differentiation, well within the range of values consistent for conspecific populations of a variety of organisms including the Pectinids *Pecten maximus* (Huelvan, 1985) and *Patinopecten yessoensis* (Yamanaka and Fujio 1983, cited by Kijima et al. 1984). Genetic distances between subpopulations of *P. fumatus* were nevertheless shown to be directly and consistently proportional to the geographical separation of the various stocks.

Major variations in the gametogenic cycle of geographically distinct populations have been described for two northern hemisphere scallop species by Barber and Blake, (1991). In *Argopecten irradians*, gametogenic timing and duration, oocyte diameter and fecundity all varied with latitude, while in *Placopecten magellanicus*, both the timing and duration of spawning activity varied latitudinally.The effectiveness of attempts to detect intraspecific genetic variation in other pectinids has varied greatly. While populations of the Queen scallop *Aequipecten (Chlamys) opercularis* have been found to show considerable genetic differentiation (Beaumont, 1982), attempts to detect genetic differences in *P. maximus* have failed (Beaumont et al., 1993). This is despite considerable evidence from reproductive studies to the contrary (Cochard and Devauchelle, 1993; Mackie and Ansell, 1983).

However, the failure to detect differences at a limited number of loci does not preclude population differences (Beaumont et al., 1993). The apparent contradiction between limited genetic differentiation in populations of *P. fumatus* (Woodburn, 1989) and observations of varying reproductive behaviour may be resolved with the assessment of a greater number of genetic loci or through reciprocal stock transplantation as attempted with *P. maximus* (Beaumont et al., 1993; Cochard and Devauchelle, 1993; Mackie and Ansell, 1993).

The importance of non genetic factors to differences in the spawning seasons of geographically distinct populations of *P. fumatus* may nevertheless override that of genetic

factors. Diet and temperature have been found to profoundly influence the development (size and condition) of gonads and gamete output (frequency of spawning and fecundity) in *P. fumatus* (see Section 5.13). Gonad development and gamete release is confined to a relatively narrow range of temperature (15± 3°C) and feeding rate (50-100% of satiation) in Jervis Bay *P. fumatus* .

While settlement of *P. fumatus* spat may occur over an extended period consistent with the duration of spawning, there is evidence to suggest that major settlement events result from gametes shed over a more limited period. In 1989/90 at Murrays Beach, Jervis Bay, four peaks were observed in reproductive condition and yet only one peak in settlement occurred in November (Fuentes, 1994). In the present study, 3 or 4 annual peaks in reproductive activity were also detected and yet no evidence of significant recruitment of advanced (16 to 20mm) juveniles was observed in Jervis Bay in 1992, 1993 or 1994 (Worthington, 1993 and pers. comm. January 1995).

Sause et al., (1987) found that despite the protracted nature of the spawning period of *P. fumatus* in Port Phillip Bay, spat collector studies indicated that spat settlement occurs over a very short period (December) and may result from major spawning later in the spawning season, rather than from earlier partial spawnings. Similar observations were made by Young et al., (1992 , unpublished manuscript) in relation to spawning and settlement of *P. fumatus* at King Island in western Bass Strait (Fig5.11.1a). A clear inference is that although reproductive indices may accurately reflect spawning activity, they are not necessarily reliable indicators of spat settlement let alone of subsequent recruitment of advanced juveniles to scallop beds.

In addition to the timing of spawning, the relative frequencies of reproductive stages in *P. fumatus* have major implications for hatchery production of this species. Relatively few scallops were found to be in either peak reproductive condition (ripe, 4.5%) or poor condition (spent, 2.0%) throughout the study. While the low number of spent scallops indicates development within the species is rapid, at least initially, it does not result in the accumulation of ripe scallops. Rather, it would appear that partial or dribble spawning has a

major influence on the relative frequency of reproductive stages of *P. fumatus* in Jervis Bay. Clearly, scallops do not remain "Ripe" for protracted periods, however, the stepped decline in reproductive condition is again suggestive of partial spawnings.

Induced and inadvertent spawning of *P. fumatus* in the hatchery observed during this study have shown that scallops macroscopically staged as low as "Developing 3" are capable of releasing viable gametes which develop beyond D-veliger. Therefore the high observed frequency of scallops in the intermediate stages of development may be a product of rapid development, frequent spawning and the ability to spawn before reaching the stage designated as "ripe." The relatively high frequency of "spawning" scallops in comparison to ripe or spent also suggests partial spawning. However, as noted by Fuentes (1994), macroscopic differentiation of spawning from atresia or even from early developing stages is not always reliable and hence a proportion of the scallops are likely to have been incorrectly staged.

The most important and encouraging aspect of this study in relation to the hatchery potential of Jervis Bay *P. fumatus,* is the demonstrated year- round ready availability of scallops with gonads in stages "Developing 2", "Developing 3 "or "Developing 4". Such stages collectively account for almost 70% of all scallops sampled (Fig. 5.11.5) and comprised a minimum of 42% and a maximum of 93% of scallops across all 27 samplings(Fig. 5.11.6) completed over the 2 year duration of the study. Scallops within this range of gonadal development can be brought into a ripe, ready to spawn condition in th hatchery within two to four weeks of collection when maintained under optimal temperature and feedig regimes (see Section 5.13)

Table 5.11.1. Macroscopic staging system for gonads in the commercial scallop, *Pecten fumatus*.

	Numerical Ranking
Spent Sperm or eggs absent or largely so. Gonad flaccid and translucent with ovarian and testicular tissue difficult to differentiate. Intestinal loop clearly visible.	1
Spawning 2 Gonad has lost turgor and ovarian tissue is mottled in appearance, presumably due to large numbers of voided acini. Intestinal loop is usually visible.	2
Spawning 1 Ovarian tissue of gonad uniform in colour interspersed with translucent specks of voided acini. Gonad turgor reduced.	3
Developing 1 Separate acini are clearly apparent; male and female portions of the gonad are distinguishable. Intestinal loop clearly visible.	4
Developing 2 Gonad less granular in appearance as acini begin to fill. Gonad increasing in turgor. Intestinal loop becoming obscured.	5
Developing 3 Very little of the intestinal loop visible (usually only a small portion of the ascending limb at the distal extremity of the gonad). Ovarian tissue appears uniform in colour and texture as acini fill.	6
Developing 4 Ovarian tissue bright, uniform in colour and turgid. Separate acini not apparent with little if any intestinal loop visible.	7
Ripe Ovarian tissue uniform in colour, bright and glossy and highly turgid. Acini not apparent and intestinal loop not visible.	8

Table 5.11.2 A comparison of *P. fumatus* collected from three areas within Jervis Bay in September 1991, including simple linear regressions describing the relationship between shell height and soft body weight, gonad weight or gonado-somatic index (GSI).

Collection Site	Depth	n	Shell height	GSI	Parasitised(%)	Regression*	Intercept	Slope	r^2	p
Murrays Beach (M2)	8-9	85	55.0[a]	24.2[a]	2(2.3)	Soft body	-17.47	0.529	0.81	<0.001
						Gonad	- 2.83	0.092	0.50	<0.001
						GSI	28.34	-0.074	0.01	0.460
Murrays Beach (M1)	15-17	111	53.4[a]	22.8[a]	2(1.8)	Soft body	-33.49	0.867	0.87	<0.001
						Gonad	- 4.62	0.130	0.74	<0.001
						GSI	28.21	-0.101	0.02	0.148
Honeymoon Bay (H)	14-15	67	66.9[b]	13.3[b]	17(25.4)	Soft body	-48.70	1.075	0.82	<0.001
						Gonad	- 4.55	0.107	0.59	<0.001
						GSI	19.13	-0.087	0.03	0.177

* Regression indicates the parameter regressed with shell height.

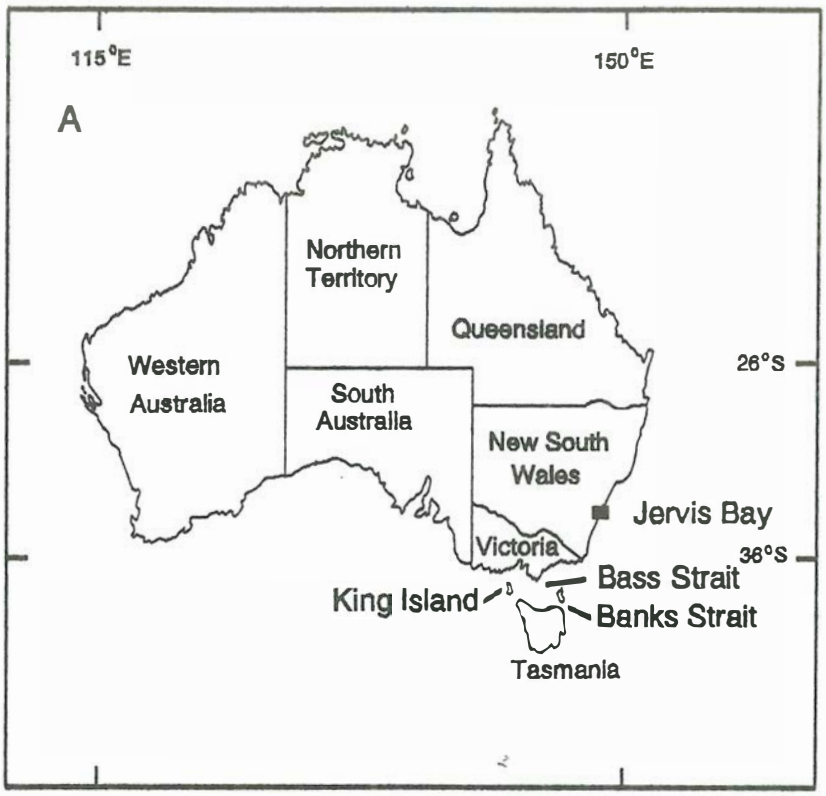
** Values with superscripts have been compared by Anova, those within columns with common superscripts do not differ significantly (P=0.05).

Table 5.11.3 Correlation matrices for the tissue weights for a 67 mm scallop predicted on the basis of regression analysis, the GSI calculated from regression estimates of gonad and soft body weight, the mean GSI for each collection and the mean macroscopic condition rating for each collection. Values are the correlation coefficient with the associated probability listed beneath in parentheses, n = 50.

	Regressed muscle weight	Regressed visceral weight	Regressed GSI	Mean GSI	Mean macroscopic ranking
Regressed gonad weight	0.36 (<0.001)	0.64 (0.008)	0.91 (<0.001)	0.93 (<0.001)	0.63 (<0.001)
Regressed muscle weight		0.59 (<0.001)	0.22 (0.109)	0.17 (0.221)	0.24 (0.088)
Regressed soft body weight			0.36 (0.009)	0.31 (0.024)	0.37 (0.008)
Regressed GSI				0.96 (<0.001)	0.61 (<0.001)
Mean GSI					0.63 (<0.001)

Fig 5.11.1 a. Location map, including distribution of *P. fumatus* in south eastern Australian states and the location of Jervis Bay and other places refered to in the text.

b. Scallops beds and collection sites, Jervis Bay, NSW



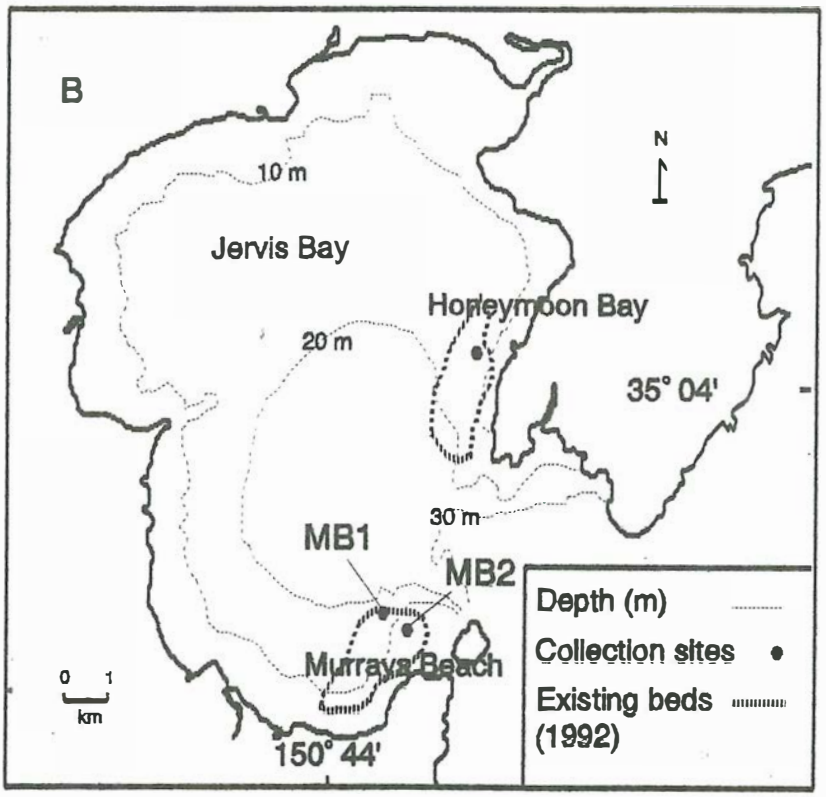


Fig 5.11.2 Box and whisker plot of shell height data of ***P. fumatus*** collected between October 1991 and October 1993.

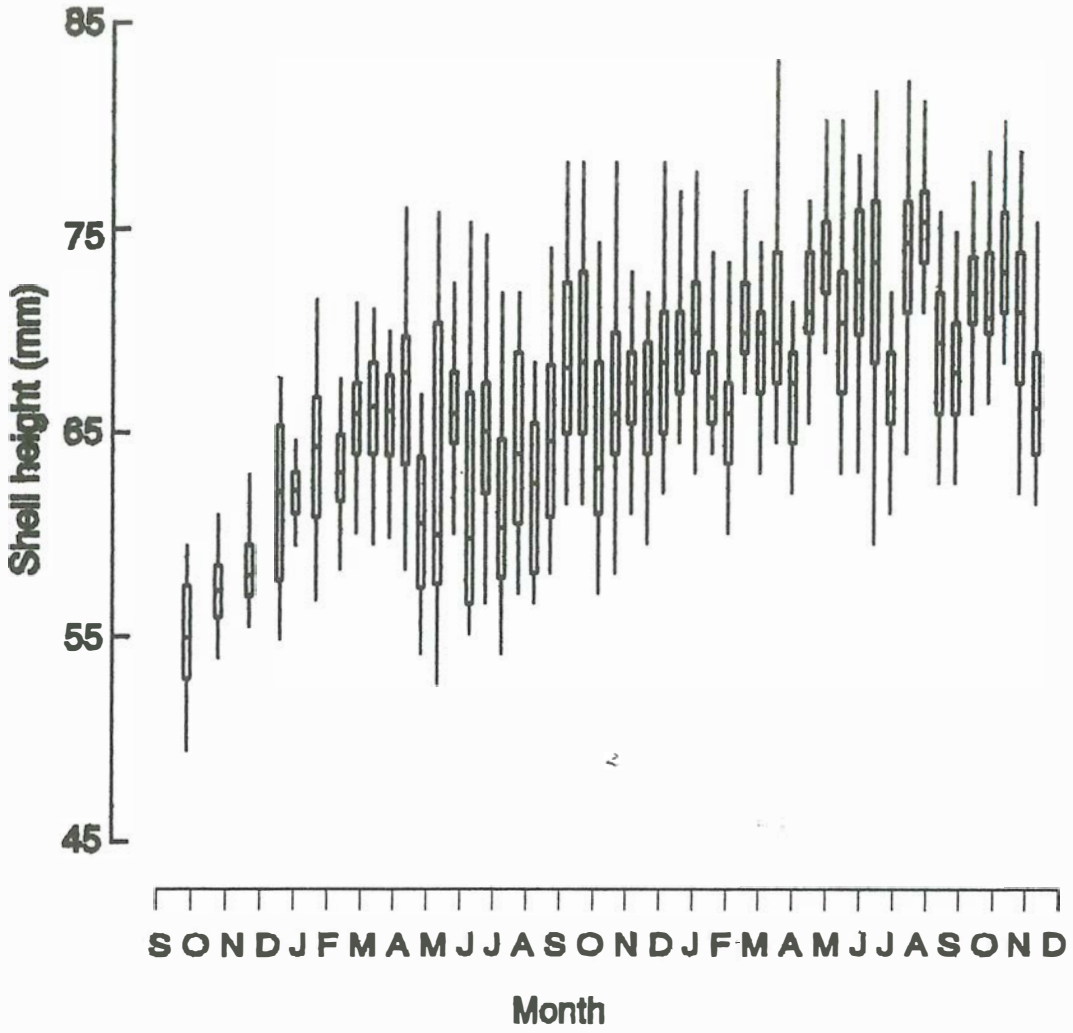


Fig 5.11.3 Seawater temperature for Jervis Bay from October 1991 to October 1993. Circles represent bottom temperature recorded at the time of collection.

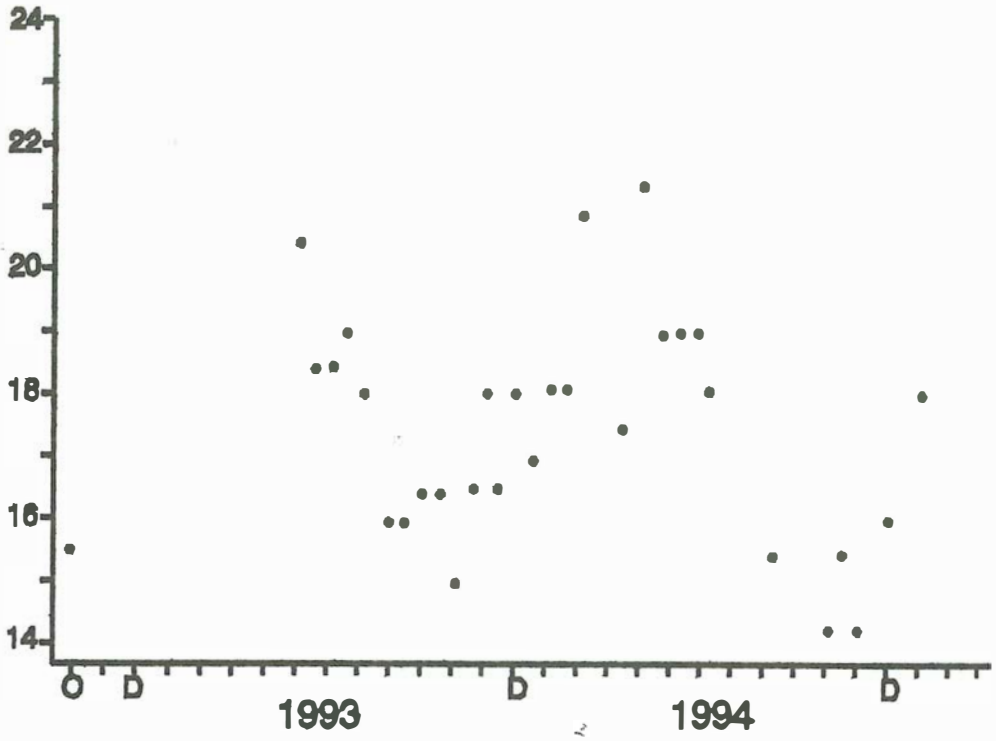


Fig 5.11.4 Mean ranking for macroscopic staging of the gonads of Jervis Bay *P. fumatus* from October 1991 to October 1993

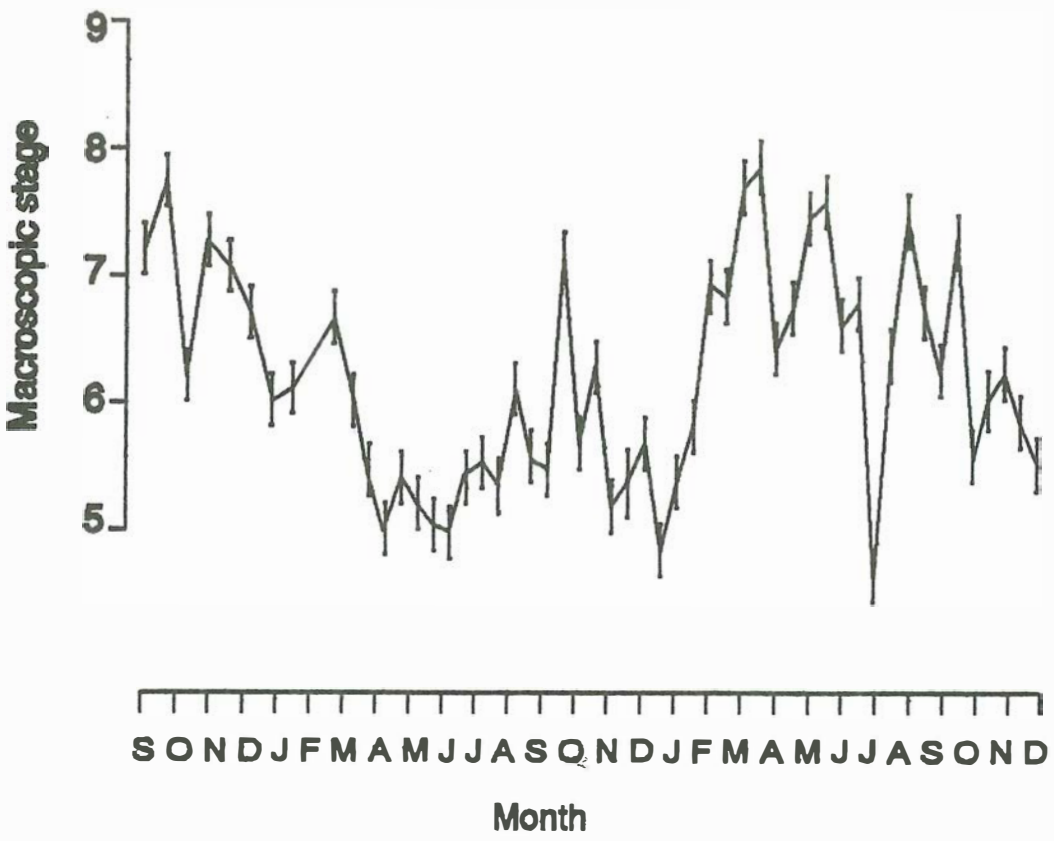


Fig 5.11.5 Overall frequency of macroscopic gonad stages in Jervis Bay *P. fumatus* from October 1991 to October 1993

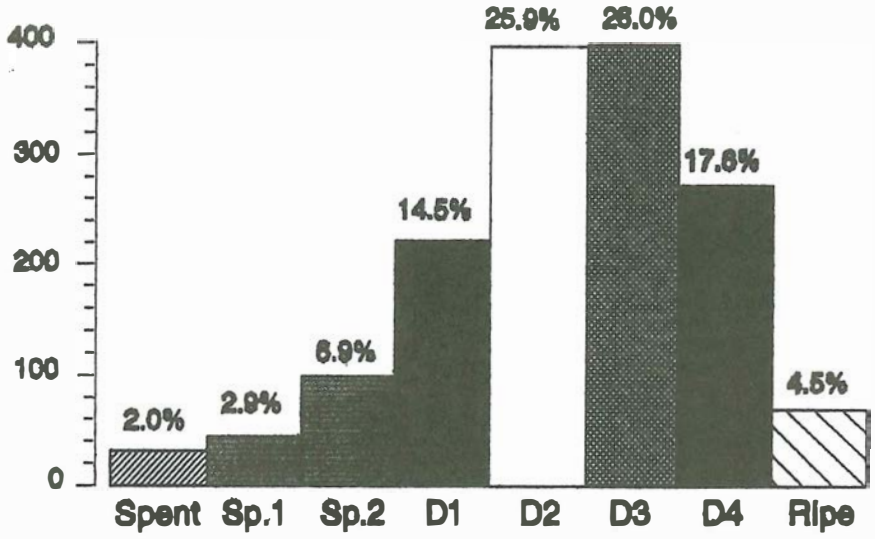


Fig 5.11.6 Relative occurrence frequency of macroscopic gonad stages in Jervis Bay *P. fumatus* for each collection completed between October 1991 to October 1993

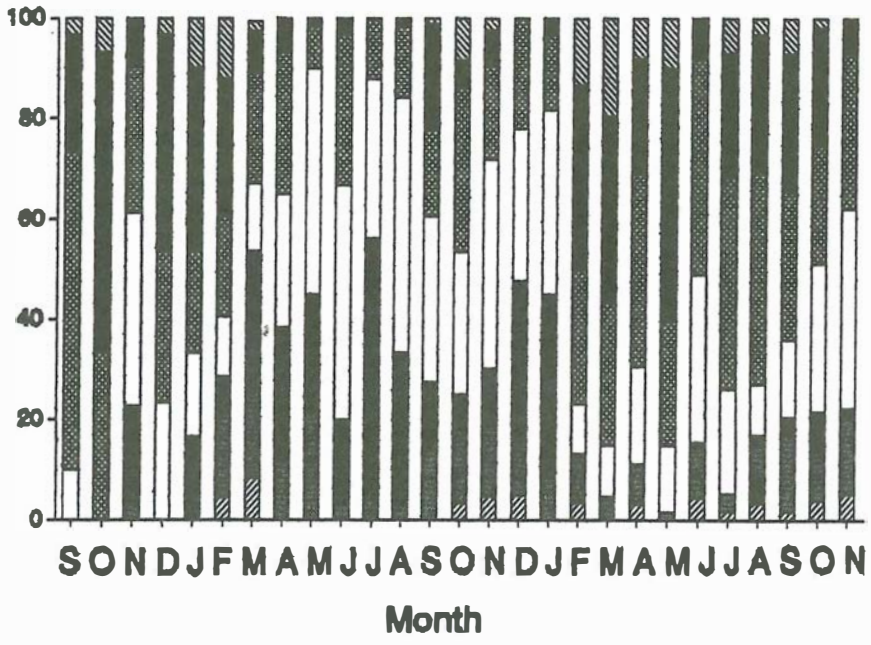


Fig 5.11.7 Box and whisker plot of GSI in *P. fumatus* collected over the period October 1991 to October 1993

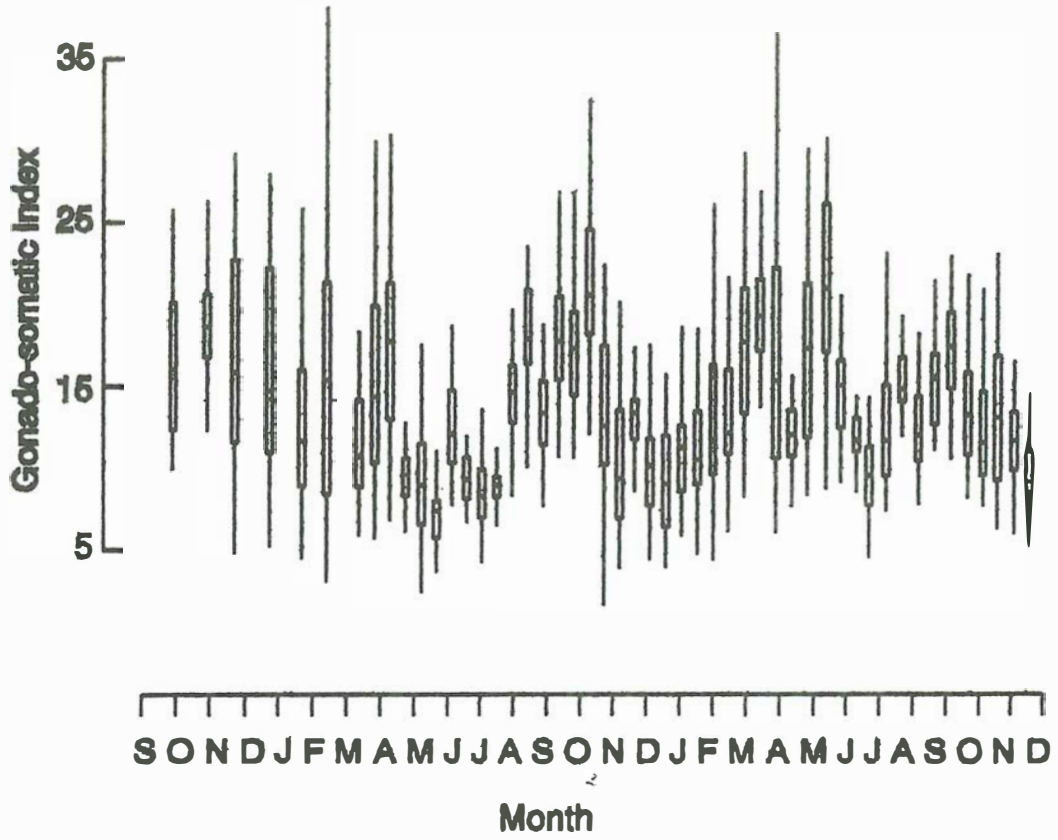
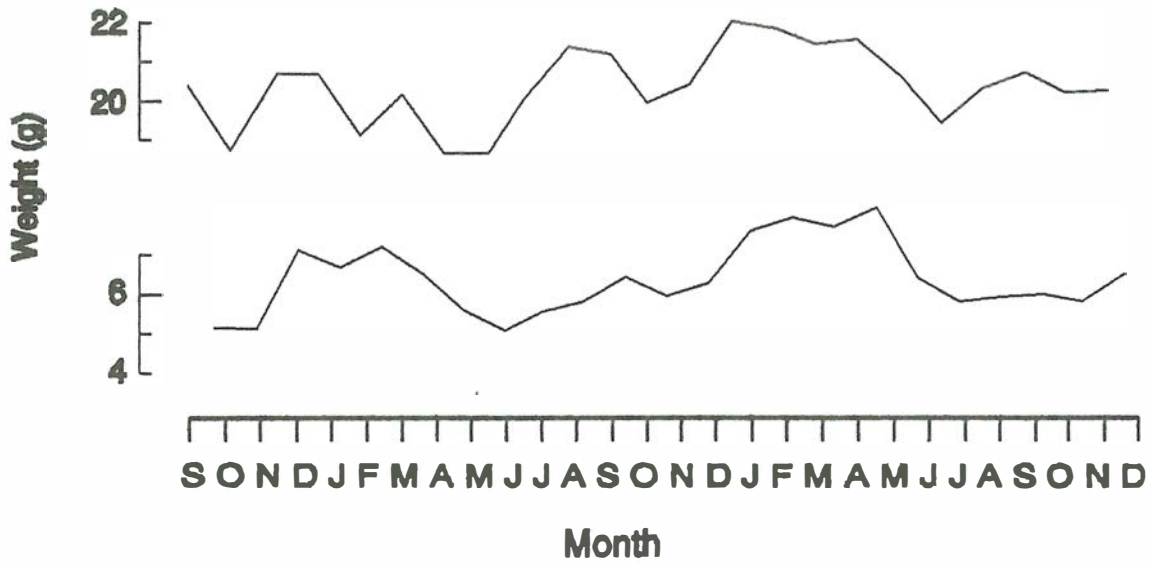


Fig 5.11.8 Seasonal variation in standardised total soft tissue and muscle weights of Jervis Bay *Pecten fumatus*.



5.12 Parasitic castration in commercial scallops (*Pecten fumatus*) and doughboy scallops (*Chlamys (Mimachlamys) asperrima*) from Jervis Bay, NSW and implications for the use of wild scallops as hatchery broodstock

Abstract

Sympatric populations of the hermaphroditic scallop, *Pecten fumatus,* and of the dioecious scallop, *Chlamys (Mimachlamys) asperrima,* from Jervis Bay, New South Wales, were sampled between October 1991 and December 1994. Over the first two years, 5.1% of adult *P. fumatus* and 4.5% of adult *C. asperrima* sampled were infected with a trematode, *Bucephalus* sp. that causes parasitic castration. A significant increase in the numbers of infected scallops occurred in spring 1993, reaching 63% in *P. fumatus* and 24% in *C. asperrima* in May and March 1994 respectively. The frequency of infection increased with scallop size, reaching 66% in *P. fumatus* greater than 80 mm in shell height and 40% in *C. asperrima* greater than 75 mm in 1994. Seasonal and inter annual variations in parasitism for the *P. fumatus* recorded over a continuous period of five years in Jervis Bay are discussed as are interspecies differences and the potential impact of the parasitic castration on reproductive potential and marketability of *P. fumatus* and *C. asperrima*.

Introduction

Many accounts exist of the impact of digenetic trematodes of the family Bucephalidae upon commercial bivalve molluscs (see Lauckner, 1983 for review). Bucephalid sporocysts and cercariae occur in bivalves, while the metacercariae and adult life stages of these parasites inhabit fishes (Lauckner 1983). The impacts of sporocysts and cercariae on their bivalve hosts are often documented in terms of their effects upon the gonad. Generally, the gonad is progressively replaced by sporocysts until castration (infertility) occurs. Following castration, the bucephalid infections may spread to other organs (Howell 1967). Other reported effects of bucephalid infections in bivalves have included increased growth (Menzel and Hopkins 1955),

increased mortality (Millar 1963; Howell 1967) and muscle weakening (Howell 1967), although, in both the oyster *Crassostrea virginica* and the scallop *Pecten fumatus* Reeve, the eating qualities of parasitised individuals are not diminished (Menzel and Hopkins 1955; Hamer unpublished data 1987).

The commercial scallop *P. fumatus* and the doughboy scallop *Chlamys (Mimachlamys) asperrima* (Lamarck) are subtidal bivalves found along the southern coast of Australia where both species have been commercially exploited (Young and Martin 1989). Both are common in Jervis Bay, New South Wales (NSW), although only *P. fumatus* have been targeted in what has been an intermittent fishery for NSW (Hamer and Jacobs 1987). In Victoria and NSW, the occurrence of the bucephalid parasite in *P. fumatus* is known to fishermen and has been monitored previously (Sanders 1966; Sanders and Lester 1981; Hamer and Jacobs 1987). However, as far as we can discern, there are no previous documented accounts of bucephalid parasitism in *C. asperrima*. This paper reports observations of bucephalid parasitism made in conjunction with a three-year study of reproductive condition of the commercial scallop *P. fumatus* and the doughboy scallop, *C. asperrima* in Jervis Bay. These observations include interannual and interspecific differences in parasite occurrence in the two scallop species.

Materials and Methods

From October 1991 until November 1993, fortnightly samples of mature scallops were collected by divers from Murrays Beach, Jervis Bay (35°04' S, 150°44' E; Fig. 5.11.1). Further scallop samples were collected monthly from February to June 1994 and intermittently from July to December 1994. On each occasion, a minimum of 30 *P. fumatus* and 30 *C. asperrima*, ranging in shell height from 55 to 85 mm and 50 to 80 mm respectively, was collected. The minimum sizes were adopted to ensure all individuals sampled had attained sexual maturity. This limitation was imposed as scallops were collected primarily for assessment of seasonal changes in the reproductive status in the respective populations.

For transport to the laboratory, scallops were wrapped in damp hessian and placed on ice.

Following dissections, which were made within 24 h of collection, the shell heights (central proximal to central distal margin of the upper left valve) were measured and the viscera, adductor muscle and gonad were weighed to the nearest 0.01 g. Comparisons of gonad and muscle weights were not made until the end of the study when sufficient parasitised animals became available within each collection. Apparent early stage bucephalid parasitism was confirmed by microscopic inspection (100x magnification).

As bucephalid sporocysts and cercariae cannot yet be identified to genus (Lasiak 1991), tissue from both scallop species was sent to Dr. R.J.G. Lester (Department of Parasitology, University of Queensland). Examination of wet mount and tissue sections from both scallop species found the bucephalid sporocysts and cercariae were indistinguishable in size and staining properties (R.J.G. Lester pers. comm. 1994).

Results

In the 3.2 years (October 1991 - December 1994) encompassed by the study, 2 459 *P. fumatus* and 1 827 *C. asperrima* were inspected for parasitism. In both species, advanced sporocyst infections of the gonad were easily discerned. Macroscopically, the presence of the bucephalid was first evident within the gonad as the tissue became vascularised or blood-shot in appearance. In *C. asperrima,* the infection appeared uniformly spread throughout the gonad, which, at least initially, retained the colour characteristic of its sex. In the early stages of infection in *P. fumatus* the male portion of the gonad commonly became transparent giving the appearance that the infection was less intense or yet to spread to the spermatogenic tissues. Only once was the infection observed in the proximal (male) portion of the gonad and not in the oogenic tissue. The colour of the parasitised gonad in *P. fumatus* varied from orange to deep red, which may reflect the age of the infection (Sanders and Lester 1981). In both species, during what was assumed to be the later stages of infection, cercariae were observed in the mantle.

Attempts to spawn parasitised scallops using intragonadal injections of serotonin (0.05 ml 10^{-3} M creatinine sulphate, Merck, Darmstadt, Germany) provided some support for

previous observations. Two spawnings were conducted involving 30 scallops of each species. In both scallop species several individuals released motile sperm. With *C. asperrima* this sperm was used to fertilise eggs that subsequently developed into D-veliger larvae. Sperm was released by *C. asperrima* with both cream and orange gonads, showing that gonad colour is not necessarily indicative of the sex of parasitised scallops. This may be a product of the darkening of *C. asperrima* gonads with the age of the infection. The suggestion that gonad colour in *P. fumatus* is a product of the age of the infection was also supported to some extent, in that sperm release was only achieved with orange and not red gonads. In both scallop species, the amount of sperm released was small in comparison to "normal" spawnings and oocyte release did not occur.

Of the scallops collected, 23.1 % of *P. fumatus* and 6.5% of *C. asperrima* (Table 5.12.1) were infected with the bucephalid parasite. Levels of parasitism ranged from 0 to 63% in *P. fumatus* and from 0 to 24% in *C. asperrima* (Fig. 5.12.1). No seasonal pattern of parasitism was apparent in either scallop species over the initial two years of the study. Low and variable rates of parasitism in the range 0-15% were recorded from October 1991 until November 1993. This result may to a large extent reflect sampling variability rather than any real change in occurrence. In the subsequent collections of scallops from February to December 1994, the relative occurrence of parasitised gonads increased sharply in both species, reaching 63% in *P. fumatus* and 24% in *C. asperrima*, by far their highest levels recorded during this study.

In June 1994, i.e. mid-term in a 12 month period of chronically high parasitism, the mean gonad weight in *P. fumatus* was significantly heavier ($P<0.05$) in parasitised than non parasitised scallops. At this time, the mean shell height of parasitised scallops was slightly but not significantly greater than that of non-parasitised scallops of the same species (Table 5.12.2). The combined weight of adductor muscle and gonad in parasitised and non parasitised *P. fumatus* were however very similar both in absolute (14.8 and 13.3 g respectively) and relative terms (51.4 and 51.9% of soft body weight respectively). By contrast, gonad, adductor muscle and soft tissue body weights of parasitised *C. asperrima* also sampled at this time all exceeded those of the non-parasitised scallops by large and statistically significant margins.

Two observations were made during the study that suggest depleted physical condition in parasitised scallops. First, it appeared that following transport to the laboratory, mortality was greater in parasitised scallops. In the March 1994 collection, a significantly greater proportion of parasitised scallops died in the 48 h post transport than non-parasitised scallops (χ^2= 4.26, df= 1, P<0.05). Secondly, upon emersion or during anaesthetisation with magnesium chloride (Heasman et al. 1994c) parasitised scallops appeared to gape more readily.

No relationship was evident between the occurrence of orange and red parasitised gonads in *P. fumatus* in the early stages of this study, possibly due to the low levels of parasitism. However, following the increase in parasitism in 1994 the frequency of orange colouration among parasitised gonads decreased from 83% in February to 65% in March and to 40% in April. Although this may have suggested a transition from orange to red with time, percentage of orange parasitised gonads returned to 65% in May and June 1994. Microscopic inspection of gonadal smears found no differences between orange and red parasitised gonads.

The frequency of bucephalid infection increased with scallop size in both *C. asperrima* and *P. fumatus* (Fig. 5.12.2). In both species, infected gonads were found in the smallest size classes of scallops collected, however, in both cases, the size classes sampled did not encompass the full size range of sexually mature scallops in Jervis Bay. A comparison of the frequency of parasitism within size classes for the two species found initial similarities between *C. asperrima* and *P. fumatus*. However, when parasitism peaked in 1994, *P. fumatus* were much more likely to be parasitised than *C. asperrima* of the same size (Fig. 5.12.2). Throughout the study no evidence was found to suggest parasitised gonad colour was related to scallop size.

Discussion

Bucephalid parasitism in P. fumatus

Over the first two years of the present study (October 1991 to September 1992 and October 1992 to September 1993) bucephalid parasitism of the gonads of *P. fumatus* from Jervis Bay remained consistently low (average 4.1% and 5.8% respectively). These percentages are similar to an overall parasitism rate of 8% reported by Hamer and Jacobs (1987) for Jervis Bay *P. fumatus* sampled during the boom catch year of 1982/83. This pattern of continuous low level parasitism however contrasted both with that of the preceding two years of 1989/90 and 1990/91, as shown in Fig. 2 by data of Fuentes (1994). It also contrasted with the final period of the present study during which parasitism increased from 10% in October 1993 to a peak of 63% in May 1994. After that it remained at levels of 50 to 60% through to the conclusion of sampling in December 1994. The reasons for this pattern are not entirely clear, however it is thought to be a product of seasonal changes in infection rates, a clearly demonstrated relationship between scallop size and age and the frequency of infection and seasonal changes in the age/size structure of the scallop population.

In an overview of five continuous years of bucephalid parasitism data available for Jervis Bay *P. fumatus* (Fig. 5.11.1), no consistent annual pattern is evident, however, when parasitic outbreaks have occurred they have typically begun in late Spring and Summer with the highest incidence in Autumn. There is even a suggestion of an Autumn peak (albeit poorly developed) in the relative incidence of parasitism in 1993. Such seasonality has been observed in *Bucephalus longicornutus* infections of *Ostrea lutaria* (mud oyster) in New Zealand (Howell 1967) and could be a product of one or a combination of factors.

Seasonality in the frequency of infection could reflect seasonal releases of infective miracidia from adult parasites infecting an unidentified definitive finfish host and/or, may reflect a period in the scallops' reproductive cycle in which increased susceptibility occurs. Clearly the parasite is only observed in reproductively mature scallops. In Port Phillip Bay *P. fumatus* The percentage of parasitised scallops remained 0 for all size classes up to 70mm (Sanders and Lester 1981), the size at which sexual maturity is attained (Sause et al. 1987).

The equivalent size in Jervis Bay stocks as observed in the present study is about 50mm which corresponds with first manifestation of bucephalid parasitism. Therefore, for seasonal changes in miracidial numbers to dramatically alter the frequency of scallop parasitism in Jervis Bay, the scallop population would have to be largely reproductively mature (ie greater than 50mm).

Further modifying the pattern of parasitic occurrence is the relationship between parasite frequency in *P. fumatus* in Jervis bay and the size/age structure of the population. Parasitism has not been observed in scallops smaller than 50mm and its occurrence increases in frequency with increasing size (Fig. 5.12.2). This is consistent with the earlier findings of Sanders(1966), in which bucephalid parasitism in Port Phillip Bay *P. fumatus* increased steadily and progressively from 4% in the 71-76mm size class up to 81% in the largest 96-100mm size class. Similarly, Bowers (1969) found that in a given year class of the cockle *Cardium edule,* larger individuals are more susceptible than smaller ones to infestation by the buchephalid *Labratrema minimus*. In *P. fumatus* the size parasitism relationship could be due to older/larger scallops having been exposed longer to the chance of miracidial infection, due to the fact that larger scallops filter greater volumes of water and thus increase the chance of miracidial contact or alternatively due to a decreasing resistance to infection with extreme age.

Regardless, the magnitude of peaks in parasitism should to an extent reflect the relative mix of size classes within adult (50 to 85mm) *P. fumatus.* The frequency of parasitism would be expected to be greatest in years when the adult component of *P. fumatus* populations are dominated by larger, older(3+ year-old) individuals. Similarly, the least prominent seasonal rises in parasitism would be expected in years in which adult component of populations are dominated by younger, smaller year classes ie 0+, 1+, and 2+ year old -stock.

The one period in five consecutive years in which there was no evidence of an autumn peak in parasitism, 1992, coincided with the period in which the mean size and age of scallops collected was at its lowest (Fig. 5.12.1). The mean size of scallops used to determine parasitic frequency during the period August 1989 to August 1991 (H. Fuentes unpub. data 1992) was

approximately 70mm. This arose from the deliberate collection of "commercial size" scallops for reproductive studies. However, the population mean for scallops in Jervis Bay in July 1991 was closer to 50mm (Fuentes 1994). Thus, when this study commenced in October 1991, the mean size of scallops collected was approximately 56mm, more accurately reflecting the mean size of the population. From Fig. 5.12.2 it is clear that parasitism in 56mm scallops is negligible, even during periods of high sustained parasitism (1994 in Fig.5.12.2), thus we observed a dramatic and sustained drop in parasitism in 1992 (Fig.5.11.1).

Between May 1992 and December 1994, little juvenile recruitment occurred and *P. fumatus* stocks in the Bay were dominated by a single year class (D. Worthington unpub. data 1995). Accordingly the mean size and age of scallops we collected increased with time. In 1993, the mean size of scallops collected was again approaching 70mm and a small autumn peak in parasitism was observed, however, the magnitude of the peak was less than that observed in 1990, 1991 and 1994 and may reflect inter-annual variations in the numbers of miracidia. In 1994, an autumn peak of the magnitude recorded by Fuentes (1994) was again evident in a 3+ year- old population averaging 75mm shell height, however the frequency of parasitism was maintained throughout that year. It is possible that this is confounded by the gradual increase in size and age of the population resulting from the failure of recruitment in the previous years .

The reported effects of Bucephalid parasitism vary in the literature but Bucephalids have commonly been shown to reduce vigour and to weaken the adductor muscle leading to debilitation and premature death in bivalves. These have included oysters (*Crassostrea spp.*), mud oysters (*Ostrea spp.),* pearl oysters (*Pinctada spp.),* cockles (*Cardium spp.*) and mussels (*Mytilis spp.* and *Perna spp.*) (Menzel and Hopkins 1955; Millar 1963; Howell 1967; Cranfield 1975; Lauckner 1983; Lasiak 1991*).* In the present study, significantly higher rates of post transportation mortality were exhibited by parasitised than non parasitised *P. fumatus*. Parasitised scallops have however been maintained within the hatchery for several months, indicating that parasitised scallops could survive long enough to maintain the high frequency of occurrence found in late 1994 without the continued presence of miracidia.

In Jervis Bay, the implications that parasitic castration has for the reproductive potential of *P. fumatus* populations are possibly more serious than those of elevated mortality in larger older scallops. Lasiak (1991) suggested that 20-32% parasitism in the population of the mussel *Semimytilus algosus* may impair its reproductive potential. Similar concern has been expressed by Fuentes (1994) concerning Jervis Bay *P. fumatus* and by Young and Martin (1989) about the high levels of parasitic castration for Port Phillip Bay *P. fumatus* reported by Sanders (1966). Fortunately *P. fumatus* in Jervis Bay attain sexual maturity by the time they reach a size of 50mm as 1+ year- olds and subsequently attain a mean size of about 75mm as 2+ year-olds. During these two years they undergo multiple annual spawnings over eight months of the year outside the warmest Summer and early Autumn months (see Section 5.11) but, as already discussed, sustain relatively low rates of parasitic infection. By the time they finally sustain high rates of parasitic castration as 3+ year-olds, they are in any case nearing the end of their natural life span (Hamer, 1987).

Non parasitised *P. fumatus* possess a large and attractive orange and cream piebald gonad, very similar to their European counterpart *P. maximus,* and are marketed "roe on". Fortunately the large, turgid, orange to red gonad of parasitised *P. fumatus* are also attractive in appearance and are not discriminated against in the marketplace. Moreover, the buchephalid parasite presents no health risk to consumers. In regard to marketable yield, Lester and Sanders (1981) showed that although the combined mean monthly weight of adductor muscle and gonad in parasitised and non-parasitised *P. fumatus* from Port Phillip Bay varies asynchronously, the seasonal range exhibited by both (16 to 22g and 18 to 24g, respectively) were very similar. From the comparative data for parasitised and non parasitised *P. fumatus* collected from Jervis Bay at the peak in parasitism in June 1994 (Table 5.12.2), it can be seen that the combined weight of adductor muscle and gonad for the parasitised *P. fumatus* (51.4% of soft tissue weight) is almost identical to that of non-parasitised scallops (51.9%). It therefore likely that bucephalid parasitism has negligible overall impact on the meat yield of *P. fumatus* on a yearly basis.

Parasitism in C. asperrima

As indicated in Figs. 5.12.1 and 5.12.2, comparative data of buchephalid parasitism in *C. asperrima* and *P. fumatus* exhibited some striking similarities. As in *P. fumatus, p*arasitism in *C.asperrima* remained low over the first two years of the study. The overall range of 0 to 12% in *C. asperrima* was very similar to that in *P. fumatus* (0 to 15%). Mean annual rates of parasitism in *C. asperrima* (6.1 and 3.2 %) were also very similar to those in *P. fumatus* (4.1 and 5.8 %). Parasitism in *C asperrima,* as in *P. fumatus,* finally exhibited a major and sustained upsurge in Spring 1993 and Summer 1994. The maximum parasitism rate of 24% reached in early Autumn (March) 1994 was however considerably lower than the rate of 63% previously reported for *P. fumatus.* Size was not thought to be a factor in the differences between the two scallop species, despite the mean size of *C. asperrima* collected in the 1994 peak in parasitism being approximately 10mm smaller than that of *P. fumatus*. A comparison of parasitism between the two scallops within size classes (Fig.5.12.2) shows *C. asperrima* is less likely to be parasitised. Lasiak (1991) found similar widely disparate levels of bucephalid parasitism in adjacent populations of mussels and suggested these could be due to differences in physiological or genetic resistance to infection. This may be the case in the current study although other factors such as the possibility of species differences in filtration rates may alter the incidence of infection.

A further factor common to both scallop species was the steady increase in the mean size from the outset of sampling until the advent of peak parasitism in Autumn 1994. The subsequent fall in mean shell height and parasitism rate in *C. asperrima* over the final eight months of the study (Fig 5.12.1) is consistent with the natural senescence of an ageing year class and the gradual entry into the sampled population of a new 1+ year class. As in *P. fumatus,* the frequency of parasitism in *C. asperrima* increased with scallop size in each year of the study (Fig. 5.12.2) while the overall incidence across all size classes increased markedly during the final year of sampling (October 1993 to December 1994).

There is no known documentation on bucephalid parasitism in other populations of *C. asperrima* throughout its distribution in southern Australia. This may be due to the paucity of

research on *C. asperrima* or due to an actual absence of the parasite in particular areas or a combination of both factors. While parasitised *C. asperrima* have not been found in extensive sampling from the D'Entrecastaux channel in southern Tasmania (Will Zacharin pers. comm. 1995), they do occur in South Australia (Craig Styans pers. comm. 1995). Observations of parasitised *C. asperrima* may also be less frequent because of the resemblance of parasitised gonads to those of a well-developed gonads. In any case, the marketing implications of parasitism are probably insignificant. As indicated by results in Table 5.12.2, the weights of adductor muscles and gonads in parasitised *C. asperrima* are both greater than those of equivalent size non-parasitised scallops with gonads representative of " average" condition throughout the year in this species.

Bucephalids are thought to be remarkably specific regarding first intermediate hosts (Lauckner 1983), however, it not yet known if the parasite poses a threat to other commercial molluscs in Jervis Bay (flat oysters *Ostrea angasi*, mussels *Mytilus edulis* and Sydney rock oysters *Saccostrea commercialis*). While Roughley (1933) has reported the presence of bucephalids in both *O. angasi* and *S. commercialis* collected in NSW, no reports have been found of adverse effects of the parasite on other bivalves in Jervis Bay.

Table 5.12.1 Comparison of parasitism in two species of scallop collected from Jervis Bay, NSW, between October 1991 and December 1994.

	Pecten fumatus		*Chlamys (Mimachlamys) asperrima*	
Time	*n*	% parasitised	*n*	% parasitised
Oct.91 to Sept.92	787	4.1	823	6.1
Oct.92 to Sept.93	729	5.8	698	3.2
Oct.93 to Dec. 94	561	52.3	245	15.6
Total Parasitism (Oct.91 to Dec. 94)		23.1		6.5

Table 5.12.2 Comparison of shell height and tissue weights of parasitised and non-parasitised scallops of the species *Pecten fumatus* and *Chlamys (Mimachlamys) asperrima* collected from Jervis Bay in June 1994.

Pecten fumatus

	Parasitised	Non-parasitised	*F*	*P*
Shell height	76.2 ± 1.34	73.2 ± 1.21	2.74	NS
Gonad weight	4.2 ± 0.23	2.9 ± 0.31	10.69	**
Muscle weight	10.6 ± 1.01	10.4 ± 0.64	0.02	NS
Soft body weight	28.8 ± 1.79	25.6 ± 1.50	1.90	NS

Chlamys (Mimachlamys) asperrima

	Parasitised	Non parasitised	*F*	*P*
Shell height	67.6 ± 1.34	65.5 ± 1.26	1.20	NS
Gonad weight	4.6 ± 0.44	1.9 ± 0.23	28.92	***
Muscle weight	7.3 ± 0.71	5.1 ± 0.51	6.15	*
Soft body weight	20.7 ± 1.45	15.3 ± 0.95	9.61	**

Values are Means ± standard errors, Shell height is in mm and weight in g. For Anova, $n = 15$ and $df = 29$ for each comparison within scallop species.

Fig. 5.12.1 Percentage frequency of parasitism in two species of scallop collected from Murrays Beach, Jervis Bay, NSW. Data for *Pecten fumatus* prior to October 1991 reproduced from Fuentes (1994). Lines with points including standard error bars represent mean shell height for each collection.

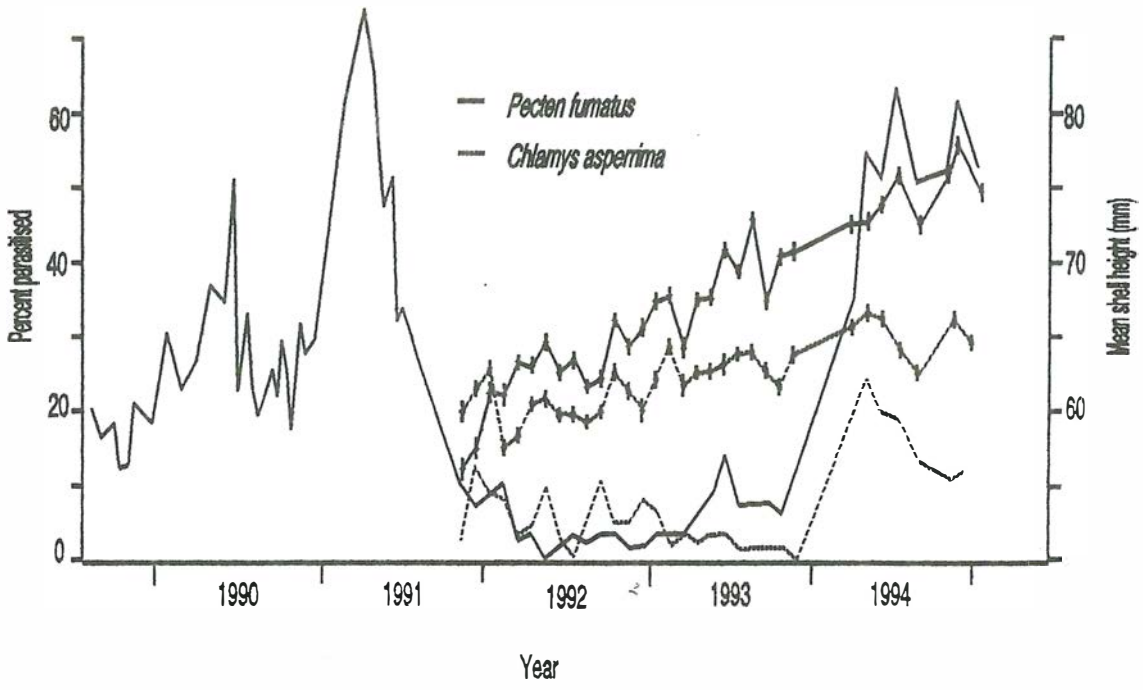
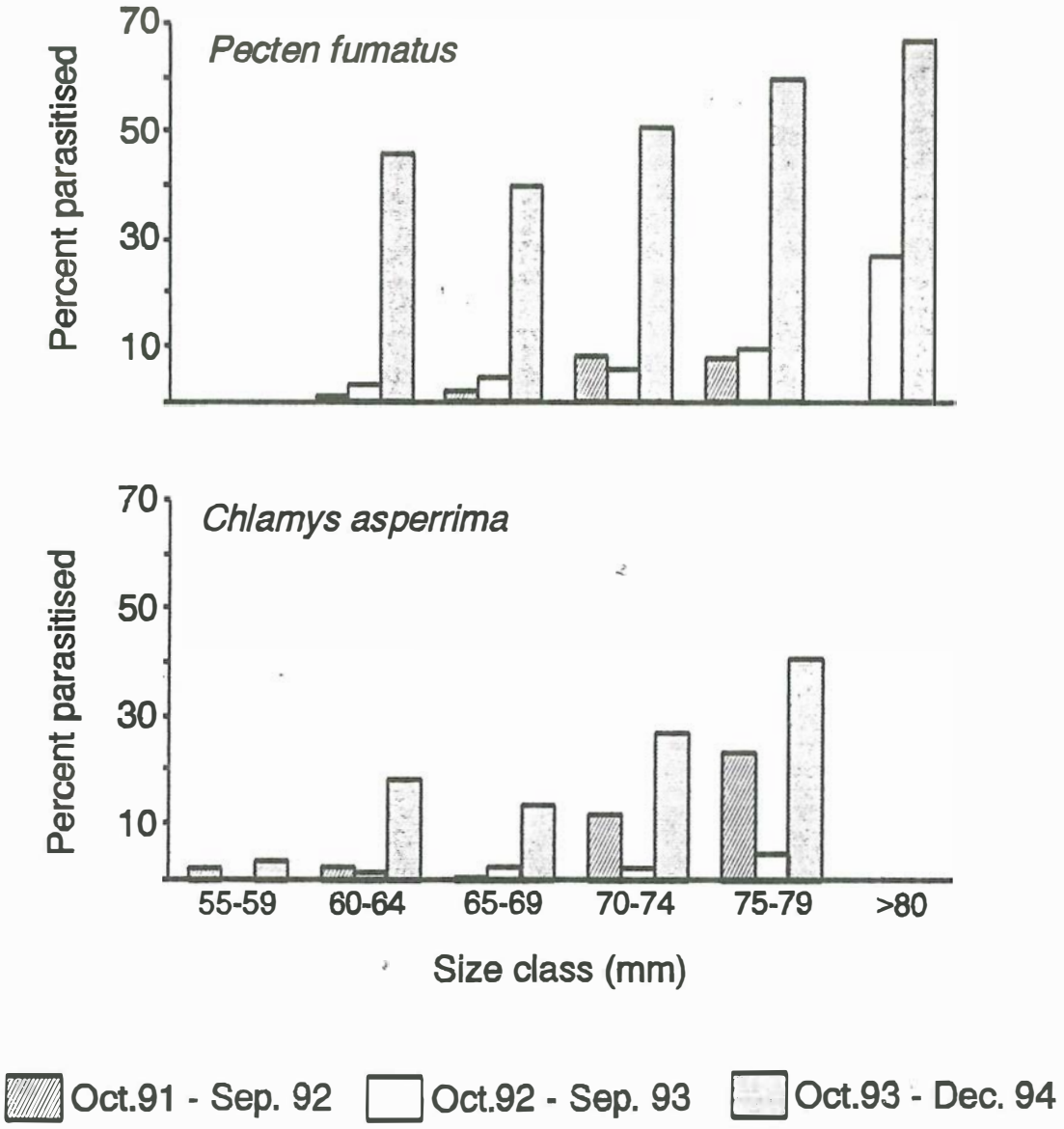


Fig. 5.12.2 Size frequency data for parasitism in scallops from October 1991 to September 1992, October 1992 to September 1993 and October 1993 to December 1994. Size classes with <10 scallops collected within each time period have been ommitted.



5.13 Development of techniques for hatchery conditioning (gonad ripening) of captive broodstock.

Abstract

Difficulty in obtaining ripe broodstock of the commercial scallop *Pecten fumatus* from wild populations in New South Wales prompted the development of hatchery conditioning protocols. Microalgal diets, feeding rates and holding temperatures conducive to rapid gonadal growth and development were identified. Results of microalgal clearance rate experiments indicated that the species *Pavlova lutheri,* Tahitian *Isochrysis* aff. *galbana, Chroomonas salina* and *Chaetoceros gracilis* are ingested by adult scallops at similar rates at 14, 18 and 21° C but more slowly at 11° C. Maximum (satiation) ingestion rate using mixed diets containing approximately equal numbers of cells of these four species, was estimated as about 6 x 10^9 cells per day for broodstock in the range 55 to 75 mm shell height. Large numbers of cells of the microalga *Tetraselmis suecica* passed through the digestive tract of scallops undigested. Egg production rate, gonad size and condition factors were all highest when broodstock were held at 15° C, lowest for scallops maintained at 21° C and of intermediate values at temperatures of 12 and 18° C. Gonad condition and egg production increased as feeding rates were raised from 12.5 to 100% of satiation at all test temperatures in the range 12 to 21°C . Frequent spawning triggered by routine handling of broodstock during these experiments emphasised the need for conditioning equipment and protocols that minimise handling and other disturbance factors. No unplanned spawning occurred at feeding rates of 25% or 12.5% of satiation at 12°C. The use low temperature and low feeding rate might therefore enable short term stockpiling of broodstock once they attain prime breeding condition. Findings of these experiments were successfully applied to a subsequent large scale conditioning and induced spawning trial.

Introduction

This aspect of project research was prompted by difficulty in obtaining ripe ready to spawn *P. fumatus,* an hermaphroditic scallop, from wild populations in NSW, as already discussed at

length in Section 5.11. This constraint was partly a result of apparent irregular seasonal patterns of gonad condition exhibited by populations of scallops at Jervis Bay (35° 04'S, 150° 44'E), the only known "permanent" scallop beds in NSW, from which broodstock were acquired. Attempts to induce apparently ripe (gonad stage 8 -see Table 5.11.1) wild *P. fumatus* to spawn following seven to ten hours transportation from Jervis Bay to our hatchery were rarely successful. This was possibly due to deleterious effects of low temperatures needed to ensure survival of *P. fumatus* during transportation. Low temperature damage of this type has been observed in another species of scallop *Argopecten irradians* (Sastry, 1979). Over the first 12 months of the present study, approximately 300 ripe *P. fumatus* were treated with intragonadal injections of serotonin and temperature shocks in an attempt to induce spawning. Egg release only occurred in about 4% of these scallops, although sperm release was common (Heasman et al.,1994b). By contrast, *P. fumatus* which had been held and conditioned in the hatchery for periods of one to several weeks frequently spawned following handling.

Temperature has been considered the primary environmental cue regulating bivalve reproduction (Sastry, 1968) and is the environmental factor most commonly cited as influencing bivalve reproduction (Sastry, 1979). It is possible to induce gametogenesis in many species of scallops outside their normal reproductive seasons by simply exposing them to appropriate temperature regimes. Establishing the correct temperatures for gonadal growth and development in *P. fumatus* were therefore seen as critical to reproductive conditioning. Providing temperatures, and in some cases photoperiods, are held within a suitable range for gonad conditioning of scallops, egg production is then likely to be dependent upon food supply (Barber and Blake, 1991). It was therefore considered important to also identify diets and feeding rates that would promote rapid reliable ripening of gonads in *P. fumatus*.

Materials and Methods

P. fumatus used in these trials were collected under permit by divers from a bed off Murrays Beach, Jervis Bay. (Fig 5.11.1b), wrapped in damp hessian, placed upon a layer of seawater ice and road freighted to the Port Stephens Research Centre (PSRC).

Experiment 1: The effect of temperature upon the consumption of algal species

Upon arrival at the laboratory, four batches of 10 *P. fumatus* were maintained within ± 0.5°C at one of four temperatures, 11, 14, 18, or 21°C. Scallops were held individually in aerated 10 l aquaria (food grade plastic buckets) for a week prior to experimentation. All scallops were starved for 24 h in 1 μm filtered seawater (35 g/kg salinity) immediately prior to use in the trial. At the commencement of the trial, all faeces and pseudofaeces were removed while replacing old seawater with new seawater filtered to 1 μm. Each scallop was then fed 2 x 10^9 cells of one of the following microalgae, *Pavlova lutheri*, Tahitian *Isochrysis* aff. *galbana, Tetraselmis suecica, Chaetoceros gracilis* or *Chroomonas salina.* Water samples were collected at frequent prescribed intervals over a total period of 30 h from each of the 40 aquaria used to house individual scallops. Algal cell density within each sample was determined by microscopic examination at 100x using an improved Neubauer haemocytometer. At the completion of the experiment, faecal samples were collected and examined at 100x to evaluate the degree to which algal cells had been digested.

Experiment 2: The effect of temperature and feeding rate upon reproductive condition of scallops

Upon arrival at the laboratory, individual *P. fumatus* were placed in separate aquaria, containing 10 l of 1 μm filtered seawater (35 ± 1 g/kg salinity) and maintained under a 14:10 h light : dark cycle. In a 4 x 4 temperature and feeding rate factorial experiment design, scallops were held within ±0.5°C at either 12, 15, 18 or 21 °C and fed the dry weight equivalent of 3 x 10^9, 1.5 x 10^9, 0.75 x 10^9 or 0.375 x 10^9 T. *Isochrysis* cells twice daily.

The highest feeding rate of 6 x 10^9 cells per day for 55-75 mm *P. fumatus* at 15 - 21°C was adopted on the basis of preliminary observations that scallops in this size range were usually incapable of fully clearing this quantity of algae each day over a succession of days, while those fed at lower levels of up to 5 x 10^9 cells per day were consistently able to do so. Thus we assumed that experimental rations of 6 x 10^9 cells per day would approximate satiation feeding; 3x10^9 cells per day, approximately 50% of satiation feeding; 1.5 x10^9 cells per day, approximately 25% of satiation feeding and 0.75x10^9 cells per day, approximately 12.5% of satiation feeding.

The diet used a combination of at least three of the following algal species, *P. lutheri*, T. *Isochrysis*, *C. gracilis* and *Chr. salina*, fed on an equal dry weight basis morning and evening (typically between 0.75 and 0.9 l of algal culture per feed). As seven replicates were run for each combination of temperature and feeding rate, a total of 112 buckets and scallops were used.

Seawater was replaced with filtered seawater of equilibrated temperature thrice weekly. Buckets were checked daily for spawning. Each week, scallops were anaesthetised in a bath of magnesium chloride (0.31 M) in seawater (Heasman et al., In Press) and gonad condition was evaluated using a rating system (Table 5.11.1) similar to that used by Mason (1983). The relative size of the scallop gonad was also rated according to a numerical scheme illustrated in Fig. 5.13.1). Where eggs had been released, fecundity was estimated by resuspending the eggs and counting duplicate 1 ml subsamples using a Sedgwick-Rafter slide. The total volume of seawater was then measured to ± 0.05 l and replaced with 10 l of new temperature equilibrated filtered seawater. Scallops were maintained under this regime for 6 weeks.

Experiment 3: Experimental conditioning

Gonad conditioning of mature *P. fumatus* was attempted on a larger scale using protocols based on the findings of Experiments 1 and 2. One hundred and twenty scallops collected from Jervis Bay were divided equally between four recirculating conditioning system

(Fig. 5.13.2) in a temperature controlled room at 15.0 ±0.5°C. These broodstock were fed to satiation as determined in Experiment 1, using the best mixed diet of algal species used in Experiment 2. Upon arrival and then at weekly intervals, 20 randomly selected scallops (five from each conditioning system) were visually assessed for gonad size and condition. Ten of these scallops were then placed individually in 1 l aerated cylindroconical plastic aquaria and exposed to three cycles of warm (20° C) and ambient (15° C) water temperatures, each cycle lasting approximately 45 min. If egg release occurred, fecundity was determined as described for Experiment 2.

Results

Experiment 1: The effect of temperature upon the consumption of different species of microalgae

Except for *T. suecica*, the rate of consumption of each algal species (Fig. 5.13.3) was not markedly different at 14, 18 and 21°C but was depressed at 11°C. In terms of cell numbers (Fig.5.13.3), the prymnesiophytes, *P. lutheri* and T. *Isochrysis*, were most rapidly consumed, being almost totally removed from the water column within 8 h. In terms of dry weight of cells (calculated from Nell and O'Connor, 1991; O'Connor et al., 1992) depleted in the first two h (Fig.5.13.4), *P. lutheri*, T. *Isochrysis* and *Chr. salina* were ingested at similar rates in the approximate range 150 to 225mg /h. The rate of ingestion of *C. gracilis* of about 40mg dry wt./h , was however markedly lower less than *P. lutheri*, T. *Isochrysis* and *Chr. salina*. Nett ingestion of *T. suecica* proceeded for the first 8 h at all temperatures but subsequently ceased (at 12 or 15°C) or began to vary greatly (at 18 and 21°C).

Microscopic examination of faecal samples from scallops fed with *T. suecica* revealed the presence of many undigested cells. This was not the case for the other algal species tested. As gut clearance time approximated six to eight h at 18 and 21°C, it is likely that resuspension of undigested *T. suecica* cells accounted for the variations in *T. suecica* cell densities recorded at these temperatures.

Experiment 2: The effect of temperature and feeding rate upon reproductive conditioning of scallops.

Fecundity.

Mean fecundity per spawning (Fig 5.13.5a) over the 6 week experiment was considerably higher for scallops held at 15°C than for those held at higher (18 and 21°C) and lower (12°C) temperatures. Mean fecundity generally increased with increasing feeding rate, being low (0 - 4.5 x 10^5 eggs/spawning) at the lowest feeding rate (approximately 12.5% of satiation) over all temperatures tested. Mean fecundity was also markedly depressed at the second lowest feeding rate (approximately 25% of satiation) except at 15°C.

Spawning frequency.

Mean spawning frequency (Fig 5.12.5b) increased with increasing temperature from 12 to 18°C but declined drastically with a further rise of temperature to 21°C. At all temperatures tested, spawning frequency increased from generally low levels (0 to 0.4 spawnings per scallop per 6 weeks) for scallops maintained at the lowest feeding rate (approximately 12.5% of satiation) to higher levels (0.5 to 1.5 spawnings per scallop per 6 weeks) when fed at a level approximating satiation.

Gonad size.

At the lowest holding temperature of 12°C, moderate increases in mean gonad size (as indicated by mean index values in Fig. 5.13.5c occurred at all feeding rates. At 15°C however, mean gonad size decreased slightly from an initial mean index score of 4.5 at the 12.5% and 25% satiation feeding rates, increased by a moderate margin at the 50% satiation feeding rate and increased markedly at the 100% satiation feeding rate to a level well above that of any other combination of temperature and feeding rate. At 18°C gonad size decreased slightly at the 12.5% satiation feeding rate, remained unchanged at the 25 and 50% satiation feeding rates and increased by a moderate margin at the 100% of satiation feeding rate.

Interim gonad size data for *P. fumatus* fed to satiation over the six week trial are illustrated in Fig 5.13.6a. These data indicate that gonad size remained constant at 12 and 18°C, increased progressively at 15°C and declined progressively at 21°C.

Gonad condition.

After six weeks at the lowest holding temperature of 12°C, gonad condition (Fig 5.13.5d) remained essentially unchanged from its initial value of 5.1 at all feeding rates. At 15°C, gonad condition again remained little changed at feeding rates approximating 12.5, 25 and 50% of satiation but had increased greatly at the feeding rate that approximated 100% satiation. After six weeks at 18°C, mean gonad condition index values had decreased markedly at feeding rates approximating 12.5, 25 and 50% satiation and remained unchanged at the 100% satiation feeding rate. At the highest test temperature of 21°C, very marked decreases in mean gonad condition occurred at all feeding rates. Indeed by the conclusion of the experiment the gonads of almost all scallops held at 21°C were classified as spent, being flaccid and translucent with the difference between ovarian and testicular tissue difficult to distinguish and the intestinal loop clearly visible. As illustrated in Fig. 5.12.6b, gonad condition index values of scallops fed at or near full satiation underwent a minor, but steady decrease at 12 and 18°C, increased steadily at 15°C, and declined during the second to fourth week of the trial at 21°C.

Experiment 3: Experimental conditioning

During the course of this experiment water quality parameters within the recirculating systems were maintained at an optimal ranges of 15 ± 1°C; 37 ± 1.5 g/kg salinity and a pH 8.1 ± 0.15. Feeding rate was also maintained at a optimum rate approximating satiation i.e. 6x 10^9 algal cells/scallop/day. Effects of conditioning period on gonad size, appearance and on the frequency and magnitude (fecundity) of response to spawning induction stimuli were as follows (values are mean±SD):

Gonad size and condition.

Gonad size (Fig 5.13.7a) increased steadily from an initial index value of 3.7 ± 0.3 to a

maximum value of 6.7 ± 0.3 at the termination of the trial 4 weeks later. Gonad condition (Fig 5.13.7b) exhibited a similar trend for the first 3 weeks increasing from an initial index value of 4.3 ± 0.2 to a maximum value of 5.6 ± 0.1 that remained unchanged (5.5 ± 0.3) over the fourth and final week of the trial.

Spawning response.

The number of scallops spawning in response to thermal stimulation (Fig 5.13.7c) increased with time spent in the conditioning system. Prior to conditioning, scallops did not spawn as either males or females, however by the fourth week, 70% of scallops released sperm and 70% released eggs in response to thermal stimuli.

Fecundity.

As with gonad condition and spawning response, fecundity (Fig 5. 13.7d) for each group of 10 scallops increased steadily over the first 3 weeks of conditioning from an initial value of 0 to a maximum value of 2.3 ± 0.5 x10^6 ova per spawning. Mean fecundity then underwent a small decline to 1.9 ± 0.5 x10^6 over the fourth and final week of the trial.

Discussion

The observed differences in the ingestion of five microalgal species by adult *P. fumatus* could be the result of one or a combination of factors. Scallops are suspension feeders, reliant upon cilia on the surface of the gills to remove suspended detrital material and phytoplankton from the surrounding seawater. As with other bivalves, feeding in scallops is a dynamic process, influenced by physical and chemical factors in the environment as well as the quantity and quality of food particles available (Ward and Cassell, 1991).

Bivalves have three types of ciliary tracts in their gill filaments; lateral (current producing), frontal (particle carrying) and latero-frontal tracts thought to be involved in particle retention (Owen and M^{c}Crae, 1976). The size of food particles has been shown to effect retention, with the suggestion that the lower limit for effective particle retention in pectinids being about 5-

7μm. This is larger than that for most other bivalves including clams, mussels and oysters (Bricelj and Shumway, 1991). Although the mechanism of particle retention by the bivalve gill is not yet fully understood, the poor retention of particles below about 5 μm by the pectinid gill has been ascribed to the poor development of the latero-frontal ciliary tracts (Bricelj and Shumway, 1991).

In the present study however, observed differences in the ingestion of different microalgal species by adult *P. fumatus* cannot be simply ascribed to particle size. Of the five species tested, the two smallest, *P. lutheri* and T. *Isochrysis*, of mean cell dimensions 6.9 x 4.9 and 8.5 x 5.4 μm respectively and the largest, *Chr. salina,* of mean cell dimensions 11.4 x 6.9 μm (Nell and O'Connor, 1991), were rapidly cleared from the water column. These results indicate that size was not a factor in the relatively poor consumption of *C. gracilis* and *T. suecica*, the cells of which average 7.4 x 5.4 μm and 9.7 x 7.1 μm respectively (Nell and O'Connor, 1991). Similarly, the possibility that *T. suecica* was poorly consumed because of its high individual cell dry weight (52 ± 11 pg) is discounted by the fact that *Chr. salina,* which has an even larger individual dry cell weight (60 ± 14 pg) was the most rapidly depleted species of all.

Scallops have the ability to regulate the amount of material ingested and have been shown to discriminate among algal species with cells of similar size (Shumway et al., 1985; Shumway et al., 1991). Le Pennec and Rangel-Davalos (1985) found that *Pecten maximus* larvae ingested and digested *P. lutheri* and T. *Isochrysis* rapidly, while *T. suecica* was poorly ingested and not digested. The lower ingestion rate of the diatom *C. gracilis* in terms of cell numbers, dry weight and cell volume by *P. fumatus* in this study may have been caused by mechanical difficulties presented by the silicate spines of *C. gracilis* or perhaps by palatability factors. Metabolites of the algae *Chaetoceros muelleri* and *Isochrysis galbana* have been shown to stimulate ingestion in *Placopecten magellanicus* (Ward and Cassell, 1991) and *Mercenaria* (Marinucci, in Epifanio, 1983) respectively, while those of other algal species have been shown to depress filtration in bivalves (Epifanio, 1983).

Poor digestion of *T. suecica* may have resulted from difficulties encountered in digesting

the theca of this alga (Epifanio, 1979). These comments may equally apply to chlorophyte microalgae such as *Dunaliella primolecta* which is inefficiently ingested and digested by *Pecten maximus* larvae (Le Pennec and Rangel-Davalos, 1985) and *Chlorella autotrophica* which is inefficiently absorbed by *Argopecten irradians concentricus* (Peirson, 1983). Regardless of the reason for its poor digestibility, *T. suecica* is clearly inappropriate for inclusion in conditioning diets for *P. fumatus.*

In two other scallops species, *A. irradians* (Kirby-Smith, 1970) and *Chlamys opercularis* (McLusky, 1973), clearance rates have been found to be independent of temperature between 10 and 20°C and to be greatly reduced at 5°C. Results of this study indicate that seawater temperatures in Jervis Bay, weekly means of which typically vary within the range 13-25°C (May et al., 1978; CSIRO, 1994), may have little effect on algal clearance rates of local stocks of *P. fumatus* . Temperatures as low as 8- 10°C are commonly encountered by more southern populations of *P. fumatus.* in Tasmania and Victoria. However, as these races are genetically distinct from Jervis Bay *P. fumatus* (Woodburn, 1989), it is possible that ingestion rates, like those of their Jervis Bay counterparts, are unaffected within local extremes of temperature. Indeed, in trials conducted at the University of Tasmania (Coleen O'Meley, pers. comm., 1993) local stocks of *P. fumatus* were found to rapidly condition at holding temperatures of 10 to 12°C. Such temperatures closely approximate those occurring from August to September in southern Tasmania when spawning activity is highest (Harrison, 1961, cited in Young and Martin, 1989) and from June to November during the scallop's spawning season in Port Phillip Bay (Sause et al., 1987b; Sanders and Lester, 1981).

Cropp (1985 and 1987) reported accelerated growth in *P. fumatus* spat from November to May, when water temperatures on the east coast of Tasmania exceeded 12°C with maximum growth occuring in January and February at 17-19°C. Reduced spat growth at low (≤13°C) temperatures recorded for Jervis Bay *P. fumatus* by O;Connor, Heasman and O'Connor(1994) by Cropp (1987), in relation to Tasmanian scallops, may result from reduced filtration at lower temperatures. As indicated in the present study (Fig 5.13.3), clearance rates of microalgae by adult Jervis Bay *P. fumatus* did not differ significantly at 15, 18 and 21°C but were reduced at 11°C especially in relation to the prymnesiophytes *P. lutheri*

and T. *Isochrysis.*

The poor gonadal growth obtained at the highest temperature (21°C) used in the present study is not surprising given that *P. fumatus* is in general confined to southern Australia (Fairbridge, 1953; Olsen, 1955). Optimal temperatures for gonadal growth vary greatly between species according to their distribution. For example, Devauchelle and Mignant (1991) suggest a range of 7 to 17° C for conditioning *P. maximus* broodstock , whereas 26 to 28° C is recommended for the tropical species *Amusium pleuronectes* (Chaitanawisuti and Menasveta, 1992). Sastry (1963) suggests that once a minimum threshold temperature for gametogenesis has been reached in *A. irradians*, the rate of development of the gametes to maturity is dependent upon temperature, while fecundity and size of the gonad is primarily determined by the amount of food available. We also found this to be the case for *P. fumatus* (Experiment 2) in which gonad size was strongly affected by dietary level while gonad condition varied more with temperature than with dietary level.

The fact that many scallops spawned one or more times during Experiment 2 was probably an artefact of the experimental design and the requirement for frequent water changes and handling of the scallops for rating. Although both 12 and 15° C. appear appropriate temperatures to condition *P. fumatus*, gonad condition and size and egg production was much higher at 15° C. The results obtained in Experiment 3, show that when handling is minimised and water quality parameters are stabilised, it is possible to rapidly condition *P. fumatus* by holding them at 15°C and feeding to satiation. Under these conditions scallops in a reproductive condition typical of the lowest mean gonad size and condition encountered in wild populations in Jervis Bay (Heasman, O'Connor and Frazer, unpublished data) were brought into prime ready-to-spawn condition in three weeks. These conditioned scallops subsequently yielded a mean of 2.3 ± 0.53 ($x10^6$) eggs in response to thermal stimuli. This figure compares very favourably with the mean fecundity of 1.2 x 10^6 eggs/spawning from ripe wild scallops, less than 4% of which spawned eggs in response to the same type of stimuli within 24 to 48 h of capture (Heasman et al, 1994a).

Fig 5.13.1 Gonad sizing criteria used for *P. fumatus*

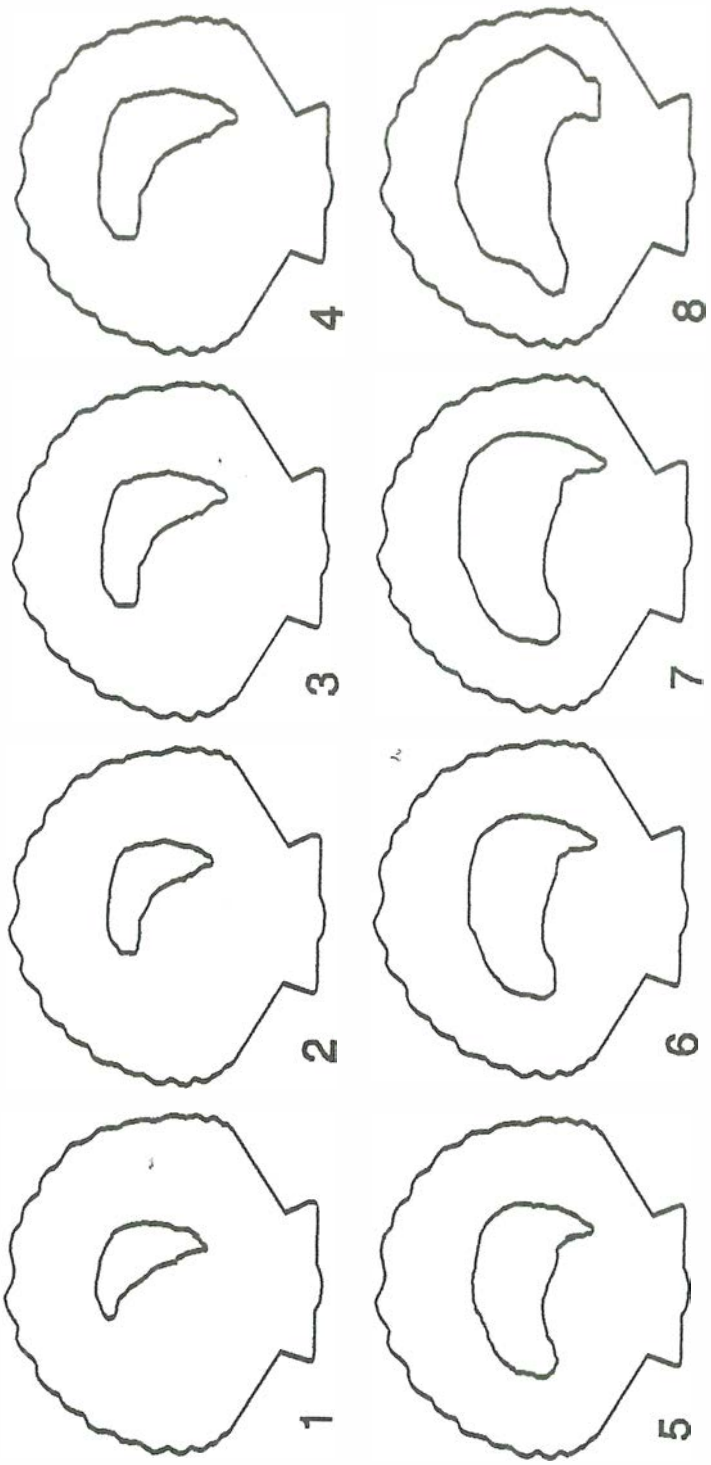


Fig 5.13.2 Scallop conditioning system with biofiltration resevoir

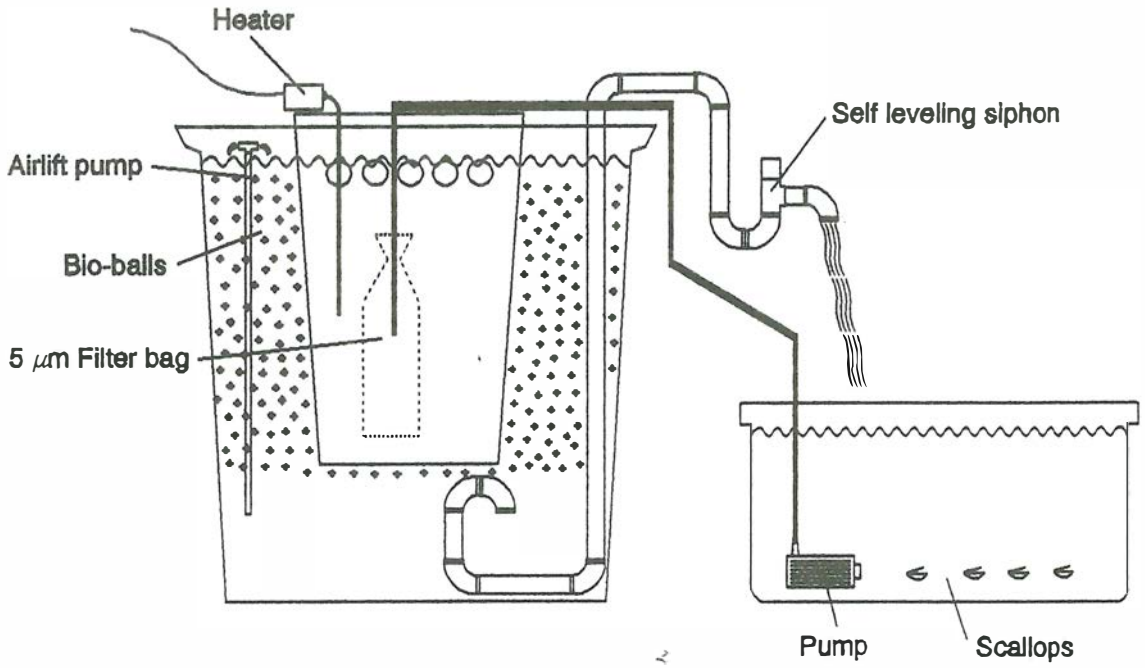


Fig 5.13.3 The effect of temperature on the consumption of five algal species by *P. fumatus*

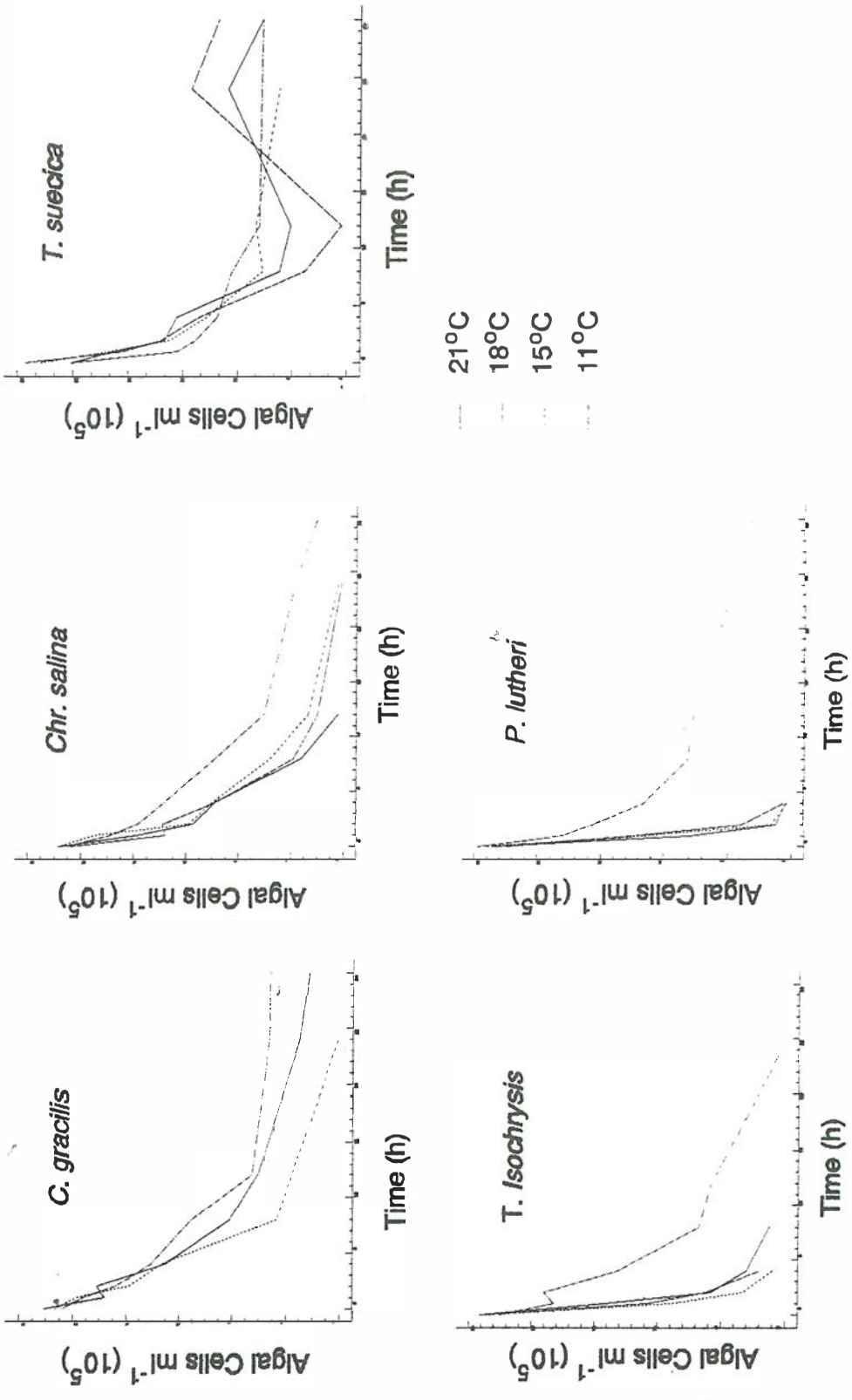


Fig 5.13.4 Cummulative consumption of five algal species used as food for *P. fumatus* on a dry weight and cell volume basis

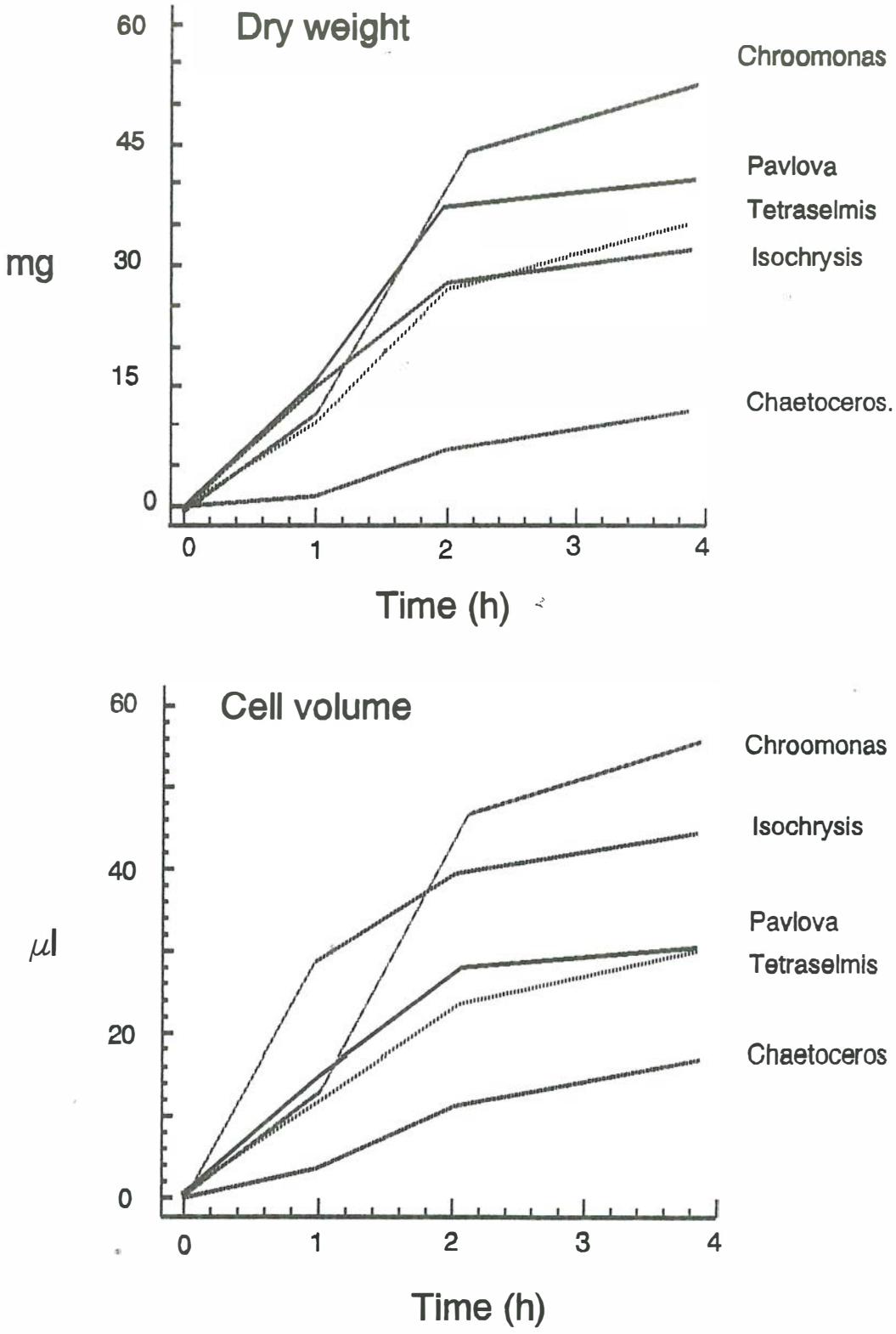


Fig 5.13.5 a. Mean number of eggs released over a six week period . b. Mean number of unplanned spawnings per animal over a six week period . c. Mean final gonad size. d. Mean final gonad condition. I is the mean initial index level.

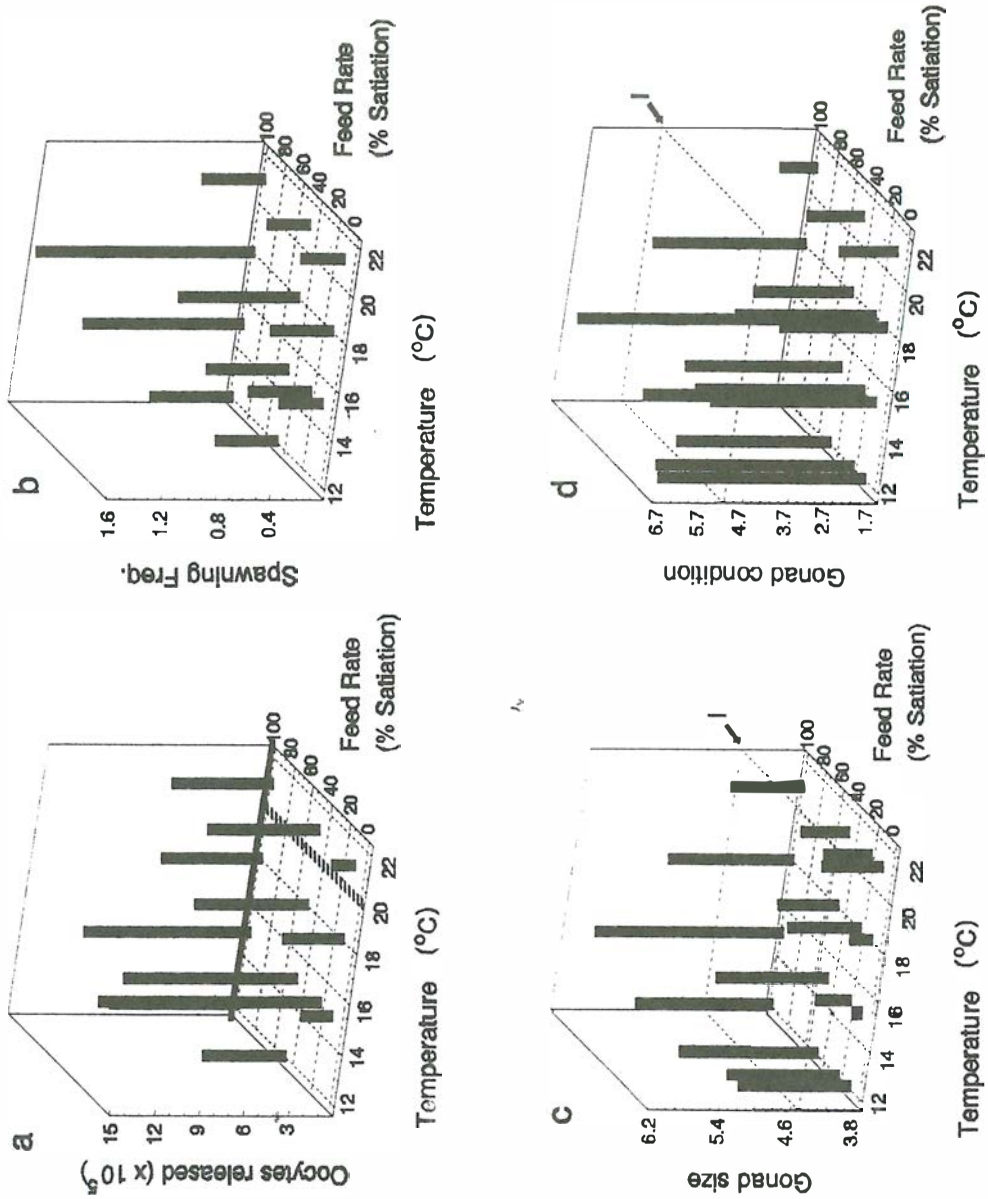


Fig 5.13.6 The effect of temperature on gonad condition and size in P. *fumatus* broodstock fed at the 100% satiation rate

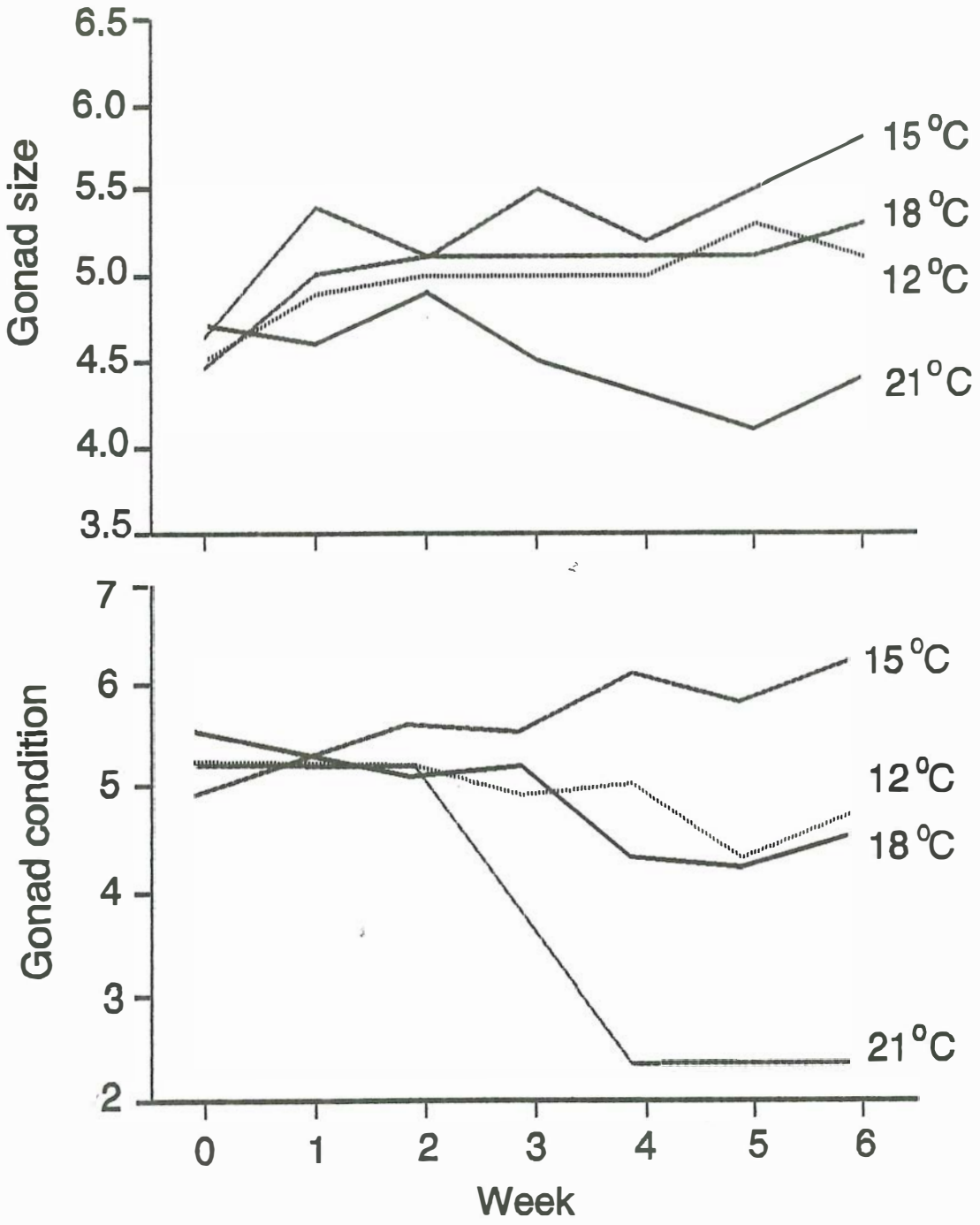
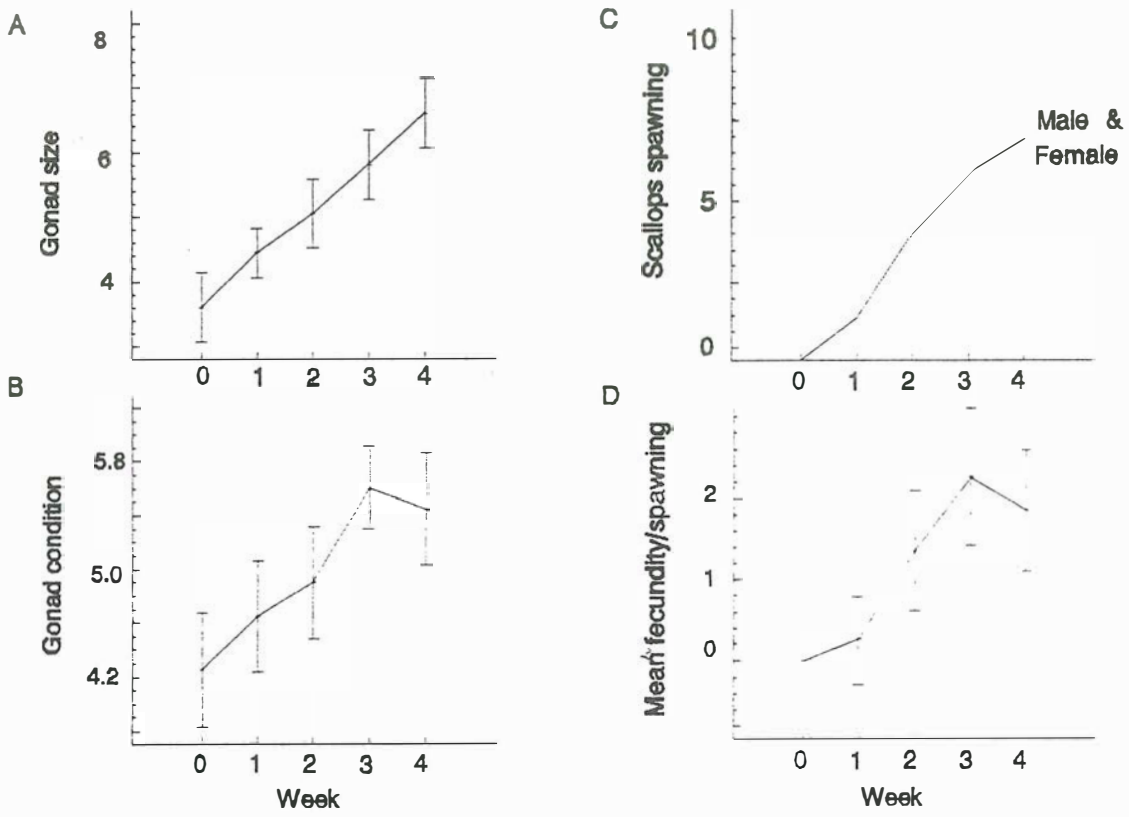


Fig 5.13.7 Mean gonad size, condition, fecundity and response to thermal spawning stimuli of twenty scallops sampled weekly from recirculating conditioning systems (± se).



5.2 Development of improved fertilization and incubation protocols

5.21 Development of fertilisation and incubation protocols to maximize the quality and yield of scallop, *Pecten fumatus* Reeve, larvae.

Abstract

Variable quality and yield (percentage development from eggs) of commercial scallop *Pecten fumatus* D-veligers prompted assessment of fertilisation and incubation protocols. Various sperm to egg ratios were tested on eggs suspended in seawater at different densities. Ratios of 1000:1 led to the highest D-veliger yield when eggs were incubated in suspension at 1/ml. With increasing egg densities, the addition of 1000 sperm/egg led to increasing average numbers of sperm visible at the periphery of each egg, indicating fewer sperm were necessary for fertilisation at higher egg densities. The time period and temperature over which released gametes were stored before fertilisation were also found to significantly affect D-veliger yield. Decreasing gamete storage temperature from 26 to 14°C increased D-veliger yield, as did a reduction in gamete storage period from 6 to 1 h. The incubation of embryos at densities in the range 5 to 50/ml did not affect D-veliger yield. A significant increase in total bacterial counts in the culture water occurred with increasing embryo stocking densities. Presumptive *Vibrionaceae* counts did not however increase significantly with increasing embryo stocking densities.

In a comparison of the viability of self and cross-fertilised embryos and larvae, fewer self-fertilised embryos developed to D-veliger stage, although survival did not differ significantly in subsequent larval rearing. Cross-fertilised larvae had attained a significantly larger size by day seven.

Introduction

Variations in growth and survival of hatchery reared scallop larvae have been attributed to many factors (Samain et al., 1992), including the techniques used during fertilisation and incubation (Loosanoff and Davis, 1963; Gruffydd and Beaumont, 1970). Gamete densities, time elapsed between gamete release and fertilisation and water temperature, have all been shown to affect rates of fertilisation in molluscs (Galtsoff, 1964; Gruffydd and Beaumont, 1970; Hahn, 1989; Hadley, 1993). Additionally, in some hermaphroditic molluscs such as the Australian commercial scallop *Pecten fumatus*, self-fertilisation is often difficult to avoid. The time between shedding sperm and eggs in *P. fumatus* can be brief (a few minutes) and in some cases broodstock apparently shed sperm and eggs simultaneously (M.P. Heasman, W.A. O'Connor and A.W. Frazer, pers. obs., 1994). Reported effects of self-fertilisation on subsequent larval viability vary considerably between species. While Beaumont and Budd (1983) reported reduced growth rates in self fertilised *Pecten maximus* larvae, Wilbur and Gaffney (1991) found no differences in growth of self-fertilised and outcrossed larvae of *Argopecten irradians*.

Prior to the current study, attempts to culture *P. fumatus* Frankish et al., (1990) followed techniques used by for hatchery production of Sydney rock oysters (*Saccostrea commercialis*) Frankish et al., (1991) or pacific oysters (*Crassostrea gigas*)(Cropp and Frankish, 1989). Using such techniques, the eggs of *P. fumatus* were fertilised as soon as possible in seawater of a similar temperature to that used during spawning, typically 24 to 26°C. Sperm to egg ratios were determined on the basis of the average number of sperm visible at the periphery of eggs and were adjusted until the ratio fell within the range 1:1 to 5:1. Embryos were stocked into tanks at approximately 5/ml.

Scallop larvae were produced using these methods although there was a high degree of variability in the production of D-veliger larvae (Heasman et al., 1994a). The aims of this study were to optimise techniques used to fertilise and incubate scallop eggs and to assess the impact of self fertilisation in this hermaphroditic species.

2. Materials and Methods

All *P. fumatus* brood stock (45-70 mm shell height) used were collected from Jervis Bay, NSW, (35°04' S, 150°44' E) by divers and maintained in a conditioning system (see Section 5.13) for a minimum of six days before experimentation. With the exception of Experiment 4, spawnings conducted during this study were induced using temperature fluctuations (after Gruffydd and Beaumont, 1970). Unless otherwise specified, all seawater was collected from a coastal site (35 g/kg salinity), filtered to 1 μm, treated with disodium ethylene diamine tetra-acetic acid (Na_2EDTA, 1 mg/kg; Utting and Helm, 1985) as a precaution against metals contamination and adjusted to a temperature of 20°C.

Experiment. 1: Sperm to egg ratio

Three separate trials were conducted to investigate effects of varying sperm and egg ratio. In each of the first two trials, eggs from three scallops were pooled and stocked at 1/ml into 1 l of gently aerated seawater in cylindro-conical containers constructed from inverted plastic soft drink bottles. Sperm from three scallops were pooled and the number of sperm/ml of suspension was determined using an Improved Neubauer haemocytometer. Sperm were added to each container within 15 min of release. In trial 1, sperm were added at a rate of 1, 5, 10, 20, 50, 100, 500 or 1000 sperm/ml. In trial 2, sperm were added at 500, 1 000, 5 000, 10 000 or 50 000/ml. In both trials a control treatment in which no sperm added, was included to assess the level of self fertilisation or extraneous fertilisation. In both trials, four replicate containers were used for each sperm concentration and control treatment. After 48 h, each container was emptied and the D-veligers retained on a 45 μm nylon mesh sieve. The number of D-veligers in each replicate was determined at 100x magnification using a stereo-microscope and a Sedgewick Rafter slide.

The objective of the third trial was to determine the effect of absolute egg density *per sé* on fertilisation success. To do this, individual scallops releasing eggs were removed from the

spawning table, rinsed and placed in one litre of fresh seawater. Following each successive release of eggs, the scallops were again rinsed and moved to a new container of sea water. Each successive batch of eggs was immediately transferred to a 15 μm nylon mesh sieve, rinsed and resuspended in fresh seawater. Each batch was then inspected microscopically and only those devoid of sperm retained. Sperm free eggs were then stocked into 1 l plastic containers at various densities in the range 10 to 1000/ml of seawater. Four replicates were prepared at each stocking rate. Sperm were then added to each container to provide a sperm to egg ratio of 1000:1, identified as near optimal from the results of the second trial. Fifteen minutes after the addition of sperm, egg samples were collected from each container and fixed in a 2% formalin seawater solution. Each sample was later examined at 100x to determine the number of sperm in a single plane of view at the periphery of 10 eggs chosen at random. Each container was then left for 3 h before determining the percentage fertilisation. This entire procedure was repeated on four occasions.

Experiment 2: Sperm and egg age and the effect of temperature on fertilisation

In a factorial design experiment, suspensions of sperm and eggs in seawater were stored separately at one of four temperatures, 14, 18, 22 or 26°C. Within each temperature, eggs and sperm were combined 1, 2, 3, 4, 5, or 6 h after spawning. Eggs were stocked at 100/ml in 10 ml of seawater in plastic test tubes and were fertilised by the addition of sperm at the rate of 1000 sperm/ml. At the time of sperm addition, a separate sample was taken and percentage sperm motility estimated. Four replicate test tubes were used at each time of fertilisation and temperature combination. Control treatments in which eggs were sampled at each of the above time intervals, but not fertilised, were also replicated in 4 test tubes to test for self or extraneous fertilisation. Following the addition of sperm to eggs, the eggs were incubated at 18°C for 2 h, at which time percentage fertilisation was determined on the basis of the number eggs having developed a polar body or undergone cell division.

An additional trial was conducted to investigate the effects of egg and sperm storage

temperatures. Egg concentrations, sperm to egg ratios and incubation protocols were the same as those described for the previous trial. Eggs held at 26°C for 4 h after spawning were mixed with sperm stored at 14°C and vice versa. Eggs were then incubated at 14 or 26°C for 2 h and the rate of fertilisation determined as previously described.

Experiment 3: Incubation

P. fumatus embryos were stocked at densities of either 1, 5, 10, 20, or 50/ml seawater in 1 l aerated cylindro-conical containers. Four replicate containers were used for each treatment. The number of D-veligers present after 48 h was determined as in Experiment 1. Total bacterial numbers and numbers of presumptive *Vibrionaceae* in each replicate were also determined after 48 h using marine agar and thiosulphate-citrate-bile-salt-sucrose (TCBS) plates.

Experiment 4: Development, growth and survival of self fertilised and outcrossed larvae.

Sexually mature scallops were placed in individual 1 l cylindro-conical containers as described for Experiment 1. They were then induced to spawn using a combination of intragonadal injection of 10^{-4} N serotonin solution (creatinine sulphate complex, $C_{14}H_{21}N_5O_6S.H_2O$, Merck, Darmstadt, Germany) and 2 to 4 thermal cycles in which temperature was raised 3-4°C and then returned to ambient. A total of 11.8×10^6 sperm free eggs from four scallops were collected as described in Experiment 1 and pooled, cross-fertilised, and divided equally into two 90 l cylindro-conical tanks. Following individual spawnings, a total of 10.8×10^6 self fertilised eggs from four scallops were pooled and also divided equally into two 90 l cylindro-conical tanks. Self-fertilised scallops were subjected to microscopic observation to ensure that the number of sperm present was not excessive (<10 sperm per egg).

After 48 h, the 90 l tanks were drained and the number of D-veligers counted. These veligers

were then used to stock eight other 90 l tanks at a rate of 5 larvae/ml. Four tanks were stocked with outcrossed larvae and four with larvae from self fertilised eggs. Larval cultures were maintained for 11 days at 21 ± 1°C and handled identically. Larval culture water was changed every 2-3 days and the number of surviving larvae in each tank was counted. At water changes, the shell length of 50 larvae from each tank were measured.

Statistical analysis

Homogeneity of variance was confirmed using Cochrans test. Data from Trials 1 and 2 in Experiment 1 were log transformed and survival data from Experiment 3 was arcsine$^{0.5}$ transformed before analysis. Treatment effects in Experiments 1-4 were tested using single factor ANOVA (Sokal and Rohlf, 1981) and differences between treatments were detected using Student Newman Keuls tests (Winer, 1971).

3. Results

Fertilisation.

Single factor ANOVA confirmed that the number of sperm used per egg during fertilisation significantly affected the numbers eggs developing to D-veliger stage ($F= 12.9$, $df= 23$, $P<0.001$ and $F= 15.1$, $df= 35$, $P<0.001$, For trials 1 and 2 respectively). The addition of sperm at ratios of less than 100:1 (Fig 5.21. 1a) and at the highest tested ratio of 50 000:1 (Fig 5.21.1b) failed to significantly increase D-veliger yields beyond the level observed in controls. In both trials 1 and 2, the greatest increase in D-veliger yield was recorded at a sperm to egg ratio of 1 000:1 (Figs 5.21. 1a & b). The number of sperm visible at the surface of each egg (Table5.21.1) was found to increase with increasing egg concentration when sperm to egg ratio was constant at 1000:1. Fertilisation was high provided that a minimum average of 0.7 sperm were visible in one plane of view on the surface of the eggs. Only in trial 3 did the percentage fertilisation differ significantly between treatments. In this case, fertilisation was significantly reduced ($P<0.05$). when numbers of visible sperm per egg fell at or below 0.4.

Fertilisation in *P. fumatus* decreased with increasing storage time of eggs and of sperm (Fig 5.21.2). Reducing gamete storage temperature prolonged the period over which fertilisation could be achieved. After 4 h, sperm held at 26°C was unable to fertilise eggs held at either 14 or 26°C. By contrast, sperm held at 14°C for 4 h was still able to fertilise eggs stored for the same period at 26°C. Fertilisation rate in the latter case was however significantly lower ($F = 83.15$, df 15, $P<0.001$) than when both eggs and sperm were stored at 14°C for 4 h.

Trends of decreasing sperm motility (Fig 5.21.3) and fertilisation rate (Fig 5.21. 2) with increasing storage time and temperature were generally similar. One notable differences was however evident between the effects of storage temperature and time on the motility of sperm as opposed to their capacity to fertilise eggs. At 26°C, sperm remained motile for one hour beyond the time at which fertilisation ceased. It is clear therefore that fertilisation ability is also limited by the viability of eggs and by sperm viability factors additional to motility.

Incubation.

Analysis of variance of the numbers of D-veligers produced when fertilised *P. fumatus* eggs were stocked at densities of from 5 to 100/ml indicated stocking rate had no significant effect upon D-veliger production ($F=2.546$, d.f.=19, $P>0.05$). Linear regression analysis was used to investigate the relationship between bacterial numbers and embryo stocking rates. Both total bacterial and presumptive vibrio counts were log transformed and homogeneity of variance was confirmed using Cochrans test (Winer, 1971). We found a significant increase in total bacterial numbers with increasing embryo stocking rates ($F= 6.67$, df= 19, $P<0.05$), however this trend was not evident in presumptive *Vibrio* counts ($F= 2.14$, df= 19, $P= 0.16$) (Table 5.21.1).

Self fertilisation.

Of the 10.8×10^6 self-fertilised eggs incubated, 2.6×10^6 (24%) developed to D- veliger stage. The 11.8×10^6 cross-fertilised eggs produced 5.57×10^6 D-veligers, representing a proportional yield of 47%, almost twice that of self-fertilised eggs. Faster growth of cross-fertilised D-veligers was apparent at the second water change (Day 4) and increased at each successive water change (Fig 5.21.4a). By Day 7 cross fertilised larvae were significantly

larger than self fertilised larvae (F=137.1, d.f. 7, $P<0.001$). Although, survival did not differ significantly between self and cross fertilised larvae, survival of self fertilised larvae remained lower throughout the experiment (Fig 5.21.4b).

4. Discussion

Fertilisation

Prior to this study, procedures for the fertilisation of *P. fumatus* eggs at our hatchery were based on those developed for routine production of the Sydney rock oyster *Saccostrea commercialis* (Frankish et al., 1991). These involved the exposure of scallops to temperature cycles ranging from ambient, typically 16 - 22°C, to temperatures 4 -6 °C above ambient. Upon spawning, scallops were removed to individual containers of seawater equal in temperature to that of the spawning table at the time of egg release. Sperm from several males were pooled, checked microscopically for motility and added until up to five sperm were visible at the surface of each egg. A delay of up to 1 h before eggs were fertilised often occured as gametes from a number of scallops were collected and pooled prior to fertilisation. Yields of D-veliger *P. fumatus* from eggs using these procedures were as poor as 10% and were commonly between 25 and 35%.

In view of the results of the present study, spawning and fertilisation protocols have been amended. Gamete storage and fertilisation in *P. fumatus* should be confined to temperatures below about 22°C and preferably around 14°C. As optimum temperature for reproductive conditioning of Jervis Bay *P. fumatus* is 14 to 15°C (Heasman et al., in prep.) the clear implication is that thermal induction of spawning be confined to a base temperature of 14 to 15°C and an upper temperature of about 22°C. In practice, regardless of the upper temperature in the thermal cycle, spawning scallops are now placed seawater of temperature <18°C. Sperm suspensions in particular are stored at reduced temperatures because, like sperm of the hard clam *Mercenaria mercenaria* (Hadley, 1993), sperm of *P. fumatus* retains viability longer at reduced temperatures. In addition, we have observed sperm is most often released before the eggs and it is commonly stored longer increasing the importance of suitable storage temperatures. Fortunately, the transfer of *P. fumatus* to cooler water following the

commencement of spawning does not interupt the continuation of spawning .

Fertilisation is now implemented as soon as practicable (generally within 20 min) after spawning. Serotonin creatinine sulphate, a fast efficient inducer of sperm release in *P. fumatus* (Heasman et al., in press) can be used to ensure that fresh sperm from several males is available to fertilise eggs. This procedure also avoids protracted storage of eggs prior to fertilisation. Based on the results of this study, the assumption that sperm motility is an indicator of fertilisation ability in *P. fumatus* appears to be sound. However, as with the doughboy scallop *Chlamys asperrima* (O'Connor and Heasman, submitted) sperm were found to remain motile in this study in some treatments beyond the time at which fertilisation ability was lost. In common with *C. asperrima*, sperm storage temperature significantly affected fertilisation success in *P. fumatus*. Sperm stored at 26°C for 4 h failed to affect fertilisation beyond control levels while sperm stored at 14°C remained viable.

Due to the number and variety of factors influencing fertilisation we consider it inappropriate to recommend a sperm to egg ratio for routine use. Rather we recommend continued adherance to a procedure of adding small volumes of sperm suspension to eggs until a minimum number of sperm are visible in one plane of view at the periphery of the egg. The mean number of sperm considered appropriate previously was up to five (Frankish et al., 1991), however, we have found far fewer sperm are needed to achieve consistantly high percentage fertilisation in *P. fumatus* eggs. For practical purposes an average of 0.7 - 1 sperm per egg is sufficient. However, on the basis of our data (Experiment 1, Trial 3) it does not appear that the use of additional sperm in the range used previously was in any way deleterious.

Incubation

Recommended procedures and equipment for incubating mollusc embryos vary with species. Gruffydd and Beaumont (1970) and Bourne et al. (1989) have used unaerated vessels, permitting fertilised eggs of the scallops *Pecten maximus*, *Crassodoma gigantea* and *Patinopecten yessoensis* to form a monolayer on the floors of flat bottomed incubator tanks. Such conditions eliminate the risk of aeration causing negatively buoyant embryos to collect

in "wind-rows" around the edge of the tank. Other workers, such as Imai (1980), have gently aerated rearing vessels to suspend bivalve embryos without incurring such problems. As *P. fumatus* embryos remain in suspension when gently aerated, we have chosen to use cylindro-conical tanks that enable higher concentrations of incubating eggs per unit volume of tank.

With regard to stocking, Imai (1980) suggested densities no greater than 30/ml. In the present study, densities as high as 50 embryos/ml were not found to significantly affect yields of normal D-veligers in *P. fumatus*. Higher egg stocking densities during incubation were found to be associated with increasing total bacterial counts. Although these elevated bacterial counts did not affect percentage development to D-veliger stage, they may impair further larval development directly or indirectly through altered species composition within the microflora. Counts of presumptive *Vibrio* bacteria, some species of which can have a teratogenic effect upon oyster embryos (Brown and Losee, 1978), and which commonly cause serious losses of *P. fumatus* larvae (see Section 5.3) did not increase with increasing egg density to 50/ml. For practical purposes we have not found it necessary to stock eggs at densities greater than 20/ml during routine hatchery operations.

Self fertilisation

To study the effects of self fertilisation in *P. fumatus* we chose to use inadvertently self fertilised eggs, rather than deliberately collecting and mixing sperm and eggs from the same scallop. Although the later technique allows greater control, we chose the former as a means of investigating the consequences of self fertilisation, as it is both common and difficult to avoid during routine spawnings with *P. fumatus*. In order to assess effect of self fertilisation rather than polyspermy, the use of self fertilised eggs was confined to batches in which low to moderate numbers of sperm (1-10) were observed at the periphery of eggs. We have observed that with *P. fumatus,* self fertilisation as indicated by polar body formation commonly occurs within 5 min of spawning and may occur even before eggs are expelled by the scallop. Consequently, polyspermy may still be a factor in batches of self fertilised eggs with apparently low numbers of attached sperm, such as used in our trials. This may have exacerbated the levels of abnormal development and caused the reduced D-veliger yields observed in the self-fertilised cultures.

For example, Stephano and Gould (1988), found greater numbers of polyspermic oyster eggs when fertilisation occurred 5-15 min after release than when the eggs were stored for 1-1.5 h before fertilisation. They also found a high correlation between the percentage of polyspermic eggs and the percentage of eggs with abnormal first cleavage. Survival of self fertilised larvae beyond development to D- veliger in our trials did not differ significantly from that of cross fertilised larvae, although reduced growth of self fertilised larvae would seem to indicate that self-fertilisation, independant of polyspermy, was yielding inferior larvae. Similar results indicating the inferiority of self-fertilised larvae have been obtained with the mussel *Mytilus galloprovincialis* (Beaumont and Abdul-Matin, 1994) and *P. maximus* (Beaumont and Budd, 1983).

Conclusions

Routine operational protocols of the hatchery have now been amended to incorporate the findings of the present study and those of associated investigations of temperature upon incubation, larval rearing, metamorphosis and early juvenile growth of *P. fumatus*. As previously stated, the base temperature for thermal induction of spawning has been changed from ambient (14 - 24°C) to a prescribed level of 14 -15°C, while the upper temperature to which broodstock are exposed to induce spawning has been reduced from 26 to 22°C. Shed gametes are now stored at 14 to 15°C. Egg batches contaminated with sperm at the time of collection are discarded on the assumption that they contain a significant proportion of inferior self-fertilised eggs. Fertilisation is now undertaken with quantities of sperm sufficient to elicit a minimum of 0.7 and a maximum of 2 sperm visible in one plane of view at the periphery of the egg. Fertilised eggs are incubated in aerated suspension at 18°C.

These amended procedures have collectively improved yields of normal D-veligers from former highly variable percentages of 10 to 40% to a higher and more consistent range of 40 to 60%.

Table 5.21.1 The effect of egg numbers on the mean (±se) number of sperm[1] visible at the surface of the egg and upon the mean (±se) percentage fertilisation.

	Trial 1		Trial 2		Trial 3		Trial 4	
Eggs/ml	Sperm/egg	Fert	Sperm/egg[2]	Fert[3]	Sperm/egg	Fert	Sperm/egg	Fert
1000	8.1±0.9^{a}	96.0±1.6^{a}			4.0±0.7^{a}	94.6±1.8^{a}	3.1±1.0^{a}	84.0±3.0^{a}
500	5.8±0.6^{b}	96.5±1.1^{a}	2.8±0.2^{b}	89.0±3.0^{a}	1.1±0.1^{b}	87.3±2.4^{a}	1.2±0.2^{b}	87.0±4.1^{a}
250	4.5±0.5^{b}	94.5±2.1^{a}						
125	2.1±0.4^{c}	94.5±1.8^{a}						
100			1.2±0.1^{b}	84.0±2.9^{a}	0.4±0.1^{b}	79.3±3.5^{b}	0.7±0.1^{b}	81.0±4.1^{a}
60	1.4±0.2^{c}	93.5±2.5^{a}						
30	1.5±0.3^{c}	94.5±1.3^{a}						
10			0.7±0.0^{b}	86.5±2.4^{a}	0.2±0.1^{b}	74.0±5.3^{b}	0.3±0.1^{b}	76.0±4.2^{a}

1 Sperm to egg ratio fixed at 1000:1 for all trials.
2 Sperm/egg indicates the mean number of sperm visible at the periphery of the egg when viewed at 100x magnification in a fixed plane of view.
3 Fert indicates mean fertilisation percentage, determined from the number of eggs extruding polar bodies or having begun cell division. Means within columns with common superscripts do not differ significantly ($P<0.05$).

Table 5.21.2 The effect of egg stocking rates during incubation on the yield of *Pecten fumatus* D-veligers, total bacterial and presumptive numbers.

Stocking density (Eggs/ml)	% D-veliger yield[1,2]	Total bacteria[1] ($x10^5$/ml)	Presumptive *Vibrios*[1] (x100/ml)
Control	-	0.9 ± 0.22	< 1
5	41.5 ± 3.1	2.3 ± 0.19	42.15 ± 16.88
10	36.8 ± 5.3	6.4 ± 1.48	36.25 ± 14.65
20	39.5 ± 5.9	3.0 ± 0.41	10.42 ± 7.86
50	41.5 ± 5.2	5.6 ± 1.45	36.85 ± 13.89

1 Values are means ± se.
2 Yield is the percentage of eggs developing to D veliger stage.

Fig 5.21.1 The effect of sperm to egg ratio on the number of eggs developing to D-veliger stage. Values are means ± se.

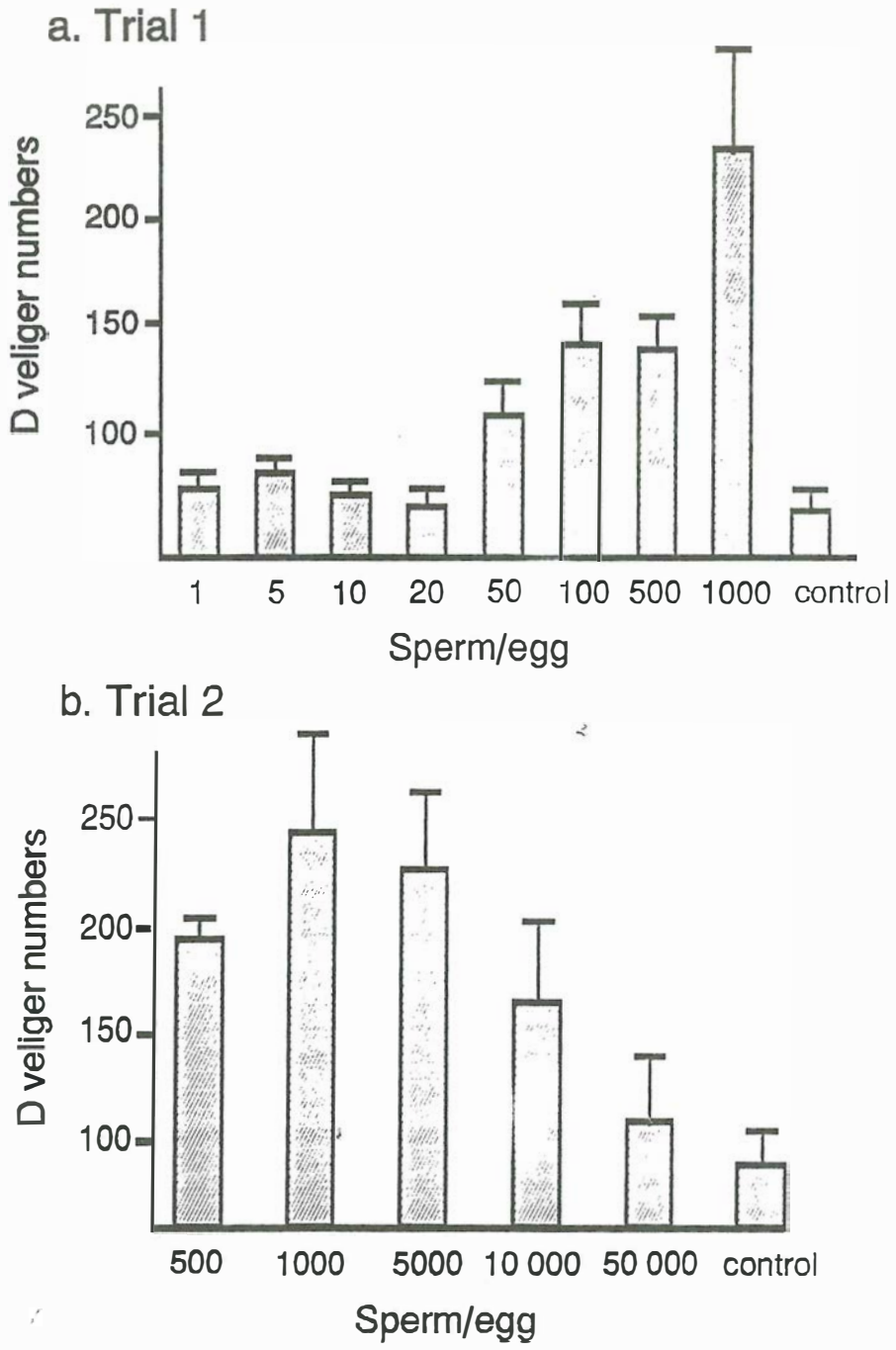


Fig 5.21.2 The effect of gamete storage temperature and time upon mean fertilisation (±se) in the scallop *Pecten fumatus*. The horizontal line line represents the mean fertilisation in the control. a, b, c and d represent the mean egg fertilisation ± se using sperm and eggs stored at 14°C, sperm stored at 14°C and eggs at 26°C, sperm stored at 26°C and eggs at 14°C and sperm and eggs stored at 26°C, in that order.

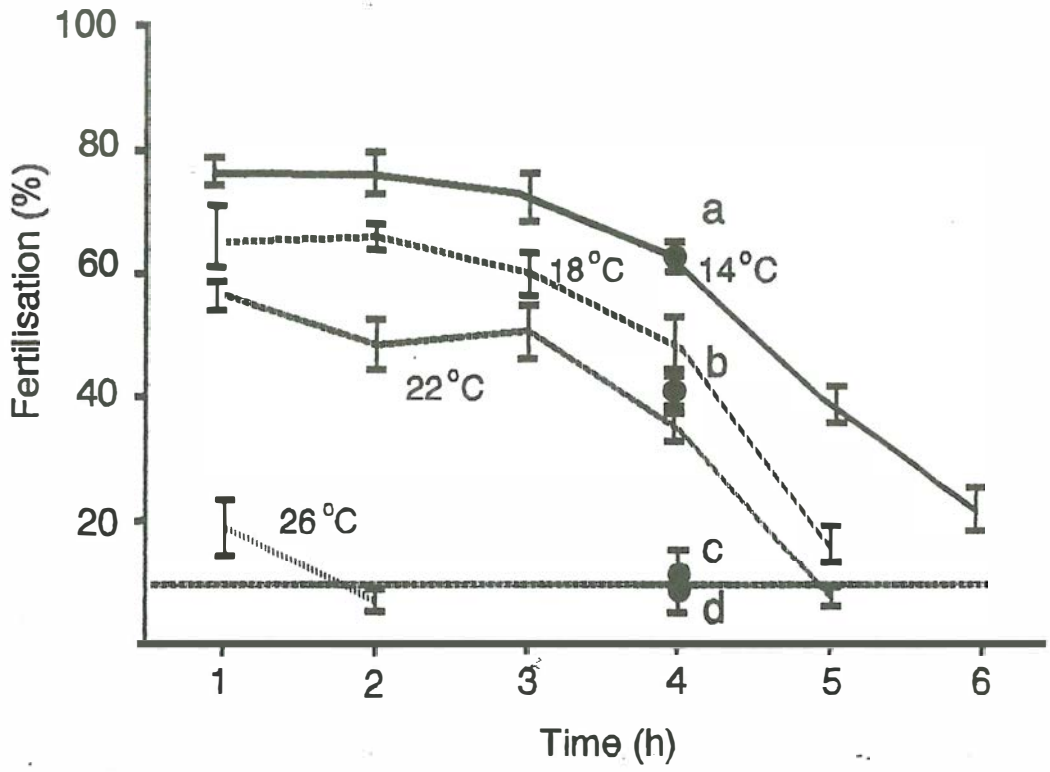


Fig 5.21.3 The effect of time and temperature upon sperm motility in the scallop *Pecten fumatus*.

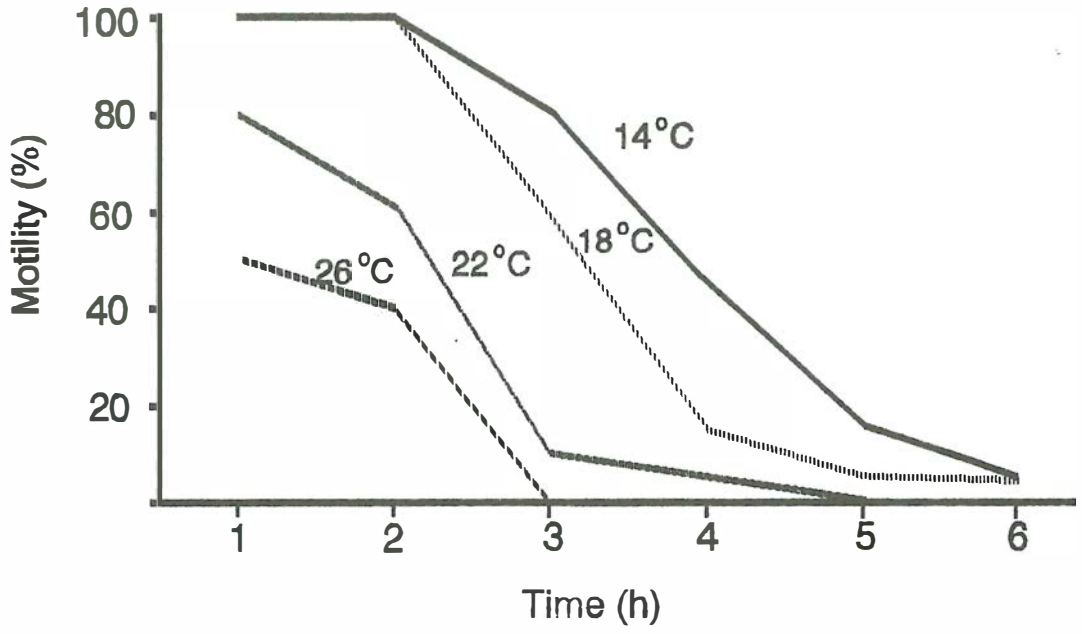
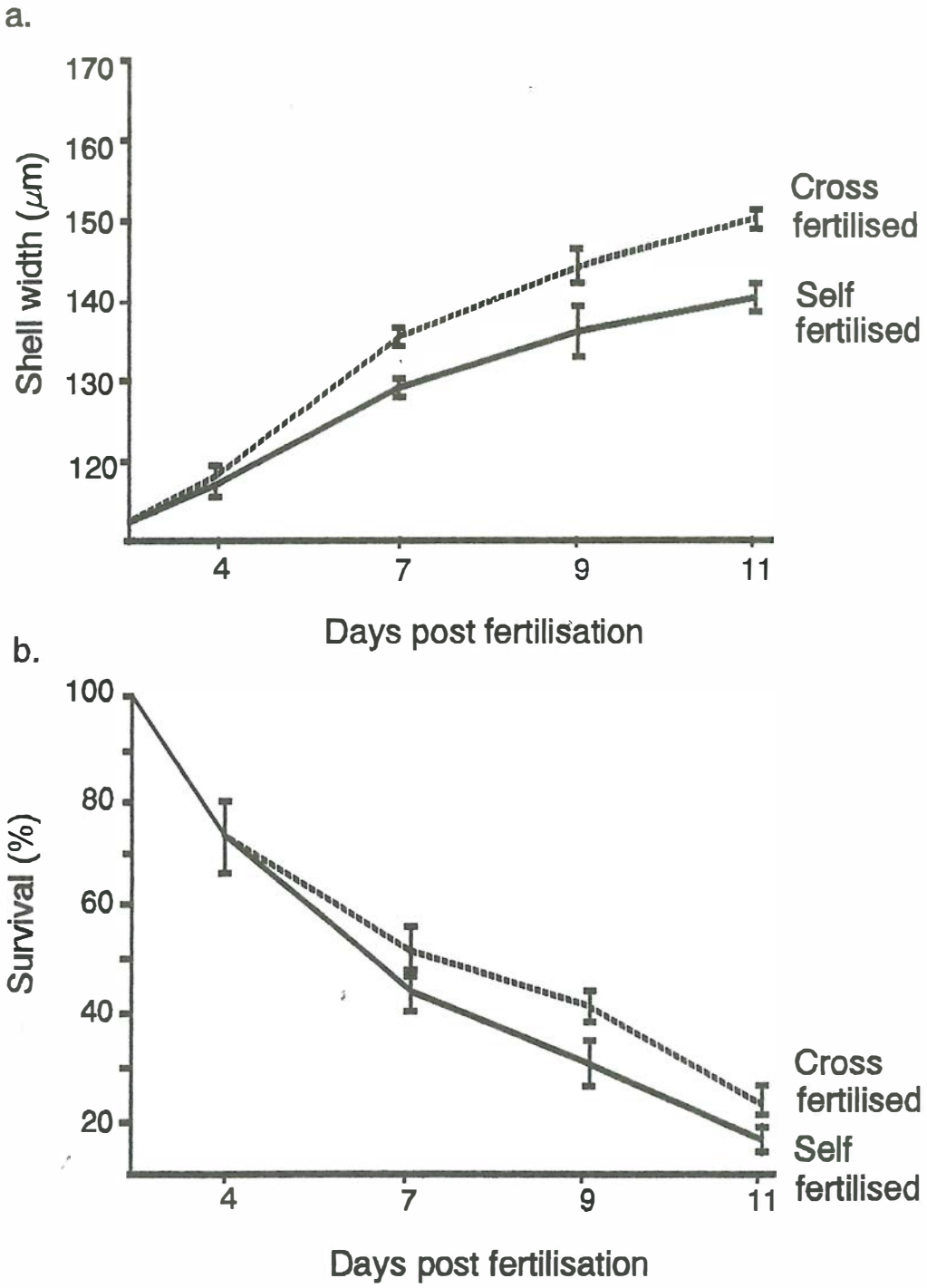


Fig 5.21.4 Growth (a) and survival (b) of self-fertilised and outcrossed *Pecten fumatus* larvae.



5.22 Growth and survival of commercial scallop *Pecten fumatus* larvae fed various algal diets.

Abstract

Growth and survival of larvae of the commercial scallop *Pecten fumatus* held at two temperatures, 16 and 22°C, and fed one of eight algal species was compared with that of unfed larvae or those fed a reference diet originally developed for rearing Sydney rock oyster larvae. The latter comprised equal amounts of *Pavlova lutheri*, Tahitian *Isochrysis* and *Chaetoceros calcitrans*. Growth on all monospecific diets, including *P. lutheri,* was considerably lower than a rate of 7.3μm/day achieved on the reference diet. Age specific survival was unaffected by temperature. However, because growth rates were generally much higher at 22° C than at 16° C, size specific mortality was much lower at 22°C, which is accordingly recommended for routine hatchery operations. *P. lutheri*, was subsequently fed in combination with each of the other eight species and growth and survival at 22°C determined. When fed in combination with *P. lutheri*, the only algal species to produce synergistic increases in growth were T. *Isochrysis* and *C. calcitrans*. With both single and combination diets, a significant negative correlation was found between growth and mortality. Eight dietary regimes all involving *P. lutheri*, *T. Isochrysis* and *C. calcitrans* but fed in differing combinations and sequences were also tested over a twelve day period. Larvae fed *P. lutheri* for three days before being fed the reference diet for nine days were significantly larger than siblings initially fed T. *Isochrysis* or *C. calcitrans.* Increasing the frequency with which all three species were fed produced greater larval shell length increases. Daily feeding of all three algal species, as traditionally practised at the Port Stephens Research Centre for larval rearing of all bivalves was thus vindicated in relation to *P. fumatus*..

Introduction

Attempts to produce significant numbers of commercial scallop (*Pecten fumatus*) in Australian oyster hatcheries during the 1980's have had mixed and usually disappointing results (Heasman et al., 1994a). A systematic series of studies was therefore undertaken to improve the reliability and efficiency of rearing of *P. fumatus* larvae by evaluating, and if necessary, refining, practices and equipment originally developed for oysters (Frankish et al., 1991).

Investigations dealing with the spawning induction, fertilization , incubation and larval rearing reported in sections 5.21 and 5.23 do not include the role of larval nutrition. The successful hatchery rearing of bivalve larvae is to a large degree dependant upon the nutritive value and physical properties of the food supplied during culture. As a result, a number of reports of the nutritive value of commonly used microalgal species to mollusc larvae have been made (Davis and Guillard, 1958; Walne, 1970). The interest in the culture of scallops has also led to the evaluation of a variety of algal species as food for scallop larvae (Whyte et al., 1989,1990; Samain et al., 1992). In Australia to date, attempts to culture *P. fumatus* larvae have involved the use of a variety of algal species without attempts to determine which are most appropriate for larval growth and survival or if the physical environment in which the larvae are cultured has an impact upon the choice of algal feed.

To decide which of the commonly available algal species should be used during further investigations of *P. fumatus* larval biology, a series of experiments was conducted to evaluate eight microalgal species alone or in combinationon larval growth and survival. These species were fed to larvae as a sole diet and in combination These test diets were evaluated against a reference diet comprising an equal blend of *Pavlova lutheri*, Tahitian *Isochrysis* and *Chaetoceros calcitrans*, formulated on the basis of previous research to improve larval rearing of Sydey rock oyster *Saccostrea commercialis* (Nell and O'Connor,1991).

The unpredictable supply of cultured algae (Coutteau and Sorgeloos, 1992) also prompted us to determine the whether or not each component of a the reference (mixed species) algal

diet needed to be fed daily or less frequently to ensure maximum growth and survival.

Materials and Methods

Scallop larvae

Scallop broodstock was obtained from Jervis Bay, NSW (35° 04'S, 150° 44'E) and spawned, using techniques as described in Section 5.21. Fertilised eggs were stocked at 5/ml in a gently aerated 90 l cylindroconical tank. After two days, D-stage scallop larvae were collected on a 45 μm nylon mesh sieve and stocked in 500 ml borosilicate glass conical flasks at a density of 5 larvae/ml. Larvae were cultured in a temperature controlled orbital shaker-incubator (Model 013422; Paton Scientific; Victor Harbor, South Australia) which rotated at 80 revolutions/min. Seawater (35 g/kg) in the flasks was changed thrice weekly, with the larvae retained on a 45 μm sieve. Throughout the study seawater was filtered using 0.2 μm cartridge filters (Cuno Pacific, Sydney, NSW) to reduce the background level of potential food items in the water. Larvae were supplied daily with 0.475 mg/l (dry weight) of the algal species or combination of species listed in Tables5.22.1 and 5.22.2. Algal cell dry weights were taken from Nell and O'Connor (1991) and O'Connor et al. (1992).

Algal culture techniques

Algal cultures were grown axenically in 2 l borosilicate glass flasks. All species were cultured in oceanic water (salinity 34-35 ‰) at 21±1°C. The nutrient medium, autoclaved f/2 beta (Guillard, 1983), was used without the addition of $Na_2SiO_3.5H_2O$. Cultures were illuminated with "White, Wattsaver" fluorescent tubes (Osram, Sydney, NSW) to an intensity of 4 000 Lux at the container surface using a 16:8 h light:dark cycle. All algal cultures were harvested in logarithmic phase of growth. In all experiments, a minimum of three batch cultures for each algal species were used. In Experiment 2, a total of five batch cultures of *P. lutheri* used over the full duration of the trial.

Experiment 1 - single algal species diets

Eight algal species (Table 5.22.1), commonly used in bivalve hatcheries were fed singly to scallop larvae cultured at one of two temperatures, 16 and 22°C. An unfed control treatment was included as was a mixed algal diet originally developed for larval rearing of the Sydney rock oyster (*Saccostrea commercialis*) based on the findings of Nell and O'Connor(1991) and subsequently adopted as a standard diet for rearing of a range of other bivalves including clams (Nell et al.,1993) and mussels, at the Port stephens Research Centre (PSRC). The latter reference diet comprises equal amounts on a dry weight basis of *P. lutheri, Chaetoceros calcitrans* and Tahitian *Isochrysis* aff. *galbana.*

Experiment 2 - combined algal species diets

P. lutheri , the single algal species promoting greatest increase in mean shell length in Experiment 1, was fed on an equal dry weight basis with each of the other 7 remaining species also evaluated in Experiment 1 (Table 5.22.2). All flasks were maintained in the shaker incubator at 22°C which had been shown in Experiment 1 to promote more rapid growth than 16°C. As in Experiment, an unfed control treatment was included as was the reference diet comprising equal amounts of *P. lutheri, Chaetoceros calcitrans* and Tahitian *Isochrysis* aff. *galbana.*

Experiment 3 - feeding regimes

The three species that produced synergistic growth increases in Experiment 2, T. *Isochrysis, C. calcitrans* and *P. lutheri,* were fed in different sequences and combinations over ten days (Table 5.22. 1). Chloramphenicol was added to the culture water at a low dose rate (1 mg/L) to ensure larval survival for the duration of the experiment (Heasman et al.,1994a) and to compensate for the loss of survival promoting factors removed by 0.2μm membrane filtration as discussed in Section 5.3. As in Experiments 1 and 2 an unfed control treatment was

included.

All experiments had four replicates per treatment l. Experiments 1 and 2 were terminated after seven days, while, Experiment 3 was continued for an extra three days to increase treatment effects. After each experiment, the larvae were preserved in seawater containing 2% formalin. The lengths (greatest shell length parallel to the hinge) of 50 veliger larvae per replicate flask were measured using a microscope and an ocular micrometer (±2.5 μm). For Experiments 1 and 2, the number of dead larvae (empty shells or shells containing necrotic tissue) encountered during the measurement of fifty larvae was expressed as a percentage and used as a measure of larval mortality.

Statistical analysis

Larval growth data was log transformed and homogeneity of variance was confirmed using Cochran's test (Winer, 1971). Larval percentage survival data was arcsin $x^{0.5}$ transformed (Sokal and Rohlf, 1981). Transformed data from Experiment 1 were analysed using two factor anova to examine the effects of algal species and temperature (16 and 22°C) and to determine if there was a significant interaction between the two factors. For Experiments 2 and 3, transformed data were analysed using a single factor analysis of variance. In both experiments, means were compared using the Student-Newman-Keuls procedure (Winer, 1971). Statistical analyses were conducted with the assistance "Statgraphics" computer based statistical package (Statistical Graphics Corporation, Rockville, MD).

Results

Experiment 1 - monospecific algal diets

When fed monospecific diets, a significant interaction ($P<0.05$) was found between effects of diet and temperature upon larval growth. Growth on all monospecific diets was

considerably lower than the maximum rate of 7.3μm/day achieved with the reference diet of *P. lutheri*, T. *Isochrysis* and *C. calcitrans* (Table 5.22.1). Of all monospecific diets, *P. lutheri* promoted fastest growth rate of 5.9 μm/day at *22°C*. As expected, unfed larvae grew the least.

Growth on *P. lutheri* was more than 50% greater than growth rate on the next two best monospecific algal diets, *C. calcitrans* and T. I*sochrysis* and 2 to 4 times faster than that on any of the remaining algal species. The four poorest performing monospecific diets, *Nannochloris atomus, Thalassiosira pseudonana, Tetraselmis suecica* and *C. gracilis,* failed to produce significantly greater growth than unfed controls. Larvae fed either *P. lutheri*, T. *Isochrysis* or *C. calcitrans* had the lowest mortality. Larval growth rate at 22°C was higher that at 16°C for both the reference diet and all nine monospecific algal diets tested and about twice as fast in most (7 out of 9) cases. Growth rate at 22 °C was in fact about twice that at 16 °C in with the reference diet and with 7 of the 9 monospecific diets.

As no significant difference (P>0.05) was found between survival of larvae reared at 16 and 22°C, the data were pooled for comparison of diet effects on survival. A significant negative correlation was found between larval shell length increase and larval mortality (r=-0.88). Larvae on diets that produced the greatest shell length increases, such as *P. lutheri* , T. *Isochrysis* and *C. calcitrans*, suffered much lower mortality than those fed poorly performing diets such as *Nannochloris atomus, Thalassiosira pseudonana* or *Tetraselmis suecica.* More than half the diets supported significantly greater (P<0.05) survival than unfed controls (Table 5.22.1)in which mortality averaged 30.4%.

Experiment 2 - combined algal species diets

Larvae fed combined diets made up of T. *Isochrysis* or *C. calcitrans* in combination with *P. lutheri* produced significantly greater (P<0.05) increases in shell length than larvae treated with solely *P. lutheri* (Table 5.22.2). While all diets produced significantly greater growth than unfed larvae, only those including the diatoms *C. calcitrans, Th. pseudonana* and *Skeletonema costatum,* produced significantly greater (P<0.05) survival. As with larvae fed a

single algal species, larvae fed a combination of algal species showed a significant negative correlation between larval shell length increases and larval mortality (r=-0.66) i.e. survival was positively correlated with growth rate.

Experiment 3 - feeding regimes

The results of Experiment 3 are presented in Table 5.22.3. Larvae fed *P. lutheri* for 3 days before switching to the reference diet were significantly larger ($P<0.05$) after 10 days than their siblings initially fed either T. *Isochrysis* or *C. calcitrans* . Increasing the frequency with which larvae were fed the three species led to progressively greater increases in shell length after 10 days, except for Dietary regime 4 (Table 5.22.3). Although each algal species was fed singly for three days in Diet 4, shell length increases were significantly greater than larvae fed a diet in which the algal species fed was alternated daily. This increase was attributed to the initial use of *P. lutheri* instead of *C. calcitrans*. The reference dietary regime in which all three species of algae were fed every day produced the best growth.
This advantage was however largely negated if daily feeding of all three species was not commenced from the outset of feeding. The relatively slow growth achieved on the reference feeding regime in Experiment 3(5.2μm/day) compared with 7.3μm/day achieved in Experiment 1 is ascribed to growth inhibiting effects of chloramphenicol as demonstrated elsewhere in this study (see Section 5.3).

Discussion

Microalgal diets of *P. lutheri* alone, or in combination with either or both of *Isochrysis* and *C. calcitrans* provided rapid growth and high survival of *P. fumatus* larvae. These same suite of algal species have previously been shown to be the best for rearing larvae of the Sydney rock oyster, *Saccostrea commercialis* (Nell and O'Connor, 1991). *P. lutheri*, which is an excellent food for scallop larvae (Peirson, 1983), contains high levels of both the 20:5n-3

fatty acid and 22:6n-3 fatty acids, whereas T. *Isochrysis* contains only high levels of the 22:6n-3 fatty acid (Brown et al., 1989). As both these fatty acids are essential for bivalve larvae (Enright et al., 1986a,b), a combination of these algal species which includes *P. lutheri* would meet the essential fatty acid of scallop larvae, although the quantity of these fatty acids in algal diets for scallop larvae need not be high (Whyte et al., 1990).

Although *P. lutheri* contains high levels of both the 20:5n-3 fatty acid and 22:6n-3 fatty acids, synergistic effects obtained with combined diets indicate that *P. lutheri* alone does not provide an optimum balance of all nutrients. This suggests that a correct balance of nutrients must be available for absorption to maximise growth, therefore a combined diet of *P. lutheri* with either T. *Isochrysis* or *C. calcitrans* should be fed daily to obtain maximal effect.

Table 5.22.1. Increase in shell length and survival of 2-day-old commercial scallop (*Pecten fumatus*) larvae[1] held at 16 o 22°C for 7 days and fed one of 10 algal diets. (Experiment 1).

Diet	Shell length increase after 7 days (μm)[2]		Percent[3] Mortality
	22°C	16°C	
P.I.C.[4]	51.5 ± 7.71^{a}	22.2 ± 9.19^{cd}	9.9 ± 7.6^{ab}
Pavlova lutheri	41.0 ± 6.70^{b}	18.0 ± 6.22^{cdc}	9.9 ± 4.7^{ab}
Chaetoceros calcitrans	26.5 ± 0.50^{c}	13.4 ± 1.00^{def}	6.0 ± 2.7^{a}
Tahitian *Isochrysis*	26.1 ± 1.16^{c}	14.0 ± 0.78^{def}	$12.1 \pm 5.9^{\pm ab}$
Tetraselmis chui	18.4 ± 3.36^{cde}	8.5 ± 1.30^{f}	13.7 ± 6.1^{ab}
Skeletonema costatum	18.0 ± 3.25^{cde}	9.2 ± 0.33^{ef}	13.1 ± 11.4^{ab}
Tetraselmis suecica	14.8 ± 2.81^{de}	7.7 ± 0.45^{f}	17.1 ± 9.7^{bc}
Chaetoceros gracilis	14.3 ± 1.62^{def}	8.5 ± 1.48^{f}	15.2 ± 11.2^{ab}
Thallassiosira pseudonana	11.8 ± 0.89^{ef}	8.6 ± 0.59^{f}	19.2 ± 5.8^{bc}
Nannochloris atomus	8.6 ± 0.59^{f}	7.2 ± 0.80^{f}	16.6 ± 6.4^{bc}
Unfed	7.6 ± 1.88^{f}	5.9 ± 0.82^{f}	30.4 ± 13.1^{d}

1 Values are means ± s.d. (n = 4).

2 For both temperatures, means with a common superscript letter do not differ significantly (P>0.05). Initial average length of larvae was 113.4 ± 2.4 μm (n = 50).

3 Mean larval survival, pooled for 16 and 22°C, means with a common superscript letter do not differ significantly (P>0.05).

4 *Pavlova lutheri*, Tahitian *Isochrysis* and *Chaetoceros calcitrans* fed on an equal dry weight basis.

Table 5.22. 2. Survival and increase in shell length of 2 day old commercial scallop (*Pecten fumatus*) larvae fed one of eight algal species in combination with *Pavlova lutheri* for seven days (Experiment 2).

Algal species	Shell length increase after seven days (μm)[1]	Percent mortality*
Pavlova lutheri\Tahitian *Isochrysis*	29.9 ± 0.83^{a}	22.1 ± 6.4^{abcd}
*Pavlova lutheri**Chaetoceros calcitrans*	29.2 ± 1.16^{a}	10.8 ± 4.4^{a}
*Pavlova lutheri**Thalassiosira pseudonana*	26.3 ± 1.48^{ab}	17.6 ± 4.2^{abc}
*Pavlova lutheri**Skeletonema costatum*	24.8 ± 2.25^{bc}	15.0 ± 6.0^{ab}
*Pavlova lutheri**Nannochloris atomus*	24.6 ± 2.24^{bc}	20.9 ± 7.3^{abcd}
*Pavlova lutheri**Tetraselmis suecica*	23.1 ± 2.19^{bc}	19.3 ± 3.4^{abcd}
*Pavlova lutheri**Chaetoceros gracilis*	23.1 ± 1.38^{bc}	21.6 ± 6.8^{abcd}
Pavlova lutheri	22.7 ± 1.58^{bc}	32.1 ± 8.4^{cd}
*Pavlova lutheri**Tetraselmis chui*	21.3 ± 0.96^{c}	8.1 ± 10.6^{bcd}
Unfed	10.5 ± 1.60^{d}	36.3 ± 6.6^{d}

[1] Values are means ± SD (n = 4). Means with a common superscript do not differ significantly (P>0.05). Initial average length of 2-day-old larvae was 110.6 ± 2.9 μm (n = 50).

Table 5.22.3. Increase in shell length of 2-day-old commercial scallop (*Pecten fumatus*) larvae fed one of eight dietary regimes for 10 days.

Diet	Day (post fertilisation)										Shell length increase
	3	4	5	6	7	8	9	10	11	12	(μm)[1]
Standard	PIC	PIC	PIC	PIC	PIC	PIC	PIC	PIC	PIC	PIC	51.9 ± 1.9^{a}
1	P	P	P	PIC	PIC	PIC	PIC	PIC	PIC	PIC	47.2 ± 1.1^{b}
2	CP	PI	IC	CP	PI	IC	CP	PI	IC	CP	47.1 ± 2.4^{b}
3	I	I	I	PIC	PIC	PIC	PIC	PIC	PIC	PIC	43.3 ± 0.9^{c}
4	P	P	P	I	I	I	C	C	C	P	41.8 ± 0.8^{cd}
5	C	P	I	C	P	I	C	P	I	C	40.9 ± 1.1^{cd}
6	C	C	C	PIC	PIC	PIC	PIC	PIC	PIC	PIC	40.2 ± 1.2^{d}
7	C	C	P	P	I	I	C	C	P	P	37.5 ± 1.7^{e}
Unfed	U	U	U	U	U	U	U	U	U	U	15.5 ± 0.6^{f}

1 Values are means ± SD (n = 4). Means with a common superscript do not differ significantly (P>0.05). Initial average length of 2-day-old larvae was 111.2 ± 2.65 μm (n = 50).

Legend P = *Pavlova lutheri*, I = Tahitian *Isochrysis*, C = *Chaetoceros calcitrans*, U = Unfed

5.23 Ontogenetic changes in optimum rearing temperatures for the commercial scallop, *Pecten fumatus* Reeve.

Abstract

Embryos, D veliger larvae and early juvenile stages of commercial scallops *Pecten fumatus* Reeve, were held at temperatures ranging from 13 to 27°C. An incubation temperature of 18°C produced the greatest yield of D veligers. Growth rate of larvae increased with increasing temperature from 2.5μm/day at 15°C to a peak of 6.5μm/day at 24°C but decreased with a further increase in temperature to 27°C. Age specific larval survival rates decreased significantly with increasing temperature in the range 15 to 27°C. However, size specific survival rate, a more meaningful measure of optimum rearing temperature, exhibited a pronounced peak value at an intermediate temperature of 21°C. On the basis of these results, the maintenance of larval rearing temperatures between 18 and 21°C is likely to provide the maximum survival of larvae to the pediveliger stage. The mean size of early stage spat (3 week post settlement; mean shell height 1.04± 0.26mm) on-reared in the hatchery, increased exponentially for 4 weeks at all temperatures tested. Growth increased from a negligible rate at 13°C to a maximum rate at 24° C. During the fifth and final week of the trial, a constraint to continued exponential growth became evident at all temperatures tested except 13°C. This result was consistent with previous observations of constrained growth of spat when maintained beyond a period of 6 to 8 weeks post-settlement (mean shell height of 2 to 4 mm.) on a standardised microalgal diet specifically developed for larvae. Survival and byssus attachment of spat was highest at temperatures supporting the highest growth rates. Possible ecological implications of these results are discussed in relation to the oceanography of south eastern Australia and variability in annual recruitment and catches. The use of byssus attachment as an indicator of favourable spat growing conditions is discussed.

Aims

The principal aim of this study was to identify optimal hatchery and nursery rearing temperatures for *P. fumatus*, embryos, larvae and spat by monitoring their growth and survival when exposed to a range of temperature representative of their natural distribution. This particular aspect of research complimented earlier investigations of the effects of temperature on reproductive conditioning, gamete storage, and fertilisation in *P. fumatus* (previously reported in Sections 5.21&5.22)

Introduction

While temperature is not the only important environmental factor influencing growth of scallops, it is one of the more measurable and controllable parameters. It also has a profound influence on survival and distribution of scallops (Nakanishi, 1977; Ventilla, 1982), including the commercial scallop *P. fumatus* Reeve (Young and Martin, 1989) both directly, through influences metabolic rate and survival (Nakanashi, 1977; Ventilla, 1982) and indirectly, through effects on the nutritional environment (Wallace and Reinses, 1985; Ito, 1991. Accordingly, seasonal temperature regimes critically influence the siting and seasonal timing of hatchery, farming and stock enhancement programs.

The geographical range of the Australian commercial scallop *P. fumatus,* as distinct from the closely related Western Australian species, *P. modestus,* Reeve 1852, (Woodburn, 1989) stretches south from central New South Wales (NSW) to Victoria, Bass Strait and Southern Tasmania and west to the Gulf of St. Vincent in South Australia. It is found at depths ranging from 7 to 60m on bottoms varying from muddy sand to coarse sand (Young and Martin, 1989). Mean monthly sea temperatures over the range of this hermaphroditic species vary from minimum winter values of 9 or 10°C in southern Tasmania to maximum Summer values

of 26°C on the central coast of NSW.

Materials and Methods

All embryos, larvae and spat used in the present study were obtained from broodstock collected by divers in Jervis Bay, NSW (35 04'S, 150 44'E) and road-freighted chilled to the hatchery within 12 h of capture as previously described. Reproductive conditioning and induced spawnings were conducted using methods described in Section 5.12. In all cases, sperm and eggs each from a minimum of five scallops were pooled before fertilisation to minimise effects of variable gamete viability.

Seawater (35 g/kg salinity) used in all experiments was filtered to 1 μm and contained 1 g/kg Na_2EDTA (disodium ethylene diamine tetra acetic acid). During Experiments 2 and 3, larvae and spat were fed mixed microalgal diets of *Pavlova lutheri* (Droop) Green, Tahitian *Isochrysis* aff. *galbana* Green (clone T.Iso) and *Chaetoceros calcitrans* (Paulsen) Takano, originally developed for the hatchery and nursery rearing of the Sydney rock oyster, *Saccostrea commercialis* (Nell and O'Connor, 1991; O'Connor et al., 1992).

Experiment 1. The effect of temperature on embryo development to D veliger stage

Eggs from five scallops were pooled in a 5 l glass beaker and thoroughly mixed with a perforated PVC plunger. The concentration of eggs within the beaker was estimated from the mean count of four replicate 1.00ml aliquots sampled during the mixing process and examined at 40x on a Sedgewick rafter slide. Sufficient sperm was then added to ensure fertilisation as described in Section 5.21 . Water temperature before and during fertilisation was held at 21± 0.5°C. Within 15 min of fertilisation, the eggs were again thoroughly mixed

as previously described. During this process, volumes containing subsamples of 5000 fertilised eggs, estimated on the basis of previously determined mean egg concentration, were collected using an adjustable automatic pipette and transferred to 1 l beakers filled with 1 μm filtered seawater (35g/kg salinity).

Replicate sets of four 1 l beakers each stocked with approximately 5000 fertilised eggs were maintained at each of five temperatures 16, 18, 21, 24 or 27 (± 0.5°C) using waterbaths fitted with thermostatically controlled immersion heaters. The five water baths were housed in a coolroom held at a constant air temperature of 14±1°C. After 48 h, the seawater in each beaker was thoroughly mixed as described above. Four replicate 5.00ml samples were taken from each beaker during mixing and the number of fully developed D veligers determined by dispersing samples on petri dishes and counting larvae with the aid of a dissecting microscope at 40x.

Experiment 2. Effect of temperature on larval growth and survival

Fertilised eggs were stocked at approximately 50/ml into a 90 L aerated polyethylene cylindro-conical rearing vessel and incubated at 21°C in 1μm filtered seawater. After 48h, sufficient D stage larvae were collected to stock 20 of the 90L cylindro-conical vessels at 5/ml. Four replicate vessels were held at each of five temperatures (15, 18, 21, 24 or 27 ± 0.5°C) by supporting them in 1000 L temperature controlled water bath (Fig 5.23.1).

Seawater in each 90 l vessel was replaced with fresh 1 μm filtered seawater every 48 h, the larvae being retained on a 45 μm nylon mesh screen. At every water change, larvae from each 90 L vessel were resuspended in 4L of seawater and a 1.00 ml sample collected. From this

sample, larval size (greatest height of shell parallel to the hinge) and survival were determined at 100x using a dissecting microscope fitted with an eyepiece micrometer that had been previously calibrated.

Because larvae as small as 185 μm had previously been found capable of settlement and attachment to the surface of the rearing tanks, thereby making samples taken from the water column potentially unrepresentative, survival data were recorded at the first appearance of 185 μm larvae in each replicate.

Experiment 3. Effect of temperature on growth, survival and byssal attachment of spat

Hatchery reared *P. fumatus* spat were maintained on 150μm polyester screens at 21° C in a downweller system until they had achieved a mean ± s.d. shell height of 1.04 ± 0.26 mm, 20 days after settlement. Forty spat were randomly allocated to each of 20 miniature downweller units specially constructed for the purpose (Fig 5.23.1). Previously described, 90 L cylindroconical rearing vessels fitted with lids were used to house individual miniature downwellers. Four such vessels were suspended in each of five 1000 L water baths maintained within ± 0.5 °C of prescribed temperatures of, 13, 17, 21, 24 or 27°C.

Seawater was replaced with fresh temperature equilibrated, 1 μm filtered seawater on alternate days. Salinities were monitored daily throughout the experiment to ensure that they did not vary outside the range 35 ± 1.0 g/kg. At weekly intervals, each miniature downweller was placed in a 45 g/kg hypersaline solution prepared by the addition of artificial sea-salt (Instant Ocean; Aquarium Systems, Sarrebourg, France) to seawater. This procedure facilitated the rapid release of byssally attached scallops without imposing traumatic injury and stress associated with mechanical methods of detaching spat (Heasman et al., 1994b and

Appendix 1). Detached spat were transferred to petri dishes and shell heights measured at 25x magnification using a dissecting microscope fitted with an calibrated eyepiece micrometer. While the number of dead spat detected in each miniature upweller was recorded on each sampling occasion, their shell heights were not measured.

Besides size and survival data, the number of spat byssally attached to the internal walls and bottom mesh of each downweller screen was monitored. This was achieved by directing a stream of seawater from a squeeze bottle directly at individual spat 5, 20, 40 minutes and 12 hours after initial stocking of the experiment and at weekly intervals immediately prior to detaching the spat with the aid of hypersaline solutions.

Results

Experiment 1. Effect of incubation temperature on embryo development to D veliger stage.

As indicated in Fig 5.23. 2, the greatest mean yield of normal D veliger larvae, 51 and 54% respectively, occurred at the lowest test temperatures of 16 and 18°C. Yield of D veligers decreased sharply with increasing temperature above 18°C falling to a mere 10% at 24°C and thence to 0% at 27°C.

Experiment 2. Effect of temperature on larval growth and survival

The growth rate of the larvae, as indicated in Fig 5.23.3A and by mean growth increment after 8 days (Fig 5.23.3B), increased markedly with increasing temperature from 15 to 24°C

but sharply decreased with a further increase of temperature to 27°C.

Age specific survival rate of larvae (Fig 5.23.4) was inversely related to rearing temperature being highest at 15° and lowest at 27°C. However, size specific survival Fig 5.23.5A), which is a much more appropriate criterion for selecting optimum rearing temperature, revealed a markedly different pattern. Survival data for larvae attaining a mean size of 150μm, the minimum mean terminal size attained by larvae over all temperatures tested, are presented in Fig 5.23.5B. These data clearly show that size specific survival increases with increasing temperature above 15°C, reaches a maximum value (in this case 40%) at about 21°C and then decreases sharply and progressively with further increases in temperature to 24 and 27° C.

Experiment 3. Effect of temperature on growth, survival and byssal attachment of spat

As indicated in Fig 5.23.6A, *P. fumatus* spat of mean± s.d. initial shell height of 1.04 ± 0.26 mm, grew exponentially at each of the five temperatures tested, i.e. 13, 17, 21, 24 and 27± 0.5°C, during the first four weeks of the trial. Exponential equations fitted to the first four weeks of growth data are provided in Table 5.23.1. The experiment was however terminated a week latter when constraints to continued exponential growth became evident at all test temperatures other than 13°C. This "stalling" of growth was not unexpected having occurred in all previous batches of *P. fumatus* spat reared at our hatchery. In every case, growth ceased in spat that had been retained in the hatchery on a standardised diet of cultured microalgae developed specifically for larvae (see previous Section 5.22) for more than 4 weeks or that had reached a mean shell height in the range 1.5 to 4mm, whichever occurred first. Exponential equations fitted to mean growth data for each test temperature (Table 5.23. 1) exhibited high R^2 "goodness-of-fit" values of 83.1 to 99.3.

Mortality rate was highest at the two lowest test temperatures with cumulative mortality at

the end of the fifth week reaching 33% at 17°C and 40% at 13°C% (Fig 5.23.7). By contrast, cumulative mortality did not exceed 8% at any of the three higher test temperatures of 21°, 24° and 27° C.. Size specific survival data presented in Fig 5.23.8 illustrate the importance of maintaining post-settlement spat above a minimum temperature of about 20°C.

As indicated in Fig 5.23.9, most spat rapidly attached to the bottom mesh or side walls of miniature downwellers screens after stocking. Peak rates of attachment were always attained within 12 h and most within 40 minutes at all temperatures tested. Short term rates of attachment did nevertheless increase with temperature, from a rate of about 77% at 13°C to over 95% at 24 and 27°C. Much greater differences in rates of byssal attachment developed over the full five week duration of the experiment. Attachment remained at or above 90% at 24°C, at or above 85% at 21°C, at or above 80% at 27°C, declined progressively to 55% at 17°C and to 24% at 13 °C. The effects of rearing temperature on rates of attachment of spat were therefore consistent with its effects on growth and survival as previously described.

5. Discussion

Temperature has a marked effect on incubation and larval development in pectinids beginning with the rate of cell division during cleavage stages that is distinctly higher at 20°C than at 15 or 10°C (Zavarzina, 1981, cited in Cragg and Crisp, 1991). While no detailed observations were made of the effect of temperature on incubation rate in the present study,

development to the straight hinge (D veliger) first feeding stage always occurred within 48h while temperature was maintained at or above 18°C. In a review of published literature, Cragg and Crisp (1991) found that time to metamorphosis in pectinids is inversely related to temperature and is described by a single regression line (Fig 5.23.10) when expressed as an Arrhenius plot. As indicated in Fig 5.23.10, equivalent data of *P. fumatus* which ranges from 14 to 15 days at 20 to 22°C (see Section 5.3 and Frankish et al., 1990) and 31 days at 13 to 15°C (Dix and Sjardin, 1975) conforms to this relationship.

Size specific survival data (Fig's 5.23.5A&B) indicate that maintenance of larvae in the hatchery at about 21°C is likely to provide the maximum yield of larvae for settlement.. This temperature corresponds with sea temperatures that o occur most commonly in early to mid Summer (December and January.) and again in Autumn (March, April or May) in Jervis Bay (May et al., 1978; CSIRO, 1994).

Most studies on the influence of temperature on scallops have focused on the tolerance limits of adult scallops (eg, Paul, 1980 A and B), with particular reference to the ecological implications. Sublethal temperature effects may limit geographic range. For example, where metabolic rate increases with increasing temperature, an increase in energy acquisition via increased phytoplankton clearance is also required to maintain a positive energy balance. Barber and Blake (1985), suggest that elevated metabolic rate in this way limits the southerly distribution of *Argopecten irradians irradians* in the United States. On the other hand, interactions between temperature and other environmental variables such as dissolved oxygen may restrict the northerly range of the same species (Voyer, 1992).

The large shift in optimum rearing temperatures from 15°C for gonad development (see Section 5.13), 16 to 18°C for fertilisation and incubation, 21°C for larval development and 24°C for growth and survival of juvenile *P. fumatus* (Figs 5.23.6B and 5.23.7) found during the course of this project were unexpected. The possible ecological significance of these

findings needs to be considered in relation to documented information on the life cycle of wild populations of *P. fumatus* in Jervis Bay and elsewhere. Studies of Jacobs(1983), Fuentes (1994) and of the current authors (See Section 5.11) have revealed multiple (3 or 4) annual peaks in gonadosomatic index in Jervis Bay *P. fumatus*. These peaks occur at 1 to 2 month intervals over an 8 to 9 month breeding season beginning in mid Autumn (April) and ending in late Spring (November)or early summer. This period coincides with mean monthly temperatures in Jervis Bay in the range 14 to 18°C (May et al., 1978; CSIRO, 1994). This temperature range is in close agreement with experimentally determined gonad conditioning range of 14 to 18°C (see Section 5.22).

Recorded catch rates for wild *P. fumatus* spat range from 2 to 80 per collector in Jervis Bay (Fuentes et al., 1992), 150 to 700 in Port Phillip Bay (Sause et al., 1987a) and 325 to 516 in South Eastern Tasmania (Hortle and Cropp, 1987). All these rates, which were recorded in breeding seasons that produced poor subsequent recruitment to their respective fisheries, are of a level considered as indicative of poor catch rates in relation to *Patinopecten yessoensis* within Mutsu Bay, Japan (Ventilla, 1982). They are also lower than average values of 1000 to 2000 spat per collector reported for *Pecten novaezelandiae* in Golden Bay, New Zealand (Bull, 1989). The catch rates in Jervis Bay are well below minimum rates of 100 to 300 per spat collector generally considered economically viable as a source of seed for farming and fisheries enhancement (Bull, 1988; Dao et al.,1985 cited in Stafford,1991) .

Throughout its range, major spat settlement in *P. fumatus* occurs over relatively short periods and from spawnings over the final 3 to 5 months (late Winter to early Summer) of breeding seasons spanning 8 to 9 months when larvae encounter rising sea temperatures (Fuentes et al., 1992; Hortle and Cropp, 1987; Sause et al., 1987a; Young et al., 1989). While this observation is in general accord with findings of the present study, the probability of *P. fumatus* larvae and spat generated from spawnings in late Winter to early Summer encountering optimal temperatures around 21°C and 24°C respectively, seems very low.

It is nevertheless conceivable that significant commercial catches that occur every 10 years or so in *P fumatus* fishery in NSW (Hamer and Jacobs, 1987) and intermittantly in Tasmania (Zacharin, 1989; Young and Martin, 1989) and Victoria (Gwyther, 1989), may coincide with unusually large and rapid increases in sea temperature in Spring or early Summer. Such events, especially if preceded by extended (3 to 6 week) periods of low and stable sea temperatures (14 to 16°C in the case of Jervis Bay) and abundant phytoplanktonic food, would promote rapid synchronous growth and ripening of gonads (See Section 5..13). This in turn may result in mass synchronised spawning at a time most conducive to high growth and survival of larvae and of spat sufficient to overwhelm natural predators.

Support for an hypothesis that booms in the *P. fumatus* fisheries in NSW, the Bass Strait and South eastern Tasmania are linked with favourable but unusual thermal sequences as described above, is provided by oceanographic studies of South Eastern Australia. Indeed such an hypothesis has already been proposed by Harris et al. 1988. The Southeast coast of Australia, including Jervis Bay, is subject to two major interrelated influences, the warm "East Australian Current " (EAC), originating in the Coral Sea (Fig 5.11.1) and flowing south along the eastern seaboard and a deeper cooler nutrient rich upwelling comprising an Ekman boundary layer. The latter is generated by the overlying EAC and driven tangentially to the right from the continental slope and across the continental shelf towards the coast.

Flow patterns of the EAC are often intense and highly variable. Between latitude 27°S (Tweed heads) and 32°S (Tuncurry/Forster), the flow often consists of strong southward currents near the edge of the shelf and equally strong northward currents further offshore. South of 32°S, the current degenerates into large anticlockwise eddies. Each year on average, four to six of these warm meandering eddies progress as far south as southern Tasmania impacting on any given section of the coast, especially during Spring and Summer (September to February), for periods of 4 weeks to 4 months (Boland, 1979).

Seawater exchange in Jervis Bay occurs mainly as a near surface inflow on the Southern side of the entrance in phase with a deeper outflow on the Northern side. Monthly flushing times for the Bay were estimated by Holloway et al. (1992) to vary from 10 to 74 days with a median 21 days. The authors also detected large pulses of cold (14 to 16°C) shelf water at a depth of 30m beneath a surface layer of warm (20 to 24°C) seawater at the Bay entrance during summer 1989/90. These cold nutrient rich seawater intrusions persisted for periods of up to three weeks at the entrance. An even more dramatic intrusion of cold (15°C) nutrient rich continental slope water was driven into Jervis Bay by a near- shore warm core eddy of the EAC in late November 1992 causing a dramatic algal (*Gephyrocapsa oceanica)* bloom which turned the whole of the Bay milky for several weeks during December (Blackburn and Cresswell, 1993).

As indicated in Fig 5.11.1, this event did apparently lower bottom temperatures within the Bay from 18 to 16°C but only fleetingly. Bottom temperature subsequently rose to a peak of only 21°C by late February 1993. This was considerably lower than peak February temperatures of 23°C in 1989 (CSIRO, 1994) and 1994 (Fig 5.11.3) and of 25°C in 1991 (CSIRO, 1994).

The warm-water eddies of the EAC and associated cold nutrient rich upwellings regularly cause coastal phytoplankton blooms along the NSW (Tranter et al., 1986) and Tasmanian coasts (Harris et al., 1988), including the Jervis Bay, in Spring and Summer. These eddies and upwellings in turn exhibit the potential to create ideal conditions for mass spawning, high subsequent spatfall rates and thence occasional high level recruitment. Harris et al. (1988) in an analysis of Tasmanian Scallop catches from the 1940's to 1960's found that years of high incidence of "Zonal Westerly Winds" (ZWW), linked to an El Nino Southern Oscillation (ENSO) cycle with a mean periodicity of 11 years, "*appear to favour good recruitment perhaps by a link between high productivity in high ZWW years, and increase in spawning and high larval survivorship*".

Apart from its possible ecological implications, the finding that *P. fumatus* spat grow and survive best at temperatures in the range 21 to 27°C and optimally at 24°C, highlights the potential benefits of greenhoused nursery systems and the need to confine nursery rearing operations to periods of the year when sea temperatures exceed about 18°C and preferably in the range 21 to 27°. This finding also raises the prospect of farming *P. fumatus* perhaps as far north as Southern Queensland ,i.e. up to 1000 km north of its natural geographic range, at mean monthly sea temperatures as high as 27°C.

Growth of pearl oyster spat retained within the hatchery and fed diets of cultured microalgae (Alagarswami et al., 1989) was significantly less than of spat in the wild or maintained in flow-through systems using natural phytoplankton. Bourne and Hodgson, 1991 report a similar finding in relation to the scallop *Patinopecten yessoensis* . This was also case with *P. fumatus* spat in the present study where an apparent barrier to continued growth, evident during the fifth week of experimentation remains unexplained and is the subject of continuing research. This problem is however easily avoided by deploying small spat on screens to field upweller systems or allowing them to bysally attach to cultch materials in mesh bags suspended from midwater longlines. Growth retardation is apparently overcome with access to wild phytoplankton rather than a standard hatchery diet specifically developed for *P. fumatus* larvae[1]. This larval diet (See Section 5.22) comprises equal amounts of three cultured species, the prymnesiophytes *Pavlova lutheri* , Tahitian *Isochrysis* and the diatom *Chaetoceros calcitrans* fed at progressively higher concentrations tailored to the needs of growing larvae.

Bysso-genesis (byssal attachment) in scallops has been proposed as a useful bioassay for potentially toxic substances (Roberts 1973, cited in Paul 1980a) and has been suggested as a possible indicator of temperature optima in the scallop *Chlamys opercularis* (Paul 1980a). Byssal attachment rates of *P. fumatus* spat within 1 to 12 hours of their transfer to new

environments (Fig 5.23.9) consistently reflected subsequent growth and mortality rates at the particular temperature involved. This finding contrasts with that of Caddy (1972) who found that the proportion of byssus attachment in *Placopecten magellanicus* did not vary over the temperature range 5 to 15°C. The ability of chemical irritants and several forms of physiological stress, especially hypersalinity, to induce rapid detachment of *P. fumatus* from settlement substrates has previously been demonstrated by the authors (Heasman et al., 1994b/ appendix 2). Detachment of *P. fumatus* spat up to 5mm, as in the mussel *Mytilis edulis* (Young, 1985), may therefore prove useful as a quick and simple indicator of suboptimal nursery and farming sites such as those subject to pollution, excessive turbidity or suboptimal pH and salinity due to coastal runoff.

Table 5.23.1 Exponential equations describing increase in shell height over 4 weeks of *Pecten fumatus* spat at temperatures of 13, 17, 21, 24 or 27°C.

Temp (°C)	Exponential Equation	r^2
13	$Y = e^{(0.0394 + 0.0559X)}$	83.86
17	$Y = e^{(-0.0234 + 0.1647X)}$	83.08
21	$Y = e^{(0.0320 + 0.2231X)}$	96.70
24	$Y = e^{(0.0348 + 0.3447X)}$	99.30
27	$Y = e^{(-0.0394 + 0.3317X)}$	97.73

Fig 5.23.1 Mini downweller system used for growing spat

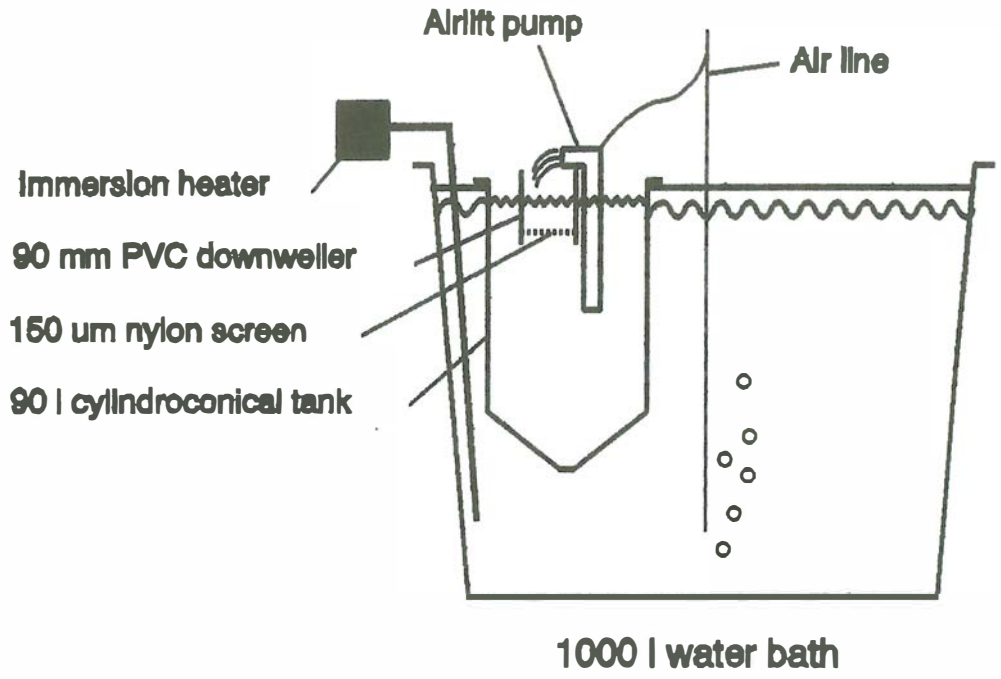


Fig. 5.23.2 D veliger yield following incubation of *Pecten fumatus* embryos at 16, 18, 21, 24 or 27 C.

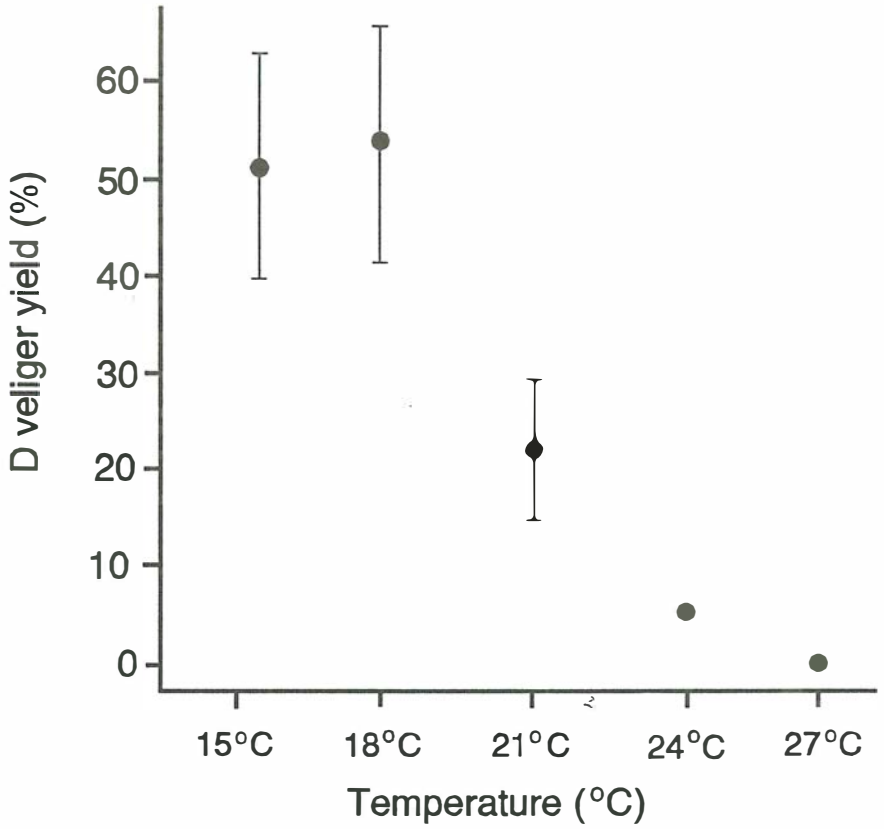


Fig 5.23.3 Mean size of *Pecten fumatus* larvae held at 15, 18, 21, 24 or 27°C (A) and mean growth increment after 9 days (B).

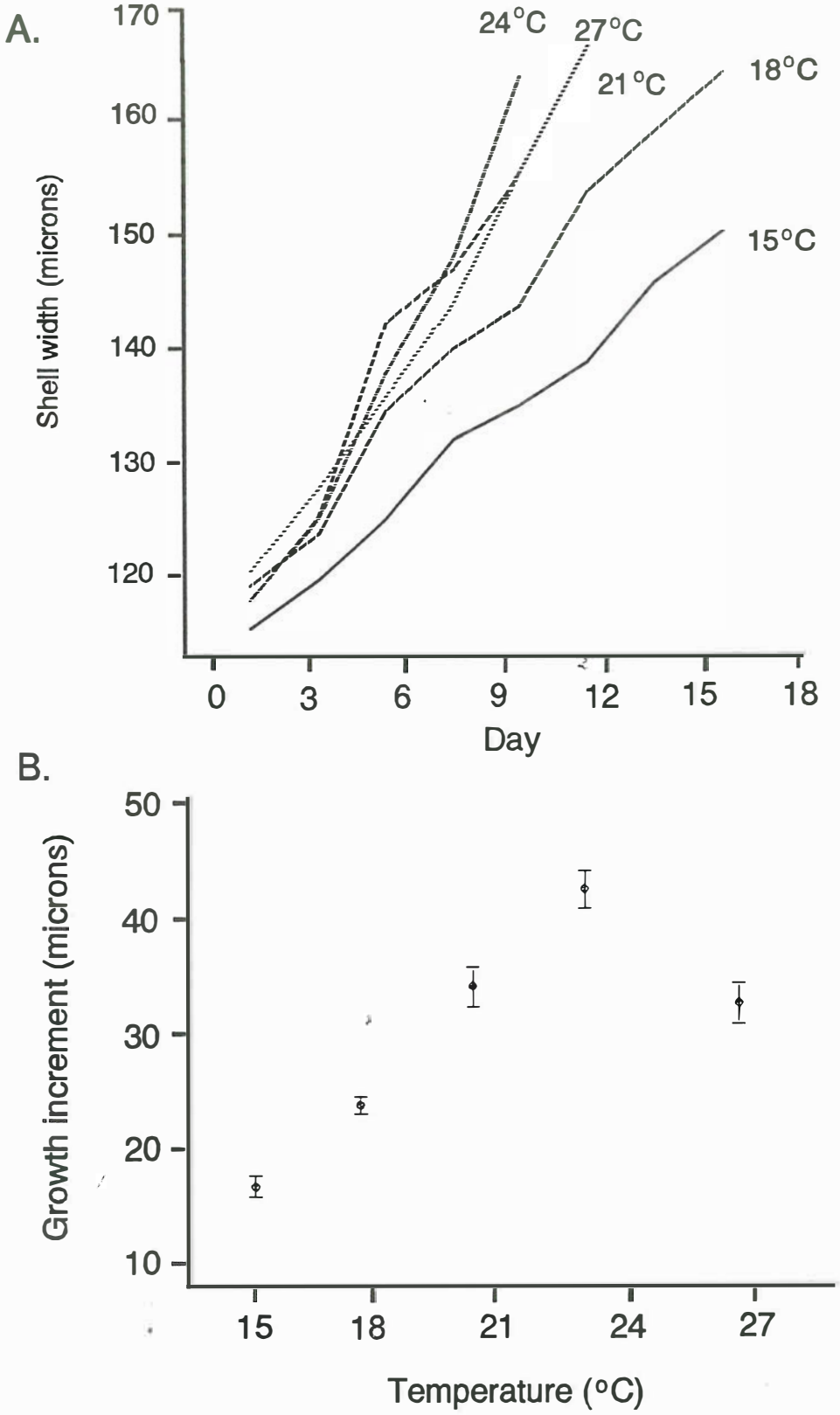


Fig. 5.23.4 Age specific survival of *Pecten fumatus larvae.*

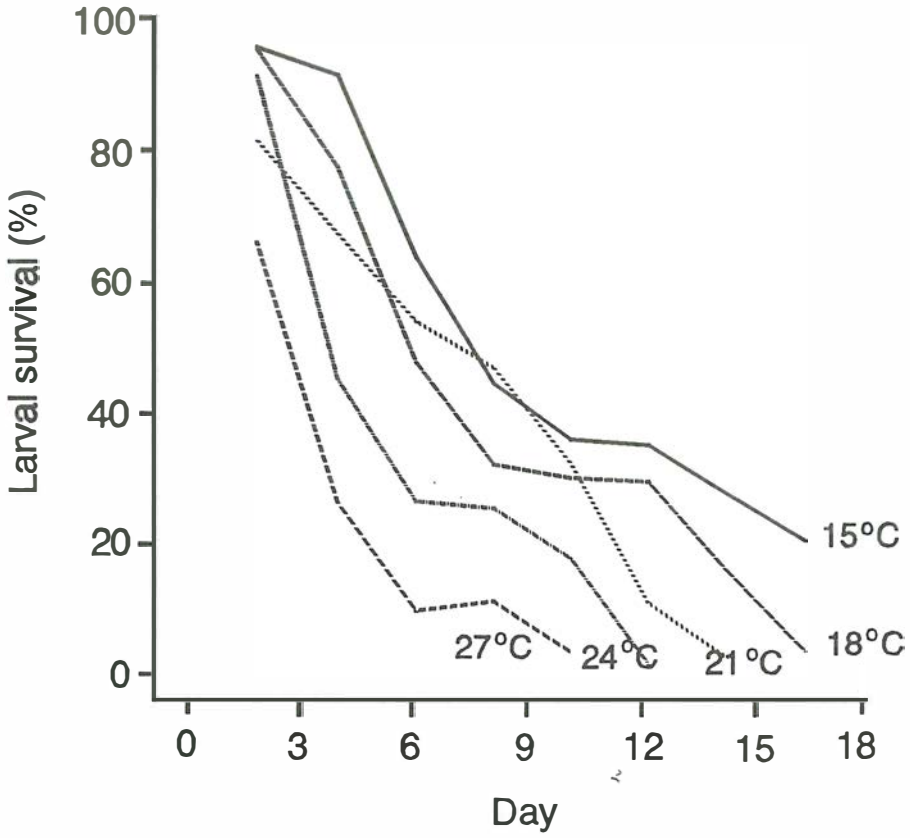


Fig 5.23.5 Size specific survival of *Pecten fumatus* larvae (A) and survival of larvae at a mean size of 160 microns (B) when held at a temperature of 15, 18, 21, 24 or 27°C.

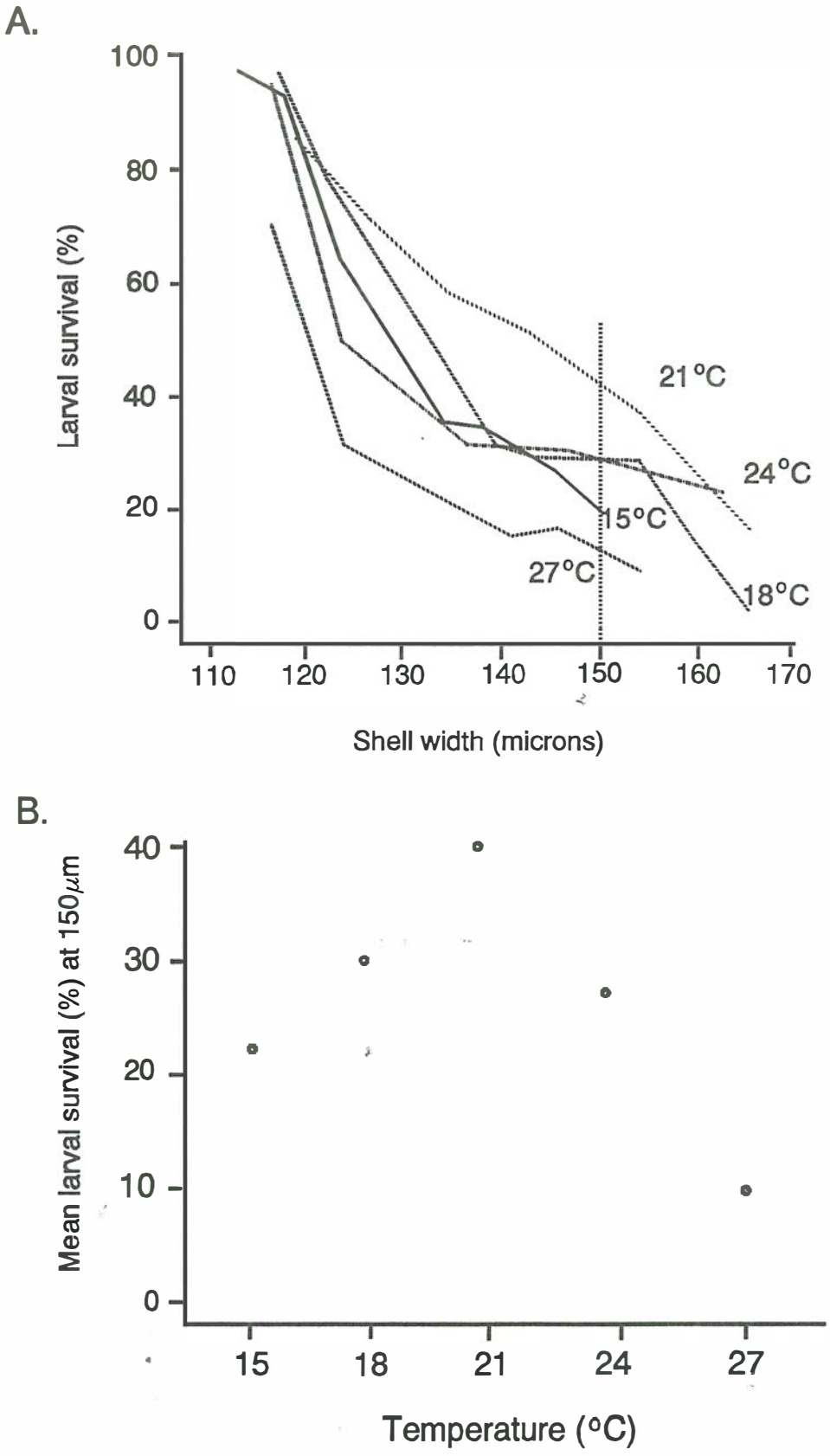


Fig. 5.23.6 Growth of *Pecten fumatus* spat held in the hatchery at 13, 17, 21, 24 or 27°C (A) and mean growth increment of *P. fumatus* spat held at these temperatures after four weeks (B).

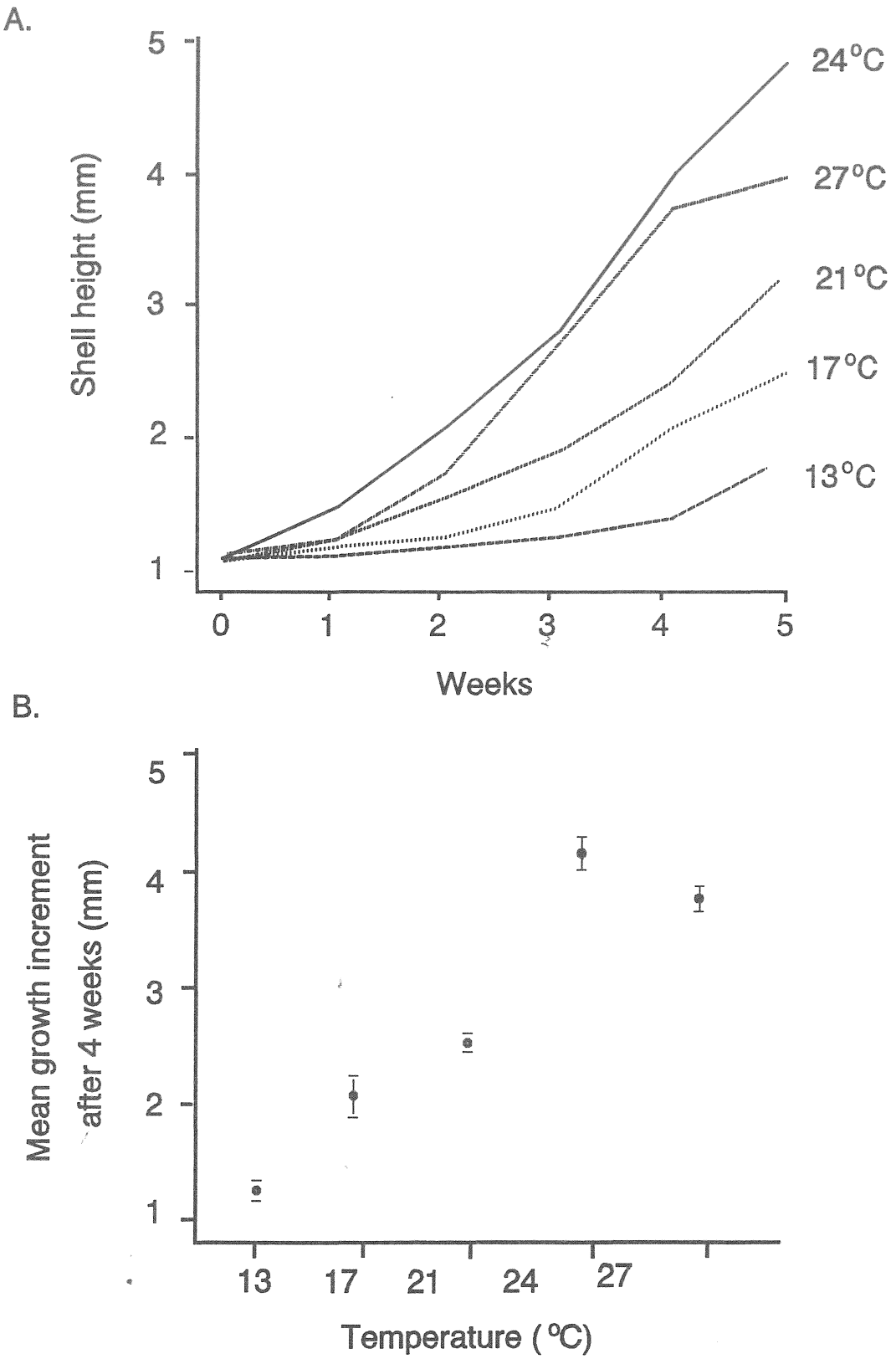


Fig. 5.23.7 Cumulative mortality of *Pecten fumatus* spat reared in the hatchery at either 13, 17, 21, 24 or 27°C.

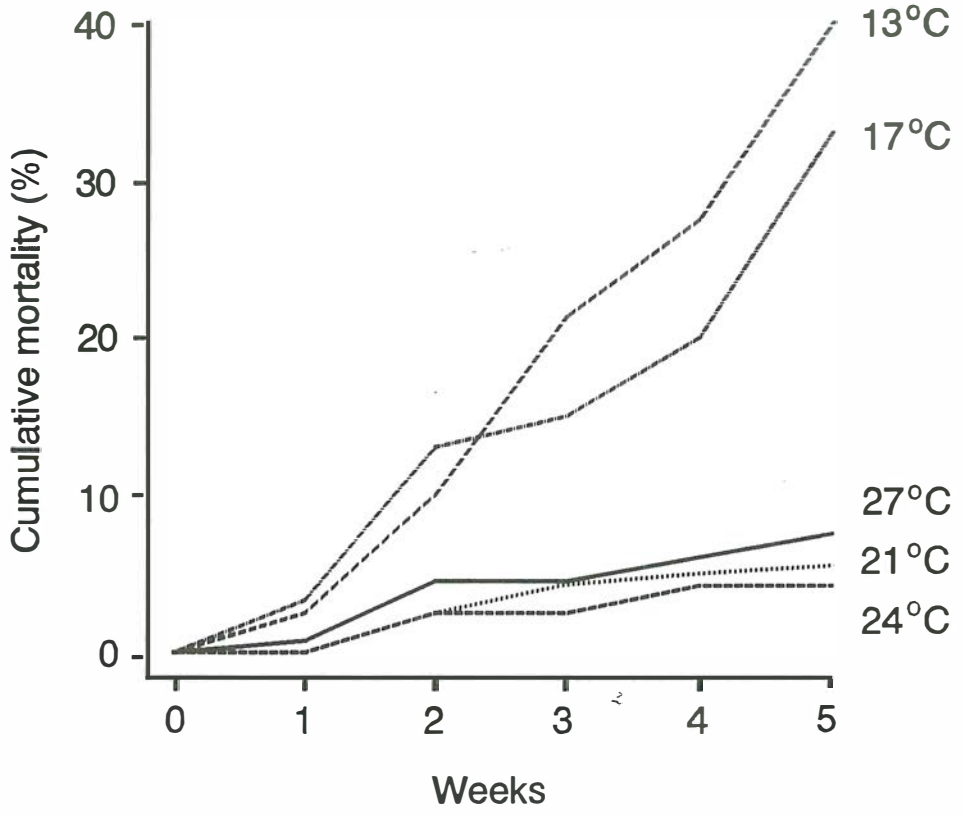


Fig. 5.23.8 Spat survival with shell height increase for *Pecten fumatus* spat held at 13, 17, 21, 24 or 27°C.

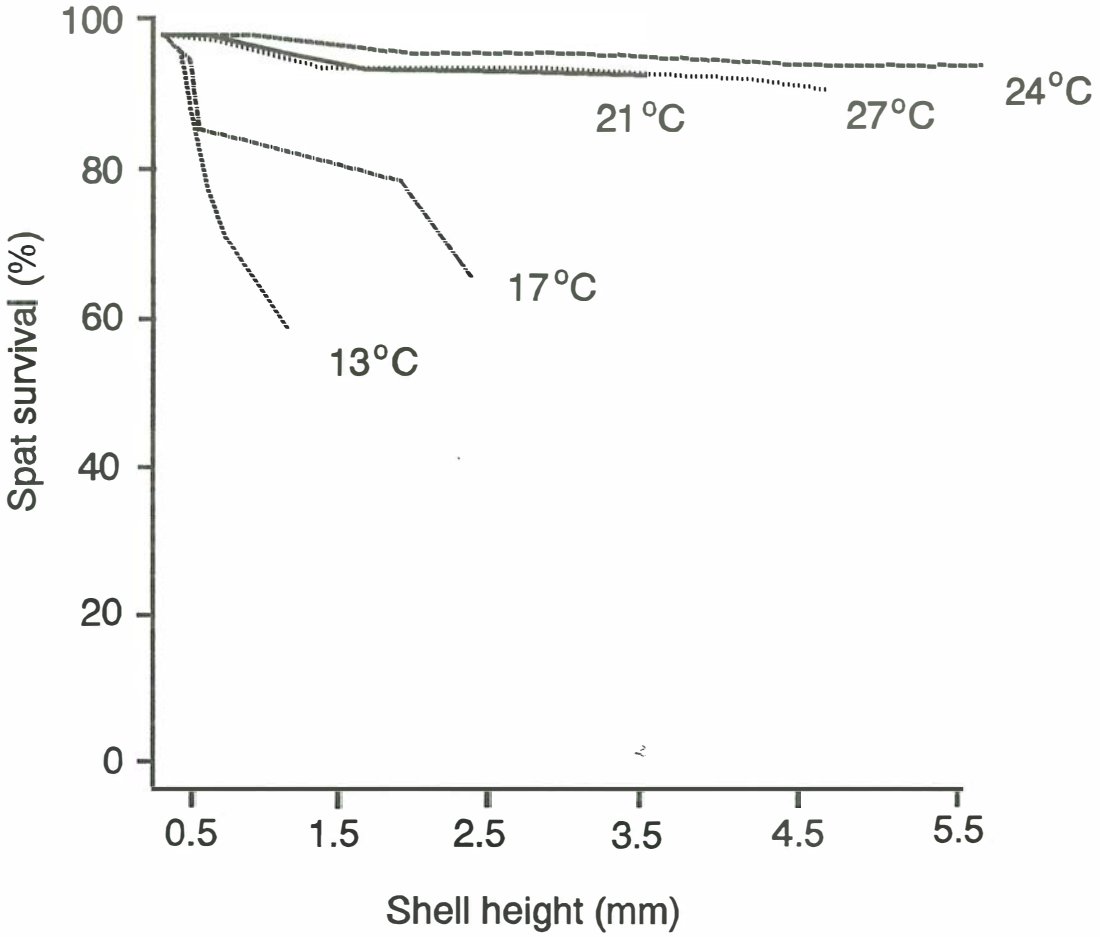


Fig. 5.23.9 Re-attachment of *Pecten fumatus* spat in the first 12 h and in the following 5 weeks when held at 13, 17, 21, 24 or 27°C.

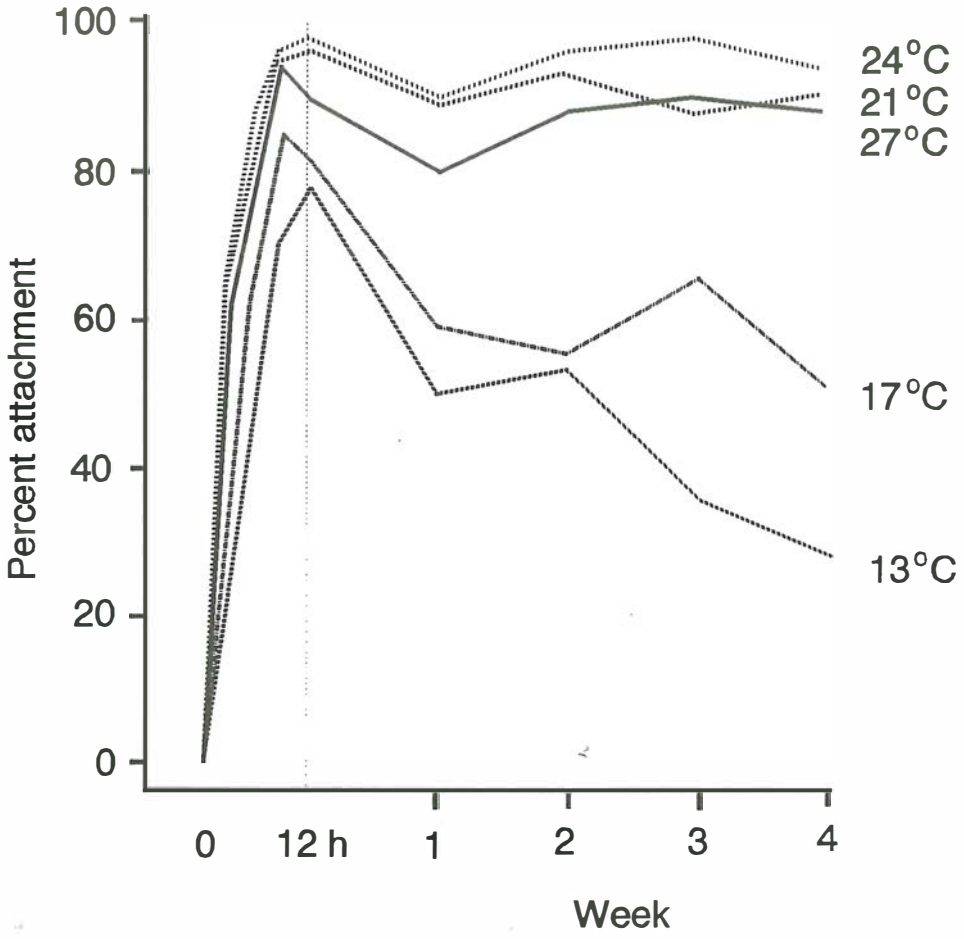
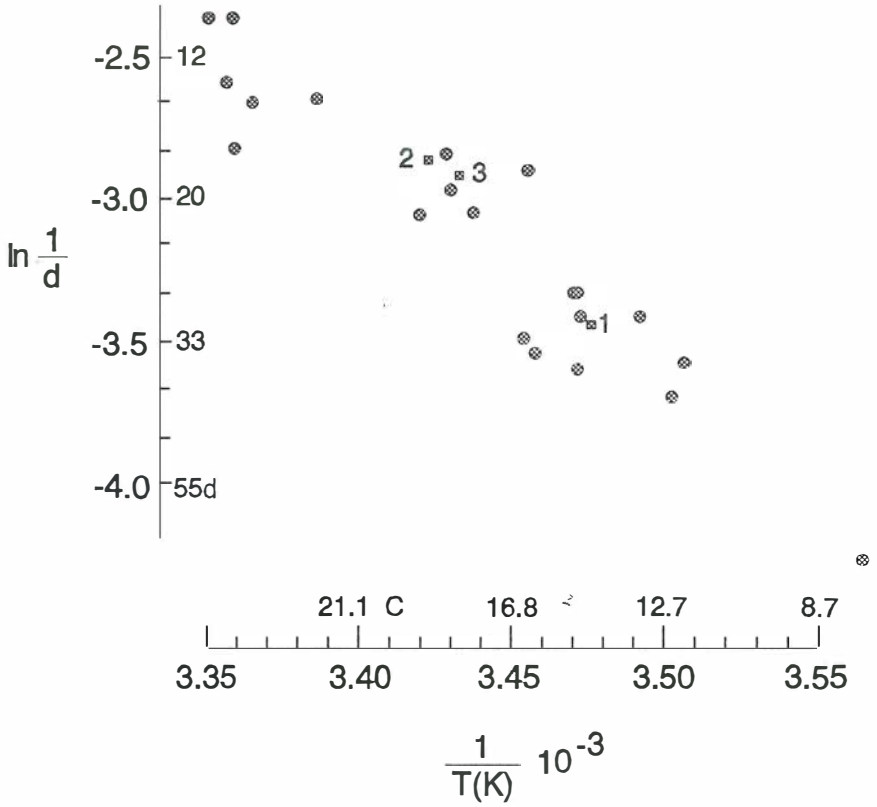


Fig 5.23.10 Arrhenuis plot of the relationship between time from fertilisation to metamorphosis and water temperature for various pectinids reared at close to optimum temperaturefor each species (redrawn from Cragg and Crisp, 1991). Data points represented by squares are for *Pecten fumatus* using data from Dix and Sjardin (1975) and Frankish et al. (1990).



	T.	D.	
1	14.15 (3.478)	31(-3.434)	Dix and Sjardin (1975)
2	18-19 (3.430)	15(-2.708)	Frankish et al. (1989)
3	18 (3.436)	18(-2.890)	Present study

5.3 Investigations of larval pathology.

Introduction

Using conventional oyster hatchery rearing techniques and equipment, Frankish et al., (1990) produced approximately 6 million settled *P. fumatus* spat at the PSRC in May 1989. Survival rate from spawning to D-veliger (first feeding) stage was about 60 % and from D-veliger to post-settlement, about 70%. Several hundred thousand settled spat were retained and reared to 10-20mm shell height at a similar rate of survival. These results however contrasted with those previously attained by Tasmanian oyster hatcheries using comparable oyster hatchery techniques and equipment (Frankish et al., 1991) and with subsequent hatchery cycles conducted at the PSRC during the remainder of 1989 and during the first half of 1990..

In attempting to meet government contracts for the supply of 4.2 million *P.fumatus* juveniles in the range 10 to 20mm, Tasmanian hatcheries were only able to supply 100 000 and 280 000 spat in 1987 and 1988 respectively. Up to this time, the largest spawning of *P. fumatus* had produced 125 million eggs but no hatchery had yet produced more than 500 000 settled spat from one batch of larvae (Cropp and Frankish, 1989).

From the outset of this project, it was considered that previous high variability in hatchery success with *P. fumatus* could have arisen through one or a combination of several factors. These included 1) variability in the quality of eggs sourced from wild spawners 2) subtle, albeit critical differences in equipment and techniques employed 3) larval nutrition factors and 4) disease(s). Whilst previous sections have dealt with factors 1,2 and 3, this section deals with investigations of disease factors.

Materials Methods and Results

Preliminary investigations

Larval rearing trials using standard techniques developed for Sydney Rock Oysters (Frankish et al., 1991) were conducted over the first year of this project (June 1991 to July 1992). Results are summarised in Fig.5.3.1. Survival to pediveliger (Day 16) ranged from 5 to 20% which compared poorly with the May 1989 result (Frankish et al., 1990) but which was in keeping with earlier results achieved by commercial hatcheries in Tasmania during 1987 and 1988, (Cropp and Frankish, 1989) and subsequent results achieved at the PSRC up to June 1990..

Larval growth rate varied considerably but was not apparently correlated to survival. Metamorphosis occurred with a shell height range of 225 to 240μm in 14 to 20 days after spawning. Batches of larvae suffering high mortality rates exhibited clinical and other disease symptoms summarised in Table 5.3.1 when examined live at 10x to 400x using conventional light microscopy and when subjected to histopathological examination by (Dr. Dick Callinan, NSW Fisheries, Wollongbar Research Station, pers. comm. 1994). These symptoms were entirely consistent those described for bacillary necrosis (= vibriosis) of bivalve larvae (Tubiash et al., 1965 and 1970; Brown ,1973; Brown and Losee,1978; Elston and Leibovitz, 1980; Elston et al., 1981; Lodeiros et al., 1987).

Brown and Losee (1978) observed that the presence of *Vibrio spp.* pathogens and of other diseased oyster (*Crassostrea virginica)* larvae did not retard the growth or time to metamorphosis of other apparently healthy larvae unless and until they too had become visibly diseased.. These observations are also consistent with those made in relation to *P. fumatus* larvae in this study and hence with the previously cited lack of apparent correlation between rates of growth and survival.

These observations drew attention to an urgent need for a small-scale experimental larval rearing system comprising sufficient treatment replication to adequately assess prospective methods of enhancing growth and survival of larvae. Such a system, comprising forty 90 l cylindro-conical rearing vessels, was commissioned in August 1992. Standard flat-bottomed

cylindrical 1000 l oyster larvae rearing vessels were utilised as controlled temperature baths. Each of the 1000 l vessels accommodated 4 of the smaller cylindro-conical rearing vessels. as described in Section 5.23 (Experiment 2) and illustrated in Fig 5.23.2 The general utility of these smaller vessels was tested and compared with that of the 1000 l vessels in September and October 1992. Growth and survival rates of sibling scallop larvae reared in both types of vessels were very similar with neither vessels type exhibiting obvious superiority.

Trial 1 - Investigation of the effects of seawater` preparation and of antibiotics on the growth and survival

Experimental treatments comprised four types of seawater preparation i.e. membrane filtration to 0.2μm absolute; filtration to 1.0μm nominal; filtration to 1.0μm nominal plus 10mg/l chloramphenicol and an unfiltered seawater control. These were combined factorially with two alternative diets, namely a standard blend of three micro algal species *(C. calcitrans,* T. *Isochrysis*, and *P. lutheri*) and the same blend of microalgae concentrated into a slurry by centrifugation (O'Connor and Nell 1991) and stored for 1 to 6 days prior to feeding. Four replicates were run for each experimental treatment. Techniques were otherwise unchanged from those used in earlier hatchery operations as described in Section 5.23 (Experiment 2).

The chloramphenicol treatment was adopted on the strength of reports (Buestel et al., 1982, Jeanthon et al., 1988; Samain et al.,1992) that larvae of the closely related European scallop *Pecten maximus,* cannot be hatchery reared with consistent success without use of such powerful broad- spectrum antimicrobials. A similar finding was reached by Lodeiros et al. (1987) in relation to bacillary necrosis of *Ostrea edulis*.

Samain et al.,(1992) found that while settlement and ageing of seawater up to 14 days and filtration to 1 µm (nominal) was beneficial to the growth of *P. maximus* larvae, growth limiting factor(s) were removed by subsequent membrane filtration to 0.2 µm. Negative effects of membrane filtration to 0.2 µm were also reported by Garland et al, (1986) in relation bacterial necrosis of pacific oyster *Crassostrea gigas* larvae. This information served as the basis for including the 0.2 µm filtered seawater treatment.

The inclusion of the microalgae concentrate diet in experimental treatments was prompted by the findings of an earlier study of Nell and O'Connor (1991) that such diets promoted superior growth and survival in Sydney rock oyster larvae.

Survival rates from D-veliger to the pediveliger stages (Fig 5.3.1) varied markedly with method of seawater preparation, being highest (70-80%) in the case of chloramphenicol treated seawater and lowest (less than 10%) for 0.2μm filtered and unfiltered seawater. Larval survival rates were reduced but growth rates were enhanced by the use of algal concentrate diets. Subsequent patterns of survival through metamorphosis were however very different with highest retention rates of 30 to 40% being exhibited in the case of 1μm filtered seawater and lowest rates of less than 1% in the case of both unfiltered and 0.2μm absolute filtered seawater treatments.

Trial 2- Investigation of the survival enhancing effects of chloramphenicol

The same experimental treatments were applied in *Trial 1* plus a "high hygiene" treatment in which larvae were doubly rinsed with 1 μm filtered seawater at each water change, experimental rearing vessels were sealed to prevent the entry and spread of bacteria via aerosols and equipment doubly cleaned and disinfected with a chlorine solution between uses. Estimates of total bacteria using marine agar plates and of presumptive *Vibrios (Vibrionaceae)* using TCBS plates, were made on alternate days until day 12 after fertilisation.

While total bacterial counts (Table 5.3.2) remained in the range 2x 10^2 to 4x 10^5 /ml all replicates across all treatments, marked differences occurred between presumptive *Vibrio* counts in the chloramphenicol treatment and all other treatments . Presumptive *Vibrio* counts associated with chloramphenicol treatment were confined to the range <1 to <100/ml while corresponding levels in all other treatments ranged from 30 to 4000 /ml ie generally one to two orders of magnitude higher. Samples were examined using genus specific and species specific monoclonal antibodies and indirect FITC-immunofluorescence microscopy by Assoc. Prof. Peter Hanna (Deakin University). This procedure showed that large numbers of

bacteria of the genus *Vibrio* were associated with the surface of larvae in all treatments except chloramphenicol. The *Vibrio* comprised the species *V. alginolyticus., V. harveyi and V. parahaemolyticus.*

Trial Series 3 - Search for alternative more benign antibiotics to chloramphenicol as a possible stop-gap method of achieving consistently high hatchery survival of P. fumatus .

A) Evaluation of *antibiotics to enhance survival through incubation i.e. from fertilisation to first feeding D veliger.*

These trials were conducted to evaluate the utility of dips and baths in combating vibriosis of *P. fumatus* larvae caused by pathogenic bacteria originating in the gonads of hatchery conditioned broodstock. Embryos were stocked at 5/ml of 1 μm filtered seawater in 1 litre cylindroconical plastic vessels within one hour of fertilisation. Experimental treatments comprised either short term exposure (dips) or continuous exposure (baths) to a range of antibiotics and an untreated control. These treatments were imposed within an hour of fertilisation . Four replicates were employed for each treatment.

Antibiotics tested included chloramphenicol, erythromycin, oxolinic acid, oxytetracycline and furazolidone. These were used at dose rates specified in Table 5.3.3 that have previously been shown to prevent or control *Vibriosis* in marine bivalve larvae. Aquacultural disinfectants commonly used in Australia (Heasman, 1992) were evaluated only as dips. These, included chloramine-T, providine iodine, benzalkonium chloride and sodium hypochlorite at concentrations specified in Table 5.3.3. This trial was repeated on several occasions to evaluate the consistency of results.

As indicated in Table 5.3.3, all antibiotic and disinfectant dips were either ineffective or reduced yields of D veligers below that of the untreated control . Of the antibiotic baths tested, furazolidone had a negative effect on survival, while oxytetracycline was ineffective. By contrast, erythromycin at 1 mg/l , chloramphenicol at both 2 and 10mg/l and oxolinic acid at 1mg/l, all produced a statistically significant improvement in survival over that of the

control treatment on at least one occasion. However no antibiotic, including chloramphenicol at 2 and 10 mg\l, increased survival beyond that of the control treatment on all occasions. This apparent inconsistency probably reflects varying quality and bacterial loads of embryos and other factors such as varying quality of seawater rather than inherent inconsistency in the effectiveness of the antibiotics against particular targeted pathogens, in this case V. *alginolyticus, V.harveyi* and *V. parahaemolyticus* .

B) Evaluation of alternative antibiotics to chloramphenicol in the enhancement of survival of P. fumatus through the hatchery cycle.

In all treatments, D veliger larvae were stocked at 5/ml into 90 l cylindroconical rearing vessels (4 replicate vessels per treatment) held at 22±0.5°C. General rearing procedures were in accordance with other standardised methods already described in *Section 5.23(Experiment 2).* Four experimental treatments comprised the continuous use of chloramphenicol at 1mg/l, oxolinic acid at 1mg/l and erythromycin at 10mg /l and an untreated control. As already demonstrated, all of these antibiotics have the potential to significantly improve the yield of D veliger larvae from fertilised eggs.

Growth and survival (Fig 5.3.3) was evaluated at day ten after fertilisation. Survival was considerably enhanced,, by chloramphenicol and by oxolinic acid but not by erythromycin. On the other hand, growth was suppressed by both chloramphenicol and erythromycin but unaffected by oxolinic acid.. FITC-immunofluorescence tests (Hanna, 1992; Chen and Hanna,1994) conducted by Dr Peter Hanna (Table 5.3.4) indicated that survival enhancing properties of oxolinic acid are probably due to inhibition of *V. alginolyticus* rather than *V. parahaemolyticus or V. harveyi.*

These results clearly establish chloramphenicol, as the most effective antibiotic for combating vibriosis and hence for enhancing survival in larval *P. fumatus*. The poor subsequent survival of larvae following their removal from chloramphenicol protection, implies obligatory use of this antibiotic well into the nursery phase. This in turn strengthens the case against routine prophylactic use of such an important but potentially hazardous medical antibiotic in hatcheries.

In view of these findings, chloramphenicol was replaced by oxolinic acid as a reference antibiotic treatment in subsequent research to find better alternative methods of preventing and combating vibriosis in *P. fumatus* larvae.

Evaluation of potentially probiotic bacteria and algae

About 95 species of microalgae within the CSIRO reference collection held in Hobart was screened by Halvey (1993) for antimicrobial properties against gram positive and gram negative bacteria. Four species were found to exhibit varying degrees of bacteriostatic activity against *Vibrio splendidus*. Cultures of three of these species, an unidentified marine *Chlorella (*code CS -195*); Synococcus sp. (*code CS-94*); code)* and *Nannochloropsis oculata (*code CS-189)-were acquired from the CSIRO Marine Laboratoriess in Hobart(Dr Shirley Jeffries-pers. comm.) and propagated at the PSRC.

In subsequent larval rearing trials only one species, *Synococcus* (at 5000 cells/ml?) was found to enhance survival rates above those of untreated controls. Results of FITC-immunofluorescence tests(Table 5.3.4 A&B) conducted by Dr Peter Hanna at Deakin University (pers. comm. 1994) indicated that survival enhancing properties of *Synococcus* (as with oxolinic acid) are due to inhibition of *V. alginolyticus* rather than *V.parahaemolyticus or V. harveyi.*

In spite of these encougaging results, subsequent larval rearing operations incorporating *Synococcus* in rearing water failed to consistently yield higher numbers of settlement stage (pediveliger) larvae than did control treaments .

Probiotic bacteria

Nogami and Maeda (1992) in combating bacillary necrosis caused by *V. anguillarum* in marine crab *(Portunus trituberculosis*) larvae, had considerable success with the use of

probiotic bacteria. Douillet and Langdon (1993 and 1994) were similarly able to significantly and consistently able to enhance the growth and survival of pacific oyster (*Crassostrea gigas)* larvae by adding an *Alteromonas* like bacterium, code named "strain CA2", to rearing water.

Written requests were sent to both research groups seeking cultures of these bacteria to evaluate their possible growth and survival enhancing properties in relation to *P. fumatus* larvae. Unfortunately both groups were unable to respond positively to these requests (Douillet, pers. comm. and Nogami -pers. comm.1994) on the basis that proprietary and marketing rights to these particular bacteria were still being negotiated with prospective commercial partners.

Investigation of alternative lower stress larval rearing techniques to combat Vibriosis in P. fumatus larvae

Preliminary evaluation of the use of large scale rearing vessels

A review of hatchery records at the PSRC in August 1994 prompted a decision to evaluate alternatives to the standard procedure of rearing scallop larvae in 1000 l or smaller vessels using alternate day water changes (batch culture). Most important was the finding that mean survival of Sydney rock oyster (S*accostrea commercialis)* larvae from D veliger to pediveliger stage reared by this standard technique over the previous four years (Table 5.3.1) had varied over a low and narrow range of 2.3 to 12.4%. These survival rates were generally below a threshold of 10%.(≡ 0.5 pediveligers /ml) commonly recognised as the lower limit of commercial viability. These same data however indicated that the use of commercial scale 20 000 l rather than experimental scale 1000 l rearing vessels provided more consistent commercially viable yields of pediveligers in the order of 12 to 14%.

At this time, scallop hatchery research had become progressively constrained by the declining breeding condition of the aging scallops from Jervis Bay. This problem was overcome in October 1994 with the use of first generation hatchery produced scallops ongrown in farming trials at Twofold Bay in a separately funded project (O'Connor, Heasman

and O'Connor, 1994). Two hundred and fifty of these scallops, which ranged from 40 to 55mm shell height, were roadfreighted to the PSRC and held in conditioning units (Fig 5.13.2). In spite of their small size (45-60mm), these scallops have already produced an average of about 1.4 million eggs and about 600 000 normal D veliger larvae per spawner after only one week of conditioning according to improved techniques developed in this study (see Section 5.13) .

Approximately 10 million D veliger larvae generated using improved , fertilisation and incubation and larval rearing protocols as described in Sections 5.21, 5.22 and 5.23. were stocked into a 20 000 l larval rearing tank half filled with 1 µm filtered seawater. Water changes were first implemented on day 4 after stocking and twice a week thereafter. Otherwise, feeding regimes and general husbandry followed a standardised protocols used to rear larvae in experimental l and 1000 l vessels described in Section 5.23 (Experiment 2). Resultant growth and survival rates to pediveliger of 15 days and 90% respectively for this batch (Fig. 5.3.1) were the highest yet recorded for this species . The possible superiority of this rearing technique nevertheless awaits confirmation. Such confirmation can only be established over in a series trials, replicated over time, in which growth and survival of sibling larvae are reared concurrently in the 20 000 l and 1000 l vessels using the same husbandry techniques.

Preliminary evaluation of flow-through (continuous) seawater exchange

Acting on a recommendation from Mr. Lindsay Goard (Technical Officer, Bivalve Hatchery at the PSRC) supported by a practical experience and advise of Mr. Daniel Liszka (who had overcome equivalent vibriosis problems whilst managing penaeid prawn hatcheries in Ecuador between 1992 and 1994), attention was focused on the possible use of flow through rather than batch water exchange as a means of enhancing hatchery survival of *P. fumatus*

In February 1995, a small number of hatchery conditioned *P. fumatus* broodstock , were

successfully induced to spawn. This was in spite of their extremely poor breeding condition when collected from Jervis Bay four weeks earlier. The resultant D veliger larvae were stocked at 1.44 larvae/ml into a standard 1000 l cylindrical rearing vessel equipped with a stand-pipe (Fig. 5.3.1). A 60 µm polyester screen was fitted to the standpipe to prevent the escape of larvae . This "flow through" rearing system was supplied with 1 µm filtered seawater The incoming seawater was gravity fed continuously from an identical 1000 l header tank that was cleaned and refilled each morning and evening. Algae was fed to the larvae via incoming seawater at prescribed rates . These were in accordance with a oyster hatchery feeding schedule developed by Frankish et al. (1991) and catered for progressive increases in the mean size of the larvae. The the amount of food was required over the total larval cycle was about twice that required with alternate day batch water exchange.

Growth rate achieved in this trial Fig 5.3.1, ranked amongst the best achieved to date . The survival rate of 21% to pediveliger stage was also very acceptable when compared with previous results achieved within a 1000 l vessels during the course of the present study but well below the 60% achieved by Frankish et al (1989) in May 1989. The merits of this rearing technique nevertheless awaits confirmation through fully replicated trials in which growth and survival of sibling larvae are reared concurrently in these same 1000l vessels operated either with batch exchange (standard "control") or with continuous flowthrough of seawater.

Discussion

The present study has revealed vibriosis as the principal factor limiting the hatchery survival of *P. fumatus* and that this disease, as in the european scallop, *P.maximus* (Samain et al., 1992) and flat oyster, *O. edulis (*Lodeiros et al., 1987) can be negated by the powerful broad-spectrum antibiotic, chloramphenicol at rates of 8 to 50mg/litre. Use of this antibiotic at 1mg/l during the present study was found to ensure very protracted and enhanced survival of larvae, even grossly malformed and stunted individuals .

Three *Vibrio* species, *V. parahaemolyticus, V. harveyi* and especially *V. alginolyticus* were consistently associated with vibriosis epizootics at the PSRC.. Nevertheless, it is highly unlikely that vibriosis per se is the primary problem. According to Elston (1990): " *Vibriosis is an opportunistic disease of the larval stages of many, perhaps all bivalve molluscs and can occur in any marine hatchery situation since the causative bacteria are ubiquitous. .....Probably all species are subject to the disease, although some (e.g. the american oyster Crassostrea virginica) may be more susceptible than others (e.g. the pacific oyster, C. gigas)... The disease is in fact regarded by most as a management disease meaning that it can be prevented and controlled by appropriate management procedures* "

These observations closely resemble those of Lodeiros et al., (1987) in relation to the flat oyster *O. edulis.* The authors concluded that bacillary necrosis in bivalve larvae does not have a particular causative agent and can be produced by one or a combination of pathogenic *Vibrio*, *Psuedomonas* or *Alteromonas* bacteria. They also concluded that such bacteria are also present in asymptomatic broods of larvae and there needs to be special stress or other predisposing factors for bacteria to proliferate and to give rise to a disease outbreak. Clinical manifestation of bacterial necrosis in this and other studies has been highly variable. This may reflect variability in the particular species mix of and abundance of pathogenic bacteria involved (in this case, *Vibrio alginolyticus* and on occasions *, V. harveyi and V. parahaemalyticus)* quality of eggs and larvae, extraneous sources of infection, etc.

Clinical disease symptoms of vibriosis depend on th the relative influence of extracellular cytotoxins produced by pathogenic strains of *Vibrio* that include a thermolabile proteinase causing necrosis and teratogenesis of the velum and other tissues plus a heat stable ciliostatic toxin causing loss of motility with or without the direct presence of bacteria on the larvae themselves Nottage and Birbeck,(1986). In common with Elston (1984) and Lodieros et al, (1987) we conclude that direct infection of tissues by bacterial cells and toxin-mediated disease are two modes of pathogenesis which interact and hence may not always be totally separated.

Monitoring of *Vibrio* levels by conventional plating techniques and use of species specific

monoclonal antibodies with indirect FITC- immunofluorescence microscopy has been proposed as worthwhile in the early detection of an impending vibriosis problem`(Elston and Leibovitz, 1980; Hanna et al.,1992). However, the low levels (in the order of 10^1 or 10^2/ml) at which elevated mortality due to vibriosis can occur naturally or be induced (Brown and Losee, 1978) and the fine line that divides the manifestation from nonmanifestation of disease, seriously undermines the value of routine monitoring. The dubious effectiveness of emergency action to combat disease,(apart from the contraversial use of broad spectrum antibiotics) further erodes the rationale for routine monitoring of bacteria.

Prevention rather than cure is unquestionably the best strategy of combating vibriosis in the hatchery rearing of *P. fumatus*. Vibrios like all other pathogens may enter the hatchery or nursery by three principal routes, seawater, brood stock or their gametes, and algal food stocks (Elston, 1990). In addition, they may be spread from other infected stock within a hatchery on contaminated equipment and utensiles or by aerosols .

In the present study, the probabiity of high levels of *Vibrio* entering via sea water was minimized by trucking raw seawater from high salinity (≥34g/kg) sites during periods of low turbidity and storing the seawater in 50 000 l settlement tanks for periods of 7 to 21 days. This strategy was originally adopted at the PSRC in 1988 after seawater collected and treated in this manner was found to consistently enhance survival of sydney rock oyster larvae beyond that previously achieved using local estuarine water. Yamamoto et al.,(1983) demonstrated that settlement and ageing of seawater results in the replacement of potentially pathogenic organic decomposer groups, especially *Vibrio* and *Alteromonas spp.,* with more benign nondecomposers Once settled, seawater used in the present study was filtered to 1μm nominal immediately prior to use. This practise was implemented on the basis of findings in the present and previous studies (Samain et al.,1992; Garland et al., 1986) that removal of smaller particles in the range 0.2μm to 1.0μm inevitably leads to reduced growth and survival of bivalve larvae.

Chlorination of seawater (followed by neutralisation) is commonly used to combat vibriosis in penaeid prawn hatcheries and is also known to differentially eliminate *Vibrio*

bacteria but not to significantly alter total bacterial counts other than during the first 24h after treament (Baticados and Pitogo, 1990). Inexplicably this same water treatment did not prove efficaceous for *P. fumatus* larvae in the present study and therefore cannot be recommended.

The very encouraging results obtained by Douillet and Langdon., (1994) in the probiotic use of an *Alteromonas* like bacterium (Strain CA-2) to enhance growth and survival of pacific oyster (*C. gigas) l*arvae provide strong grounds for evaluating this bacterium in relation to *P.fumatus* and other vibrio susceptible species. Accordingly, efficacy trials will be commenced as soon as commercial owners of the bacterium are willing to make innocula available for this purpose.

Samples of all three standard species of algae routinely used to feed scallop larvae (see Section 5.22) tested totally negative to the presence of any of *Vibrio* bacteria when subjected to genus specific and species specific monoclonal antibodies with indirect FITC-immunofluorescence microscopic examination by Dr.Deschen Chen and Assoc. Prof. Peter Hanna,`Deakin University, (pers.comm. July 1994). The complete absence of *Vibrio* is attributed to the chlorination seawater used in bulk production of these algae at the PSRC (O'Connor and Diemar, 1991) and subsequent generation of secondary bacteriostatic byproducts (chloramines, bromates and bromoforms). As this method of algae food production is *Vibrio* free, its continued use is recommended.

Altered larval rearing equipment and techniques appraised in this study have produced encouraging initial results when combined with improved broostock conditioning, spawning induction, fertilization and incubation protocols. The latter include strict adherence to upper temperature limits of about 18°C during incubation and 21°C during larval rearing (See Section 5.23). Seasonal manifestation of vibriosis in hatcheries has been linked to the onset of high temperatures in oysters (Elston, 1990) and flat oysters (Lodeiros et al., 1987).

During the formative stages of this project it was postulated that generally poor and highly

variable rates of survival achieved with *P.fumatus* larvae were in part due to deficiencies of standard Sydney rock oyster culture techniques (Frankish et al.,1990) when applied to *P. fumatus.* A recent review of the efficacy of these techniques also exposed them as inadequate in relation to sydney rock oysters (Table 5.3.1) when used in conjunction with experimental scale 1000 l flat bottom rearing vessels. The latter were adopted as a standard larval rearing vessel at the PSRC in 1989. This was to satisfy the need for replication in experimental treatments, in particular those of an oyster genetics selection program that required 6 separate lines of oyster larvae to be reared simultaneously.

During the concluding stages of this project, the use of a commercial scale (20 000l) rearing vessel and of a standard 1000l experimental vessel operated with continuous flow through rather than batch exchange of seawater were evaluated.. Both produced high yields of pediveligers and rapid growth. The apparent advantages of continuous water exhange in circumventing vibriosis is tentatively ascribed to the elimination of harvesting and handling trauma and stress that is imposed every 2 to 3 days with batch water exchange. Highly elevated survival rates were achieved with both sydney rock oyster and scallop larvae when reared in 20 000 l tanks using standard batch feeding and seawater exchange regimes. This result ise tentatively ascribed to the lower surface to volume ratio of 20 000 l vessels, more stable temperatures and probable lower susceptibility to vibrio transfer from elsewhere in the hatchery via personnel, contaminated equipment and aerosoles.

Inherent limitations imposed on the PSRC bivalve hatchery operations by competing demands of a broad and diverse array of bivalve research projects have almost certainly exaccerbated "in-house"vibriosis problems. Paramount amongst these is the restriction of only one dry-out disinfection of the hatchery wet floor per year and a sharing of common floor and air space by up to four different bivalve species and various life stages thereof. These limitations are in turn largely attributable to the fact that the facility was not originally intended for research . To the contrary, it was specifically designed and commissioned as a commercial hatchery to supply NSW oyster farmers with an alterative source (to wild spat) of single seed sydney rock oysters. In this context, bivalve larval rearing systems and husbandry regimes that overcome vibriosis problems at the PSRC are likely to be at least as

efficacious when adopted by commercial hatcheries.

In an overview of bivalve hatcheries in the the UK, Ireland, France, New Zealand, Australia and the USA, Holliday (1986), concluded that rearing systems fell into one of two categories. The first, comprising high volume low larval density (1 to 10/ml) systems were used throughout the USA but most successfully by the the Whisky Creek hatchery in Oregon, which coincidentally uses 20 000l reaing tanks. The second category comprised low volume (100 to 1000l) high larval density (30 to 50/ml) systems originally developed at Conwy in Wales. The latter employ continuous feeding and water flow at the rate of one or more water exchanges every 24h. Findings of the present study are thus consistent with "overseas experience"that with appropriate equipment, operating systems and husbandry, vibriosis can be circumvented, even in the most *Vibrio* susceptible species. These findings also and raise serious questions regarding the continuing dependence of european scallop (*P. maximus)* hatcheries on routine prophylactic use of chlorampenicol (Samain et al., 1992) to prevent vibriosis .

Table 5.3.1 Sequential Symptoms of Larval Vibriosis in *Pecten fumatus*

1	Abnormal development (shape and morphology) and reduced growth rate of affected larvae
2	Reduced swimming activity often with abnormal swimming patterns. Patches of sluggish larvae appear and disappear on bottom of rearing vessels. First appearance of pale green 10 -20 micron spherical vesicles within and around upper digestive tract.
3	Cessation of swimming resulting in the formation of large patches of moribund larvae which fail to resuspend.
4	Decreased filter feeding rate of batch with gut contents of larvae pale and reduced
5	Decreased intervalvular movements
6	Bacteria sometimes but not always apparent
7	Swarms of bacteria sometimes but not always present around velum and shell margins
8	Portions of damaged ciliated velum hanging loose or detaching completely and "swimming away"
9	Cessation of internal movements
10	Abundant bacterial activity and extensive necrosis
11	Proliferation of ciliates as secondary invaders

Table 5.3.1 Summary of Sydney rock oyster hatchery runs at the Port Stephens Research Centre 1992 - 1994

Date	Operation	Larval tanks used	Eggs	D Veligers (% yield)	Pediveliger (% yield)	Spat (% yield)	Advanced spat (% yield)	Comments
Aug '90		6 x 1 000 l	?	25.4 x 10^6	2.9 x 10^6 (12.6%)	1.5 x 10^6 (52%)	0.52 x 10^6 (36%)	65% of spat died (mass mortality)
Oct '91	Dryout							
Jan '92	Mass selection	12 x 1 000 l	?	61 x 10^6 (?%)	8.43 x 10^6 (7.2%)	6.0x10^6 (70%)	Very high (>90%)	Described as good larval result (no antibiotics).
Oct '92	Dryout							
Jan '93	Mass selection	12 x 1 000 l	96 x 10^6	70.5 x 10^6 (73%)	3.97 x 10^6 (5.7%)	3.42 x 10^6 (86%)	Very high (>90%)	Generally poor growth rate of larvae. Antibiotics used at some point in all 1000 l tanks.
Oct '93	No dryout							
Jan '94	Mass selection	6 x 1 000 l	80 x 10^6	41 x 10^6 (52%)	1.39 x 10^6 (3.4%)	Very low yield Run aborted	-	Poor growth and survival of larvae.
Feb '94	Triploid project							Severe water quality problems reduced yields of setting spat. Mass mortality recurred perhaps triggered by overstocking and/or grading and counting operations on early spat held in the hatchery.
	Diploids	1 x 20 000 l	60 x 10^6	36 x 10^6 (60%)	4.48 x 10^6 (12%)	2.52 x 10^6 (50%)	0.8 x 10^6 (35%)	
	Triploids	3 x 20 000 (½ filled)	55 x 10^6	43.2 x 10^6 (79%)	6.0 x 10^6 (14%)	2.6 x 10^6 (43%)	0.8 x 10^6 (35%)	
April '94	Mass selection (2nd trial)	12 x 1 000 l	96 x 10^6	60 x 10^6 (63%)	1.365 x 10^6 (2.3%)	0.065 x 10^6 (4.8%)	Very low	Very poor larvae growth and survival. Very poor set rate.
Equivalent shellfish data for culture (Bicheno, Tasmania)		10 000 l tanks	?	?	5 - 8%	30-40% raised from 15-20%	Very high >90%	Set induced with epinephine open system upwellers used within 4 days of set no grading of spat until in 2° nursery.

Table 5.3.2 The effect of various treatments upon total bacterial numbers and *Vibrio* numbers in *Pecten fumatus* larval cultures over ten days.

Chloramphenicol

Replicate	Day 2	Day 4	Day 6	Day 8	Day 10
A. Total	$<10^4$	$2x10^4$	$4x10^3$	$3x10^5$	$5x10^4$
A. *Vibrio*	$<10^2$	10^2	$<10^2$	1	1
B. Total	$<10^4$	10^5	$2.5x10^4$	$8x10^4$	$3.8x10^5$
B. *Vibrio*	$<10^2$	$<10^2$	10^2	2	1
C. Total	$<10^4$	$5x10^4$	$6.5x10^4$	$6x10^4$	$7.5x10^4$
C. *Vibrio*	$<10^2$	$<10^2$	$<10^2$	<1	20
D. Total	$<10^4$	10^4	10^5	$2.5x10^4$	$1.3x10^5$
D. *Vibrio*	$<10^2$	$<10^2$	$<10^2$	2	30

1μm filtered seawater (control)

Replicate	Day 2	Day 4	Day 6	Day 8	Day 10
A. Total	$<10^4$	$9x10^4$	10^4	$1.1x10^5$	$4x10^4$
A. *Vibrio*	10^2	$1.5x10^3$	$3x10^2$	30	90
B. Total	$<10^4$	$2.3x10^5$	$3.8x10^4$	$5x10^4$	10^5
B. *Vibrio*	10^2	$1.5x10^3$	$1.7x10^2$	$3x10^2$	10^2
C. Total	$<10^4$	$<10^4$	$9x10^4$	$3x10^4$	$2.5x10^4$
C. *Vibrio*	$<10^2$	10^2	$5x10^2$	80	$4x12^2$
D. Total	$<10^4$	$2x10^4$	$7x10^4$	$8x10^4$	10^5
D. *Vibrio*	10^2	$8x10^2$	$6.6x10^2$	40	20

0.2μm filtered seawater

Replicate	Day 2	Day 4	Day 6	Day 8	Day 10
A. Total	$<10^4$	10^4	$1.2x10^5$	$11x10^4$	$25x10^4$
A. *Vibrio*	$<10^2$	$8x10^2$	$4x10^2$	10^2	$2x10^2$
B. Total	$<10^4$	$3.3x10^5$	$3x10^4$	$11x10^4$	$5x10^4$
B. *Vibrio*	10^2	$3.5x10^3$	$2.9x10^2$	10^2	25
C. Total	$<10^4$	$7x10^4$	$5x10^3$	$5x10^5$	$1.5x10^4$
C. *Vibrio*	10^2	$3x10^2$	$1.3x10^2$	$3x10^3$	20
D. Total	$<10^4$	$2x10^5$	$1.4x10^4$	$1.4x10^4$	$2.8x10^4$
D. *Vibrio*	10^2	50	$3x10^2$	$1.2x10^2$	20

Algal concentrate fed

Replicate	Day 2	Day 4	Day 6	Day 8	Day 10
A. Total	$2x10^5$	$2.5x10^4$	$2.3x10^3$	$2.1x10^5$	$8.3x10^4$
A. *Vibrio*	$2x10^2$	10^3	$1.1x10^2$	$4x10^2$	$4x10^2$
B. Total	$<10^4$	10^4	$2x10^3$	$3.5x10^4$	$4.1x10^4$
B. *Vibrio*	10^2	$9x10^2$	$1.5x10^2$	30	20
C. Total	$5x10^4$	10^4	$7x10^3$	$4x10^4$	$1.1x10^5$
C. *Vibrio*	10^2	$9x10^2$	$1.3x10^2$	$>10^3$	50
D. Total	$<10^4$	10^4	10^4	$1.5x10^4$	$3.4x10^4$
D. *Vibrio*	10^2	$8x10^2$	$4x10^3$	402	$2.3x10^2$

Table 5.3.2 (cont.)

High hygiene

Replicate	Day 2	Day 4	Day 6	Day 8	Day 10
A. Total	$<10^4$	$2x10^4$	$4x10^3$	$2.7x10^4$	$3.6x10^4$
A. *Vibrio*	$<10^2$	$3.1x10^3$	$2.2x10^2$	10^2	90
B. Total	$<10^4$	$8x10^4$	$2x10^3$	$5x10^4$	$3.8x10^5$
B. *Vibrio*	10^2	90	90	$5x10^2$	80
C. Total	$<10^4$	$2.3x10^5$	$1.2x10^5$	$8x10^4$	$15x10^4$
C. *Vibrio*	$3x10^2$	$1.4x10^3$	$5.4x10^3$	$2x10^2$	50
D. Total	$<10^4$	$3x10^4$	$3x10^4$	$14x10^4$	$3.5x10^4$
D. *Vibrio*	$<10^2$	$2.2x10^2$	$1.3x10^2$	$4x10^2$	30

Table 5.3.3 Treatments used in attempts to improve embryo survival to D veliger stage.

Treatment	Exposure method	
	Bath	Dip
Erythromycin	10 ppm*	100ppm, 10 min
Chloramphenicol	2 ppm*	10ppm, 10 min
	10 ppm*	50ppm, 10 min
Oxolinic Acid	1 ppm*	10ppm, 10 min
	10 ppm	-
Furazolidone	2.5 ppm†	-
Oxytetracycline	10 ppm	-
Chloramine T		5ppm, 30 seconds
		5ppm, 5min†
Providone iodine		1ppm, 1 min
		10ppm, 2 min†
Benzalkonium chloride		1ppm, 5 min†
		1ppm, 10seconds
Chlorine		3ppm, 5 min
		10ppm, 5 min†

* significantly improved D veliger yields in some trials.
† significantly decreased D veliger yields.

Inter trial variation was great, no treatment consistantly significantly improved the yield of D veligers

Tables 5.3.4 A&B
A. Sample analysis 9 February 1994

6 samples recieved; all four were formalin fixed.
At the time of arrival it was not known that the samples were formalin fixed. Attempts to culture bacteria from centrifuge concentrated samples were made on TCBS selective media.

Results

Sample	Growth	Cells/ml	Genus test	Species test
Holmes 25	none	N/A	*Vibrio*	*V. alginolyticus*
Holmes 100	none	N/A	*Vibrio*	*V. alginolyticus*
Nan 25	none	N/A	*Vibrio*	*V. alginolyticus*
Nan 100	none	N/A	*Vibrio*	*V. alginolyticus*
Oxolinic acid	none	N/A	negative	negative
Control	none	N/A	negative	negative

Conclusions
All sample of Holmes and Non contained *V. alginolyticus,* particularly samples Nan (25 and 100) in which the larvae had serious internal infections.

B. Sample analysis 16 March 1994

5 samples recieved; all four were formalin fixed
The analyses for the presence of vibrio were performed after centrifuging the samples and washing the pellet 3 times in PBS. Analyses were baesd on FITC-immuno fluoresence tests

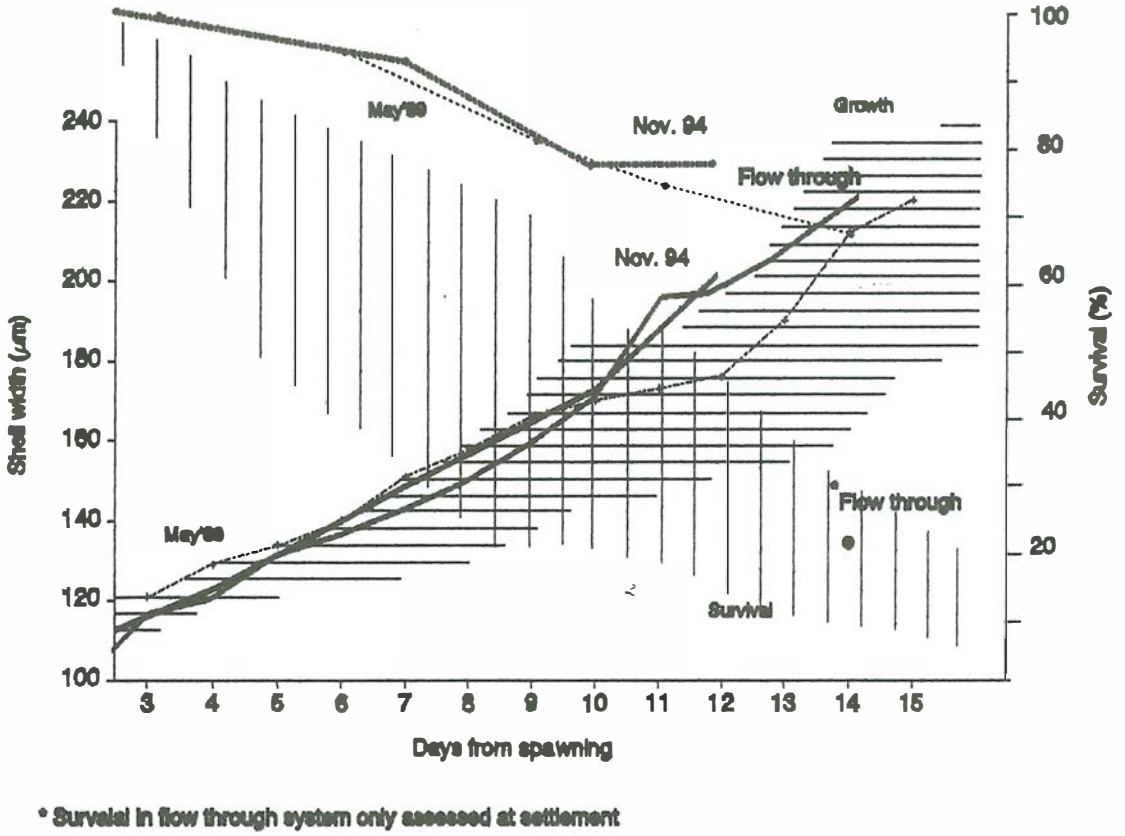
Results

Sample	Genus test	Species test		
		V. alginolyticus	*V. harveyi*	*V. parahaemalyticus*
1	++	+	++	+
2	+++	–	+++	–
3	+++	–	+++	+++
4	–	–	–	–
5	++++	++	++++	+++

Only a few larvae appeared to have *Vibrio* attached, except for sample 5 in which attachment was more noticeable. It appeared that most of the bacteria present in the samples were associated with debris of unknown origin. That is, the debris could have been decomposing alga or larvae.

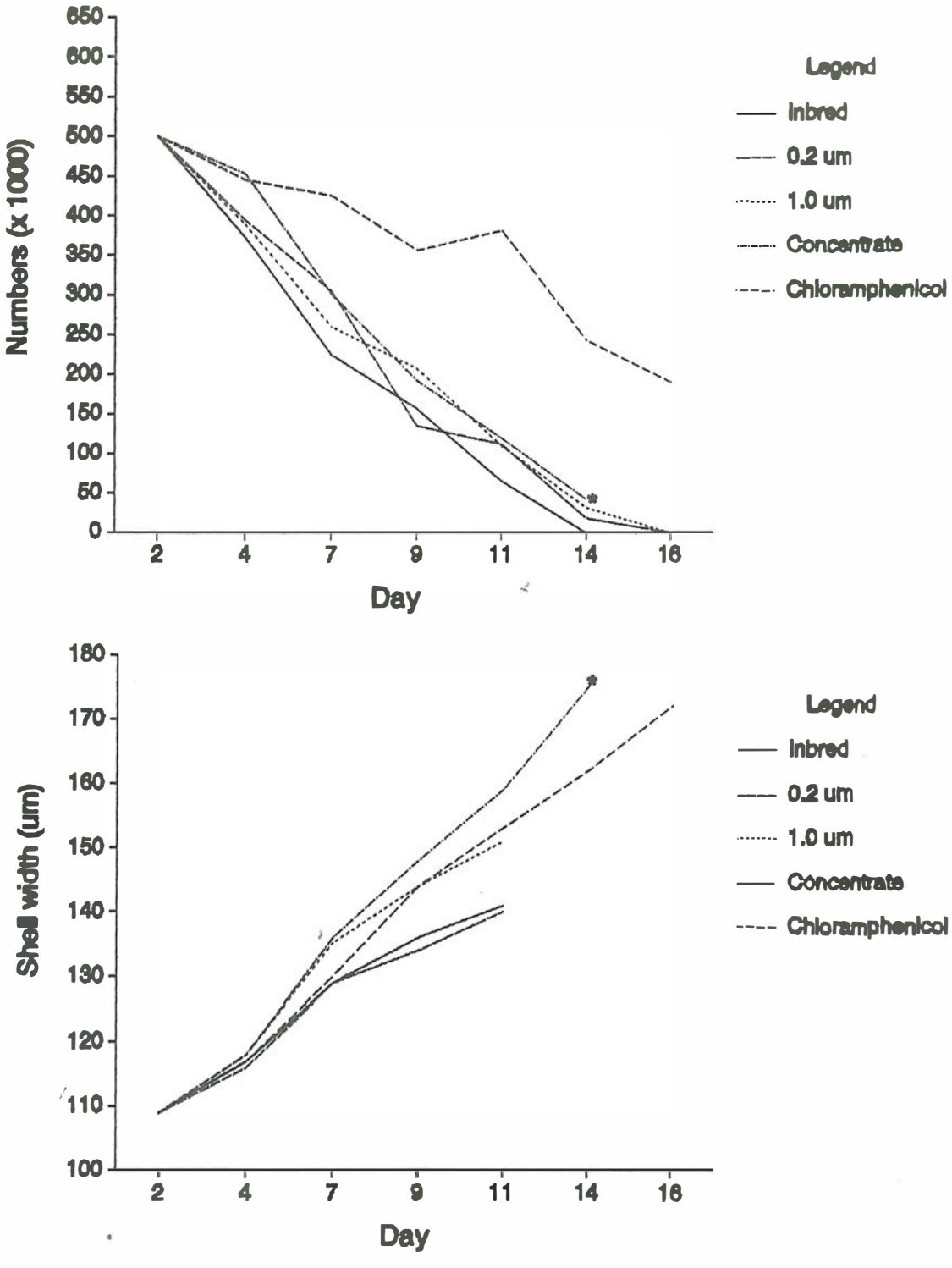
Conclusions
Sample four clearly showed no signs of *Vibrio*. The *Vibrio* present in the other four samples were a mixture of several bacteria including *V. alginolyticus, V. harveyi and V. parahaemolyticus.*

Fig 5.3.1 Growth and survival of various batches of *Pecten fumatus* larvae over four years. Horizontal line shading represents the range of survival rates recorded in seven hatchery cycles during the first year of this project, while the vertical line shading represents the range of mean growth rates recorded over the same cycles.



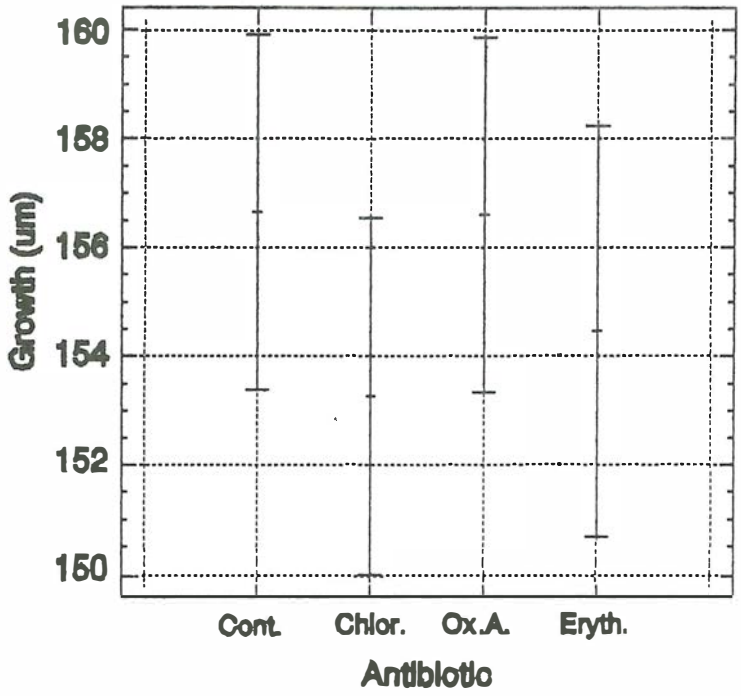
* Survival in flow through system only assessed at settlement

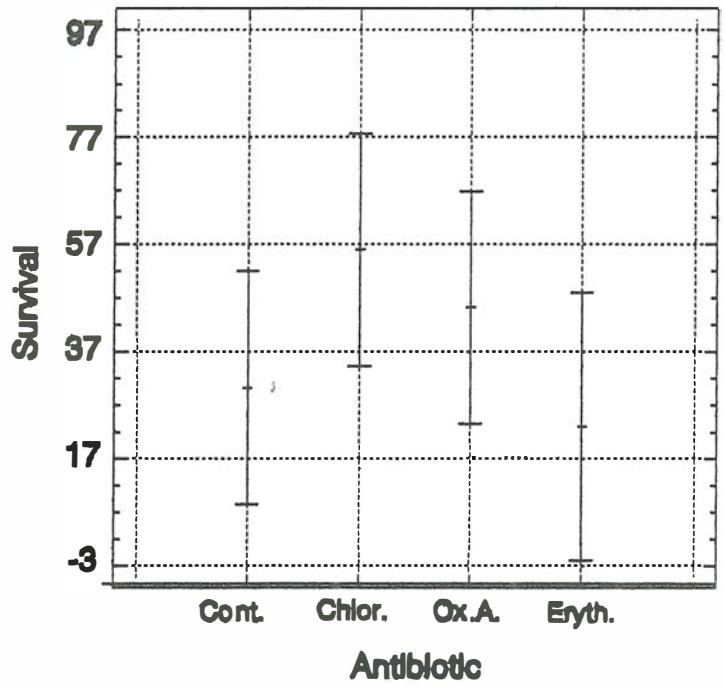
Fig 5.3.2 Growth and survival of larvae reared using several water treatments.



*** Concentrate larvae put to set day 14. Rearing temperature 20-22°C.**

Fig 5.3.3 Growth and survival of *P. furnatus* larvae with the addition of antibiotics to the culture water. 10 day trial 9/11/93, 22oC.





5.4 Nursery Rearing

Abstract

A tiered (stacked screen) upweller nursery system, was developed as an interim cost effective method of producing large numbers of 10 to 15mm scallop spat for fisheries enhancement and farming. A miniature experimental version of this system was built to determine combinations stocking density and seawater flow rates that would ensure high operating efficiency without seriously compromising growth rate. In the absense of screen rotation, mean growth rates were greatest for spat held on the first (most upstream) of eight screens making up each stack and exhibited a progressive decline in successive downstream screens at all densities tested. The mean biomass gain for spat on screens within stacks that were rotated daily was significantly greater ($F=9.434$, $df=5$, $P<0.05$) than `that exhibited by spat held on screens retained in fixed positions within stacks. Rotation of screens was shown to overcome some of the adverse effects of screen position within stacks and was able to significantly increase nett biomass gains per unit of upweller space and per unit of seawater flow. However the reason for this was not clear. Surface area density per sé is a critical factor determining growth rate of *P. fumatus* spat on field upweller screens. In the absense of other growth limiting factors such as food, the upper limit of stocking rate at which maximum growth rate is maintained lies at about 100% screen coverage. However, only marginal reduction in growth occurs at stocking rates of up to 300% screen coverage. Although maximum growth rates recorded for *P. fumatus* spat coincided with flow (l/min) to biomass(Kg) ratios of 20 to 30 : 1 such ratios are inherently inefficient. Reuse of seawater via tiered upweller screens enabled a doubling or trebling of yields per unit of flow. Regular rotation of screens must however be coupled to increased vigilance and use of other safeguards such as alarm systems plus increased cleaning, predator removal and other maintence operations. Otherwise, cost advantages of stacked screen systems over single (monolayer) systems may be more than offset by occasional catastophic losses.

Introduction

While hatchery production of *P. fumatus* has been deemed to be economically viable (Cropp

and Frankish, 1990), several technical constraints to high efficiency production still exist. These include, development of reliable techniques for controlled settlement of larvae on to mesh or fibrous materials that are stuffed into long- line spat bags. Such methods are the subject of continuing research. Frankish et al., (1989) found that large numbers of *P. fumatus* larvae avoid setting on mesh substrates within spat collector bags when placed in hatchery rearing vessels, choosing instead to settle on the walls of the vessels.

As an interim solution to this problem, use was made of culch-free settlement technology previously applied to the European scallop *Pecten maximus* (Delaunay et al., 1993). "Competent" larvae were transferred onto 450mm downweller screens fitted with 160µm mesh. Following settlement and metamorphosis, spat were retained within the hatchery at densities of 10 000 to 100 000 per 450mm diameter downweller screens (Frankish et al., 1991) until large enough to be retained on 500μm mesh upweller screens. They were then transferred to a field upwelling system to feed on wild phytoplankton thereby reducing labour requirements and freeing-up hatchery space.

As with the Japanese scallop *Patinopecten yessoensis* (O'foighil et al., 1990) and the purple hinge rock scallop *Crassodoma gigantea* (Leighton and Phleger, 1981) the move to a natural diet has been found to be beneficial for *P. fumatus*. Spat deployed to field upwellers have been found to outgrow siblings held in the hatchery on downweller screens and maintained on a standard blend of three cultured algae *(Pavlova lutheri*, Tahitian *Isochrysis* and *Chaetoceros calcitrans*) previously identified as supporting optimum growth and survival of the larvae of Sydney rock oyster (Nell and O'Connor, 1991) and of *P. fumatus* (See Section 5.22)

Healthy *P. fumatus* spat attach to screens by byssal threads and may be retained at high densities to a size of 5-8 mm at which their byssus gland becomes vestigial. At this time the rapidly growing spat adopt a benthic habit necessitating horizontal orientation as a monolayer. As a consequence, they become much more susceptible to physical disturbance and density dependent reductions in growth. Food availability and waste product removal can be improved by increasing water flow rates, however, growth and survival may be impaired by the scallops competing for food and space and by physically damaging each other.

Concerning the latter, overcrowding of detached spat on can lead to inadvertent "biting" where swimming scallops lock valves damaging the mantle and other soft tissues. This soft tissues damage results in reduced growth, increased susceptibility to secondary infection by microbial disease agents, shell deformities and involuntary gaping and hence increased susceptibility to a variety of predators that gain access to the screens as settlement stage larvae via incoming seawater. These predators commonly include flatworms, polychaetes and crabs. As a result, spat densities in upweller screens need must be continually monitored and periodically reduced in order to maintain the health and growth at "acceptable levels" within practical limits of cost.

Tackling equivalent problems in relation to 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.

An adaption of this principle, involving the use of multi tiered (stacking) upweller screens, was developed during the current study with two objectives in mind. The first was to determine optimum combinations of stocking density and seawater flow rate for scallop spat held in a field upwellers at a high salinity site within Port Stephens. The second objective was to assess the practical utility of stackable upweller screens for large scale nursery production of 10 to 15mm spat.

Materials and Methods

An experimental upweller system was installed at Tomaree Headland, a high salinity site adjacent to the entrance to Port Stephens (32° 43'S; 152° 11'E)(Fig.5.11.1). The unit (Fig 5.4.1) comprised a twin compartment 2.2 x 1.2 x 0.5m fibreglass tank 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. Five mini-upweller modules were installed in one of the upweller compartments. Each module accomodated eight sets of miniature screens constructed from PVC plumbing components.

Each of the 40 sets of screens comprised a 90mm PVC pipe outlet manifold to which stacks of up to nine interlocking screens, made from 90 mm PVC endcaps 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 thence 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 eight screen from escaping through the overflow port.

The flow rate of seawater upwelling through each stack of miniature screens was regulated in two stages. The first stage, controlling combined flow rate through the entire 40 stacks, was achieved by adjusting flow into the main chamber of the upweller unit with a 50mm PVC ball valve. The second stage enabled regulation of flowrates through individual mini upweller stacks. This involved the use of a simple adjustable weir comprising a 20mm 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 exhalent (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 (one fortieth of the total rate entering the common inlet manifold) or to differing rates, 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 100mL 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 psuedofaeces by back flushing (from top to bottom)with running sea water.

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

Experiment 1 The effects of spat density and screen level upon growth.

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 were combined in fixed positions to form each mini- upweller stack. Five replicate stacks at each density were randomly placed among the 5 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 2 weeks

Experiment 2 the effect of rotation of screen order.

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 screens in each of four replicate stacks were stocked at 1.8 g screen^{-1} (14.4 g stack^{-1}). Each day, the bottom screen (position 1) was rotated to the top of the stack (position 8). Growth of scllops on rotated screens 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.

Experiment 3 The effects of spat crowding.

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 from 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 sack 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 precieved 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± 0.5l min^{-1} $cylinder^{-1}$, which was not expected (at least initially) to be limiing 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) $^{-2}$

Statistical analysis

Homogeneity of variance in each case was confirmed using Cochrans test (Winer, 1971) and data were analysed using ANOVA. Means were compared using Student-Newman-Keuls 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 $\chi^{0.5}$ 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 and 3, seawater temperature ranged

from 17 - 22°C and salinity was in the range 33.0 to 35.0 g kg^{-1}.

Two factor ANOVA was conducted on the effects of density and screen level upon spat weight increases in Experiment 1. A significant interaction between the factors ($F=2.016$, $df=49$, $P<0.001$) was demonstated. Spat weight increase (Fig.5.4.2) was greatest in the first screen in each stack and exhibited a progressive decline over successive screens at all densities tested. Biomass dependent decline in growth rate, as indicated by the negative slopes of lines linking data points in Fig 5.4.3 A&B were very similar for all initial stocking densities in the range 0.6 to 7.2 g/screen. Slopes of lines linking data of spat stocked at the two lowest initial densities of 0.3 and 0.45 g/screen were however less pronounced . Nevertheless, least squares linear regressions fitted to the latter (Table 5.4.1) exhibited very small negative slopes that were significantly less than 0 at all but the lowest socking rate of 0.3 g per screen.

When stocked at a density of 1.8 g/screen, at which screen position effects could be expected , mean ± sd survival (77±?%)`of spat on screens subjected to daily rotation in Experiment 2 did not vary significantly ($F= 1.452$, $df=5$, $P>0.05$) from that of spat on screens in fixed positions (72±?%). 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.

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 5.4.4, the effects of surface area stocking density *per sé* were clear. At the completion of week 1, size of spat stocked initially at100% screen area was very marginally below that of spat initially stocked at 50%. Corresponding surface areas at the end of week one were 88% and 175% screen area. By this time, spat stocked at higher initial rates of 150% or more of available screen area had reached a screen coverage of 250 to 630% and growth was less than at lower stocking rate treatments..

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 density of 300% and had fallen slightly behind in biomass gain.

It is clear from the results of Experiment 3 (Fig. 5.4.4) 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 absense 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%..

The relative (proportional) increase in biomass from week to week varied greatly in Experiment 3 (Fig.5.4.5), . 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 much unexpectedly much lower 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, while variable, showed a similar range for each week. Thus salinity and temperature are not thought to have caused the reduced growth in week 2.

Discussion

Upwelling systems have been used to culture a variety bivalve molluscs (Bayes, 1981; Rodhouse and O'Kelly, 1981;Manzi et al.,1986; Spencer et al., 1986; Malinowski and Siddal,1989) and growth within such systems has been explained in terms of several variables. Food availability in particular has been found to be the major factor limiting growth and production. The effect of water flow rate on growth of hatchery reared *Ostrea edulis* and *Crassostrea gigas* was found by Spencer (1988) to depend on the amount of food in the water with maximum feeding and growth rate coincident with flow rates that produced about a 20% filtration of suspended material passing through upwelling screens.

Very similar findings were made in relation to clam (*Mercenaria mercenaria*) spat by Manzi et al. (1986). Growth rates were consistently reduced as phytoplankton filtration rate (as measured by reduction of chlorophyll levels) rose above 20%. Such rates of removal generally coincided with seawater flow (l/min) to biomass (Kg) ratios in the order 15 - 30 : 1, depending on relative concentrations of phytoplankton, but did rise as high as 60:1 during periods of particularly low food density. Comparable flow to biomass ratios of 25:1 can be derived from data provided by Rhodes and Widman (1980) for raceway reared scallop *(A. irradians*) spat. Maximum growth rates recorded in the present study pertaining to *P. fumatus* spat on the first (lowest) screen within non rotated stacks (Experiments 1 and 2) also coincided with flow to biomass ratios of 20 to 30: 1. An important inference of the above is that any attempt to maximize growth rate by adjusting seawater flow to a rate at which spat filter out only 20% of available food, constitutes an inherently inefficient use of pumping operations and of capital infrastructure.

Even if maximisation of growth were to become a legitimate target, (as in the production of relatively small numbers of high value species such as pearl oysters (*Pinctada* spp)), or when stock must be grown to a minimum critical size to successfully overwinter, assessment of available food and its rate of depletion is problematic. Unfortunately, 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, is not always clear let

alone measurable. Numerous studies of juvenile bivalve growth have used chlorophyll levels as a measure of available food. However this may discount significant contributions from direct uptake of dissolved substances and other potential particulate food sources such as detritus and associated bacteria and yeast.

The site of the present study on Tomaree Headland lies only 400m from the entrance to mouth of Port Stephens, and is subject to large and rapid changes in physiochemical characteristics of seawater.. This is especially so during the summer months when the central and southern NSW coast is affected by upwelling of deep cold (14 to 16°C) nutrient rich seawater generated by eddies that split from the southward flowing East Australian current (see fuller discussion in Section 5.23). This cold nutrient rich water may displace or mix in a complex way with warmer coasal water or surface layers of the East Australian Current, depending on local coastal geography, prevailing wind patterns, tidal amplitudes etc..

This complex oceanography may in turn generate large and rapid fluctuations in the quality and concentrations of available food. .Phtyoplankton level changes as great as an order of magnitude may occur as a consequence (Hallegraef, 1981). Although measurement of available food within seawater pumped through the upweller units at Tomaree Headland was deemed beyond the scope of this study, varying food availability may well account for the 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). Beninger (1991) has suggested that when particles of >10 μm in size exceed a threshold concentration, undesirable particles (unpalatable, toxic, unwieldy or inorganic) are rejected with increasing efficiency as particle concentration increases.

It was thought that the threshold for particle rejection may correspond to the threshold for the formation of psuedofaeces, however Lesser et al. (1991), found *P. magellanicus* juveniles exhibited particle selection at food concentrations below the level at which psuedofaeces were formed. Importantly, the same study found particle selection in juvenile *P. magellanicus* was not based upon particle size alone and suggested selection may have resulted from chemosensory cues determining which cells were cleared or ingested.

Within stacked screen upweller nursery systems of the type used in the present study, 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 amongst a complex array present in raw incident seawater. To control the amount of material ingested, bivalves may vary the time spent filtering, vary the particle clearance (filtration) rate or vary the rate at which filtered particulate matter is rejected as pseudofaeces rather than injested (Foster-Smith, 1975).

While the former conrtol methods are unlikely to adversely affect scallops in subsequent (downstream) stacked screens, the rejection of excess food as pseudofaeces by upstream scallops may deny that food source to those further downstream. However, while pectinids undoubtedly produce pseudofeaces, Bricelj and Shumway (1991) contend that scallops primarily regulate ingestion through reductions in clearance rate rather than pseudofaeces production. In addition, spat located on the first screen within upweller stacks were not exposed to waste products nor to possible deleterious effects associated with decreased oxygen and pH or with an accumulation of pathogens and parasites emanating from upstream screens.

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 maximized 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 nett biomass gains per unit of volume within upwellers and per unit of seawater flow.The reason for this is not however clear. A possible explanation of observed advantages of regular screen rotation may arise from the fact that pectinids, being typical suspension feeding bivalves, exhibit decreasing efficiency with wich 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 nett 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 up to 200% the available screen surface area whilst maintaining high mean growth rates . Rhodes and Widman (1980) reported that *Argopecten irradians* could be grown from a mean shell -height of 5mm to 10mm at stocking rates up to 170 000 scallops per $12m^2$ 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 Experiment 3 in the present study are comparable with those of Manzi et al. (1986), who found that upflow systems used to rear the clam *M. mercenaria* were far more space efficient than traditional raceway nursery systems, supporting the production of more than five times the biomass of animals per unit area. Results of Experiments 2 and 3 are also comparable to 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 absense of screen rotation .

Results of the present study also indicate that very high nett biomass gains, and hence

overall operational efficiency of stacked screen upwellers, can be attained at surface area stocking rates up to about 400 % of avaliable screen area without seriously compromising mean growth rate. However, as possible long- term deterimental effects, such as irreversible shell deformity, were not evaluated, stocking rates beyond 200% of available screen area may not be advisable .

Use of tiered water reuse nursery systems does nevertheless have a number of practical disadvantages. Unlike monolayer systems, stock within stacked screens cannot be inspected at a glance. Tiered upwelling systems are also more prone to water flow restiction caused by multiple rather than single layers of mesh and associated biofouling. They are also more 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 maintence operations. Otherwise, cost advantages of tiered systems over monolayer systems may be more than offset by occasional catastophic losses.

Fig 5.4.1 Experimental upweller unit

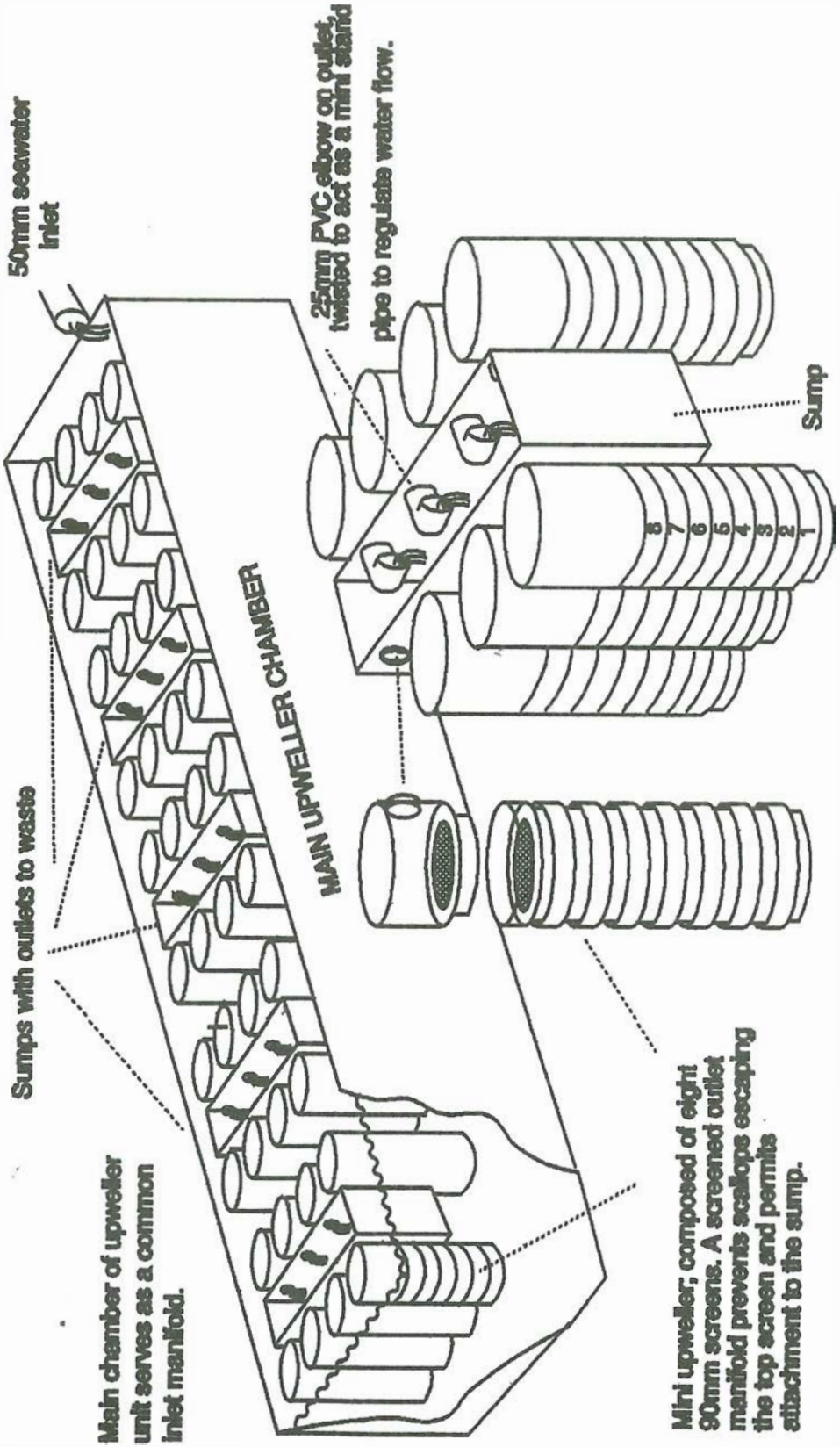


Fig 5.4.2 Biomass increase in commercial scallop spat held at different levels within stacks of upweller screens. Positions 1-8 indicate the order in which screens recieved seawater. *Initial stocking density in g/screen.

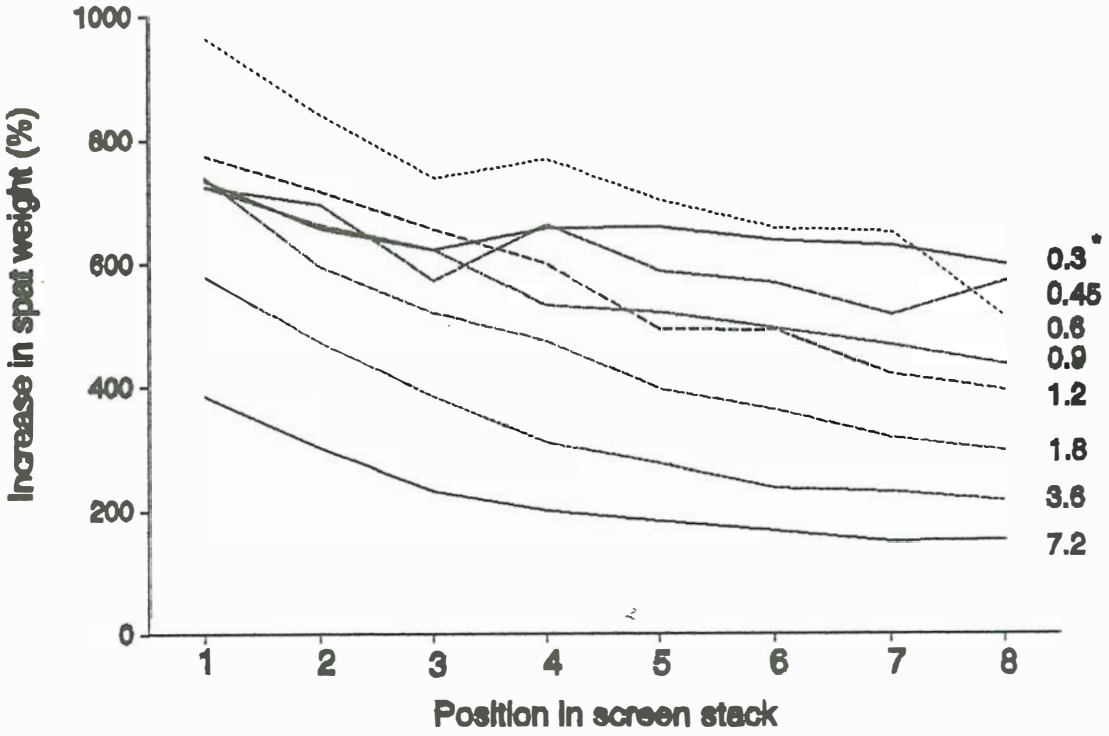


Fig 5.4.3 A & B Biomass increase of the commercial scallop held at one of eight densities in stacksvr of eight upweller screens. Initial mean weight of spat 26±1 mg. Values are means ± se for (A) spat held in the first screen to recieve water in each stack and (B) for the total stack.

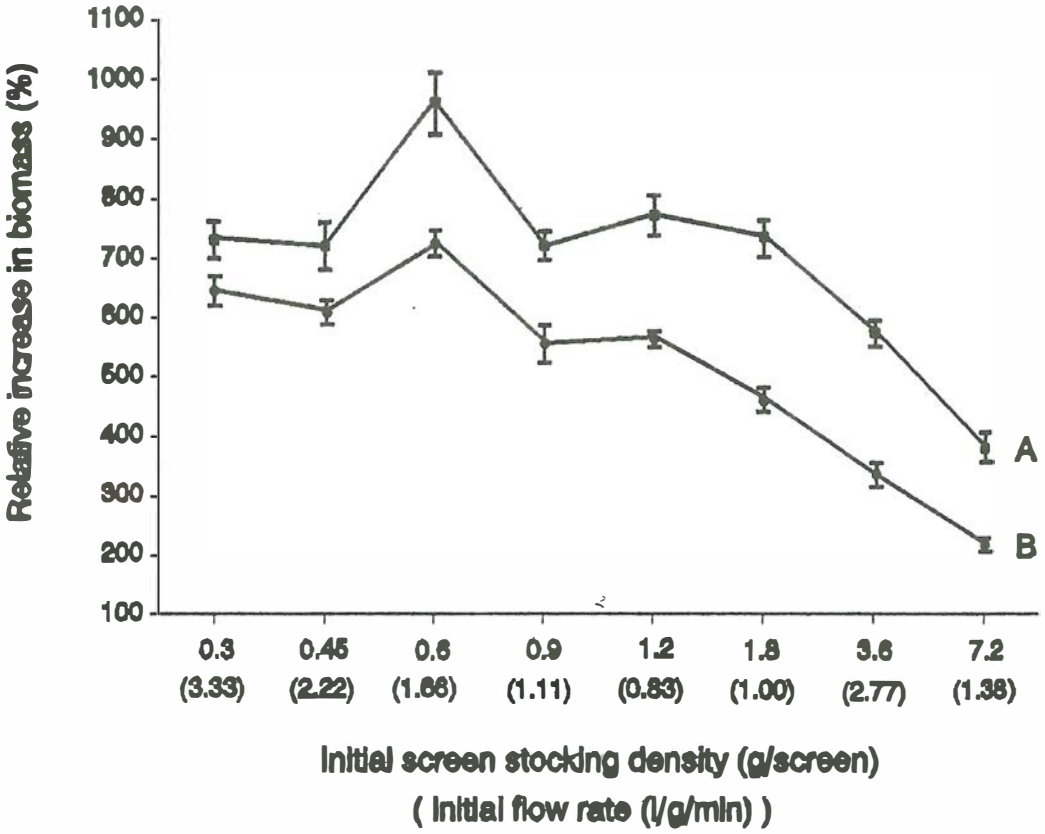
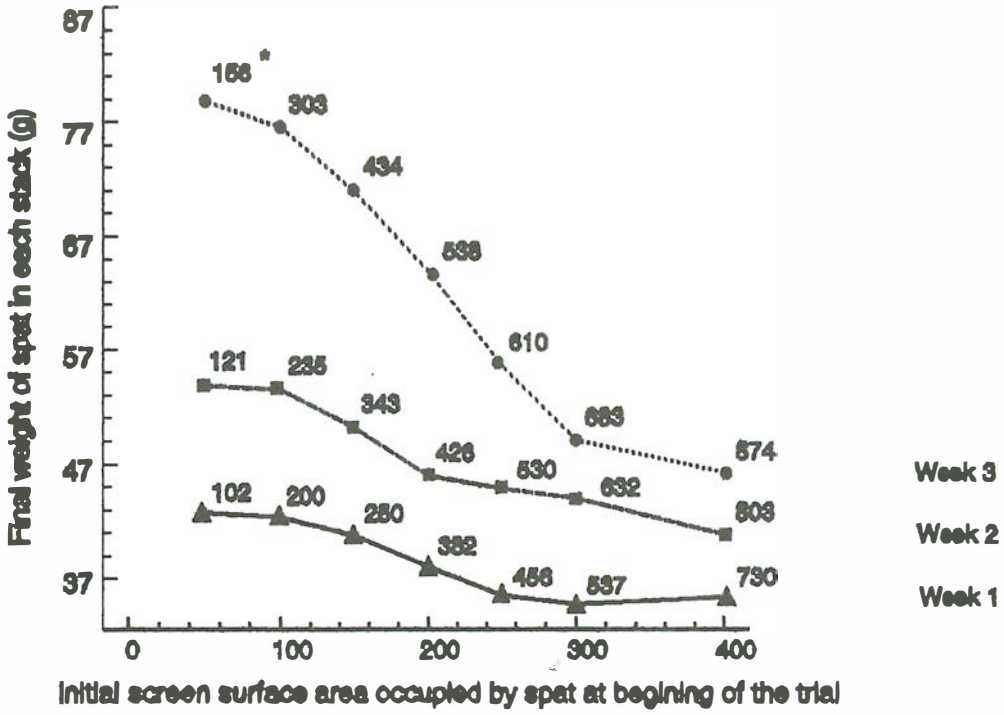
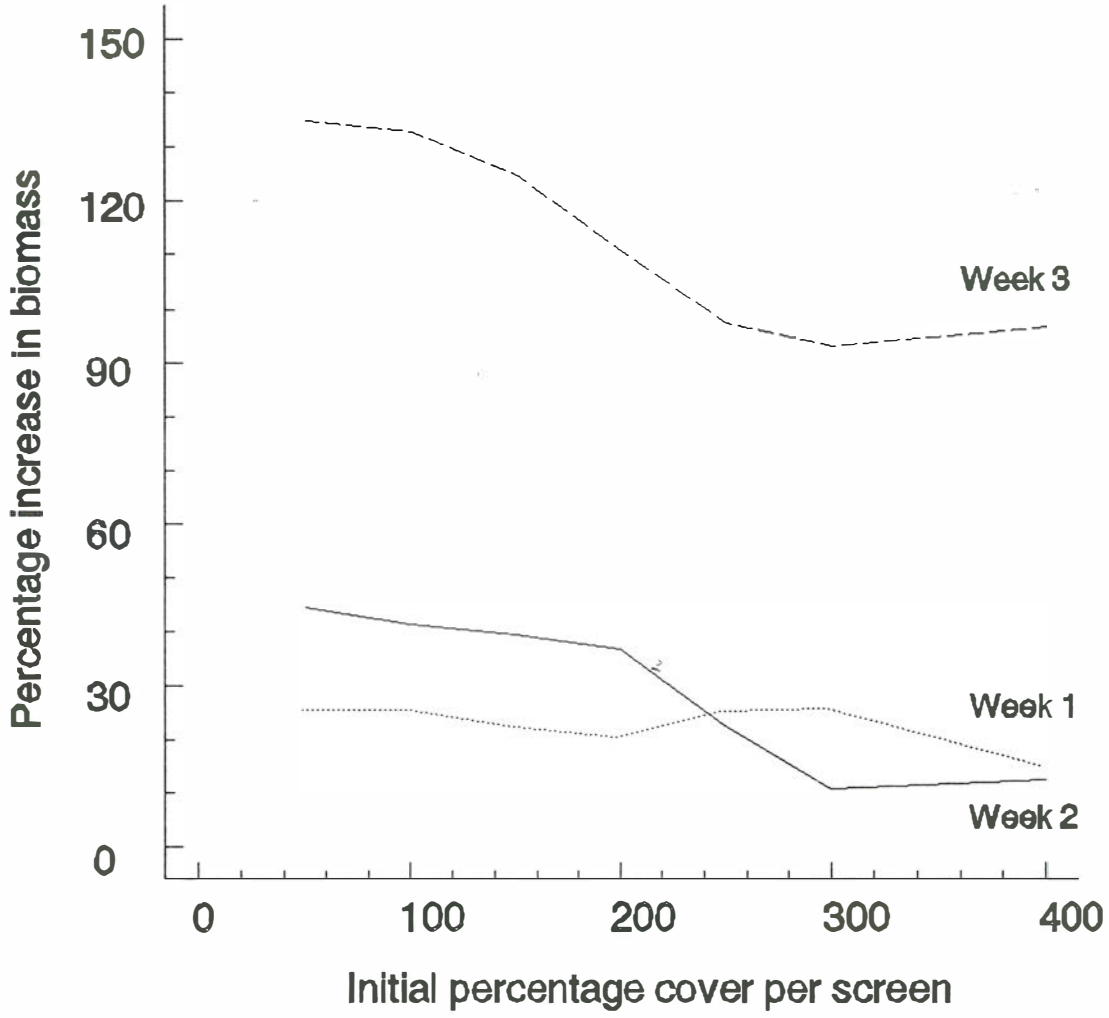


Fig 5.4.4 Weight increase over time of 14..4 g of commercial scallop spat stocked at various densitieswithin stacks of upweller screens.



* Estimated % of available screen area occupied by spat at the end of each week at each biomass/surface are stocking rate.

Fig 5.4.5 Weekly biomass increaseof commercial scallop spat held in two or more screens held within an eight screen stack. Initial stocking density 14.4 g/stack.



REFERENCES

Alagarswami, K., Dharmaraj, S., Chellam, A. and Velayudhan, T.S., 1989. Larval and juvenile rearing of the black-lip pearl oyster, *Pinctada margaritifera* (Linnaeus). Aquaculture, 76:43-56.

Barber, B.J. and Blake, N.J., 1985. Substrate catabolism related to reproduction in the bay scallop *Argopecten irradians*, as determined by O/N and RQ indexes. Mar. Biol., 87(1): 13-18.

Barber, B.J. and Blake, N.J., 1991. Reproductive physiology. In: S.E. Shumway (Editor), Scallops: Biology Ecology and Aquaculture. Elsevier, Amsterdam, 377-420.

Barber, B.J., Getchell, R., Shumway, S., and Shick, D., 1988. Reduced fecundity in a deep-water population of giant scallop, *Placopecten magellanicus*, in the Gulf of Maine, U.S.A.. Mar. Ecol. Prog. Ser., 42: 207-212.

Bartrom, A., 1987 Japanese scallop production. Catch, Nov: 11-14

Baticados M. L. C. and Pitogo, C. L., 1990 Chlorination of seawater used for shrimp culture. Israel J. Aquac. (Bamidgeh), 42: 128-130.

Bayes, J.C., 1981. Forced upwelling nurseries for oysters and clams using impounded water systems. In: C. Claus, N. de Pauw and E. Jaspers (Editors), Nursery Culturing of Bivalve Molluscs. Eur. Maricult. Soc. Spec. Publ. 7, Bredene, Belgium, pp. 73-83.

Bayne, B.L., 1983 Physiological ecology of marine mollusc larvae. In: A.S.M. Saleuddin and K.M. Wilbur (Editors), The Mollusca, Vol. 3. Academic Press, New York, pp. 299-343.

Beaumont, A. R. and Abdul-Matin A. K. M., 1994. Differences in morphology, survival and size between Self- and cross-fertilized larvae of *Mytilus galloprovincialis*. J. Mar. Biol. Ass. U.K., 74: 445-448.

Beaumont, A. R. and Budd, M. D., 1983. Effects of self fertilisation and other factors on the early development of the scallop *Pecten maximus*. Mar. Biol., 76: 285-289.

Beninger, P.G., 1991. Structures and mechanisms of feeding in scallops: Paradigms and paradoxes. In: Shumway, S.E and Sandifer, P.A. (Editors) An International Compendium of Scallop Biology and Culture. The World Aquaculture Society, 331-340.

Blackburn, S.I. and Cresswell, G., 1993. A coccolithophorid bloom in Jervis Bay, Australia. Aust. J. Mar. Freshwater Res., 44: 253-260.

Boland, F.M., 1979 A time series of expendable bathythermograph sections across the East Australian Current. Aust. J. Mar. Freshwater Res., 30: 303-313.

Boland, F.M., B.J. Barber, and N.J. Blake., 1985. Substrate catabolism related to reproduction in the bay scallop *Argopecten irradians*, as determined by o/n and RQ indexes. Mar. Biol., 87(1): 13-18.

Bourne, N. and Hodgson, C.A., 1991. Development of a viable nursery system for scallop culture. In: Shumway, S.E and Sandifer, P.A. (Editors) An International Compendium of Scallop Biology and Culture. The World Aquaculture Society, 273-280.

Bourne, N., Hodgson C. A., and Whyte, J. N. C., 1989. A Manual for Scallop Culture in British Columbia. Canadian Tech. Rep. Fisheries and Aquatic Sciences N° 1694. 209 pp..

Bowers, E.A., 1969. *Cercaria Bucephalus haimeana* (Lucaze-Duthiers, 1854) (Digenea: Bucephalidae) in the cockle *Cardium edule* L. in South Wales. J. Nat. Hist., 26: 813-826.

Brand, A.R., 1991. Scallop ecology: distributions and behaviour. In: S.E. Shumway (editor) Scallops: Biology, Ecology and Aquaculture. Developments in Aquaculture and Fisheries Science, 21. Elsevier, Amsterdam. pp. 517-584.

Broom, M.J. and Mason, J., 1978. Growth and spawning in the pectinid *Chlamys opercularis* in relation to temperature and phytoplankon concentration. Mar. Biol. 47, 277-286.

Bricelj, V.M., Epp, J. and Malouf, R.E., 1987. Intraspecific variation in reproductive and somatic growth cycles of bay scallops *Argopecten irradians*. Mar. Ecol. Prog. Ser., 36: 123-137.

Bricelj, V. M. and Shumway, S., 1991. Physiology: Energy Acquisition and Utilisation. In: S.E. Shumway (Editor), Scallops: Biology Ecology and Aquaculture. Elsevier, Amsterdam, pp. 305-346.

Brown, A., 1972. Experimental techniques for preserving diatoms used as food for larval *Peneaus aztecus*. Proc. Nat. Shellfisheries Ass., 62: 21-25.

Brown, C. 1973. The effects of some selected bacteria on embryos and larvae of the American oyster, *Crassostrea virginica.* J. Invertebr. Pathol., 21: 215-223.

Brown, C. and Losee, E., 1978. Observations on natural and induced epizootics of vibriosis in *Crassostrea virginica* larvae. J. Inv. Path., 31: 41-47.

Brown, M.R., Jeffrey, S. W., and Garland, C.D., 1989. Nutritional aspects of microalgae used in mariculture : a literature review. C S I R O, Marine Laboratories Report, Hobart, Tas. 44pp.

Bucaille, D. and Kim, N., 1981. Influence de la chloration sur la fixation, le detachement, la croissance des larves pediveligeres et le jeune naissain de moules, et sur la gametogenese des adultes, Influence des Rejets Thermiques sur le Milieu Vivant en Mer et en Estuaire. pp. 385-411.

Buested, D. Cochard, J.C., Dao, J. C. and Gerard, A., 1982. Production artificielle de naissain de coquilles Saint -Jacques *Pecten maximus* L. Premiers results en rade de Brest. Vie Mar., 4: 24-28.

Bull, M. F., 1988. Scallop farming in Japan. In: Scallop Industry study mission of Canada to Japan. Reference infomation II.Zengyoren. (National Federation of Fisheries Cooperative Associations), Tokyo.

Bull, M.F., 1989. The New Zealand scallop enhancement project cost and benefit.. *In:* Dredge, M.L.C., Zacharin, W.F. and Joll L.M.,(eds.) Proceedings of the First Australasian Scallop Workshop, Hobart, Australia, pp 154-165.

Caddy, J.F., 1972 Progressive loss of byssus attachment with size in the sea scallop,*Placopecten magellanicus* (Gmelin). J. Exp. Mar. Biol. Ecol., 9:179-190.

Castagna, M., 1975. Culture of the bay scallop, *Argopecten irradians*, in Virginia. Mar.Fish. Rev., 37: 19-24.

Chaitanawisuti, N. and Menasveta, P., 1992. Preliminary studies on breeding and larval rearing of the Asian moon scallop (*Amusium pleuronectes*). J. Aquaculture Trop., 7 : 205-218.

Chen, D. and Hanna, P.J., In Press. Immunodetection of specific *Vibrio* bacteria attaching to tissues of the giant tiger prawn *Penaeus monodon* (Fabricius, 1798.) Dis.Aquat. Organisms.

Claus, C., 1981. Trends in nursery rearing of bivalve molluscs. In: C. Claus, N. de Pauw and E. Jaspers (Editors), Nursery Culturing of Bivalve Molluscs. Eur. Maricult. Soc. Spec. Publ. 7, Bredene, Belgium, pp. 1-33.

Cochard, J.C. and Devauchelle, N., 1993. Spawning, fecundity and larval survival and growth in relation to controlled conditioning in native and transplanted populations of *Pecten maximus* (L.): evidence for the existence of separate stocks. J. Exp. Mar. Biol. Ecol., 169: 41-56.

Coleman, N., 1989. Spat catches as an indication of recruitment to scallop populations in Victorian waters In: M.L.C. Dredge, W.F Zacharin, and L. M. Joll. (Editors) Proceedings of the Australasian Scallop Workshop, Hobart, Australia, pp. 51-60.

Cranfield, H.J., 1975. Molluscs: Bluff oysters (1). New Zealand Nature Heritage 5 (71): 1965-1970.

Cragg, S.M. and Crisp, D.J., 1991. The biology of scallop larvae. In: S.E. Shumway (Editor). *Scallops: Biology Ecology and Aquaculture.* Dev. Aquaculture and Fish. Sci., 21:.75-132.

Cropp, D.A., 1985. Scallops thrive in TFDA culture program. Aust. Fish., 44(1): 16-18.

Cropp, D.A., 1987 Feasibility of scallop culture in Tasmania. Tasmanian Sea Fisheries. Tech. Report 15

Cropp, D.A. 1989. Scallop culture in the Pacific region. In Dredge, M.L.C, Zacharin, W.F. and Joll, L.M. (Editors), Proceedings of the Australasian Scallop Workshop. Hobart, Australia, 134-153.

Cropp, D.A. and Frankish, K., 1989. Cost comparison of hatchery and naturally produced spat for the scallop *Pecten fumatus* Reeve. In Dredge, M.L.C, Zacharin, W.F. and Joll, L.M. (Editors), Proceedings of the Australasian Scallop Workshop. Hobart, Australia, 196-225.

CSIRO 1994. Jervis Bay baseline studies: Final Report, CSIRO, Division of Fisheries, Jervis Bay, NSW, Australia.1282 pp.

Daniel, C. and Wood, A.S., 1980. Fitting Equations to Data: Computer Analysis of Multifactor Data. 458 pp. John Wiley & Sons, New York.

Dao, J. C., Buestel, D. and halary, C., 1985. not sur l'evolution compare'e des coquilles Saint -Jacques d'origine differente en Bretagne nord. 5th Pectinid Workshop. La Corogne, May 1985.

Davis, H. C. and Guillard, R. R., 1958. Relative value of ten genera of microorganisms as food for oyster and clam larvae. Fish. Bull. . Fish.Wildlife Service, U.S., 58: 293-304.

Delaunay, F., Marty, Y., Moal, J. and Samain, J.F., 1993. The effect of monospecific algal diets on growth and fatty acid composition of *Pecten maximus* (L..) larvae. J. Exp. Mar. Biol. Ecol., 173 : 163-179.

Devauchelle, N. and Mignant, C., 1991. Review of the reproductive physiology of the scallop *Pecten maximus,* applicable to intensive aquaculture. Aquat. Living Resour., 4: 41-51.

Dix, T. G. and Sjardin, M.J., 1975. Larvae of the commercial scallop *Pecten meridionalis* from Tasmania, Australia. Aust. J. Mar. Freshwater Res., 26: 109-12.

Dorange, G., Paulet, M., Le Pennec, M. and Cochard J.C., 1989. Critereshistologiques d'evaluation de la qualite des ovocytes emis par *Pecten maximus* (Mollusque, Bivalve). C. R. Acad. Sci., Paris, 309: 113-120.

Douillet, P. and Langdon, C. J. 1993. Effects of marine bacteria on the culture of axenic oyster *Crassostrea gigas (*Thumberg) larvae. Biol. Bull. 184: 36-51.

Douillet, P. and Langdon, C. J. 1994. Use of a probiotic for the culture of larvae of the pacific oyster *Crassostrea gigas (*Thumberg) . Aquaculture, 119: 25-40.

Dredge, M.L.C., 1981. Reproductive biology of the saucer scallop *Amusium japonicum balloti* (Bernardi) in central Queensland waters. Aust. J. Mar. Freshwater Res., 35: 775-87.

Elston, R.., 1990 Mollusc diseases. - Guide for the shellfish farmer. Washington Sea Grant Program. Univ. Washington Press. Washington, USA. 73pp.

Elston, R. A. 1984. Prevention and management of of infectious diseases in intensive mollusc husbandry. J. World Mar. Soc. 15: 284-300.

Elston, R. and Leibovitz, L 1980. Pathogenesis of experimental vibriosis in larval American oysters, *Crassostrea virginica*. Can. J. Fish. Aquat. Sci. 37 : 946-978.

Elston, R., Leibovitz, L., Relyea, D. and Zatila, J., 1981. Diagnosis of vibriosis in a commercial oyster hatchery epizootic: diagnostic

Enright, C. T., Newkiirk, G. F., Craigie, J.S. and Castell, J. D. 1986a. Evaluation of phytoplankton as diets for juvenile *Ostrea edulis L.* J. Exp. Mar. Biol. Ecol., 96: 1-13.

Enright, C. T., Newkiirk, G. F., Craigie, J.S. and Castell, J. D. 1986b. Growth of juvenile *Ostrea edulis* L. fed *Chaetoceros gracilis* Schütt of varied chemical composition. J. Exp. Mar. Biol. Ecol. 96:15-26.

Epifanio, C.E., 1979. Growth in bivalve molluscs: nutritional effects of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* (Gmelin) and the hard clam *Mercenaria* (L.). Aquaculture, 18: 1-12.

Epifanio, C.E., 1979. Growth in bivalve molluscs: nutritional effects of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* (Gmelin) and the hard clam *Mercenaria* (L.). Aquaculture, 18: 1-12.

Epifanio, C.E., 1983. Plankton and Yeast as foods for juvenile bivalves: A review of research at the University of Delaware. In: G.D. Pruder, C. Langdon and D. Conklin (Editors), Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and physiological approaches to shellfish nutrition. October 27-29, Rehoboth Beach, Delaware, 292-304.

Fairbridge, W.S., 1953. A population study of the Tasmanian "commercial" scallop *Notovola meridionalis* (Tate), (Lamellibranchiata, Pectinidae). Aust. J. Mar. Freshwater Res., 4: 1-40.

Frankish, K., Goard L. and W. A. O'Connor. 1990 Hatchery scallop culture success in NSW. Austasia Aquac. Mag., 4(9): 10-11.

Frankish, K., Goard, L.J. and O'Connor, W.A., 1991. The development of hatchery rearing techniques for the production of Sydney Rock Oyster *Saccostrea commercialis*. BWFCRS. Dept. Agri. & Fish., Internal Report, 26pp..

Fuentes, H. R., 1994. Population biology of the commercial scallop (*Pecten fumatus*) in Jervis Bay, NSW. Memoirs of the Queensland Museum 36(2), 247-259.

Fuentes, H.R., Williams, R.J., Diver, L.P. and Smith, A.K., 1992. Jervis Bay Marine Ecology Study, Project 6: Commercial Molluscs of Jervis Bay, Final Report, September 1992. NSW Fisheries, NSW, Australia, 193 pp..

Gabbot, P.A. 1975. Storage cycles in marine bivalve molluscs: a hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In: H. Barnes (Editor), Ninth European Marine Biology Symposium, Aberdeen University Press, Aberdeen, Scotland, pp. 191-211.

Garland, C. D., Cooke, S.L., Cooke, T.A. McMeekin, T.A. and Valentine, J.E., 1986. Effects of 0.2μm filtered seawater as a culture medium on fertilized eggs and larvae of the Pacific oyster,*Crassostrea gigas.* Aust. J. Mar. Freshw. Res., 37: 713-720.

Galtsoff, P.S., 1964. The American Oyster *Crassostrea virginica* Gmelin. Fishery Bulletin of the Fish and Wildlife Service, US Govt.Printing Office, Washington, D.C., Vol. 64: 440 pp.

Gibbons, M.C. and Castagna, M. 1984. Serotonin as an inducer of spawning in six bivalve species. Aquaculture, 40: 189-191.

Grant, A. and Tyler, P.A. 1983. The analysis of data in studies of invertebrate reproduction. I. Introduction and statistical analysis of gonad indices and maturity indices. Int. J.of Invertebrate Rep., 6: 259-269.

Griffith, G.W., Murphy Kenslow, M.A. and Ross, L.A., 1973. A mass culture method for *Tetraselmis* sp - A promising food for larval crustaceans. Proc. Ann. Meet. World Mariculture Soc., 4: 289-294.

Gruffydd, Ll. D. and Beaumont, A. R., 1970. Determination of the optimum concentration of eggs and spermatozoa for the production of normal larvae in *Pecten maximus* (Mollusca, Lamellibranchia). Helgoländer wiss. Meeresunters, 20: 486-97.

Gruffydd, LI. D., 1976. The development of the larva of *Chlamys islandica* in the plankton and its salinity tolerance in the laboratory (Lamellibranchiata, Pectinidae). Astarte, 8: 60-67.

Gwyther, D. 1989 History of management in the Victorian scallop industry. In: M.L.C. Dredge, W.F Zacharin, and L. M. Joll. (Editors) Proceedings of the Australasian Scallop Workshop,Tas. Gov. Printer, Hobart, Australia, pp. 12-20.

Gwyther, D., Cropp, D.A., Joll, L.M. and Dredge, M.L.C., 1991. Australia. In: S.E. Shumway (Editor), Scallops: Biology Ecology and Aquaculture. Elsevier, Amsterdam, pp. 835-851.

Gwyther, D. and McShane, P.E., 1988. Growth rate and natural mortality of the scallop *Pecten alba* Tate in Port Phillip Bay, Australia, and evidence for changed in growth rate after a 20-year period. Fish Res., 6: 347-361 .

Hadfield, A.J. and Anderson, D.T., 1988. Reproductive cycles of the bivalve molluscs *Anadara trapezia* (Deshayes), *Venerupis crenata* Lamark and *Anomia descripta* Iredale in the Sydney region. Aust. J. of M.and Freshwater Res., 39: 649-60.

Hadley, N.H., 1993. Effects of hard clam hatchery management practices on productivity and on broodstock quality. World Aquaculture, 24(3): 30-31.

Hallegraeff, G.M., 1981. Seasonal study of phytoplankton pigments and species at a coastal station off Sydney: Importance of diatoms and the nanoplankton. Mar. Biol., 61: 107-18.

Hallegraeff, G.M. and Jeffrey, S.W. 1993. Annually recurrent diatom blooms in spring along the New South Wales coast of Australia. Aust. J. of Mar. and Freshwater Res., 44: 325-34.

Halvey, S. 1993. The use of algae for biological control of animal diseases in marine aquaculture. Unpublished report. November- December 1986. Revised by Dr. Shirley Jeffery, Nov. 1993. pp. 1-9.

Hamer, G., 1987. Scallop Biology and Management. NSW Agriculture and Fisheries. Agfact F1.0.2. Agdex 486/00.

Hamer, G. and N. Jacobs. 1987. The biology, fishery and management of the commercial scallop (Pecten fumatus) in Jervis Bay, New South Wales. Wetlands Aust., 6(2): 39-47.

Hanna, P.J., Altmann, K., Smith, A., Moon, P., Hammond, L.S. 1992. Development of monoclonal antibodies for rapid identification of epizootic *Vibrio* species. J. Fish. Dis. 15: 63-69.

Harris, G.P., P.Davies, M. Nunez and G. Meyers. 1988. Interannual variability in climate and fisheries in Tasmania. Nature, 333: 754-57.

Harrison, A.J. 1961 Annual reproductive cycles in the Tasmanian scallop *Notovola meridionalis*, Unpublished B.Sc.(Hons.) thesis, University of Tasmania, Hobart.

Heald, D., 1974. Project: scallops: *Pecten.* Research Report 1973-1974, Western Fisheries Research Committee Research meetings, 250.

Heasman, M. P. 1992 Rationale for regulation and registration of aquacultural chemicals and drugs in Australia. Prepared for the "Working Group on Aquaculture."

Heasman, M.P. and Nell, J.A. (1991) Scope for enhancement of scallop *(Pecten fumatus)* fisheries in NSW using hatchery reared broodstock. In: Hill, B.J. and N. Preston (eds.), Proceedings of Marine Reseeding Workshop, CSIRO Marine Lab., Cleveland, Qld. Sept. 1991.

Heasman, M.P., O'Connor, W. A. and Frazer, A.W., (in prep.a) Temperature and nutrition as factors in conditioning broodstock of the commercial scallop *Pecetn fumatus* Reeve. Aquaculture,

Heasman, M.P., O'Connor, W.A. and Frazer, A.J.W., 1994a. lmproved hatchery and nursery rearing techniques for *Pecten fumatus* Reeve. Memoirs of the Queensland Museum, 36(2):352-356. Brisbane. ISSN 0079-8835.

Heasman, M.P., O'Connor, W.A. and Frazer, A.J.W., 1994b. Detachment of commercial scallop, *Pecten fumatus*, spat from settlement substrates. Aquaculture, 123:401-407.

Heasman, M.P., O'Connor, W.A. and Frazer, A.J.W., 1995 (In Press)c. Induction of anaesthesia in the commercial scallop *Pecten fumatus* Reeve. Aquaculture

Heasman, M.P., W.A. O'Connor, and A.W. Frazer. (submitted) Effects of fertiization and incubation on the quality and yield of larvae scallop, *Pecten fumatus* Reeve, larvae. Aquaculture Research, .

Heasman, M.P., W.A. O'Connor and A.W. Frazer. (in prep.a) Investigations of the scallop, *Pecten fumatus* Reeve, in Jervis Bay, NSW, Australia, as a source of hatchery broodstock. Aquaculture,

Heasman, M.P., O'Connor, W.A.and Frazer, A.W. J., (in prep.b) Bucephalid trematode infections in commercial scallops *(Pecten fumatus)* and doughboy scallops *(Chlamys asperrima)* in Jervis Bay, NSW. J. Fish Diseases

Holloway, P.E., G. Symonds and R. Nunes Vaz. 1992. Observations of circulation and exchange processes in Jervis Bay, New South Wales. Aust. J. Mar. Freshwater Res., 43 : 1487-14.

Holliday, J.E., 1986. International developments in oyster hatchery technology. Brackish Water Fish Culture Research Station, Salamander Bay, NSW, NSW Agriculture & Fisheries Misc. Rep. No. 1, 101 pp.

Hopkins, S. H., 1954. The American species of trematode confused with *Bucephalis (Bucephalopsis) haimeanus*, Parasitology 44, 353-370.

Hortle, M.E. and Cropp, D.A., 1987. Settlement of the commercial scallop *Pecten fumatus* Reeve 1855, on artificial collectors in eastern Tasmania. Aquaculture, 66: 79-9

Howell, M., 1967. The trematode *Bucephalus longicornutus* (Manter, 1954) in the New Zealand mud-oyster, *Ostrea lutaria*. Trans. Roy. Soc. N.Z., 8: 221-237

Huelevan, S., 1985. Variabilitè gènètique de poulations de *Pecten maximus* L. en Bretagne. Ph. D. Thesis, Universitè de Bretagne Occidentale, France.

Imai T., 1980. The rearing of larval and seedling bivalves. In: Aquaculture in Shallow Seas, Rotterdam (ed. by A.A. Balkema) pp. 579-598. Translation of Senkai Kanzen Yoshoki (Senkai Yoshoku no Shinpo). Koseisha Koseiku Publishers. Tokyo. 1971.

Ito, H., 1991. Japan. *In:* S.E. Shumway (editor) Scallops: Biology, Ecology and Aquaculture. Developments in Aquaculture and Fisheries Science, vol. 21. Elsevier, Amsterdam, pp. 1017-1055.

Jacobs, N., 1983 The growth and reproductive biology of the scallop *Pecten fumatus(*alba) in jervis Bay , NSW and the hydrology of jervis Bay. Mar. Sci. Hons Thesis, University of NSW, 55pp

Jeanathon,C. Prieur, D. and Cochard, J.C., 1988. Bacteriological survey of antibiotic-treatrd sea waters in a *Pecten maximus* hatchery. Aquaculture, 71 : 1-8.

Kijima, A., Mori, K., and Fujio, Y., 1984. Population differences in gene frequency of the Japanese scallop *Patinopecten yessoensis* on the the Okhotsk Sea Coast of Hokkaido. Bull.Jap Soc. Sci. Fish. 50: 241-248.

Kirby-Smith, W.W. 1970. Growth of the scallops, *Argopecten irradians concentricus* (Say) and *Argopecten gibbus* (Linne), as influenced by food and temperature. In: S.E. Shumway (Editor), Scallops: Biology Ecology and Aquaculture. Elsevier, Amsterdam, p.315.

Krantz, G.E., Baptist, G.J. and Merritt, D.W., 1983. Three innovative techniques that made Maryland oyster hatcheries cost-effective. J. Shellfish Res., 3: 1, 95-96.

Lasiak, T., 1991. Bucephalid trematode infections in mytilid bivalves from the rocky intertidal of southern Chile.

Latrouite, D. and Claude, S. 1979. Essai de culture suspendue de la coquille Saint Jacques *Pecten maximus*: influence de la transplantation sur le development gonadique. I.C.E.S., C.M. 1979/F:16: 10

Lauckner, G., 1983. Diseases of Mollusca: Bivalvia. In: O. Kinne, Diseases of Marine Animals. Biologische Anstalt Helgoland, Hamburg, Germany, pp. 632-655.

Leighton, D.L. and Phleger, C.F., 1981. The suitability of the purple hinge rock scallop to marine aquaculture. Calif. Sea Grant College Prog., Sea Grant Pub. Tech., T-CSGCP-001, 85 pp.

Le Pennec, M. and Prieur, D., 1977. Les antibiotiques dans les elevages de larves dde bivalves marins. Aquaculture, 12: 15-30

Le Pennec, M. and Rangel-Davalos, C. 1985. Observations en microscopie a epifluorescence de l'ingestion et de la digestion d'algues unicellulaires chez des jeunes larvae de *Pecten maximus* (Pectinidae, Bivalvia). Aquaculture, 47: 39-51.

Lesser, M.P., Shumway, S.E., Cucci, T., Barter, J. and Edwards, J., 1991. Size specific selection of phytoplankton by juvenile filterfeeding bivalves: comparison of the sea scallop Placopecten magellanicus (Gmelin, 1791) with Mya arenaria Linnaeus, 1758 and Mytilus edulis linnaeus, 1758. In: Shumway, S.E and Sandifer, P.A. (Editors) An International Compendium of Scallop Biology and Culture. The World Aquaculture Society, 341-346.

Lodeiros, C., Bolinches, J. Dopazo ,C. P. and Toranzo A. E. 1987. Bacillary necrosis in hatcheries of *Ostrea edulis* in Spain. Aquaculture, 65 : 15-29.

Loosanoff, V. L. and Davis, H. C., 1963. Rearing bivalve molluscs. Adv. Mar. Biol., 1:1-136.

MacDonald, B.A. and Thompson, R.J. 1988. Intraspecific variation in growth and reproduction of latitudinally differentiated populations of the giant scallop *Placopecten magellanicus.* Biol. Bull., 175: 361-371.

Mackie, L.A. and Ansell, A.D. 1993. Differences in the reproductive ecology in natural and transplanted populations of *Pecten maximus*: evidence for the existence of separate stocks. J. Exp. Mar. Biol. and Ecol., 169: 57-75.

McLusky, D.S., 1973. The effect of temperature on the oxygen consumption and filtration rate of *Chlamys (Aequipecten) opercularis* (L.)(Bivalvia). Mason, J. 1983. Scallop and queen fisheries in the British Isles. Fishing News Books Ltd, Surrey.

Mcshane, P.E. and Lester, R.J.G., 1984. The occurence of a larval nematode in *Pecten alba* Tate from Port Phillip Bay, Victoria. Marine Science Laboratories Technical Report No. 37.

Malinowsky, S. M. and Siddall, S.E., 1989. Passive water reuse in a commercial scale hard clam, *Mercenaria mercenaria,* upflow nursery system. J. Shellfish Res. 8(1):241-248.

Manzi, J.J., Hadley, N. H. and Maddox,M.B., 1986. Seed Clam, *Mercenaria mercenaria(*Linne') culture in an experimental-scale upflow nursery system. Aquaculture, 54: 301-311.

Mason, J., 1983. Scallop and queen fisheries in the British Isles. Fishing News Books, Farnham, UK. 143pp.

Matsutani, T. and Nomura, T. 1982. Induction of spawning by serotonin in the scallop *Patinopecten yessoensis* (Jay). Mar. Biol. Lett., 3: 353-358.

Matsutani, M. and Nomura, T. 1986. Pharmological observations on the mechanism of spawning in the scallop *Patinopecten yessoensis*. Bull. Jap. Soc. Sci. Fish., 52(9): 1589-1594.

May, V., Collins, A.J. and Collett, L.C. 1978. A comparative study of epiphytic algal communities on two common genera of seagrasses in eastern Australia. Aust.J. Ecol., 3: 91-104.

Menzel, R. W. and Hopkins, S. H., 1955. The growth of oysters parasitised by the fungus *Dermocystidium marinum* and by the trematode *Bucephalus cuculus*. J. Parasitology, 41: 333-342.

Millar, R. H., 1963. Oysters killed by trematode parasites. Nature 197, 616.

Minchin, D., 1992. Induced spawning of the scallop, *Pecten maximus*, in the sea. Aquaculture, 101: 187-190.

Nakanishi, I., 1977. Studies of the effect of the environment on the heart rate of shellfishes. 1. effect of temperature, salinity and hypoxia on the heart rate of scallops. *Bull. Hokkaido Reg. Fish. Res. Lab.*, 42: 65-73.

Nell, J.A. and Gibbs, P.J., 1986. Salinity tolerance and absorption of L- methionine by some Australian bivalve molluscs. Aust. J. Mar. Freshwater Res., 37: 721-727.

Nell, J.A. and O'Connor, W.A., 1991. The evaluation of fresh algae and stored algal concentrates as a food source for Sydney rock oyster *Saccostrea commercialis* (Iredale & Roughley) larvae. Aquaculture, 99: 277-284.

Nogami, K. and Maeda, M. 1992. Bacteria as biocontrol agents for rearing larvae of the crab *Portunus trituberculatus*. Can. J. Fish. Aquat. Sci., 49(11): 2373-2376.

Nottage, A. S. and Birbeck, T. H., 1986. Toxicity to marine bivalves of culture supernatant fluids of the bivalve- pathogenic *Vibrio* strain NCMB 1338 and other marine vibrios. J. Fish. Dis., 9:249-256.

O'Connor, W. and Diemar, J., 1991. Techniques for the mass culture of marine microalgae. New South Wales, Agric.Fish. Advis. Bull. No. 4, 3pp. ISNN 1036-0867

O'Connor, S., Heasman, M.P. and O'Connor, W.A., 1994. Scallop nursery rearing and farming trials at Bungo Bay, Two Fold Bay, NSW. Progress report 1993-94. Report to the Office of Labour Market Adjustment, 24 pp.

O'Connor, W.A. and Nell, J.A., 1991. The potential of algal concentrates as food for the production of Australian bivalves. In G.L. Allan and W. Dall (Editors), Proc. Aquaculture Nutrition Workshop, 15-17 April 1991, NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, Australia, pp.

O'Connor, W.A., Nell, J.A. and Diemar, J., 1992. The evaluation of 12 algal species as food for juvenile Sydney rock oysters *Saccostrea commercialis* (Iredale & Roughley). Aquaculture, 108: 277-283.

O'Connor, W.A., and Heasman, M.P., (Submitted). Spawning induction and fertilisation in the doughboy scallop *Chlamys asperrima*. Aquaculture,

O'Foighil, D., Kingzett, B., O'Foighil, G. and Bourne, N., 1990. Growth and survival of juvenile Japanese scallops, *Patinopecten yessoensis*, in nursery culture. J. Shellfish Res., 9(1): 135-144.

Owen, G. and M^{c}Crae, J.M., 1976. Further studies on the laterofrontal tracts of bivalves. Royal Soc.

Olsen, A.M., 1955. Underwater studies on the Tasmanian scallop *Notovola meridionalis* (Tate). Aust. J. Mar. Freshwater Res., 37: 392-409.

Paul, J.D., 1980a. Upper temperature tolerance and the effects of temperature on byssus attachment in the queen scallop *Chlamys opercularis* (L.). J. Exp. Mar. Biol. Ecol., 46: 41-50.

Paul, J.D., 1980b. Salinity-temperature relationships in the queen scallop *Chlamys opercularis. Mar. Biol.*, 56: 295-300.Aquaculture in Shallow Seas, Rotterdam. pp. 579-598. Translation of Senkai Kanzen Yoshoki (Senkai Yoshoku no Shinpo). Koseisha Koseiku Publishers. Tokyo. 1971.London. B., 194: 527-544.

Peirson, W.M., 1983. Utilisation of eight algal species by the bay scallop *Argopecten irradians concentricus* (Jay). J. Exp. Mar. Biol. Ecol., 68: 1-11.

Rhodes, E.W. and Widman, J.C., 1980. Some aspects of controlled production of the bay scallop (*Argenopecten irradians).* Proc. World Maricult. Soc., 11:235-246.

Rodhouse, P. G. and O'Kelly, M., 1981. Flow requirements of the oysters, *Ostrea edulis* L. and *Crassostrea gigas* (Thunberg) in an upwelling column system of culture. Aquaculture, 22: 1-10.

Roughley, T.C., 1933. The life history of the Australian oyster (*Ostrea commercialis*). Proc. Linnean. Soc. New South Wales, 58: 279-333.

Samain, J. F., Séguineau, C., Cochard, J. C., Delaunay, F., Nicolas, J. L., Marty, Y., Galios, R., Mathieu, M. and Moal, J., 1992. What about growth variability for *Pecten maximus* production. Océanis, 18(1): 49-66.

Sanders, M. J., 1966. Parasitic castration of the scallop *Pecten alba* (Tate) by a bucephalid trematode. Nature, 212(5059): 307-308.

Sanders, M. J. and Lester, R. J. G., 1981. Further observations on a bucephalid trematode infection in scallops (*Pecten alba*) in Port Phillip Bay, Victoria. Aust. J. Mar. Freshwater Res., 32: 475-478.

Sastry, A.N., 1963. Reproduction of the bay scallop, *Aequipecten irradians* Lamarck.Biol. Bull. (Woods Hole), 125: 146-153.

Sastry, A.N., 1968. The relationships among food, temperature and gonad development of the bay scallops *Aequipecten irradians* Lamarck. Physiol. Zool., 41: 44-53.

Sastry, A.N., 1979. Pelecypoda (excluding Ostreidae). In: A.C. Giese and J.S. Pearse (Editors), Reproduction of Marine Invertebrates, Academic Press, New York, NY, pp. 113-292.

Sause, B.L.; Gwyther, D.G. and Burgess, D., 1987. Larval settlement, juvenile growth and the potential use of spatfall indices to predict recruitment of the scallop *Pecten alba* tate in Port Phillip Bay, Victoria, Australia. Fish. Res., 6: 81-92.

Sause, B.L., Gwyther, D., Hanna, P.J. and O'Connor, N.A. 1987. Evidence for a winter spring spawning of the scallop *Pecten alba* (Tate) in Port Phillip Bay, Victoria. Aust. J. Mar. Freshwater Res., 38: 329-37.

Sause, B.L., Gwyther, D., Hanna, P.J. and O'Connor, N.A., 1987. Evidence for the winter-spring spawning of the scallop *Pecten alba* in Port Phillip Bay, Victoria. Aust. J. Mar. Freshwater Res., 38: 329-37.

Sato-Okoshi, W., Sugawara, Y., and Nomura,T., 1990. Reproduction of the boring polychaete Polydora variegata inhabiting scallops in abashiri Bay, north Japan. Mar. Biol., 104: 61-66.

Sause B. L., Gwyther D., Hanna P.J. and O'Connor N.A., 1989. Evidence for Winter-Spring spawning of the scallop *Pecten alba* (Tate) in Port Phillip Bay, Victoria. Aust. J. Mar. Freshwater Res., 38: 329-337.

Shafee, M.S. 1980. Application of some growth models to the black scallop *Chlamys varia* (L.) from Lanveoc, Bay of Brest. J. Exp. Mar. Biol. and Ecol., 43: 237-250.

Shumway, S.E. 1991. Scallops: Biology, Ecology and Aquaculture, S.E.Shumway (editor). Dev. Aquaculture and Fish. Sci., vol. 21. Elsevier, Amsterdam

Shumway, S.E. and Cucci, T.L., 1987. The effects of the toxic dinoflagellate *Protogonyaulax tamerensis* on the feeding and behavior of bivalve molluscs. Aquatic Toxicology, 10: 9-27.

Shumway, S.E., Cucci, T.L., Gainey, L. and Yentsch, C.M., 1985. Particle selection, ingestion /and absorption in filter feeding bivalves. J. Exp. Mar. Biol. Ecol., 91: 77-92.

Shumway, S.E., Cucci, T.L., Lesser, M.P., Bourne, N. and Bunting, B. 1991. Particle selection by three species of scallops. J. Shellfish Res., 10: 273.

Spencer, B.E., 1988. Growth and filtration of juvenile oysters in experimental outdoor pumped upwelling systems. Aquaculture, 75:139-158.

Spencer, B.E., Akester, M.J. and Mayer, I., 1986. Growth and survival of seed oysters in outdoor pumped upwelling systems supplied with fertilized sea water. Aquaculture,55:173-185.

Sokal, R.R. and Rohlf, F.J. 1981. Biometry. W.H. Freeman and Company, New York, NY, 895 pp.

Stafford, B., 1990. A report on development of government supervised co-operative scallop husbandry fishing industry in Tasmania. Compiled by Brian Stafford & Associates. Commisssioned by the Tas . Dept. of Primary Industry.

Stephano, J. L. and Gould M., 1988. Avoiding polyspermy in the oyster (*Crassostrea gigas*). Aquaculture, 73: 295-307.

Stephens, P.J., 1978. The sensitivity and control of the scallop mantle edge. J. Exp. Biol., 75: 203-221.

Stewart, M., Barlow, C.G. and Longhurst, C., 1987. Reliable food production essential for rearing of barramundi larvae. Aust. Fish., 46 (7): 22-24.

Tettelbach, S.T. and E.W. Rhodes. 1981. Combined effects of temperature and salinity on embryos and larvae of the northern bay scallop *Argopecten irradians*. Mar. Biol., 63: 249-256.

Thompson, R.J. 1977. Blood chemistry, biochemical composition and the annual reproductive cycle in the giant scallop, *Placopecten magellanicus*, from southeast Newfoundland. J. Fish. Res. Board Canada, 34(11): 2104-2116.

Tranter D.J., D.J. Carpenter and G. S. Leech. 1986. The coastal enrichment of the east Australian current eddy field. Deep Sea Res., 33:1705-1728.

Tubiash, H. S., Chanley, R.E. and Leifson, E., 1965. Bacillary necrosis, a disease of larval and juvenile bivalve mollusks. Etiology and epizootiology. J. Bacteriol., 90: 1036-1044.

Tubiash, H. S., Colwell, R.R. and Sakazaki, R., 1970. Marine vibrios associated with bacillary necrosis, a disease of larval and juvenile bivalve mollusks. J. Bacteriol., 103(1) : 271-272

Ursin, E., 1963 On the incorporation of temperature in the Von Bertalanffy equation. *Medd.Dan. Fisk. -Havunders.* 4: 1-16.

Utting, S.D. and Helm, M.M., 1985. Improvement of sea water quality by physical and chemical pre-treatment in a bivalve hatchery. Aquaculture, 44: 133-144.

Ventilla, R.F., 1982. The scallop industry in Japan. Advances Mar. Biol., 20: 309-382.

Velez, A., Alifa, E. and Azuaje. O., 1990. Induction of spawning by temperature and serotonin in the hermaphroditic tropical scallop *Pecten ziczac*. Aquaculture, 84: 307-313.

Voyer, R.A., 1992. Observations of the effect of dissolved oxygen and temperature on respiration rates of the bay scallop *Argopecten irradians*. *Northeast Gulf Sci.*, 12(2): 147-150.

Wallace, J.C. and T.G. Reinses. 1985. The significance of various environmental parameters for growth of the Iceland scallop, *Chlamys islandica* (Pectinidae), in hanging culture. Aquaculture, 44: 229-242.

Walne, P.R., 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea, Crassostrea, Mercenaria,* and *Mytilus*. Fisheries Investigation London, Series II. 26(5

Ward, J.E. and Cassell, H.K., 1991. Stimulatory effects of microalgal chemical cues on the clearance and ingestion rates of *Placopecten magellanicus*. J. Shellfish Res., 10(1): 292.

Watson, R.H., 1986. Instant food for bivalve hatcheries. Aquaculture Digest. Sept., pp. 6-9.

Watson, R.H., Jones, G.G. and Jones, B.L., 1986. Using centrifuged algae for feeding oyster larvae. J. Shellfish Res., 5: 136 (abstract).

West, G.J., 1990. Methods of assessing ovarian development in fishes: a review. Aust. J. Mar. and Freshwater Res. 41: 199-222.

Whyte, J. N. C., Bourne, N. and Hodgson, C.A., 1990. Nutritional condition of rock scallop, *Crassadoma gigantea (Grey), larvae* fed mixed algal diets. Aquaculture, 86: 251-263.

Wilbur, A.E. and Gaffney, P. M., 1991. Self fertilization in the bay scallop, *Argopecten irradians*. J. Shellfish Res., 10(1): 274.

Whyte, J.N.C. and Carswell, B.L., 1983. Chemical aid for shucking the Pacific oyster, *Crassostrea gigas*. Can. Tech. Rep. Fish. Aq. Sci., No. 1238: 38 pp.

Woo, L.O.L. and Woodburn, L., 1981. Stock assessment of the commercial scallop *Pecten alba* (Tate) in Port Phillip Bay, Victoria, January 1981. Commercial Fish. Rep., 3, 1.

Woodburn, L., 1989. Genetic variation in southern Australian Pecten. In: M.L.C. Dredge, W.F.Zacharin and L.F. Joll (Editors) Proceedings of the Australasian Scallop Workshop, Taroona, Tasmania, Australia. 1988, pp. 226-240.

Worthington, D., 1992. A survey of the scallop population in Jervis Bay. Unpublished Report, Fisheries Research Institute, N.S.W. Fisheries, Cronulla, N.S.W. June, 1992. 26pp.

Worthington, D., 1993. A survey of the scallop population in Jervis Bay. Unpublished Report, Fisheries Research Institute, N.S.W. Fisheries, Cronulla, N.S.W. June, 1993. 28pp.

Yamamoto, H., Ezura, Y. and Kimura, T. 1983 Changes of bacterial flora in stored seawater and incubated seawater inoculated with *Vibrio parahaemolyticus.* Bull. Jap Soc. Sci. Fish. 49(2): 295-300.

Yamanaka, R. and Fujio, Y., 1983. Fish. Genet. Breed. Sci. 8: 33-37.

Young, P.C. and Martin, R.B., 1989. The scallop fisheries of Australia and their management. Critial Rev. Aq. Sci., 1: 615-38.

Young, P.C. and R.B. Martin, 1990. The scallop industry to reach new heights. Austasia Aquaculture Magazine, 2(4): 8-10.

Young, P.C., McLoughlin, R. J., West, G. and Martin, R. B, (Unpublished manuscript) 1991. Reproduction of the scallop *Pecten fumatus* in Bass Strait, Australia. CSIRO Div.Fisheries, Marine Lab. Hobart, Tasmania. 20pp. 9 Figs.

Young, P.C., Martin, R. B., McLoughlin, R. J. and West, G., 1989. Variability in spatfall and recruitment of commercial scallops *(Pecten fumatus)* in Bass Strait. In: Dredge, M. L. C., Zacharin, W.F. and Joll, L.M. (Editors) Proceedings of the first Australasian scallop workshop, Tas. Gov. Printer, Hobart, Australia. pp. 80-91.

Zacharin, W.F., 1989. Scallop fisheries management: The Tasmanian experience. In: M.L.C. Dredge, W.F. Zacharin and L.M. Joll, (Editors) Proceedings of the first Australasian Scallop Workshop, Tas. Gov. Printer, Hobart , Australia. pp.1-1

7. 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 15mm 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. 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. Concerning the former, Tascallops during the current (1994/95) wild spat collection were in fact able to harvest only 20 million. This was well short of their minimum target of 30million and the size range (5 to 30mm) greatly exceeding the optimum range of 10 to 20mm (Scott Crawford-Tascallops Pty. Ltd pers. comm. April '95).

With respect to the NSW scallop fishery, enhancement of the current 1 in 10 to 15 year Jervis Bay fishery to an annual fishery of say 300 tonnes (10% of peak natural production) would return direct revenue of at least $1million. 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 $4million 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.

9.0 FURTHER DEVELOPMENTS

The most significant further development in this project has been the funding and initiation in October 1994, of a follow-up three year FRDC project (94 / 084)"Enhancement of farming of scallops in NSW using hatchery produced seedstock. " It is anticipated that this project will pave the way to enhancement of the Jervis Bay scallop fishery and to the establishment of a private sector commercial scallop hatchery, nursery and farming industry in NSW.

At the time of writing, considerable further progress has been made in achieving rapid synchronised metamorhosis of competent pediveliger larvae using the neurochemical inducers L-Dopa , epinephrine and gamma amino butyric acid (GABA). Considerable recent progress has also been made in the efficient transfer of recently settled spat into traditional longline spat collector bags.

10.0 STAFF

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

Full-time Project staff
Senior Technical Officer.....Mr. Wayne O'Connor
Technical Officer.....Mr. Allan Frazer

Support Staff (NSW Fisheries, PSRC)
Technical Officer (Bivalve hatchery operations)....Mr. Lindsay Goard
Technical Officer (Microalgal production).....Mr. John Diemar
Technical assistant.. (Microalgal production)..... ..Mrs. Geraldine.Want
and Mr. David Keith

11.0 FINAL COST

Contribution from FRDC.....$350 001.36
Contrutions from NSW Fisheries.....$371 500.00

Total Costs.....$721 501.36

See following statement of Receipts and Expenditure

FISHERIES RESEARCH AND DEVELOPMENT CORPORATION

Statement of Receipts and Expenditure for the period 1 July 1994 to 31 March 1995

Name of Research Organisation	FRDC Project Number	Title of Project
NSW FISHERIES	91/53	Evaluation of hatchery production of scallop Pecten Fumatus

Budget Summary	1991-92	1992-93	1993-94	1994-95(1)
Original Budget	125,653.00	122,916.00	111,478.00	0.00
Current Budget (2)	125,653.00	122,916.00	111,478.00	0.00

Summary of receipts and Expenditure for the Project since commencement

	1991-92	1992-93	1993-94	1994-95(1)
Balance B/F	0.00	15,660.75	23,089.64	220.67
FRDC Funds (Plus)	124,653.00	122,916.00	83,609.00	
Expenditure (Minus)	108,992.25	115,487.11	106,477.97	
Returns (3)				
Balance C/F	15,660.75	23,089.64	220.67	
Funds Receivable	1,000.00	0.00	27,869.00	

Details Financial Year to 31 March 1995

Funds Available

Balance Brought Forward from previous year	220.67
Total funds received from FRDC during Financial year 1994-95	0.00
Funds available for FY 1994-95	220.67

Allocation FY	*Less Expenditure*	
0.00	Salaries	16,115.06
0.00	Travel	1,871.97
0.00	Operating	1,057.00
0.00	Capital	0.00
	Refund	
0.00	Balance as at 31 March 1995	(18,823.36)

Notes

(1) Use this column for the final year ONLY regardless of the lenght of the project

(2) Total current budget shall not exceed total original budget without approval in writing from FRDC

(3) Refunds should only be paid at the completion of the project together with the final audited statement.

(4) ACTUAL EXPENDITURE ONLY. Commitments shall NOT be included

(5) Show allocation for the current financial year

Comments:

Final payment of $28,869 to be received.

12.0 DISTRIBUTION OF REPORT

NSW Fisheries will distribute copies of this report as follows:

. 10 Copies including 1 unbound to FRDC together with a copy on 3.5" floppy disc.

. 2 copies to the NSW Fisheries, Fisheries Research Institute, Library

. 2 copies to the NSW Fisheries, Port Stephens Research Centre, Library.

. 1 copy each to State Dept. of Fisheries/ Primary Industries Libraries in Tasmania, Victoria, South Australia and Western Australia.

. 1 copy to the National Fishing Industry Council, Unit 1 6 Phipps Pl. DEAKIN ACT 2600.

13.0 APPENDICES (Attached)

APPENDIX 1

IMPROVED HATCHERY AND NURSERY REARING TECHNIQUES FOR *PECTEN FUMATUS* REEVE

M.P. HEASMAN, W.A. O'CONNOR & A.W. FRAZER

Heasman, M.P., O'Connor, W.A. & Frazer, W.A. 1994 08 10: Improved hatchery and nursery rearing techniques for *Pecten fumatus* Reeve. *Memoirs of the Queensland Museum* 36(2): 351–356. Brisbane. ISSN 0079-8835.

Fortnightly sampling of a population of the hermaphroditic scallop *Pecten fumatus* in Jervis Bay was initiated in July 1991. Results over the first 18 months showed that wild stocks constitute a poor and unpredictable source of ripe ready-to-spawn broodstock for hatchery use. This prompted development of hatchery conditioning protocols. The most rapid development of gonads occurred when broodstock were held at 15°C and fed to satiation ($6x10^9$ cells scallop^{-1} day^{-1} on a diet of approximately equal amounts of at least 3 of 4 microalgae, namely: *Chaetoceros calcitrans, Pavlova lutheri,* Tahitian *Isochrysis* and *Chroomonas salina*). Intragonadal injection of serotonin at 0.05ml of a $0.5x10^{-4}$N solution per scallop reliably induced sperm release within 5–25min over a broad (12–24°C) temperature range. Survival from fertilisation to D-veliger stage was substantially improved by incubating eggs in suspension at up to 100 ml^{-1} in aerated cylindro-conical vessels. Survival to metamorphosis on Day 16 ranged from 5–20%. Rates up to 70% were achieved with experimental scale cylindro-conical rearers when seawater was prefiltered to 1m or when antibiotics were used. Post-settlement retention rates of 10-50% were achieved by transferring pediveligers onto cylindrical downweller screens fitted with 160μm polyester mesh. Growth of 5–10mm juvenile scallops maintained in an upweller nursery unit located at a site at the entrance to Port Stephens was found to increase with increasing seawater flow rates up to 40ml g^{-1} biomass min^{-1} and to be suppressed when the surface area of scallops approached 100% that of the screens on which they were stocked. Mean growth rates of 2.8 mm week^{-1} were exhibited over the size range 5–25mm when maintained at low density in screens or lantern cages suspended from a long line at 20–24°C. Small spat in the hatchery grew faster with increasing temperature in the range 12–27°C but ceased growing at 1.5–3mm.

M. P. Heasman, W. A. O'Connor, and A. W. Frazer, NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, New South Wales 2301; 9 March 1994.

The NSW scallop fishery is spasmodic and confined to Jervis and Twofold Bays (Fig.1). Peak annual catches of 1000–3000 tonnes live weight occur only once in 10 years with insignificant catches in intervening years (Fuentes et al., 1992). If higher and more consistent scallop yields are to be achieved in NSW, the central problem of low and variable annual recruitment of juveniles, must be addressed. Wild caught *Pecten fumatus* spat in Jervis Bay (and probably elsewhere in southern NSW) is likely to be low and unreliable in most years (Fuentes et al.,1992).

The importance of reliable, low-cost hatchery and nursery rearing techniques for *P. fumatus* and a successful pilot hatchery trial in May 1989 prompted a 3 year, Fishing Industry Research and Development Corporation (FRDC) funded, research project at the Brackish Water Fish Culture Research Station (BWFCRS) from July 1991.

The 1989 pilot study gave encouraging results using wild scallops from Jervis Bay as spawning stock and conventional hatchery rearing techniques and equipment (Frankish et al., 1991). Approximately 6 million settled spat were being produced at estimated survival rates from spawning to D-veliger (1st feeding) stage of c.60% and from D-veliger to post-settlement, of c.70%. Several hundred thousand settled spat were retained and onreared to 10–20mm shell height at a similar rate of survival (Frankish et al.,1990). These results contrasted with those previously attained by Tasmanian oyster hatcheries using comparable techniques and equipment. In attempting to meet Tasmanian government contracts for the supply of 4.2 million *P. fumatus* juveniles in the range 10–20mm, the hatcheries were only able to supply 100000 and 280000 in 1987 and 1988 respectively. Up to this time, the largest spawning of *P. fumatus* had produced 125 million eggs but no hatchery had produced more than 500000 settled spat from one batch of larvae (Cropp & Frankish,1989).

From the outset of this project, in 1991, it was considered that previous high variability in hatchery success with *P. fumatus* could have

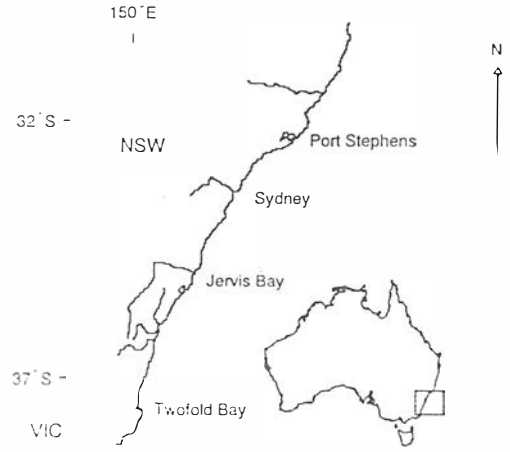


FIG. 1. Central and southern New South Wales.

arisen through one or a combination of several factors. These included: variability in the quality of eggs sourced from wild spawners; subtle, but critical differences in equipment and techniques employed, especially in relation to settlement and metamorphosis of pediveligers; disease(s) and larval nutrition factors.

MATERIALS AND METHODS

Evaluation of Wild Stocks of *P. fumatus* as a Source of Ready to Spawn Broodstock

Fortnightly sampling of a Jervis Bay population of *P. fumatus* was initiated in July 1991. On each occasion 120–150 scallops in the range 55–90 mm shell length were collected by a professional diver. All collections made between 7–8am were road freighted (insulated from an underlying layer of ice which maintained them at 10–15°C) to BWFCRS within 8–10hrs. They were immediately stocked into a lantern cage suspended in a 1000l holding tank at ambient temperature (16–22°C). The following morning 40 randomly selected scallops was measured, subjected to a macro-visual staging of gonad condition (Fuentes et al.,1992) and then dissected to determine gonad somatic index (GSI)[1].

$$GSI = \frac{Weight\ of\ gonad}{Total\ shell\ free\ drained\ weight} x100$$

Of the remaining 80–110 scallops, the 10 individuals exhibiting highest apparent gonad condition (degree of ripeness) were subjected to induction of spawning stimuli within 72h of capture. Induction of spawning stimuli comprised the exposure of scallops to 3 thermal cycles in which temperature was raised 3–8°C above ambient over periods of 45–60 minutes.

Release of sperm and eggs was recorded for this hermaphroditic species with fecundity being determined in the case of egg releases.

Gonad Conditioning Protocols for Captive Broodstock

Attempts to condition broodstock in the hatchery were conducted between July and September, 1991. Wild scallops with medium to high gonadal development attained a ripe, 'ready to spawn', condition in 4–6 weeks during July and August at 16–19°C and fed to satiation. Gonad condition regressed as temperatures rose above 20°C in September and October.

These results prompted construction of a controlled temperature broodstock conditioning facility at the BWFCRS to develop techniques that would enable controlled ripening, stockpiling and induced spawning of captive broodstock throughout the year. This facility, commissioned in April 1992, comprised 4 water baths held at 12.0±0.5; 15.0±0.5; 18.0±0.5 and 21.0±0.5°C respectively, each accommodating 36 x 10 l plastic aerated aquaria to accommodate individual scallops. Experiments to identify appropriate microalgal diets and satiation feeding levels were initiated in June 1992. Subsequent trials to identify optimum combinations of holding temperature at 100, 50, 25 and 12% of satiation feeding levels were conducted in July 1992.

Spawning Induction and Incubation Protocols

A series of trials was conducted to determine whether intergonadal injection of the neurotransmitter serotonin is more effective than temperature shocks in triggering spawning of ripe *P. fumatus*. To establish optimum dosage rates, 0.05ml serotonin was injected at concentrations of 10^{-6} to 10^{-3} N. Time to spawning of sperm and eggs was recorded as was fecundity in the case of released eggs. Fertilised eggs were stocked into 1l cylindroconical vessels and incubated at densities of 1–100 eggs ml^{-1} to gauge the effect of stocking density on survival to the 'D' veliger stage 48h after fertilisation.

Larval Rearing Techniques

Standard hatchery techniques and equipment for Sydney rock oysters (*Saccostrea commercialis*) larvae (Frankish et al.,1991) were used over the first 12 months. Consistently poor hatchery survival achieved by these means and lack of suitable facilities with which to conduct

replicated trials to evaluate alternative rearing techniques, prompted construction of a small scale bivalve larvae rearing system in August 1992. Ten standard 1000l flat-bottomed cylindrical oyster larval rearing vessels were utilised as controlled temperature baths, each accommodating 4x80l cylindroconical rearers. As with the 1000l vessels, the smaller units were made of rotationally moulded polyethylene.

Experimental treatments comprised four types of seawater preparation ie. filtration to 0.2μm absolute; filtration to 1.0μm nominal; filtration to 1.0μm nominal plus 10mg l^{-1} chloramphenicol and an unfiltered seawater control. These were combined factorially with two alternative diets, namely, a standard blend of 3 microalgal species (*C. calcitrans*, Tahitian *Isochrysis*, and *P. lutheri*) and the same blend of microalgae concentrated into a slurry by centrifugation and stored for 1–6 days prior to feeding. The chloramphenicol treatment was to evaluate the claim that a closely related European scallop *P. maximus*, cannot be hatchery reared with consistent success without use of such powerful broad spectrum antimicrobials (Samain et al.,1992) and hence whether microbial pathogens posed a significant constraint to hatchery success with this species.

Inclusion of chloramphenicol as an experimental treatment should not be construed as an endorsement of its use. To the contrary, identification of alternatives to use of broad spectrum antibiotics that will ensure consistently high survival of hatchery reared *P. fumatus* has become the most important single objective of this project.

Larval Settlement and Early Nursery Rearing Protocols

Post settlement recovery of *P. fumatus* pediveligers using conventional plastic mesh catch (culch) materials and by transferring pediveligers directly onto downweller screens fitted with 160μm polyester mesh were compared. Subsequent growth and survival was monitored for post-larvae retained on downweller screens in the hatchery and for those transferred to upwellers screens and lantern cages at Tomaree Head, adjacent to the mouth of Port Stephens (32° 44'S; 152° 11'E; Fig. 1).

A trial to determine optimum stocking and water flow rates for small juvenile *P. fumatus* reared on upweller screens at Tomaree Head was initiated on Christmas Eve 1992. A total of about 20000 spat averaging 5.6mm and 30mg were stocked at 8 different densities (5 replicates per density) using 40 miniaturised upweller units each consisting of vertically nested stacks of 8 interlocking screens.

The salinity tolerance of 1–2mm juvenile scallops was investigated in the laboratory as were interactive effects of salinity and temperature on growth and survival.

Specialist Handling Techniques for Early Juvenile and Adult Scallops

Mechanical methods such as seawater jets and scrapers used to dislodge small (10mm) bysally attached *P. fumatus* from culch materials and nursery screens were found to cause injury and subsequent high mortality. To address this problem, the effectiveness of a number of irritant chemicals and physiological stress factors to induce detachment of 2–4mm juveniles was evaluated in fully replicated trials.

A comparable series of trials was also conducted to test the anaesthetic properties of a range of chemical compounds on mature *P. fumatus* of 55–75mm shell height. The aim was to identify quick and simple techniques of reducing stress and subsequent inadvertent spawning caused by routine handling and assessment of gonad status of hatchery conditioned scallops.

RESULTS AND DISCUSSION

Wild Stocks of *P. fumatus* in NSW as a Source of Ripe Brood Stock

Fortnightly sampling of a Jervis Bay population of *P. fumatus* (July 1991–December 1992) revealed that this potential source of ripe 'ready to spawn' broodstock is nearly always unproductive and unpredictable. Even when breeding condition was highest, as indicated by peak in mean gonad-somatic index values of 18–21%, very few individual scallops, including those in a ripe condition (large, turgid, glossy and richly coloured gonads) responded positively and predicably to conventional spawning induction stimuli. Of 300 (10/collection) apparently ripe scallops subjected to spawning induction stimuli over the first 18 months of the project, less than 4% were successfully induced to spawn eggs. Moreover, seasonal peaks in breeding condition, were not consistent from year to year. For example, recorded mean GSI values were highest between December 1991 and March 1992 (Summer) but were continuously low during May-September 1992 (late Autumn-mid Spring). This pattern varied from that of chronically low mean GSI values recorded by Fuentes et al. (1992) through Summer and early Autumn of the two previous years and coin-

cided with unseasonally high winter sea temperatures and unseasonally low summer sea temperatures.

Hatchery Conditioning Protocols

Experiments to identify appropriate microalgal diets, feeding rates and temperatures for gonadal growth were undertaken in June 1992. Results of microalgal clearance rate experiments indicated that *Pavlova lutheri*, Tahitian *Isochrysis* aff. *galbana, Chroomonas salina* and *Chaetoceros gracilis* are ingested by adult scallops at similar rates at 14, 18 and 21°C but more slowly at 11°C. Satiation feeding rate using diets containing approximately equal numbers of cells of these 4 species, was estimated as about $6x10^9$ cells per day for broodstock of 55–75mm shell height. Cell densities of *Tetraselmis suecica*, another microalga commonly used to feed bivalves, declined over the first 8h of the experiment but then fluctuated, indicating resuspension of undigested cells from the faeces.

In a subsequent 6 week conditioning experiment, egg production rate associated with inadvertent spawning, along with gonad size and condition factors, were found to be highest when broodstock were held at 15°C, lowest for scallops maintained at 21°C and of intermediate values at 12°C and 18°C. Across all these temperatures, feeding of individual scallops with twice daily algal rations of $3x10^9$ cells (100% satiation) and $1.5x10^9$ cells (50% satiation) produced higher gonad ratings and egg production rates than rations of $0.75x10^9$ cells (25% satiation) or $0.375x10^9$ cells (12.5% satiation).

Frequent inadvertent spawnings triggered by handling emphasised the need for conditioning equipment and protocols that minimise handling and other disturbance factors. No inadvertent spawning however, occurred at feeding rates of 25 or 12.5% of satiation at 12°C. Use of low temperatures combined with reduced feeding rates might therefore enable stockpiling of broodstock at prime reproductive condition.

Opportunistic use was made of near ideal water temperatures (14–16°C) in August and early September 1992 to condition 100 broodstock. Scallops were held in lantern cages suspended in 20,000l tanks in the bivalve hatchery at BWFCRS and fed to satiation on a diet comprising equal amounts of the previously cited 4 microalgal species. To reduce the incidence of inadvertent spawning, rations of microalgae were drip fed into the tanks. The impact of water changes was kept to a minimum and handling of stock was totally avoided. No inadvertent spawnings were recorded over this period.

After 4 weeks of conditioning, 30 scallops were randomly selected and subjected to attempted thermal induction of spawning. Of these, 10 spawned as males and 12 as females, the latter yielding 25 million eggs and thence 13 million D-veliger larvae.

Spawning Induction, Fertilization and Incubation Protocols

Attempts to induce spawnings in ripe *P. fumatus* sampled fortnightly from Jervis Bay, were almost always unsuccessful. This result was originally ascribed, at least in part, to inadequacy of a standard thermal spawning induction stimulus (exposure of scallops to successive temperature rise cycles of 3–8°C above ambient) to trigger spawning. This misconception was corrected when development of effective gonad conditioning protocols yielded broodstock that spawned viable eggs rapidly in response to the same thermal induction techniques.

A dose of 0.05ml intragonadal injection of a $0.5x10^{-4}$N serotonin solution induced sperm release within 5-25 minutes over a broad range of temperature (14–22°C) in scallops with moderate to high gonad development. As with thermal shock technique however, induced spawning of eggs using serotonin was found to be effective only in suitably conditioned broodstock.

Injection of serotonin was nevertheless found to have a distinct advantage over the thermal induction of spawning. In being able to induce spawning of individual scallops held in isolation from one another. This in turn enables better control over the timing and extent (sperm to egg ratios) of fertilisation and the reduction of self fertilisation in hermaphoditic species such as *P. fumatus*. This attribute may prove particularly useful if applied to induced triploidy programs in the future. Use of serotonin induction of spawning has been extended to Sydney rock oysters at the BWFCRS in anticipation of this application.

Results of preliminary incubation trials conducted in November 1992 indicated that survival from fertilisation to D-veliger can be substantially improved by use of aerated cylindroconical vessels in which eggs are kept in suspension rather than allowing them to settle in a monolayer on the floor of conventional flat bottom oyster larvae rearing vessels. Suspended incubation also enables eggs to be stocked at high densities (up to 100 ml^{-1}) without apparent impairment to survival.

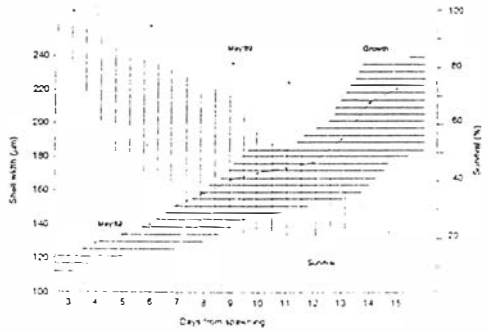


FIG.2. Summary of the growth and survival of hatchery reared scallops (*Pecten fumatus*) at BWFCRS, Salamander Bay. Dotted lines represent the results of the May 89 larval batch. Shaded areas indicate the range of results obtained from 6 larval batches conducted since July 91.

Larval Rearing Techniques

Larval rearing trials conducted over the first year of this project (Fig. 2) used standard 1000l flat-bottomed cylindrical tanks, used previously at the BWFCRS for the hatchery production of Sydney rock oysters (Frankish et al.,1991) and for the pilot hatchery production of *P. fumatus* in May 1989 (Frankish et al.,1990).

Survival to metamorphosis (Day 16–18) was 5–20% compared very poorly with the May '89 result but was in keeping with earlier results achieved by commercial hatcheries in Tasmania in 1987–1988 (Cropp & Frankish,1989). Larval growth rate varied and was not correlated with survival. Metamorphosis was attained at shell height of 225–240μm 14–20 days after spawning.

The first trial to systematically address the problem of low hatchery survival was undertaken in October 1992. Survival rates from D-veliger stage to the onset of metamorphosis (Fig. 3) varied with method of seawater preparation, being highest (70–80%) with chloramphenicol treated seawater and lowest (less than 10%) for 0.2μm filtered and unfiltered seawater. Larval survival rates were reduced but growth rates enhanced by algal concentrate diets. Subsequent patterns of survival through metamorphosis were however different with highest retention rates of 30–40% with 1μm filtered seawater and lowest rates of less than 1% with both unfiltered and 0.2μm absolute filtered seawater treatments.

These results highlighted the importance of continued research to identify seawater preparation, management protocols and feeding/stocking regimes that will enable consistent attainment of commercially acceptable hatchery survival rates. These are generally considered as nett yields of 0.2–1 of settled spat ml^{-1} of rearer volume (L. Goard, pers. comm.). The major challenge faced by continuing research is achievement of satisfactory yields of settled spat without the use of antibiotics.

Larval Settlement and Early Nursery Rearing Protocols

Of 7 larval rearing cycles completed during the first half of this project, 3 yielded significant numbers of spat. Approximately 15,000 spat were produced in November 1991; 30,000 in March 1992 and 200,000 in October/November 1992. Survival rates through settlement varied over the broad range 5–50%. Lowest survival was associated with the use of traditional plastic mesh culch materials deployed directly into larval rearing vessels. Much higher (10–50%) survival rates have however been consistently achieved by transferring pediveligers into downweller screens fitted with 160μm polyester mesh just prior to settlement.

Once settled onto downweller screens, subsequent mortality of spat was negligible. Growth of spat maintained in the hatchery is highly temperature dependent, increasing from c.15–150μm.day^{-1} with rising temperature over the range 12–27°C. Growth abruptly stalled as hatchery held spat attained a size of 1.5–3mm.

P. fumatus spat transferred to a longline unit at

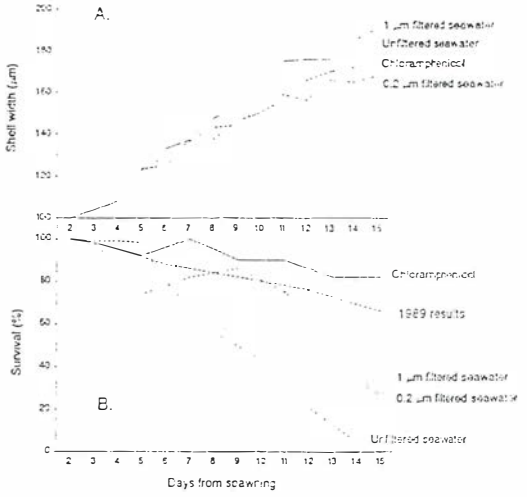


FIG.3. Effect of water treatment on growth and survival of *Pecten fumatus* larvae.

Tomaree Head, grew at mean rates of 2.8mm week^{-1} for sustained periods of up to 7 weeks at 20–23°C. This growth rate is higher than the previously highest summer rates of 1.7mm week^{-1} (Cropp,1985) for equivalent size *P. fumatus* glued to tapes suspended from midwater longlines in Tasmania.

A trial to determine optimum stocking rates of juvenile scallops reared in field upweller unit at Tomaree Head was initiated on Christmas Eve 1992. About 20,000 spat averaging 5.6mm and 30mg were stocked at 8 different densities (5 replicates per density) into 40 miniature upweller units, each of nested stacks of 8 interlocking screens.

Under prevailing conditions, including mean daily sea temperatures of 18–22°C and salinities of 34–35g.kg^{-1}, growth rate of spat of 6-10mm shell height and 30–150mg live-weight, was constrained by flow rates of below about 40ml g biomass^{-1} min^{-1}. For scallops in this size range, suppression of growth due to crowding coincided with a stocking density of about 0.3 g.cm^{-2} representing a surface area stocking rate approximating 100% of available screen area.

Salinity tolerance of 1–2mm juvenile *P. fumatus* was identified as a narrow range of 32–38mg ml^{-1} outside of which significant mortality occurs within 72h. These results were consistent with salinity tolerances reported for adult *P. fumatus* (Nell & Gibbs,1986).

Specialist Handling Techniques

Hypersaline baths (45 g.kg^{-1}) and exposure to air (emersion) for 2 hours were effective in inducing more than 95% of 1–3mm spat to detach from nursery screens. Hypersaline baths created by the addition of an artificial sea salt to seawater produced greater spat detachment after 2h than those created by equivalent additions of sodium chloride. The rate of detachment in hypersaline baths was unaffected by increasing temperature from 20–26°C, but was depressed at 11°C. Addition of magnesium chloride (27g.kg^{-1}) to seawater and reduction of seawater pH to 2 were also effective in increasing spat detachment rate, but not as effective as hypersaline baths or air exposure. With the exception of spat exposed to seawater containing 115mg.kg^{-1} available chlorine, no significant mortality and 95% reattachment occurred within 24h of all detachment methods tested.

Of 14 compounds tested, only chloral hydrate, Mg Cl_2 and Mg SO_4 induced anaesthesia in adult scallops within 1h. Mg SO_4 was excluded from further testing due to high postanaesthesia mortality. Doses of 4g.l^{-1} Chloral hydrate at 4g.l^{-1} (0.024M) or $MgCl_2$ at 30g.l^{-1} (0.31M) were most suitable on the basis of time to and recovery from anaesthesia. Neither anaesthetic caused mortality nor increased spawning activity. Mg Cl_2 reduced inadvertent spawning triggered by routine handling and maintenance activities. Time to anaesthesia for both agents was found to be affected ($P<0.05$) by water temperature.

ACKNOWLEDGEMENTS

We thank staff of the BWFCRS for assistance in manuscript preparation, Dr John Nell, Stephen Battaglene and John Holliday for editorial comments and Wayne Walker and John Kelly for collection and delivery of broodstock from Jervis Bay. This study was part of Fisheries Research and Development Corporation grant 91/53.

LITERATURE CITED

CROPP, D.A. 1985. Scallops thrive in TFDA culture program. Australian Fisheries 44(1): 16-18.

CROPP, D.A. & FRANKISH, K. 1989. Cost comparison of hatchery and natural scallop spat for the scallop *Pecten fumatus* Reeve. Pp. 196-225. In Dredge, M.L.C., Zacharine W.F. & Joll, L.M. (eds), 'Proceedings of the Australasian Scallop Workshop, Taroona, Tasmania, July 1988' (Tasmanian Government Printer: Hobart).

FRANKISH, K., GOARD, L. & O'CONNOR, W. 1990. Hatchery scallop culture success in NSW. Australasian Aquaculture Magazine 4(9): 10-11.

FRANKISH, K., GOARD, L. & O'CONNOR, W. 1991. 'The development of hatchery techniques for the production of Sydney rock Oysters (*Saccostrea commercialis*)'. Internal Report, NSW Fisheries, Brackish Water Fish Culture Research Station, 28p.

FUENTES, H.R., WILLIAMS, R.J., DIVER, L.P. & SMITH, A.K. 1992. Commercial molluscs of Jervis Bay. Pp. 000-000. In 'Jervis Bay marine ecological study. Final Report'. (NSW Fisheries, Fisheries Research Institute: Cronulla).

NELL, J.A. & GIBBS, P.J. 1986. Salinity tolerance and absorption of L- methionine by some Australian bivalve molluscs. Australian Journal of Marine and Freshwater Research 37: 721-727.

SAMAIN, J.F., SEGUINEAU, C., COCHARD, J.C., DELAUNAY, F., NICHOLAS, J.L., MARTY, Y., GALOIS, R., MATHIEU, M. & MOAL, J. 1992. What about growth variability for *Pecten maximus* production? Oceanis 18: 49-66.

APPENDIX 2

ELSEVIER

Aquaculture

Aquaculture 123 (1994) 401–407

Technical Paper

Detachment of commercial scallop *Pecten fumatus*, spat from settlement substrates

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Abstract

The ability of chemical irritants and several forms of physiological stress to induce *Pecten fumatus* spat to detach from settlement substrates was evaluated. Hypersaline baths (45‰) and exposure to air (emersion) for 2 h were both found to be effective in inducing more than 95% of spat to detach from mesh settlement screens. Hypersaline baths, created by the addition of an artificial sea salt to seawater, produced greater spat detachment after 2 h than those created by equivalent additions of sodium chloride. The rate of detachment in hypersaline baths was unaffected by increasing temperature from 20°C to 26°C, but was depressed at 11°C. Addition of magnesium chloride (27 g/kg) to seawater and reduction of seawater pH to 2 were also effective in increasing spat detachment rate, but not as effective as hypersaline baths or air exposure. With the exception of spat exposed to seawater containing 115 mg/kg available chlorine, no significant mortality and >95% reattachment occurred within 24 h of all detachment methods tested.

1. Introduction

A research project to develop hatchery production techniques for the commercial scallop, *Pecten fumatus*, was initiated at the Brackish Water Fish Culture Research Station (BWFCRS), in July 1991 (Heasman and Nell, 1991). Technical obstacles to efficient mass-production included the need to improve methods of dislodging byssally attached spat of ⩽15 mm shell height from substrates in settlement and nursery rearing systems.

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Nursery rearing protocols developed at BWFCRS employ conventional 450 mm diameter cylindrical downweller and upweller screens, fitted with monofilament polyester mesh. To reduce screen fouling and to offset increasing biomass, growing spat need to be frequently graded and transferred onto screens of successively larger surface area and mesh aperture size. Mechanical methods such as seawater jets and scrapers, originally employed to dislodge byssally attached spat from substrates, were found to cause injury and subsequent high mortality, particularly in spat < 5 mm shell height.

The aim of this study was to identify methods by which scallop spat could be induced to detach from substrates in response to exposure to mild irritants and other non-injurious stress factors. Bourne et al. (1989) listed a number of such methods in relation to hatchery-reared Japanese scallops (*Patinopecten yessoensis*). These included exposure to chlorine, increased salinity and air exposure.

2. Materials and methods

General

Hatchery-reared *P. fumatus* spat (2–4 mm shell height) that had been settled on screens (0.16 mm apertures) and housed in a hatchery downweller system operated at 20 ± 1 °C and 34‰ salinity were exposed to the following array of experimental treatments thought likely to cause their detachment from the screens. Prior to the start of each experiment, any loose individuals were gently rinsed from screens leaving only firmly attached spat at densities ranging from 700 to 9000 per screen, i.e. 0.44–5.66 spat/cm^2.

Treatment 1 (control)

A "control" treatment was used to gauge the effect of repetitive rinsings of screens on spat detachment. As with all other treatments described below, screens were placed in 350-litre seawater baths (34‰ salinity) at 20°C from which they were briefly removed after cumulative intervals of 5, 10, 15, 30, 60, 90 and 120 min. On each occasion, any spat that had detached since the previous removal were gently rinsed from the screen with seawater, counted and transferred to a clean screen in the downweller system where they were appraised for survival and re-attachment 24 h later.

Treatments 2, 3, 4 and 5 (hypersaline baths at 11, 20 and 26°C)

The same general procedures described for treatment 1 were repeated at each of three temperatures, 11, 20 and 26°C (treatments 2, 3 and 4 respectively) using seawater baths in which salinity had been raised to 45‰ with artificial sea salt (Instant Ocean; Aquarium Systems, Sarrebourg, France) and also at 20°C (treatment 5), using a hypersaline bath in which salinity was raised to 45‰ with the addition of sodium chloride (rock salt, from Pacific Salt, Yennora, NSW, Australia) to seawater. Salinity was measured using an inductive salinometer (Yeo-Kal, Sydney, Australia).

Treatment 6 (magnesium chloride)

Sufficient magnesium chloride (27 g/kg) was added to seawater baths to again obtain salinometer registrations of 45‰. This concentration of magnesium chloride had previously been found to induce rapid anaesthesia in *P. fumatus* broodstock (Heasman et al., unpublished data, 1993).

Treatments 7 and 8 (chlorine and reduced pH)

In treatment 7, sufficient sodium hypochlorite solution was added to a seawater bath at 20°C to bring it to a level approximating 115 mg/kg available chlorine. This was equivalent to a 250 mg/kg solution of sodium hypochlorite in seawater, cited by Bourne et al. (1989) as suitable for inducing detachment of Japanese scallop (*Patinopecten yessoensis*) spat. The figure 115 mg/kg available chlorine was based on the premise that the strength of added sodium hypochlorite solution had remained at 125 g/kg available chlorine, claimed by the supplier (Albert Oakey Pty. Ltd., Sydney, NSW) as a minimum level at the time of packaging. In treatment 8, the pH of the seawater bath was reduced to 2 using hydrochloric acid. Detached spat in treatments 7 and 8 received an additional thorough rinse in seawater before being returned to the hatchery downweller system.

Treatment 9 (air exposure)

Screens were removed from the downweller system, rinsed free of loose spat and exposed to air at room temperature (19°C) for 2 h. The numbers of spat washed from the screens after 30 min, 1 h and 2 h were recorded, as were the numbers of spat that remained attached to the screens.

With the exception of the chlorine treatment (treatment 7), three replicate trials were run for each treatment. Those spat that had failed to detach within 2 h in hypersaline baths (treatments 2 to 5), were retained in the same baths and re-examined after 24 h to determine if longer-term exposure caused mortality.

Statistical analysis

As the numbers of attached spat varied greatly between individual downweller settlement screens, results of various treatments were compared on the basis of percentage of spat detached after 2 h. Mean percentage detachment values were arcsin $\sqrt{\times}$ transformed to meet the assumptions of normality and homogeneity of variance and analysed using single factor analysis of variance. Homogeneity of variance was confirmed by Cochran's test (Winer, 1971). Differences between treatments were evaluated for significance using the SNK test (Sokal and Rohlf, 1981).

3. Results

All treatments induced significantly greater ($P<0.05$) spat detachment than the control treatment 1 (Fig. 1). With the sole exception of the chlorine treatment (treatment 7), spat survival and re-attachment after 24 h exceeded 95%.

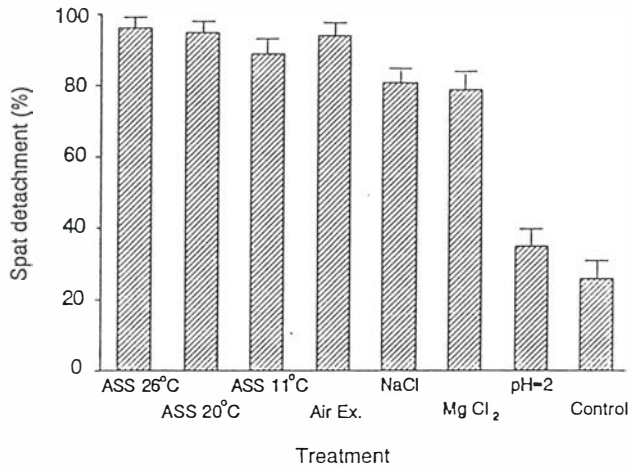

Fig. 1. Percent of *Pecten fumatus* spat (2–4 mm shell height) detached from settlement screens after 2 h exposure to an array of hypersaline solutions, to the air and to low pH. Bars represent mean detachment ± s.d. for each treatment. $n=3$ for all treatments. ASS denotes hypersaline (45 g/kg) solutions of seawater plus artificial sea-salts; Air Ex. = air exposure; NaCl = hypersaline (45 g/kg) solutions of seawater plus sodium chloride; $MgCl_2$ = hypersaline (45 g/kg) solutions of seawater plus magnesium chloride; pH = 2 = seawater the pH of which was reduced to 2 with hydrochloric acid; Control = unadulterated seawater (35 g/kg).

Although available chlorine at 115 mg/kg in seawater induced more than 95% spat detachment within 5 min, total mortality was apparent within 15 min.

When results of artificial sea salt hypersaline baths at different temperatures (treatments 2 to 4) were compared, no significant differences ($P>0.05$) in detachment were detected between trials run at 20 and 26 °C. Rate of spat detachment was however significantly reduced ($P<0.05$) at the lower test temperature of 11 °C.

Hypersaline baths made up with artificial sea salts at 20 and 26 °C (treatments 3 and 4) produced significantly greater ($P<0.05$) rates of spat detachment over 2 h than all other methods tested with the exception of air exposure (Fig. 1). However, as indicated in Fig. 2, initial detachment rate with air exposure was considerably slower than with hypersaline baths. There was no apparent tendency for screens stocked at higher densities to respond differently in terms of spat detachment rate (Fig. 2). This result was not unexpected in that volume of treatment baths (350 litres) was very high in relation to biomass of spat, which even in the most densely stocked screens was less than 20 g.

Sodium chloride and magnesium chloride mediated hypersalinities (treatments 5 and 6 respectively) were significantly less effective in inducing spat detachment than either artificial sea salt mediated hypersalinity or air exposure, while reduction of seawater pH to 2 (treatment 8) produced comparatively poor results (Fig. 1).

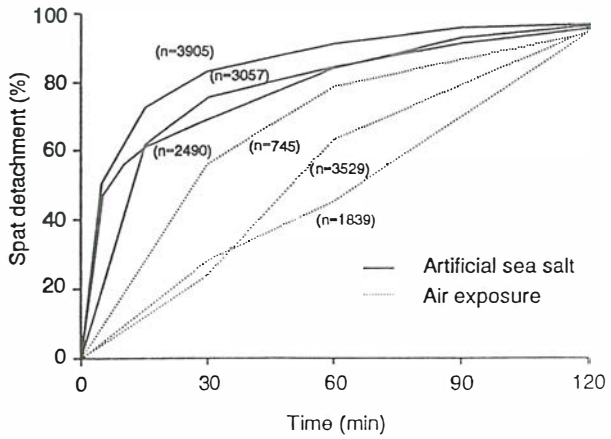

Fig. 2. Detachment rate of *Pecten fumatus* spat when exposed to air at 19 °C or placed in artificial sea salt hypersaline baths at 20°C. n = the number of spat on that replicate screen prior to treatment.

4. Discussion

Exposure of *P. fumatus* spat to hypersaline seawater followed by gentle rinsing was found to be a safe and effective means of removing them from settlement screens. Induced detachment almost certainly occurs as an avoidance response of spat to hypersalinities. Indeed, they exhibit stress response (retraction of mantle tissues and shell gaping) within 24 h of exposure to salinities above 40‰. Both spat (Heasman et al., unpublished data, 1992) and adult *P. fumatus* (Nell and Gibbs, 1986) die if exposure to such salinities is extended to 48 h.

Failure of sodium chloride mediated hypersalinities to induce detachment of the magnitude or speed of equivalent artificial sea salt solutions infers that tonicity (osmolarity), as indicated by conductivity salinometer measurements, was not the only factor inducing detachment. This result has implications for research on salinity tolerance in *P. fumatus* which appears to vary considerably with the specific method (in this case, choice of particular brand of artificial sea salt) used to raise seawater salinity to test levels.

The response and tolerance of *P. fumatus* to several methods of induced detachment differed markedly from that reported for *Patinopecten yessoensis* (Bourne et al., 1989). Air exposure, as practised by Bourne et al. (1989), was highly effective in inducing detachment. However, the 30-min exposure period used with *Patinopecten yessoensis* resulted in a cumulative detachment rate of less than 50% when applied to *P. fumatus* held at 20°C (Fig. 2). Species differences were also observed with the use of magnesium chloride which failed to induce spat detachment in *Patinopecten yessoensis* and yet was successful with *P. fumatus*. This apparent disparity could nevertheless have resulted from a higher dose rate (27 g/kg seawater) used in this study than that (10 g/kg seawater) used by Bourne et al. (1989).

Further experimentation with chlorine solutions to induce spat detachment was

abandoned when spat used in the first replicate trial of treatment 7 suffered catastrophic mortality. While *Patinopecten yessoensis* apparently tolerates exposure to 115 mg/kg available chlorine in seawater for 30 min without ill effects (Bourne et al., 1989), *P. fumatus* juveniles die rapidly at this concentration. Much lower concentrations of 0.5 mg/kg available chlorine, found to cause detachment in pediveligers of the mussel species *Mytilus edulis* and *M. galloprovincialis* without adverse side-effects (Bucaille and Kim, 1981), could also be effective in relation to *P. fumatus* spat.

Future choices between air exposure and hypersaline baths as the preferred detachment method for routine hatchery use may alter according to circumstance. While costs of artificial sea salt, associated equipment and bath preparation time are relatively high, the initial rate of detachment is faster and less variable. Attendant risks would also appear lower with artificial sea salt baths in that exposure for up to 24 h did not cause mortality of spat. Although air exposure is inexpensive and simple, its effectiveness is likely to alter according to factors such as air temperature and humidity, which in turn impose risks of spat desiccation and temperature stress.

The value of *P. fumatus* spat for ongoing research at BWFCRS prevented the determination of tolerance limits to chlorine and low pH, and of the combined effects of air temperature and humidity on spat desiccation. All these factors warrant further study. In the meantime, however, use of artificial sea salt hypersaline baths has been adopted as the standard procedure at BWFCRS for inducing *P. fumatus* spat to detach from settlement and nursery screens when stocked at densities of up to $30/cm^2$.

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References

Bourne, N., Hodgson, C.A. and Whyte, J.N.C., 1989. A Manual for Scallop Culture in British Columbia. Canadian Technical Report of Fisheries and Aquatic Science, No. 1694, 215 pp.

Bucaille, D. and Kim, N., 1981. Influence de la chloration sur la fixation, le détachement, la croissance des larves pédivéligères et le jeune naissain de moules, et sur la gamétogenèse des adultes. In: Influence des Rejets Thermiques sur le Milieu Vivant en Mer et en Estuaire. Electricité de France, Paris, pp. 385–411.

Heasman, M.P. and Nell, J.A. 1991. Scope for enhancement of scallop *(Pecten fumatus)* fisheries in NSW using hatchery reared broodstock. In: B.J. Hill, and N. Preston (Editors), Proceedings of Marine Reseeding Workshop, CSIRO Marine Laboratories, Cleveland, Qld., September, 1991 (unpublished manuscript).