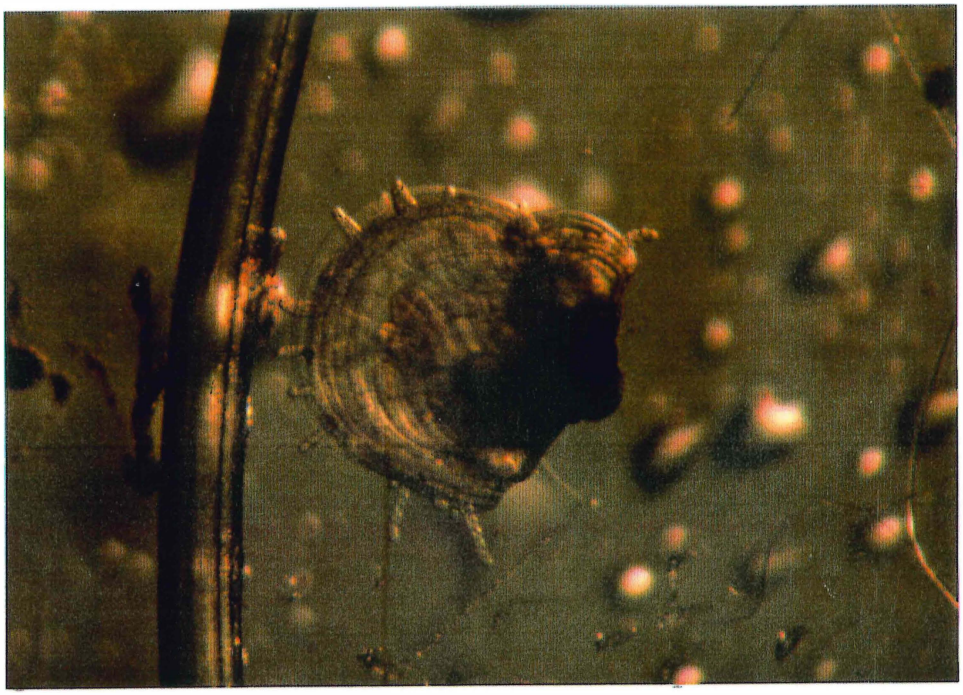
Aquaculture of the saucer scallop *Amusium balloti*

FIRDC 89/58 WSF1Z



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Cover Photograph:

Recently settled cultured spat of the saucer scallop
(*Amusium japonicus balloti* (Bernardi, 1861)
Western Australia.

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This paper constitutes the final report for the project "Aquaculture of the saucer scallop *Amusium balloti*". The research project (FIRDC 89/58 WSF1Z) discussed herein was conducted at Western Seafarms Ltd, Carnarvon, WA., and funded by the Fishing Industry Research and Development Trust Fund, Department of Primary Industry, Canberra.

Abstract

Adult saucer scallops (Amusium japonicus balloti) were obtained from trawlers operating in Shark Bay, Western Australia and maintained in 6,000 L and 12,000 L pools using filtered seawater which was heated in winter. Adults were fed daily with cultured microalgae to improve gonad condition. Induced spawnings were conducted, over a 3 year period, in all months from April to December. Adults were induced to spawn by a water temperature increase. Up to 4.1 million pediveligers were placed into settling tanks from one spawning. The optimum larval culture temperature was 22.18 ± 0.94 °C and settlement was achieved 12 days after spawning at a size of 212 µm. Batches of settled spat regularly exceeded 0.5 million with the highest count attained of approximately 1.4 million spat sampled 17 days after metamorphosis commenced. Spat were reared in a land-based nursery system for up to 155 days before being transferred to a sea-based longline system. Although the scale was restricted in this study the potential for large scale commercial hatchery culture of A. balloti has been demonstrated.

Contents

Background

The saucer or swimming scallop occurs in commercial quantities from southern Queensland northwards and from the southern coast of Western Australia northwards. However, significant fisheries only exist or have existed in the regions around Fraser Island, Hervey Bay, Yeppoon, Bustard Heads, Round Hill and Lady Musgrave Island in Queensland. In Western Australia the major fisheries have been or are around the Abrolhos Islands and in Shark Bay.

As with most scallop fisheries worldwide, annual catch fluctuations have been dramatic and resembled a typical boom-bust pattern. Even so, neither the Queensland nor Western Australian fisheries have existed at the 'bust' level for lengthy periods. Minimum annual catches in both states have been about 80-110 t of meat in past years whilst peak catches have been around 800 t of meat in Queensland and 3,000 t of meat in Western Australia.

The feature that makes this history somewhat remarkable is that this scallop species recruits to the fishery at approximately one year of age and is also reproductively active at that stage. The bulk of fishery catches in both states appear to consist of mainly 1+ (years old) animals. The fact that the saucer scallop is a "*highly fecund, broadcast spawner, with a long breeding season*" (Joll, 1987; p. 1) probably explains why these fisheries have survived in the face of intensive fishing effort. The nature of their habitat and the fishing technique (trawling) means that non-catchable stocks exist in and around reef's and are often left untouched from year to year. Additionally it seems that animals are spread over vast areas of seabed at densities below economically viable levels. Both these aspects imply that numbers of adult scallops are present from year to year and possibly responsible for the long term survival of the fisheries.

Success with collection of juvenile scallops (spat) in Tasmanian waters during the mid-1980's provided technology which could be used for the saucer scallop and potentially for stock enhancement work. Unfortunately early research on spat collection of saucer scallops suggested that this technique may be not be successful for that species (Dredge, 1988; Sumpton, et al., 1990).

Development of hatchery scallop production techniques (FIRTA 84/93) also in Tasmania provided sufficient information on which to base a proposal for the hatchery production of saucer scallop spat. An application for funding was submitted to FIRDC for consideration in early 1989. Funding to Western Seafarms Ltd was approved and the project commenced in July 1989 at Carnarvon (Shark Bay) Western Australia.

Introduction

The Objectives for this project were:

(A) To establish larval rearing techniques for scallop spat (*A.balloti*) under commercial hatchery conditions.

(B) To establish suitable nursery culture techniques.

(C) To assess the release of spat onto the seabed.

(D) To examine the health of larvae, juveniles and adults.

The overall objective of the project was to establish suitable techniques to serve as a basis for development of appropriate large scale culture systems.

The development of commercial hatchery production techniques for *Amusium japonicus balloti* (Bernardi, 1861) in Western Australia was the primary aim. Integral aspects of this were the conditioning of broodstock, development of a suitable larval diet, identification of an optimum settling technique and a satisfactory nursery rearing method. These aspects are addressed separately in the report.

Larval development and hatchery production for a number of species of Australian scallops has been documented (Dix and Sjardin, 1975; Dix, 1981; Rose & Dix, 1984; Cropp & Frankish, 1988; Rose et al. 1988; Rose et al. 1988 and Cropp, in press). However, virtually all of those papers were on *Pecten* or *Chlamys sp.*, some of which were the basis of significant commercial fisheries in southern Australian waters. *Amusium japonicus balloti* is the target species for significant trawl fisheries in central Queensland waters (Williams and Dredge, 1981) and Shark Bay, Western Australia (Joll, 1989). It is commonly known as *A. balloti* (Young and Martin, 1989), the saucer or swimming scallop and has been commercially caught from southern Western Australia, through to the Northern Territory and from south Queensland northwards. It is a tropical-subtropical species which appears to prefer a water temperature of 19-24°C and is found on medium to coarse sandy mud bottom.

A. balloti has already been the subject of a number of studies on natural spat settlement and recruitment, McDuff, 1975; Kettle, 1984; Dredge 1981; Campbell, 1987 and Sumpton et al. 1990. In recent years there have been attempts to artificially culture *A. balloti* in a hatchery (Rose et al. 1988 and Connolly, 1990).

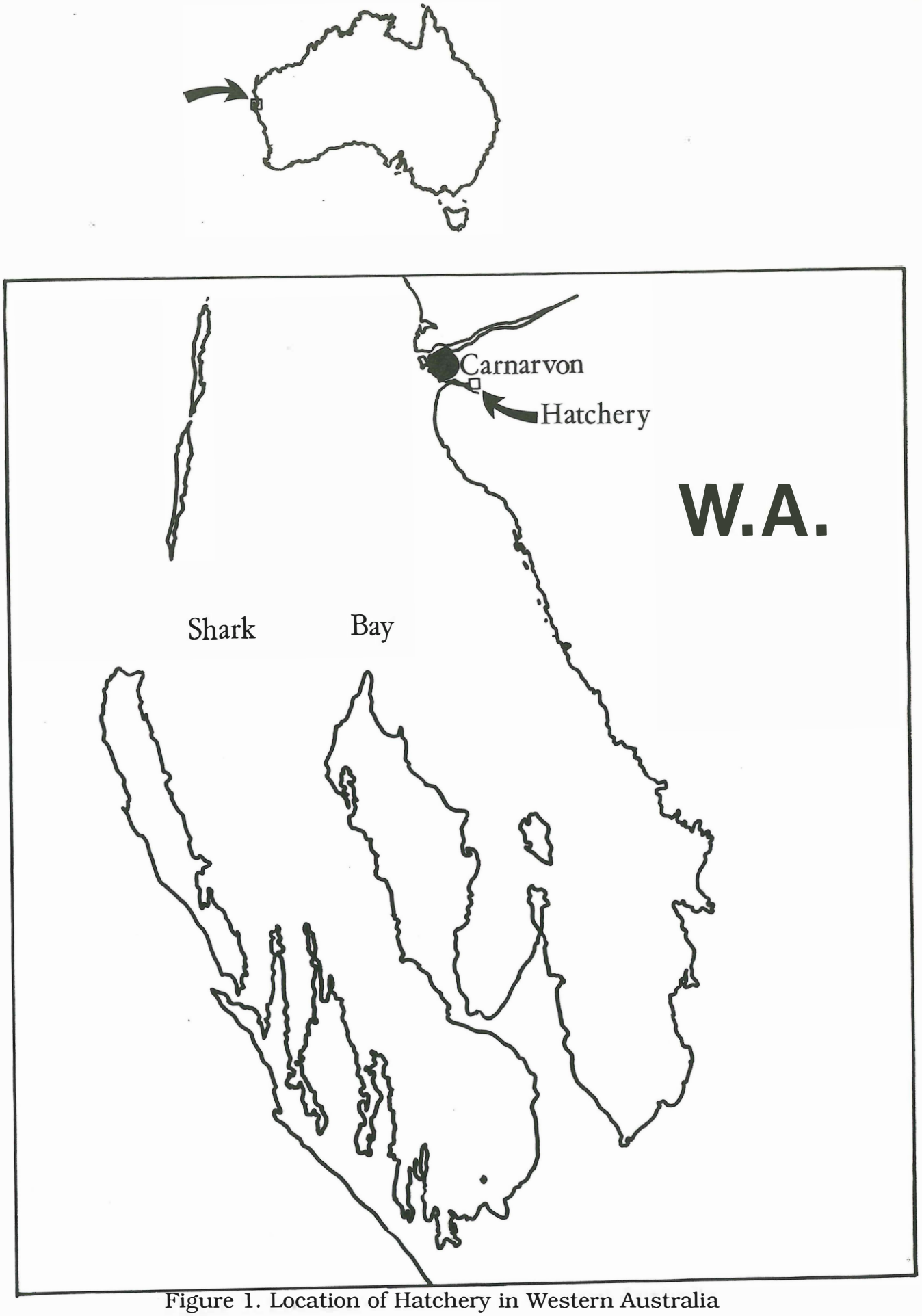

Figure 1. Location of Hatchery in Western Australia

These studies were hindered by the tendency of the metamorphosing larvae not to exude a strong byssal thread (Rose et al. 1988, Dredge, 1981). The attachment was also found to be for a short time period only (Rose et al. 1988), unlike Pecten or Chlamys species studied elsewhere in Australia (Dix and Sjardin, 1975; Rose and Dix, 1984; Sause et al. 1987; Hortle and Cropp, 1987 and Cropp, in press). The initial attempts at hatchery rearing of *A. balloti* (Rose et al. 1988 and Connolly, 1990) did not fully utilise recent improvements in broodstock conditioning and larval rearing techniques (Gwyther et al., 1991; Cropp, 1988a) and were either not successful or had very limited results. Those improvements were implemented and further developed in this study.

The ability of scallop spat to attach to collectors has been utilised in many areas overseas and for numerous different species (Cropp, 1988b). It has allowed industries to develop due to the availability of large amounts of spat. This spat can be grown to harvestable size using a number of techniques (Cropp, 1988b, 1988c), some of which were applicable to *A. balloti.* However the unusual post-settlement behaviour of *A. balloti* made the development of a unique nursery system necessary. Unfortunately the cost-effectiveness of the land-based system is questionable on a large scale.

While not all unsuccessful trials have been mentioned in this report it should be noted that the techniques detailed herein were determined over a three year Research and Development Project and the procedures discussed at length were the most successful and are recommended as a starting point for any future work on this species.

Broodstock

Scallops for this study were collected from trawlers (scallop and prawn) operating in Shark Bay, Western Australia between April and October of each year from 1989-91. The broodstock were obtained from the sorting trays and immediately placed into either small portable tanks (120-250 L) containing aerated water which had been cooled to 2°C below ambient using saltwater ice or into steel mesh baskets in the vessels circulating tanks. Scallops held in the vessels tanks were placed into small portable tanks upon arrival in port.

They were transported in the tanks, by road, to a nearby hatchery facility (Figure 1. Carnarvon, Western Australia) and maintained in 6,000 L and 12,000 L above-ground swimming pools.

Water in these pools was preferably kept at approximately 18-20°C for a period of usually 5 days prior to a spawning being attempted with some of the animals. In mid-winter, 2 KW electrical immersion heaters were used to maintain the water temperature. The hatchery is located approximately 5 km south of the Carnarvon township and port at a position of approximately 113° 50' E and 24° 50' S.

Saltwater for the hatchery was sucked from a nearby mangrove-lined creek (Oyster Creek). It passed through a sand filter before being stored in a 20,000 L fibreglass tank. Salinity was checked daily using a refractometer. When required the water was pumped through a series of 'Cuno' cartridge filters in the series: 20 µm, 10 µm, 5 µm, 2 µm and finally 1 µm. Broodstock pools were filled with 20 µm filtered water, larvae tanks with 1-20 µm filtered water, depending on the daily water quality and research requirements (thorough filtering for dirty water), and 1 µm filtered water was used for algal cultures.

During the broodstock holding period, 50% of the pool volume was changed at least every second day and on occasions daily. The normal stocking rate of broodstock was at least 100 animals per 6,000 L pool and 200 animals for the 12,000 L pool. Initially, volumes of a non-axenic algae culture, *Tetraselmis suecica* were added daily in sufficient quantity to establish a food cell density in the holding pool of 30,000-40,000 cells/mL. After early gonad conditioning work exhibited poor results, the algae species was changed to another non-axenic algae, *Chaetoceros gracilis*. This species is acknowledged to have a higher nutritional value than *T. suecica* (Brown et al., 1989) and is also reasonably easy to grow in large volumes out-doors. When available, this diet was supplemented by non-axenic *Chaetoceros calcitrans*, *Pavlova lutheri* and Tahitian *Isochrysis (aff.) galbana*. *T. suecica* was used occasionally, as available, as a minor component of the diet.

Gonad condition of the scallops was monitored visually on a regular basis. This was achieved by inserting a blunt knife between the valves whilst the animal was respiring and carefully lifting gill tissue to

inspect the gonad underneath. When well developed or mature gonads were apparent a spawning was attempted. In most spawnings, 4-10 males and 10-20 female scallops were used. Sexes of live animals were easily distinguished by the creamy white gonad colour of the males and the bright orange colour of the female gonad. Only animals with well developed gonads were selected for use in each spawning attempt. Trials in 1989 showed that a forced spawning of broodstock was possible but in batches where the gonads were immature, poor larval results were evident.

Broodstock were maintained in the holding pools during most of each year, but control of the water temperature was essential for the animals to survive during the coldest part of winter and the hottest part of summer. For these respective periods when ambient water temperatures fell to 12-13°C a heater was required or when ambient water temperatures rose to 28-29°C, a cooler was necessary.

If the water temperatures were not controlled the metabolic rate of the scallops would slow until they died due to cold water in winter or in summer they would spawn out before dying due to heat stress. Gonad condition was maintained through most months except the hottest (January-March) when spawnings were not attempted. Attempts were made to retain broodstock over summer but due to the difficulties and expense it was deemed more efficient to obtain new stock from scallop trawlers as soon as they were available (April-May). Broodstock held in December was then not retained for the next season's spawning. It was generally necessary to condition the gonads of this broodstock prior to a spawning. This situation varied depending on the collection site in Shark Bay. There was clear evidence of a variation in the spawning date of isolated patches of scallops within the fishing grounds; water temperatures apparently varied marginally at these sites. Trawlers tended not to fish areas where spawning was occurring or had just finished and therefore adults with developing gonads were often available off the vessels.

Spawning

Scallops to be spawned were gently cleaned and air-dried for ten minutes before being placed into water, on a glass-walled table, at either the same or less than the broodstock pool temperature. Water

temperature in the broodstock pools was 19-23°C in summer and 17-19°C in winter respectively (with the assistance of heaters). Scallops were left to acclimatize to the water on the spawning table before any spawning inducement was attempted. This generally involved a period of 30-60 minutes. Two aerator stones were set-up on the table to maintain the level of dissolved oxygen, especially when the water temperature was raised. A 5 KW immersion heater placed in a header tank was used to heat water for the spawning table. The water was circulated between the spawning table and header tank using a small submersible electric pump.

Water temperature on the spawning table was increased gradually up to approximately 24.5°C over a period of 1-2 hours. If spawning did not occur the heater was switched off and the water allowed to cool gradually. In warmer months saltwater ice was used to decrease water temperature when necessary. Additionally, several mL of a sperm solution, extracted from a male in the broodstock pool, would be added when the water temperature was approaching 24.5°C. On numerous occasions, spawning commenced before sperm was added to the water.

In other trials, gradual spawning could be induced over a period of hours by placing scallops in a 3 L transparent plastic bowl, immersed in clean saltwater and positioned in direct sunlight. Solar heating of the water resulted and when the temperature attained approximately 24°C spawning commenced in animals with ripe gonads. Unfortunately the slow increase in water temperature (and probable decrease in dissolved oxygen) was generally sufficient to cause the death of some broodstock after spawning.

During the usual indoor spawning procedure, spawning animals were removed from the table water, placed in a 3 L bowl and immersed in clean saltwater, at the same temperature as the spawning table water. The 3 L container was placed on the table, the water of which acted as a temperature bath. Females were allowed to spawn for approximately 20 minutes in these 3 L bowls before the resultant eggs were transferred to 20 L plastic buckets through a 120 μm screen. The screen was used to separate faecal matter and detritus from the eggs. Males were also allowed to spawn in their 3 L containers for 20-30 minutes before the sperm solution was replaced with clean water. Sperm older than 30 minutes was discarded.

Sperm was added to the 20 L buckets in the ratio of several mL per 5 million eggs or 5-8 mL of concentrated sperm solution for 15-20 million eggs. This was assessed visually in the first instance and qualified by microscopic examinations of the sperm solution and counts of the fertilized eggs. This day was referred to as Day 0 when monitoring larval development.

Eggs and sperm were mixed in each 20 L bucket using a small plunger. This consisted of a perforated plastic disk of 150 mm in diameter, attached to one end of a 500 mm section of 25 mm diameter PVC pipe (Cropp, in press). Ten minutes after mixing, a sample was taken to determine the number of sperm/egg; if this revealed a non-satisfactory fertilization rate, more sperm solution was added and mixed with the plunger. Twenty minutes after the first sperm was added, a sample of 0.5-0.8 mL of eggs was taken using a 1 mL plastic pipette. The plunger was used to lift eggs from the bottom of the bucket and distribute them evenly through the water as the sample was being collected. Batches of eggs that were deemed to be of poor quality (unsatisfactory shape, size and stage of maturity) were discarded.

The sample of eggs was drained from the pipette onto a Sedgwick-Rafter counting cell (with cover-slide) and placed under a 'Ziess' dissecting stereo microscope. The total number of eggs in the sample was counted and thus the total egg count in the 20 L bucket was evaluated. The number of sperm/egg was also checked again if necessary. The average size of the eggs was determined using an eyepiece graticule.

The fertilized eggs were poured gently into a pre-filled (and often heated) 4,000 L larvae tank with very gentle aeration stemming from an air stone in the centre at the bottom of the tank. Larvae tanks were heated, when necessary, by convection using coils of black polyethylene tubing filled with fresh water. A thermostat controlled hot water cylinder was used as a header tank to maintain the temperature whilst a small 800 W electric pump was used to circulate the water through the piping. The 18 mm tubing was coiled at least twice around the inside of each larval tank and was connected to the main (insulated) circulation line near the ceiling of the larvae-tank shed. The piping was ducted up over the top of each larval tank for easy removal and cleaning, rather than through the walls.

In warmer months a refrigerated water cooler unit was connected to the system and tanks were cooled by convection when necessary.

Larval Rearing

A 20 μm screen was used to obtain a small sample from the surface of each larval tank on Day 1, approximately 24 hours after the eggs had been fertilized. The larvae/trochophores were examined and measured to determine developmental stage. This was repeated on Day 2 at a similar hour. When a high percentage of 'D' shaped larvae were present, the tank was drained through a 45 μm screen. Only swimming 'D' larvae were retained. Larvae and debris remaining on the bottom of the tank were discarded.

Larvae present in the 45 μm screen were washed carefully into a 20 L plastic bucket. A 0.5-0.8 mL sample was taken as described earlier and the number of larvae counted. The total number of larvae per tank was then evaluated. All larvae were then poured from the bucket into another pre-filled 4,000 L larvae tank. A mixed diet of cultured algae was then added to each larval tank. The diet was composed of approximately equal portions of high quality *Chaetoceros calcitrans*, *Pavlova lutheri* and Tahitian *Isochrysis (aff.) galbana.*

The tank changing procedure used on Day 2 was repeated every two days until settlement with the screen size increased in conjunction with the larval size. This technique also ensured that smaller slow growing larvae were gradually eliminated from the batch.

The feeding rate was initially 15,000 cells/mL for most batches of larvae and gradually increased to 25,000 cells/mL at metamorphosis. This regime was reduced slightly during the project to 10,000 cells/mL at day 2 and 15,000 cells/mL at metamorphosis. Water quality and temperature were monitored daily; any ambient variations were controlled with filters and the water heating/cooling system. Reduced salinities in mid-winter (28 ppth.) were adjusted by adding hypersaline water from a bore; high salinities in summer were reduced by the addition of freshwater from the town water supply.

Settlement

Prior to the appearance of pediveligers, mesh spat collectors were constructed. These consisted of a red mesh bag with a drawstring at the top and filled with approximately four metres of old (hardened) monofilament shark netting.

The bags were 780 mm long and 360 mm wide with mesh measuring 0.9 x 0.9 mm internally or 1.2 mm diagonally (Cropp, in press). One corner of the bottom of each of five to ten collectors was then tied into a 3.5 m length of 8 mm polyethylene rope. The collectors were spaced evenly along the rope. A small piece of polystyrene foam was attached to one end of the rope and a 1 kg ceramic weight (inert in saltwater) to the other. This assemblage of collectors was termed a 'dropper'. The droppers were washed and then soaked in saltwater for at least 4 hours before being immersed in a larvae tank.

When a large percentage of the larvae in a particular tank were observed to exhibit an active and probing (motile) foot, droppers were placed in the tank. The number of droppers/collectors immersed in each tank was determined by the number of pediveligers present. This day was referred to as the settlement day for each batch of larvae.

The tanks then had a portion of water drained every day, second day or third day depending on the density of larvae/spat in the tank. After this water was drained through a 100 μm screen any larvae caught in the screen were returned to their respective tanks. Each tank was then re-filled with water of a suitable temperature and salinity. If necessary, a spare 4,000 L tank was filled (with temperature and salinity adjusted) prior to draining the spat tanks. Water was then pumped from the spare tank into each drained spat tank. This was particularly important in mid-winter when ambient water temperature was very low (eg. less than 18°C), or mid-summer when ambient water temperature and salinity were very high, eg. 29°C and 42 ppth. These parameters were adjusted in the spare tank until water conditions were similar to that in the tank to be filled.

Algal cell density within each spat tank was increased up to 30,000 cells/mL as the scallop spat grew. Acceptable algal density was determined by regular microscopic observation of the stomach of spat.

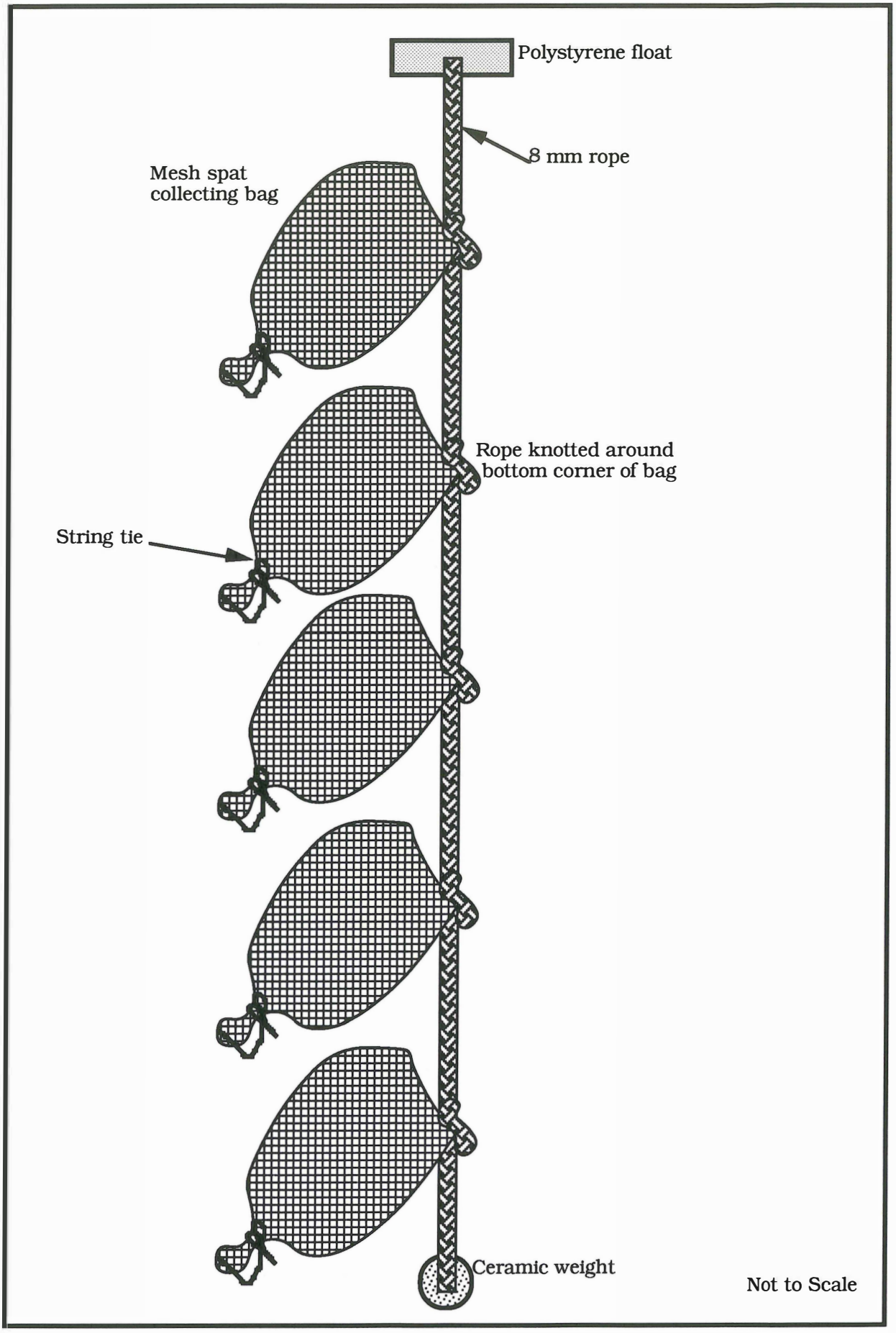

Figure 3. Diagram of spat collector dropper.

The stomach condition was easily monitored visually through the shell. A visual assessment was also made daily of the un-eaten amount of algae remaining in each spat tank.

Larval sizes were generally recorded every second day from a sample of 10 larvae while spat size was assessed from a sample of at least 10 spat, periodically as they grew. Total larval counts were also recorded every second day and spat counts periodically by sub-sampling collectors. Height of the shell was taken as the perpendicular distance from the tip of the umbo to the distal margin of the shell. The maximum measurement taken at a right angle to this and from one shell margin to the opposing margin, is referred to as the length.

Nursery Culture

Nursery culture was considered to be the stage after metamorphosis or settlement had ceased to occur, that is, all animals present were spat. During the transition period through metamorphosis numbers decreased considerably. However, the extent of this loss was difficult to determine without considerable handling of newly settled spat. If these spat were handled (counted) then high mortalities could be expected, hence this was avoided.

Extremely high numbers of spat were settled in collectors (up to 46,000) in addition to those that settled on the tank walls and floor. Initial trials indicated that spat on the tank floor (about 10% of settled numbers) would grow well for up to several weeks but an increase in faecal matter and algal detritus on the tank floor would eventually kill them. Even with regular water exchange and tank cleaning it was found to be essential that spat be removed from the tank floor regularly and placed inside mesh collectors suspended in the water column. Although this was somewhat time consuming it did give satisfactory results. The spat characteristic that made this work more important was the lack of a firm and long term byssal attachment. Settled spat retained a strong and active foot which allowed them to crawl around the substrates, detach and swim in the water column at will. If collectors were suspended on a longline at sea after settlement, then any spat detaching would be lost to nature. In 1989 spat collectors were transferred to a sea-based sub-surface longline (offshore). Spat were not transferred until they were on

average larger than the mesh size of the enclosing collector; hence spat losses due to byssal detachment should have been minimal. This was confirmed, in part, by the recovery of greater than 6,000 dead scallop spat of 3 $^{+}$ mm in size from damaged collectors deployed on the longline. This was an average in excess of 600/collector compared to the deployment number of about 1,000/collector.

The longline was located in Shark Bay approximately 5 km North-west of the Carnarvon township. The depth of water was 13-14.5 m, with a water current of approximately 1.3 knots over a sandy bottom. Monitoring was difficult due to reliance on a Fisheries Patrol vessel which had many other commitments and due to frequent rough weather. Limited reliable data was available because of interference and vandalism on the longline. Longline culture was discontinued after its deliberate destruction in mid 1990, but two small samples of spat were obtained prior to this. The first sample in March 1990 revealed a size of 16.7 $\pm$ 2.78 mm and a range of 13.3-21.2 mm at a post-settlement age of 177 days; a growth of about 94 µm/day. This was lower than the annual growth rate predicted by Joll (1987). The second and final sample at day 269 (June 1990) yielded spat of 50.0 $\pm$ 5.16 mm and a range of 44 - 56 mm (n=4) in size. The apparent growth rate for the period from March to June was 33.3 mm or 362 µm/day which was biased due to the small final sample size (n=4). Even so, Joll (p37, 1987) did comment that substantial growth occurred in wild saucer scallops between March and May in Shark Bay. Overall the growth from settlement to day 269 averaged 185 µm/day or 67.56 mm at one year of age. This is less than the predicted growth rate and respective size of one year old wild scallops in Shark Bay.

Subsequent nursery culture on land allowed for more control of environmental conditions but due to necessary stock density requirements (available tank space) only small numbers of spat were retained for a long term nursery stage. The intention was to grow spat on land to a manageable size for handling (10 + mm) before transferring them to a sea-based culture system. It was obvious from trials in 1989 that small spat were highly susceptible to suspended fine silt and fouling of sea-based mesh grow-out cages. Culture of spat to 10+ mm in size on land (in filtered water) should therefore

improve the subsequent survival as tolerance levels generally increased as spat grew.

Results

Temperature and water quality trial for larvae

In an early attempt (August-September 1989) to ascertain the temperature regime and water preferences of larvae a small scale trial using 4 polyethylene 200 L bins was conducted. The aim was to rear 4 batches of larvae produced from the spawning of 2 male and 2 female adults. Spawned eggs were fertilized in a 20 L bucket and then carefully divided into 4 similar batches of which one was poured into each of the pre-filled and pre-heated 200 L bins. The varying culture conditions involved a warm rearing phase (2 bins), 24°C initially to the preferred temperature of 19.5 ± 1°C at settlement, and a stable 'cool' rearing phase, (2 bins) 19.5 ± 1°C from eggs to settlement. Ambient water temperature in Shark Bay during the natural reproductive cycle (early April to December/January; Joll, 1987) apparently varied from 19-24°C. Water filtration criteria was also included in this trial, with one of each pair of bins using water filtered to 20 µm and one filtered to 5 µm using the Cuno filter cartridges. Salinity was maintained at a constant level for all bins and similar numbers of fertilized eggs were poured into each. Thus the non-replicated trial involved 4 bins with the following water conditions:

Table 1. Culture details for 200 L bins.

Bin	Temp. (°C)	Salinity (ppth)	Filtered Water	No. eggs (10^6)
E1	24	35	20	1.6
E2	20.3	35	20	1.52
E3	20.2	35	5	1.65
E4	24	35	5	1.64

Water conditions were maintained throughout the larval period as shown, except for water temperature. By day 15 (12th September) all bins were at 19.5 ± 1°C.

As expected, larval growth was slightly more rapid in the bins at 20°C. By day 14 the first pediveligers were present in E1; pediveligers were present in all bins on day 15. E2 produced the highest survival, 9.45% at day 14, E3-2.65%, E1-2.34% and the lowest survival was E4 at 0.38%. The warmer initial culture temperature resulted in larvae approximately 9 μm larger in size at the 14 day stage (206 against 197 μm). A consideration of all results allowed the preferred culture conditions to be ranked from best (1) to worst (4). The ranking would be **1**: E2; **2**: E3; **3**: E1 and **4**: E4.

Overall it appears that the higher culture temperatures allow rapid larval development but may also result in higher mortalities due to possible bacteria build-up in conducive conditions. Preferred water filtration of 20 μm was initially apparent however subsequent (to day 14) high mortalities in E2 as compared to E3 cloud this issue. Thus the objectives of larval culture would need to be identified before one culture regime became preferable to another. In the present study the filtration level was based upon the daily water quality; increased filtration was implemented when water quality declined.

Larval Culture

Larval culture results improved significantly over time as the husbandry techniques improved. The culture success based upon the percentage survival of the respective number of fertilized eggs, number of 2 day old veligers and number of pediveligers per batch improved markedly each year (Table 2).

A number of survival indicator percentages can also be extrapolated from the data in Table 2; these are shown in Table 3. The success rate or efficiency of spawnings underwent some major changes over the three year period. The average productivity per female did not change greatly although a slight decrease is shown. The underlying reason behind this statistic was the testing of different spawning techniques which were not always successful (eg. solar spawning, cold water shock); this artificially lowered the spawning figure per female.

Table 2. Average annual results for each of the development stages per spawning batch.

Year	No. of Batches	No. Females	No. Fert. eggs (10^6) (F)	No. 'D' veligers (10^6) (D)	No. Pediveligers (P)	Pediveliger Size (µm)	Days to settlement
1989	19	13.26	27.4005	1.4298	68,658	202.45	15.40
1990	11	12.91	21.9327	0.8896	496,667	212.82	14.30
1991	5	9.00	18.5000	2.7420	1,765,000	211.00	11.75

NB: No. Fert. eggs = Number of fertilized eggs (F);
No. 'D' veligers = Number of 'D' shaped larvae (D)
No. Pediveligers = Number of pediveligers (P)

The number of females used per spawning was clearly reduced each year and this led to a smaller total number of fertilized eggs being used per batch. However, past that stage, marked improvements were evidenced in the percentage of larvae surviving from one phase of the larval cycle to the next. The increase in the statistic D in 1991 was considerable and is indicative of well developed gonads and a successful spawning technique. The extremely high figure (64 %) of 'D' shaped larvae surviving to the pediveliger phase is evidence of the high standard of larval and algal culture conducted in the hatchery. Overall, the survival from fertilized eggs to pediveligers was 9.5 % in the third year (1991).

Table 3. Average annual percentages of larvae at each of the development stages per spawning batch.

Year	No. of Batches	No. Fert.eggs (10^6) per female	No. 'D' veligers (D) (10^6)/F x 100	No.Pediveligers (P)/D x 100	P/F x 100
1989	19	2.0664	5.2182 %	4.8019 %	0.2506 %
1990	11	1.6989	4.0560 %	55.8304 %	2.2645 %
1991	5	2.0556	14.8216 %	64.3691 %	9.5405 %

The improved culture techniques also resulted in an increase, up to 7.3 µm/day, in the average daily growth rate from day 2 to pediveliger (Table 4); this was a marked improvement on rates achieved at the beginning of the project in 1989.

Table 4. Average annual size and growth rates of larvae per batch.

Year	No. of Batches	Egg Size (µm)	Size of 'D' larvae, day 2 (µm)	Pediveliger size (µm)	Days to settlement	Daily growth of larvae, day 2 to settlement (µm/day)
1989	10	75	114.56	201.78	15.40	5.6636
1990	5	75	119.70	214.38	14.30	6.6210
1991	4	75	123.62	211.00	11.75	7.3089

NB: Only batches where an accurate Day 2 and pediveliger size were available are documented in this table, hence the batch and pediveliger difference to Table 3. Pediveliger size is taken as the larval size on settlement day.

The survival and growth rate of larvae from a batch (referred to as HB3/91) spawned on 24 July 1991 is shown in Figures 3 and 4. This could be classified as one of the most successful batches of the entire 3 year program as 1.4 million spat were present on Day 28 and 1.089 million on Day 34 after spawning. For the production of this batch, five male and 10 female scallops were placed on the spawning table on 24 July 1991 and all but one female spawned. A total of 29.8 million fertilized eggs resulted or an average of 3.3 million per female. Larvae were reared in larvae tanks at a salinity of 35 ppth and an average temperature of 22°C $\pm$ 1 °C. The algal diet was composed of approximately equal portions of *C. calcitrans*, *P. lutheri* and Tahitian *I. (aff.) galbana* at a density increasing from 10,000 cells/mL on day 2 up to 15,000 cells/mL at day 12 (settlement) and then increasing further to 25,000 cells/mL for settled spat. To allow for later comparison, a breakdown of the daily diet has been shown below (Fig. 4) for algal cells per larvae.

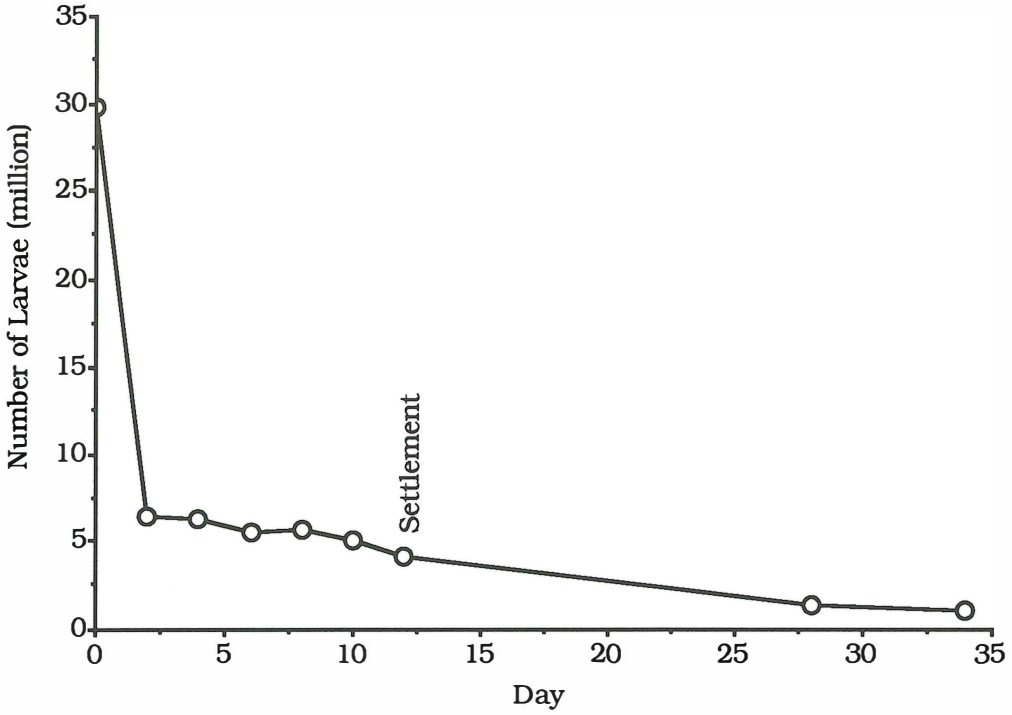

Figure 4. Number of eggs, larvae and spat during the culture phase,for HB3/91.

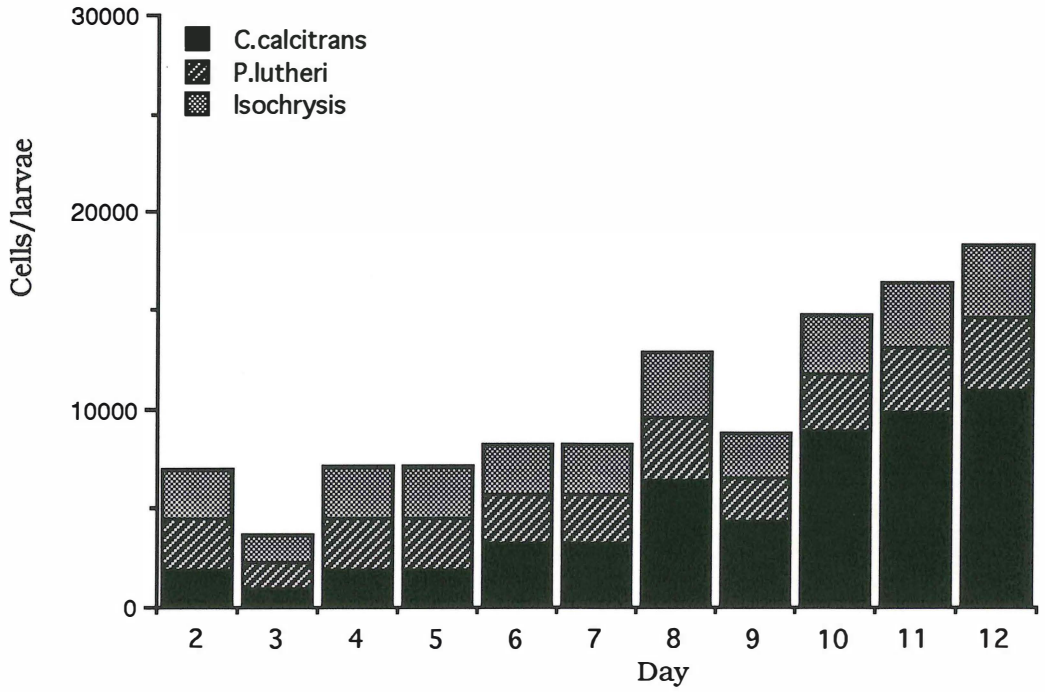

Figure 5. Algal diet for larvae during the culture phase for HB3/91.

As mentioned earlier, 4.1 million pediveligers were put in to settlement tanks, 1.4 million spat were present at day 28 (41,333 ± 4,509 spat/collector plus 160,000 loose spat) and (31,500 ± 3,697 spat/collector plus 144,000 loose spat). A total of 30 mesh spat collectors were used for this batch. The shell of spat gradually changed from opaque to white as they grew (greater than 4 mm), a feature which has not been documented previously. It is also an aspect to be considered when attempting to identify naturally occurring spat collected in tropical and sub-tropical areas.

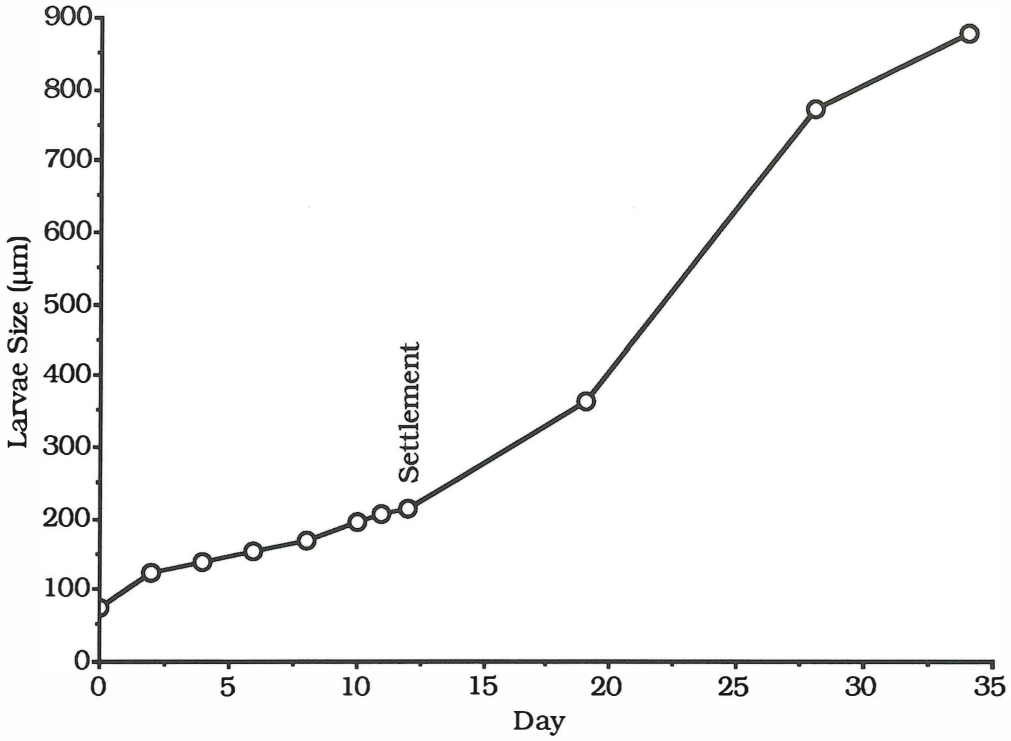

Figure 6. Growth of *A. balloti* larvae and spat, for HB3/91, shell height.

Water temperatures for the October 1991 batch shown overleaf (referred to as HB5/91) were maintained at 23-25°C (mean 23.9°C). Occasional temperatures in excess of the predicted 25°C safe limit (26°C) were caused by cooling system malfunctions and resulted in high spat mortalities.

The situation regarding optimum water temperature for the culture of larvae was further clarified in the 1991 batches, two of which are shown here (July and October). The July batch were cultured at

22.18 ± 0.94 °C and the October batch at 24.87 ± 0.39 °C. The July batch commenced settlement on day 12 and 212 µm while the October batch commenced settlement on day 11 at 212 µm. The reduction of the larval period by one day (October batch) was not worthwhile given the comparatively poor survival rates in relation to the 'cooler' July batch. The October survival rate from fertilized eggs to 'D' larvae was 8.69%, from 'D' larvae to pediveligers it was 35.37% and from fertilized eggs to pediveligers it was only 3.07%. The comparative July figures were 21.62%, 64.06% and 13.85% respectively. It should be noted that feeding and general culture regimes were similar for each of the batches. Thus, the difference between respective survival rates is dramatic and further supports results from the initial water temperature and quality trial. Inherent in this is the assumption that the reproductive condition of each group of broodstock was the same and that larvae produced at different times of year have equivalent development potential. Joll (1987, p.33) suggested that the early and latter parts of the breeding season "*do not appear to produce the bulk of the recruitment (at least in the years under study)*", but there could be other environmental explanations for this situation.

For the October batch shown overleaf 3,950 scallop spat were transferred from the nursery system to a sea-based (inshore) longline 166 days after they were spawned (155 days ex-settlement). Their size at that stage (late March 1992) was 15.59 ± 1.59 mm. This gave an overall growth rate of 100.6 µm/day from settlement, which was slower than the growth rate exhibited by spat grown (over a similar period) at sea in 1989-90. Again, this appears to be lower than the annual growth rate shown for natural (seabed) scallops by Joll, 1987, suggesting that *A. balloti* may prefer seabed conditions to mid-water.

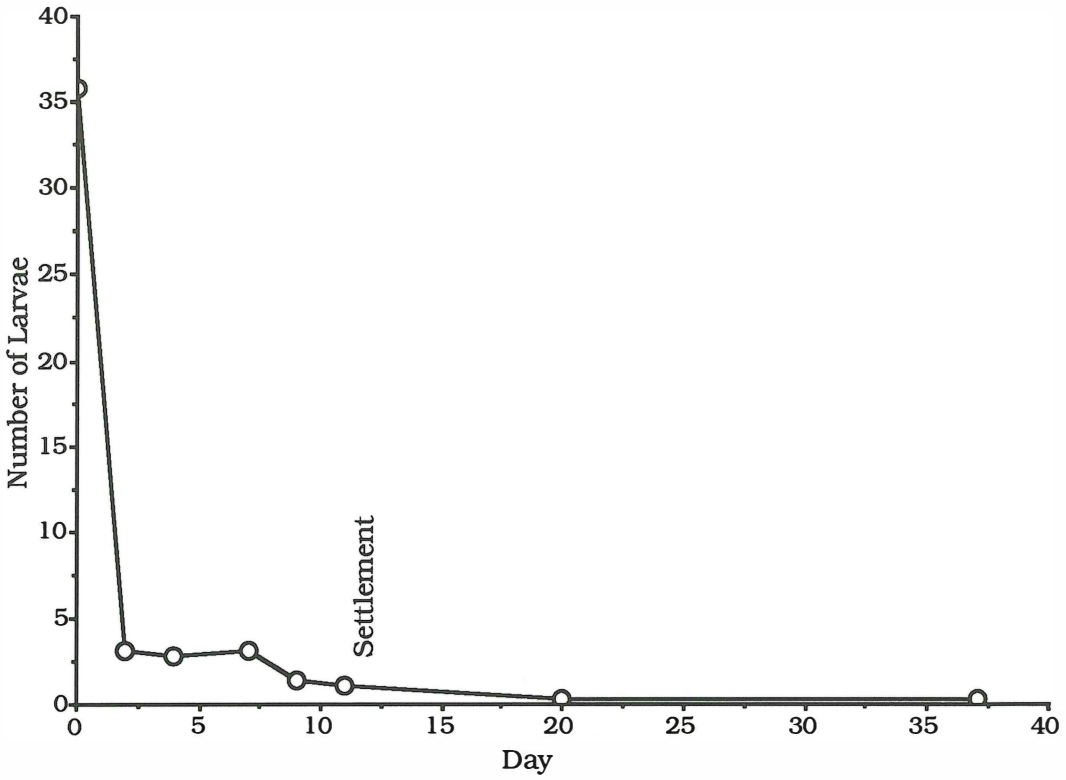

Figure 7. Number of eggs, larvae and spat during the culture phase, Oct. 1991 (HB5/91).

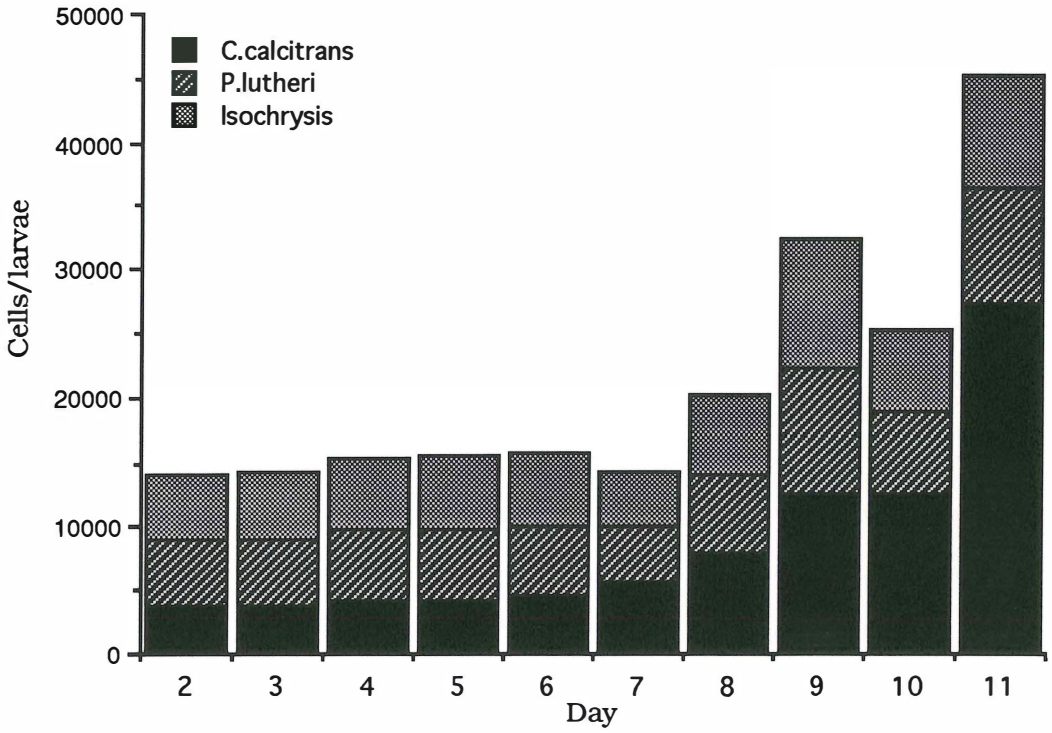

Figure 8. Algal diet for larvae during the culture phase, Oct. 1991 (HB5/91).

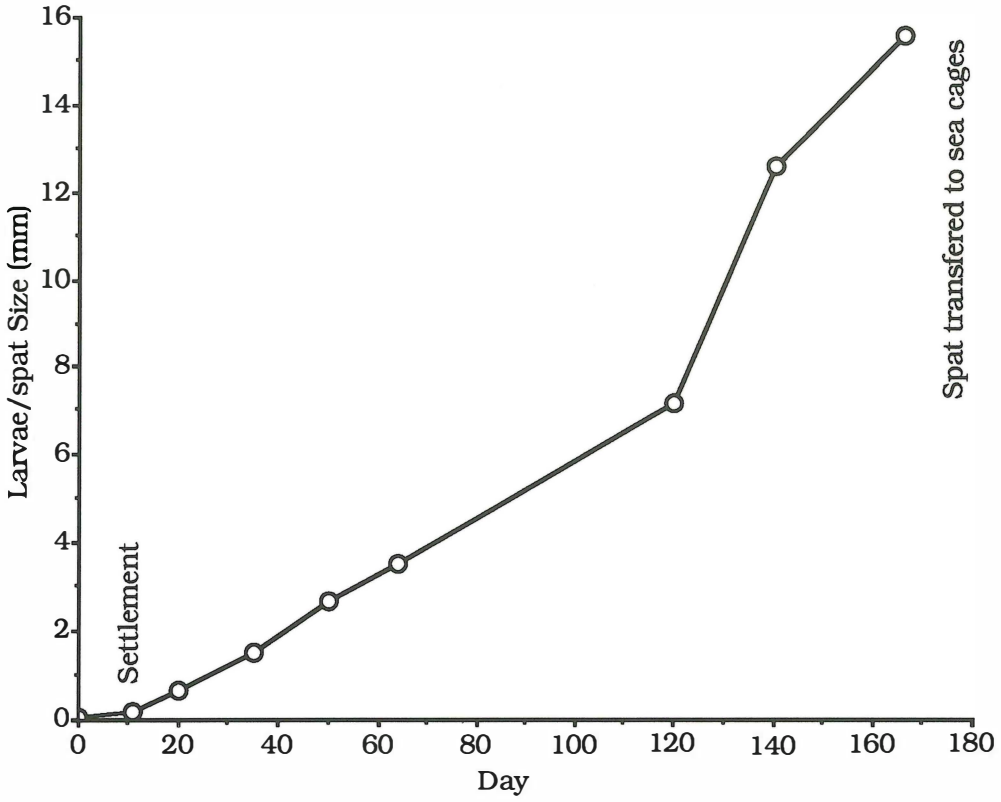

Figure 9. Growth of *A. balloti* larvae and spat, Oct. 1991-March 1992 (HB5/91); shell height.

Assessment of the release of spat onto the seabed.

Given that the release of cultured spat onto the seabed was possible in early 1990, extra funds were sought both from FIRDC and the W.A Government for the operation, including further grow-out to be conducted in liaison with W.A. State Fisheries. At this time the financial status of the State Government was very poor and the natural fishery had experienced a significant recruitment. No funds were provided for extra grow-out trials hence this aspect could not be pursued to the desired extent.

However, research on nursery grow-out techniques has provided considerable insight into any future reseeding of this species. Subsequently the following recommendations are put forward.

1. That designated release areas be protected from trawling;
2. That longline culture of spat settled in mesh collectors appears to be viable provided that collectors are deployed only after spat become larger than the collector mesh size;

3. That spat be released from collectors onto the seabed at greater than 10 mm in size;
4. That an intermediate nursery stage where spat are cultured in cages on longlines prior to release appears to be not economically or biologically viable on a large scale;
5. That examination of bottom sediments and water quality occur before a release site is selected - high levels of fine silt is to be avoided as is shallow water exhibiting an extreme temperature range, ie. less than 18°C or greater than 25°C or salinity greater than 37 ppth.
6. That culture and release of spat not be conducted at present as it would not be economically viable, given the buoyant state of fishery production and the low market value of scallops.

Health of larvae, juveniles and adults

As part of the animal health study for this project, various samples of healthy and moribund larvae were collected from a number of batches of larvae and preserved in 2.5% glutaraldehyde fixative. In addition, some live samples were taken and examined. Preserved larvae were double-embedded (via agar into paraffin wax or Epon resin), sectioned and stained (at the Animal Health Laboratories, W. A.), then photographed and examined. Resin sections were prepared for electron microscopy by lead citrate and uranyl acetate staining.

Subsequently, three penultimate causes of larval death were pinpointed:

- Digestive gland degeneration suggestive of toxin ingestion or exposure.
- Filamentous bacterial infections secondary to other insults including the above.
- Systemic vibriosis as an isolated event.

The virus-like agents visualized in the electron microscope could not be associated with disease processes but required further monitoring,

as similar viruses cause velar disease in oysters. No further evidence for the presence of the virus-like agents was found.

The one outbreak of vibriosis (bacillary necrosis) reflected an isolated incident of reduced hatchery hygiene at one point in the rearing process. This could be traced back to a batch of algal feed that was found (after it was used) to be contaminated. Clearly, only high quality algae, devoid of *Vibrio sp.* and preferably devoid of bacteria, should be fed to larval *A. balloti.*

Studies during 1990 identified the need for continued observation of digestive gland and velar changes as there was damage suggestive of toxin exposure. In a further attempt to analyse the digestive system and its possible breakdown, larval samples were collected from one 1991 batch (day 12) every 4 hours over a 24 hour period. From 1200 hours until 2400, the amount of pigment (feed) in the digestive gland increased, then it decreased. (Daily provision of algae to the larvae occurred at approximately 1100 hours). Over the same time the degree of vacuolation also increased, and continued to do so up to 1200 hours on the second day. The cell architecture became less distinct over the last 12 hours. Thus at the end of the 24 hour cycle, the gland histology differed from that at the start, with more vacuolation and some cell degeneration. These changes may have related more to the feeding cycle rather than a diurnal cycle. The degree of change was neither pathological nor as great as occurs in other tidal shellfish species (Langdon, pers. comm., 1991).

At this stage of development the larvae were becoming pediveligers and morphological changes could be expected. However, if the changes noted above were due to the feeding cycle then it is possible that a once daily feeding is not the best strategy for healthy larval development; hence a twice daily feeding regime (possibly at a higher cell density) may eliminate any degeneration of the digestive gland that occurs due to a lack of available food cells. Larvae in this trial were from a batch of 4.6 million being cultured at 21.5-22.5 °C in a 4,000 L tank, ie. 1.15 larvae/mL. The food (algal) cell density was 15,000 cells/mL which meant that the ratio of algae cells per larvae was 13,043. Further research is necessary to clarify the situation regarding feeding regimes and digestive gland degeneration.

Significant storage of lipids by late stage larvae, was observed, and considered to be sufficient for metamorphosis to occur. Spat settlement results have confirmed this expectation. No diseases or significant bacterial infections were observed in adults used as broodstock or in spat cultured through the hatchery. Broodstock were found to be infected with a parasitic nematode, but during this study no detrimental effects on the broodstock, larvae or subsequent spat were detected. Also, no visible incidence of the parasite was noted in larval or juvenile scallops, nor was any reproduction or dispersal of the parasite evidenced.

During the study detailed here, larval samples were prepared and provided to James Cook University (JCU) of North Queensland for an Honours project. Samples of fed and starved larvae were analysed by JCU for carbohydrate, protein and lipid content. The biochemical level and form of energy storage was identified in the larvae and this was related to its energy demands. Lipids were identified as the most abundant biochemical fraction in early *A. balloti* larvae; more than 60% of the total organic matter. Protein and carbohydrate accounted for about 20% and 15% respectively of total organic matter. Larval lipid reserves were clearly a form of stored energy. Levels of lipid decreased rapidly from day 6 to 10 of larval life then less rapidly to day 14 before increasing from day 14-16. However protein levels increased through to day 14 (to about 50%) before decreasing sharply to 32% of total organic matter at day 16. It thus appears that the larvae was drawing on stored lipids during major stages of development and then accumulating it prior to the non-feeding metamorphosis period. Proteins appear to be heavily used during the late pediveliger stages prior to metamorphosis (Connolly, 1990). In these JCU trials, metamorphosis did not occur before day 16.

In conclusion, the successful production of numerous batches of settled spat is indicative of good husbandry techniques; however the presence of some larvae exhibiting digestive gland breakdown should be examined further if and when further work is conducted on this species. Even with the successful larval work detailed above, survival rates could still be improved upon considerably. In a commercial situation this would reduce the costs of production.

Discussion and Conclusions

Major determining factors in the improvement of culture statistics for *A. balloti* were:

(i) changes made to the broodstock conditioning technique;
(ii) changes to the water filtration system;
(iii) variations made in the larval culture conditions;
(iv) changes to post-settlement conditions.

The broodstock were maintained in the holding pools at ambient temperature in 1989, hence the water temperature suffered considerable day-night fluctuations. The main diet species in 1989 was initially *T. suecica* as this species is relatively easy to grow in large volumes outdoors and it is of some nutritional value. However, larval results from 1989 tended to indicate that this algal species was not a satisfactory diet to ensure adequate gonad development. In 1990, the broodstock diet was centred on *C. gracilis* which has a higher nutritional value than *T. suecica* (Brown et al., 1989). This diet was supplemented with high quality *C. calcitrans*, *P. lutheri* and Tahitian *I. (aff.) galbana* when possible. A more careful control of food cell density was maintained. Also, more stringent temperature control was ensured by the use of immersion heaters and insulation mats over the pools at night.

No selection of broodstock for high growth rates was possible as they were naturally occurring animals of unknown age (within each year class). Only two year classes (1 and 2 year olds) were generally available for broodstock, but the larger and healthier looking animals were usually selected for each spawning; provided the gonads were ripe. Thus, whilst no conscious attempt at specific selection for broodstock exhibiting increased growth rates was intended, it appears that this has occurred to some extent.

In the USA, Heffernan et al. (1991 and 1992) recently found (for *Mercenaria mercenaria* and *Argopecten irradians* respectively) that there was a negative larval response to selection of adult broodstock for increased growth rates. These findings also place some doubt on the benefit of culling small larvae during hatchery culture, as occurred to some extent during this program on *A. balloti.* The anomaly had previously been raised by Heffernan et al., 1991 who presented results concurring with earlier work by Newkirk and

Haley, (1982) on *Ostrea edulis*. At the time of writing, no comparative papers on *Amusium sp.* appeared to exist nor could any similar work on other Australian bivalves be located.

In the present study, 'fecundity' counts were based upon eggs that were assessed as satisfactory for fertilization and thus counted; unsatisfactory egg batches were discarded and not counted. Criteria for the selection of satisfactory eggs increased from 1989-1991. A smaller percentage of spawned eggs from each female were actually fertilized even though an improved gonad conditioning technique meant that eggs were more developed than in 1989.

The larval, spat and broodstock diets were relatively similar in that they all composed the species *C. calcitrans*, *P. lutheri* and Tahitian *I. (aff.) galbana*. These species were combined with *C. gracilis* for the spat diet and with *C. gracilis* and *T. suecica* for the broodstock diet. Based upon studies by Whyte (1987) and Brown (1990, 1991) the nutritional value of such a diet, for each of the life stages, could be regarded as high even though changes in the relative composition of each were made. Brown (1991) noted the importance of a mixed diet where some species appear to have high nutritional qualities and others have low. The nutritional value of various algal species is difficult to define with respect to scallop larvae. The assessment, by Whyte (1987) of available energy, calculated from lipid, protein and carbohydrate constituents, allowed him to rank six species of algae commonly used in mariculture, according to their energy levels. Of relevance to the present study was the fact that Tahitian *I. (aff.) galbana* was ranked number one (highest) and *C. calcitrans* was ranked number three.

Commonly assessed phases of the larval rearing stage have been shown as percentages which clearly indicate that culture in 1989 was markedly less successful than culture of larvae in 1990 and especially 1991. An average survival figure of 14.8% from eggs to D-shaped larvae and 64.4% from D-shaped larvae to pediveligers compares very favourably with data from Canadian research (Thompson, et al., 1985) on the Japanese scallop *Patinopecten yessoensis*. Larval rearing of this species produced survival rates for corresponding phases of 10% and 10%.

In Australia, Rose et al. (1988) recorded a growth of 5.2 μm/day for *A. balloti* larvae from the first D stage to the umbonal veliger, then 6.3 μm/day until the pediveliger stage. Larvae in the study documented here attained an overall average (for 1991) of 7.3 μm/day for the period from the first D-shaped larvae (day 2) to pediveliger. The batch spawned on 24 July 1991 gave an overall growth rate of 8.7 μm/day for the same phase. Larval rearing trials with *Amusium pleuronectes* in the Philippines failed to perfect culture techniques and obtained low survival percentages in all the life stages (del Norte, 1991).

Rearing trials with *A. balloti* in Queensland (JCU) Australia by Connolly (1990), suggested that larvae could not be cultured outside certain latitudes due to excessively high water temperatures (> 25°C) experienced in the warmer months (from October onwards). However, the recent work in Western Australia has shown that larvae can be cultured through to settlement in at least all months from May to December inclusive. Ambient water temperatures in the area exceeded 25°C during the period but this was controlled in larvae tanks by using the refrigerated convection cooling system. The lack of successful batches in the warm period from January-April was determined by a lack of available broodstock; the trawl fishery was closed during that period. Even so, the research indicated, that with the use of broodstock conditioning, it should be possible to successfully culture *A. balloti* larvae through to settlement irrespective of the ambient water temperature. However the cost of maintaining broodstock and producing the larvae by artificially controlling larval water temperature may be prohibitively high.

The optimum larval culture temperature was 22.18 ± 0.94 °C and settlement was achieved 12 days after spawning at a size of 212 μm. High survival rates were produced by this batch which was cultured in July 1991; which is mid-winter with ambient water temperatures of about 17-18°C.

This three year study on *A balloti* has demonstrated a potential, for the first time, to produce large numbers of spat of this species in a commercial hatchery situation. Unfortunately, neither the economic nor the fishery status in Australia is conducive, at present, to further develop the culture of this species.

Recommendations for culture of *A. balloti*

1. Broodstock be held in tanks at 19-20°C for at least one week before spawning;
2. Algae of high quality and nutritional value (eg. *Chaetoceros gracilis*) be fed to the broodstock daily after a water exchange;
3. Do not unduly stress broodstock during spawning;
4. Collect the eggs and sperm within 30 minutes of spawning;
5. Ensure a mixed high quality algal diet is supplied to larvae on at least a once daily basis-further research may indicate that twice daily feeds are preferable;
6. Provide a large volume of suitable substrate for initial settlement of spat and ensure good water movement;
7. Do not leave settled spat on the floor of tanks for more than one week unless they are in an an area of good water movement and low silt/detritus levels;
8. Culture of post-settlement spat is preferable in mid-water, this can be achieved at sea or on land once spat are greater than 2 mm in size-ie. retained within mesh collectors;
9. Longline culture of spat is possible under certain circumstances, growth rates in both mesh collectors and lantern cages were acceptable but lower than for wild spat;
10. Land-based nursery culture is possible but expensive, and not likely to be commercially viable on a very large scale;
11. Release of spat onto the seabed appears to be more feasible than mid-water cage culture through to harvest in Shark Bay.
12. Nursery culture of spat up to at least 10-15 mm in size is recommended prior to reseeding. This could be achieved via a sea-based or land-based nursery system, depending on the site, quantity of spat and funding availablity.
13. Further work on the impact of one daily feed for larvae verses repetitive feeds, including examination on the state of the digestive gland.

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