Marking scallops for release and recapture

T Lucas, P Palmer, S Wang, R Scoones and E O'Brien

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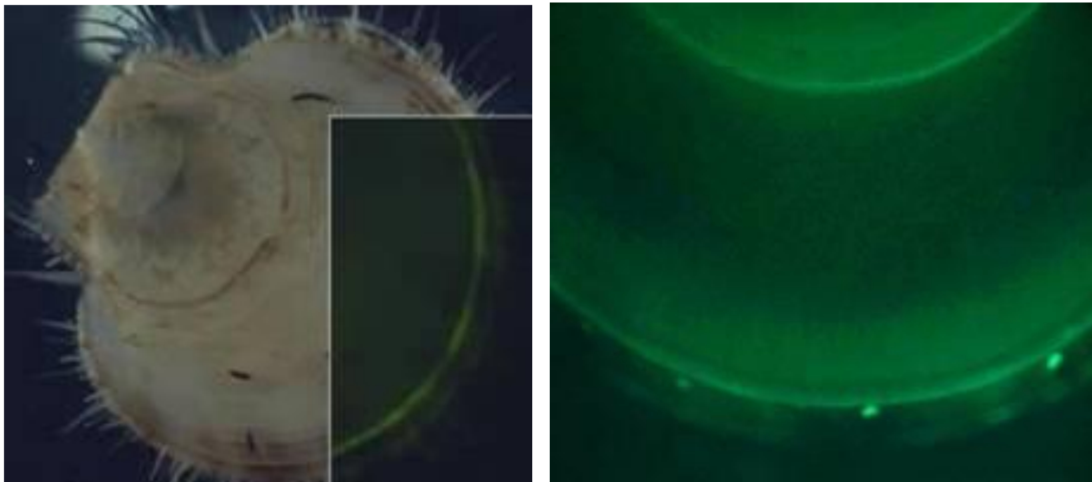

Project number 2005/016

Final Report - July 2008

Queensland Government
Department of Primary Industries and Fisheries

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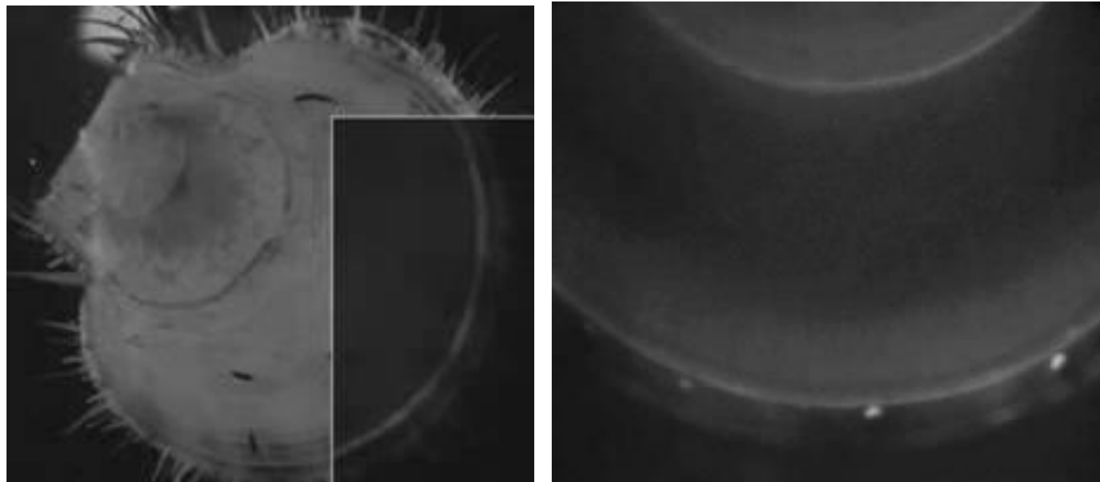

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NON-TECHNICAL SUMMARY

2005/016 Marking scallops for release and recapture

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Objectives

To develop a method to mark hatchery reared saucer scallops to distinguish them from animals derived from wild populations.

Outcomes achieved
Juvenile saucer scallop (*Amusium balloti)* shells have been successfully marked *en masse* using 3 chemicals, namely alizarin red S, calcein and oxytetracycline (OTC). Considering spat survival, mark quality and mark duration collectively, the most successful chemical was OTC. Scallop spat immersed for three days in 200 or 300 mg L^{-1} OTC resulted in good mark incorporation and high survival. Tris was an effective means of buffering pH change during OTC treatment, with no apparent adverse effects to the scallops. The marks from OTC treatment were still visible in live scallops for at least 10 months, even with exposure to natural filtered light during that period. A second discernible shell mark was added 27 days after the first with no evident toxicity to the scallops. A simulated seabed system was designed which provide marked improvements in scallop juvenile survival and growth. Advice on shell marking has been given to QSS by DPI&F, and the first commercial trials have now commenced, with initial results showing successful marking of juvenile scallops at QSS. This research will allow the industry to monitor the survival, growth and movement of specific cohorts of deployed scallops. This will provide valuable feedback to assess the value of the ranching venture, to optimise release strategies, and to develop improved species management plans.

The saucer scallop *Amusium balloti* is a valuable fisheries resource for Queensland, however wild catches are known to fluctuate dramatically between seasons. Methodology has now been developed to stabilise catches by supplementing natural recruitment with hatchery-produced spat at 2-5 mm. This project aimed to help identify the hatchery-produced spat upon recapture so that the success of the re-seeding strategy could be evaluated. Furthermore, identification of hatchery-produced scallops enables feedback, which will help to optimise spat deployment strategies. The perfect marker would incorporate into the shell in a rapid, reliable way, be easily identified upon recapture 8-12 months later, be non-toxic for both the scallops and human consumption, and be approved and affordable for commercial use.

Three chemicals, namely alizarin red S, calcein and oxytetracycline (OTC), were tested in this study for mark quality, mark retention and toxicity to scallops. Marks were identified and visually assessed for brightness using an epifluorescence microscope. Of the three chemicals, OTC was identified as producing a brighter, more persistent mark than calcein, and lower mortality than alizarin red S. Fluorescence incorporated into the scallop shells was still visible after 10 months of culture in controlled conditions. The efficient use of pH buffers was refined for use in OTC marking procedures.

In a separate experiment, scallops were OTC-marked twice, one month apart with negligible mortality, and were subsequently reared in a simulated sea-bed environment with managed sand substrate. This demonstrated that the brightness of the initial marks was comparable to new marks, and that 'bar codes' could be created with multiple shell mark patterns. Multiple marks have potential to be used to distinguish between cohorts or size classes of cultured spat. A healthy sand substrate was found to prevent fouling of new shell growth and resulted in higher survival and growth than in previous experiments.

This study determined that immersing scallops for 3 days in OTC at 200 or 300 mg L^{-1} can produce reliable fluorescent shell marks with negligible mortality. This chemical exposure was generally conducted in low light conditions, with minimal temperature and pH fluctuations, and in the presence of adequate levels of several species of cultured microalgae in the seawater so that scallops continued to feed for maximum chemical uptake. Marks can persist for at least 10 months in living scallops, and this indicates that the methodology will be useful for industrial application. Our commercial partners Queensland Sea Scallop Ltd are currently undertaking preliminary commercial trials.

To further test the utility of this shell marking system, marks were also applied to juvenile pearl oysters *Pinctada imbricata*. Marks were clearly visible in some individuals, however autofluorescence from the oyster shell made categorical detection of marks difficult. Mark brightness ratings were, however, significantly higher (P<0.01) in marked treatments than in unmarked controls.

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1. BACKGROUND

The project was initially proposed in support of the FRDC Project (No. 2002/048) entitled "Enhancement of saucer scallops (*Amusium balloti*) (Bernardi) in Western Australia" as well as the scallop enhancement program being conducted by Queensland Sea Scallops Ltd in Hervey Bay in Southern Queensland. To determine optimal methods and sizes for release the first season of spat deployment in FRDC 2002/048 saw approximately 12 million spat of different sizes (0.5 mm to 4.0 mm) released onto a number of locations on the seabed offshore from Geraldton, WA. The results of high intensity trawling over the reseeding site between eight and 20 months after these deployments revealed higher catches in one location than anywhere in the nearby surrounding waters, but the distribution of the catches relative to those on adjacent seabed areas did not permit researchers to be categorical in asserting that any of them had origins in the hatchery stock released during 2002. A minor but widespread natural recruitment of scallops had contributed to the problem of determining the origins of the scallops recovered. In the absence of a significantly higher localised density of scallops, only a permanent identifying mark was considered likely to have allowed the researchers to be certain as to whether the scallops had wild or hatchery origins.

In Australia, only two companies (one in WA and one in Qld) have been involved with the sea ranching of saucer scallops. Both have highlighted the development of an identifying mark as a research priority and both have been involved in developing and supporting this research.

As outlined by Rothlisberg and Preston (1992), tagging methods used in reseeding programs ideally should 1) be able to mark small individuals, 2) be detectable in all subsequent life history stages (especially the adult), 3) be unique to the local population, 4) be suitable for identification of individuals or cohorts, 5) be inexpensive to apply and detect, 6) be harmless to the tagged animals and subsequent consumer, and 7) be acceptable to the public. A review of scientific literature regarding tagging methods which adhered to these requirements revealed immersion marking as the preferred option, and three likely chemicals for this application, namely, alizarin red S, calcein and oxytetracycline (OTC). Since the commercial objectives of saucer scallop sea ranching require deployment of juveniles at around 4 mm shell length, the chemical tags would likely need to be applied just prior to deployment.

The ability to identify hatchery reared scallops was therefore considered worthy of development and potentially valuable in determining the overall success of saucer scallop reseeding in Australia as well as being a research tool to estimate survival, dispersal and growth rates in reseeded areas and in the wild fishery.

2. NEED

The ability to distinguish hatchery-produced scallops from wild recruits was found necessary to determine the survival and optimal size, site and time of deployment to the seabed in the previous scallop reseeding project FRDC 2002/48. Furthermore, to enable rigorous evaluation of commercial marine reseeding ventures, a means of identifying the released scallops upon recapture is required. Successful labelling of juvenile scallops will also allow researchers to estimate other biologically relevant factors that could be used in species management plans, such as growth rates, dispersal and migration patterns.

An identifying mark provides far greater sensitivity and accuracy than statistical-based assessment. There are a number of other reported methods that could be used to discern hatchery produced animals, but the methods we have chosen are relatively inexpensive and easy to apply to animals no greater than 4 mm shell length (commercial release size). An alternative marking method, molecular analysis of scallop tissue, was evaluated with qualified opinions sought, but was rejected on the basis of high expense and processing time compared with testing cheaper alternatives with demonstrated utility in other species.

3. OBJECTIVES

To develop a method to mark hatchery reared saucer scallops to distinguish them from animals derived from wild populations.

Detailed Objectives

The project Milestones guided activities towards the overall objective listed above. These were as follows:

1. By June 2006 : Successful testing of a range of chemical labelling methods on juvenile scallops at Bribie Island Research Station.
2. By June 2007 : Successful testing of retention of labels in scallops at 8 to 12 months in tanks at Bribie Island Research Station.

Revised due date for Milestone 2 following approved project extension.

2. By 20 December 2007 : Successful testing of retention of labels in scallops at 8 to 12 months in tanks at Bribie Island Research Station.

4. METHODS

4.1 Developing a shell marking system for hatchery reared saucer scallops

This scallop shell marking research was carried out with a logical sequence of short- and longer-term experiments which addressed chemical immersion methods and the rearing environment necessary for mark evaluation. This included three preliminary chemical exposure experiments (Experiments 1, 2 and 3). These experiments identified several inadequacies in the marking and rearing systems that were initially applied. Progressive testing and improvements in the rearing environment are documented in Section 4.2. Two more detailed mark retention trials (Experiments 4 and 6) applied a range of improved methodologies, and several short-term trials with focus on the use of pH buffers for immersion solutions are collectively described as Experiment 5.

All scallops used for this project were obtained from Queensland Sea Scallops (QSS Ltd). Small spat (approx 4 mm diameter) were sourced from their hatchery at Burnett Heads, and scallop "buttons" larger than 10 mm shell height (~ diameter) were sourced from the wild via their hatchery holding facilities. Experiments were conducted at the Bribie Island Aquaculture Research Centre (BIARC), Queensland, Australia, following acclimation of transferred animals in various seawater systems. Scallop shells were marked by immersing live scallops in seawater solutions containing the various chemicals which were incorporated into new shell growth over a number of days.

Similar supplemental research directed at marking the shells of Akoya pearl oyster (*Pinctada imbricata*) shell was conducted to further test marking methods and rearing facilities for an alternative bivalve species. The long-term mark retention and survival results from the scallop marking experiments are presented separately in Sections 4.4 and 5.5.

All mark detection was done with a Nikon Microphot FXA epifluorescence microscope using a super-high pressure mercury lamp with three different light wavelength filters. The type of light required to view marked shells was dependant on the marking chemical used. For OTC, ultra-violet (UV) was achieved with a DM455 filter (Nikon), for alizarin red S, green light was produced with a XF102-2 filter (Omega Optical), and for calcein, a DM510 filter (Nikon) produced blue light.

Safety aspects for each chemical were also taken into account. Calcein is not dangerous under normal operating conditions and is rated at level one for all hazard categories by Chemwatch (2007). Alizarin is a suspected carcinogen, and is rated a level two hazard under the categories of toxicity, body contact and chronic. OTC is a broad spectrum antibiotic with level two hazard ratings for toxicity and chronic categories in Chemwatch. To avoid harm from any of these substances, thick PCV gloves, eye protection, a lab coat and a dust mask were used during handling. Careful handling of the powdered forms of these substances was particularly important to avoid inhalation. The diluted chemicals were treated after use by slowly dripping the solution through an active carbon filter before disposal.

4.1.1 Experiment 1 - Using OTC, calcein and alizarin red S to mark large and small juvenile scallops

This first experiment sought to mark a small number of scallops and assess survival using chemicals and concentrations that had previously been successful in other molluscan species (Hidu and Hanks, 1968; Newell and Hidu, 1982; Jackson, 1990; Pirker and Schiel, 1993; Day *et al.*, 1995; Kaehler and McQuaid, 1999; Moran, 2000; Eads and Layzer, 2002).

Three treatments each containing 5 large (~15 mm) and 10 small (~2 mm) animals were used, namely:

1. OTC 500 mg L^{-1}
2. Alizarin red S 20 mg L^{-1}
3. Calcein 50 mg L^{-1}

The animals were placed freely in plastic tubs with 16 L of filtered (20 µm) seawater containing marking chemicals and cultured microalgae. The microalgae consisted of an equal blend of three microalgae species, being *Isochrysis galbana*, *Pavlova lutheri* and *Chaetoceros muelleri,* at a final concentration of 30,000 cells mL^{-1}. The solutions were gently aerated on a continuous basis and replaced after 24 h. The total exposure period was 72 h. After treatment, the scallops were placed into clean aerated tubs of seawater with a similar concentration of microalgae. After three days survival was assessed by inspection under the microscope. Scallops were then maintained in aerated tanks with a continuous (3 × tank volume per day) exchange of filtered seawater and a continuous similarly metered supply of these microalgae until the surviving scallops were used for Experiment 3, 20 days later.

4.1.2 Experiment 2 - Testing two concentrations of OTC, calcein and alizarin red S using 5 mm spat

The aim of Experiment 2 was to further test the three previously identified chemicals on scallop spat. Two concentrations were used for each chemical (Table 1), and 50 pat were used in duplicate for each treatment. The average (± se) size of scallop spat at the beginning of the experiment was 5 ± 1.3 mm. During chemical exposure, these were housed in screen chambers made from 250 mm PVC pipe with 500 µm mesh bottoms. These were partially submerged in the chemical dilutions, inside rectangular plastic trays (527 mm long x 381 mm wide x 140 mm deep). The total volume of water used in each treatment was 16 L, and the volume inside the screen was 1.8 L. One air stone was placed inside each screen chamber to provide gentle circulation and aeration (Figure 1).

Table 1. The chemicals and concentrations used to mark *A. balloti* spat over 48 hours in Experiment 2

Chemicals	Concentrations
OTC	500 & 750 mg L^{-1}
Calcein	25 & 50 mg L^{-1}
Alizarin red S	10 & 20 mg L^{-1}
Control	0

Figure 1. Chemical immersion setup for Experiment 2

Filtered (20 µm) seawater (27-29ºC, 34-35 ppt) was used for all treatments and the control. The pH of all treatments was adjusted to 7–8 immediately prior to the addition of spat using $NaHCO_3$ (bicarb), whereas the control was left unadjusted at pH 8.0-8.2. Spat were treated for 48 h, with water and chemicals renewed after 24 h. This renewal was undertaken by removing the screen and placing in freshly premixed chemical baths. Three species of microalgae were added to each experimental unit at a feeding rate of 10,000 cells mL^{-1} for each species (as in Experiment 1).

After chemical exposures, spat in screen chambers were rinsed and placed into a 500 L tank with filtered seawater and a similar concentration of microalgae. Water was renewed daily by flushing seawater through the tank for 90 min at 500 L h^{-1}. Fresh microalgae was supplied after each water exchange. The screen chambers were operated as down-wellers by attaching airlifts to each of the screen chambers. Airlifts consisted of 4 mm air tube delivering air (6 psi) to 30 mm air stones inside the base of vertical 19 mm pipe with top elbow to direct water flow. The scallop spat were given 7 days to stabilize prior to the first shell mark and mortality assessments.

4.1.3 Experiment 3 - Double-cross marking with OTC and calcein

Twenty days after the first marking trial (Experiment 1), a small number of juveniles that had previously been marked with calcein and OTC were again immersed in these chemicals in our first attempt at double marking. Scallops which had previously been marked with calcein were re-marked with OTC and vice-versa. Since the scallops treated with alizarin red S in the first trial had died, alizarin red S was not included in Experiment 3. The details of the chemicals used in this cross-marking trial are summarised in Table 2.

The scallops were placed into screens in 16 L tubs containing filtered seawater, marking chemicals (calcein or OTC) and microalgae as described for Experiment 2. Gentle aeration was again provided for each treatment inside the screen chambers. The treatment lasted for 48 h with no water exchange. After the treatment, all screen chambers and scallops were moved into a single raceway tank. The screen chambers were operated as downwellers by attaching an airlift to each of the screen chambers as previously described. Unfiltered (raw) seawater was supplied to the scallops without microalgal supplementation. Survival and retention of the chemical marks was assessed after one week.

Table 2. The chemicals and concentrations used for double-cross marking *A. balloti* spat

<table>
<tr><td>Chemicals used in Experiment 1</td><td colspan="2">OTC 500 mg L^{-1}</td><td colspan="2">Calcein 50 mg L^{-1}</td><td colspan="2">Control (no mark)</td></tr>
<tr><td>Size (mm)</td><td>4-6</td><td>14-25</td><td>4-6</td><td>14-25</td><td colspan="2">14-25</td></tr>
<tr><td>Number of animals used</td><td>8</td><td>5</td><td>7</td><td>5</td><td>12</td><td>12</td></tr>
<tr><td>Chemical used in Experiment 3</td><td colspan="2">Calcein
50 mg L^{-1}</td><td colspan="2">OTC
500 mg L^{-1}</td><td>Calcein
50 mg L^{-1}</td><td>OTC
500 mg L^{-1}</td></tr>
</table>

4.1.4 Experiment 4 - Marking spat using OTC and calcein with a range of concentrations and exposure times

This fourth experiment explored a wider range of concentrations and exposure times for OTC and calcein as applied to 2-5 mm scallops. Treatments compared the marking success and toxicity of long exposures at low concentration with shorter exposures at higher concentrations.

Sixty spat were placed inside each of two screens per treatment and control (Table 3) and these were immersed in marking solutions for either two or seven days. Scallops in control screens were subjected to all handling stresses and conditions associated with marking for seven days, but without the chemicals.

Table 3. Chemical treatments used in Experiment 4

Abbreviation	Immersion period (days)	Chemical	Concentration (mg L^{-1})
2-Cal-50	2	Calcein	50
7-Cal-10	7	Calcein	10
7-Cal-20	7	Calcein	20
2-OTC-500	2	OTC	500
2-OTC-750	2	OTC	750
7-OTC-250	7	OTC	250
7-OTC-500	7	OTC	500
Control	7	0	0

The chemical immersion protocol was similar to that used in Experiment 2, using mesh screens that were partially submerged in shell marking solutions and gentle aeration (Figure 1). Seawater, microalgae and marking chemicals were mixed and replenished daily, and adjusted to a pH between 7 and 8 using bicarb. After treatment, scallops were placed in clean screens in 264 L raceway tanks. Microalgae was continuously drip-fed from bag cultures and mixed with 1 μm filtered seawater in a header tank to a final concentration of 30,000 cells ml^{-1}. This was then

supplied constantly to the scallops at 0.5 L min^{-1}, which was equal to 2.6 times the volume of the tank per day.

After one week, mortality and mark brightness were evaluated. All mark assessments were undertaken using a 'blind' selection and observation procedure so that expectations associated with treatment labels did not bias the results. Mark brightness was rated between zero and five according to Day *et al.* (1995) as shown in Table 4. Unlike the assessment used by Day *et al.* (1995), we did not score mark length because it was not informative in scallops (ie. it was either 0 or 5). These scallops were then grown at BIARC for 10 months to determine mark longevity. The details regarding the long-term maintenance and monitoring of these animals is presented in section 4.2.

Table 4. Mark brightness scale for quantifying observed fluorescence from Day *et al.* (1995)

Mark brightness rating	Description
0	Not detectable
1	Just detectable
2	Dull
3	Bright
4	Intense, thin
5	Intense, thick

4.1.5 Experiment 5 - Testing Tris and bicarb to stabilise pH during OTC exposure

The aim of these trials was to optimise pH conditions for scallop marking, under the assumption that minimal pH change away from the basline level of seawater (8.0-8.2) during chemical exposure would help maintain normal scallop feeding activities. This would result in maximum shell growth and chemical uptake, and minimise stresses which could lead to mortalities.

The preliminary experiments in the project used sodium bicarbonate (bicarb) to buffer against the acidic effect of OTC in seawater. This had been added to the chemical baths without specific quantification whist monitoring and attempting to maintain the pH at levels ranging from 7 to 8. However, this caused heavy precipitation and yellow froth from airstones in the water baths, and caused concerns regarding the possible low availability of OTC in solution. Additionally, the variable high mortalities previously noted may have been attributable to gill fouling by the precipitation of chemicals during lengthy exposure periods. The arduous task of measuring and manually adjusting the pH in multiple water baths also caused significant uncertainty with regards to pH levels across experimental designs and over time. It was also considered that the resultant pH fluctuations in themselves may have placed unnecessary stresses on the fragile juvenile scallops during chemical exposure.

To address these concerns, a series of short-term trials were designed to:

a) Measure the concentration of bicarb required to more effectively adjust the pH of OTC solutions at the planned concentrations of 100, 200 and 300 mg L^{-1}. Observe and qualitatively assess precipitation at all concentrations.
b) Test Tris as an alternative pH buffer, at the concentration recommended from previous research in fish otolith marking of 0.6 g per 1 g OTC (Dan Willett, QDPI&F pers. comm.). Compare precipitation observations with those for bicarb.
c) Describe the typical changes in pH over time, as each solution is used for 24 h exposure periods.

All trials were conducted indoors at a controlled temperature of 23.5°C. The first trial involved OTC dissolved in three beakers of 500 mL seawater at concentrations of 100, 200 and 300 mg L^{-1}. Each solution was then divided into two portions of 250 mL for use with either Tris and bicarb as the buffering agent. The pH was measured at the start of the trial and regularly after that using a hand held digital pH meter (pHScan BNC, Eutech Intruments) which had been calibrated prior to the beginning of the experiment. Bicarb was added to each of the three bicarb treatments in small increments initially, stirred vigorously and the beakers were visually inspected to ensure dissolution of powder before recording the pH. Increasing quantities of bicarb were added to the solution until the pH failed to increase substantially. In each of the three Tris treatments, Tris buffer was only added once, at the recommended dose of 0.6 g per g OTC. The pH was measured after initial addition of chemicals, and then all six solutions were left overnight with vigorous fine bubble aeration (30 mm airstone). These solutions were visually assessed for precipitation and the pH was tested after 16 h and 24 h.

The second trial built on the results of the first by further investigating the effect of time on the pH of the highest concentration (300 mg L^{-1}) of OTC planned for use in shell marking experiments. Treatments comprised unbuffered solutions and solutions buffered with the previously determined optima of either 1.2 g L^{-1} bicarb or 180 mg L^{-1} Tris. Plain seawater was used as a control. Solutions were vigorously aerated and pH was recorded at 0 h, 17 h and 24 h.

A third trial within this series directed at buffers aimed at determining an amount of bicarb able to provide pH 8 in 300 mg L^{-1} OTC solution with minimal fluctuation of pH through time. This was necessary because we had thus far failed to achieve the desired pH of 8 without the effect of time. Concentrations of 1.2, 2.4, 3.6, 4.8, 6.0, 9.6, 13.2, 17.2, 21.2 and 41.2 g L^{-1} bicarb were tested.

4.1.6 Experiment 6 - Double-marking spat using OTC at three concentrations and buffered with Tris or bicarb

This sixth and final shell marking experiment tested the survival and mark quality of scallop spat immersed in various OTC solutions. This chemical had proven the most reliable in terms of marking success. It was also preferred due to survival improvements thought possible by replacing bicarb with more stable Tris-buffered solutions, and because it (unlike the other chemicals) was approved for use by the Australian Pesticides and Veterinary Medicines Authority (APVMA).

In selecting the levels of OTC to be tested, consideration was given to using lower concentrations than had produced the unacceptably high mortalities in previous trials

(eg: less than 500 mg L^{-1}), and concentrations close to 250 mg L^{-1} which had produced clearly visible shell marks in Experiment 4. The levels of bicarb and Tris used were the previously determined optima derived in Experiment 5.

All chemical exposures were performed over three days, because the seven day exposure period tested in Experiment 4 also appeared to cause excessive mortalities, either from the chemicals themselves of from the handling and manipulations that were inherently necessary. Excessive exposure periods were also considered somewhat cumbersome and impractical given that solutions would likely need to be renewed each day. A summary of the treatments that were applied is given in Table 5. Each treatment and the control were replicated four times, with two replicates housed in each of two separate raceways that were managed in similar ways. A detailed description of the improved rearing conditions used in this final experiment is given in Section 4.2

Table 5. Summary of spat marking treatments for Experiment 6. Each treatment was replicated four times

Treatment name	OTC conc. (mg L^{-1})	Buffer
100 Bicarb	100	Bicarb
100 Tris	100	Tris
200 Bicarb	200	Bicarb
200 Tris	200	Tris
300 Bicarb	300	Bicarb
300 Tris	300	Tris
Control	0	0

Spat were obtained from QSS Ltd one week prior to commencement of the trial. During that acclimation week they were maintained in 20 µm filtered seawater with equal proportions of five species of microalgae, namely *Isochrysis galbana*, *Chaetoceros muelleri*, *Pavlova salina*, *Pavlova lutheri* and *Proteomonas sulcata* at a final concentration of 30,000 cells mL^{-1}. The water was constantly flowing at 750 mL min^{-1} and heated to a minimum of 21°C using four 300 W aquarium heaters per tank.

The room used for chemical treatments was temperature controlled to 23°C to reduce temperature stress. 200 L of seawater used for chemical immersion was acclimatised in the room for 24 h before five species of microalgae were added in equal proportions to a final concentration of 30,000 cells mL^{-1}. This was fully mixed and then divided into three containers, each holding 60 L. OTC was mixed at three concentrations before each OTC solution was divided into two lots of 30 L for addition of bicarb (2 × OTC mass) or Tris (0.6 × OTC mass). Finally, each of the six solutions was well mixed and then distributed into four 6 L portions in 15 L buckets. The buckets were used for holding small mesh screens made from 100 mm PVC pipe with 2 mm plastic mesh 20 mm from the bottom. Each screen and bucket housed 40 scallop spat during immersion. The screens were also fitted with 13 mm airlifts for circulation and aeration. To avoid excessive frothing, open-ended airlines were used in preference to airstones. The pH and temperatures were measured inside the six tubs prior to distribution into buckets, and again after 24 h in each bucket/ replicate. Fresh dilutions of marking chemicals were prepared each day in this way, and screens holding the scallops were transferred directly into new bucket preparations at 24 h intervals. The process was repeated three times providing a 72 h total exposure time.

To best gauge the impact of the overall marking process, controls were not subjected to any of the handling stresses associated with chemical immersions, and were instead left in raceways and maintained without disturbance as described above for the acclimation week. A completely randomised design was applied to the distribution of scallops among screens, and to the arrangement of treatments in the immersion marking room.

Scallops were allowed to recover for one week in the raceways with flowing, microalgae-supplemented seawater, before mark assessment was performed using procedures described in Section 4.1.4. To apply a second shell mark this marking procedure was repeated 27 days after the first chemical immersion began. Scallops were subjected to the same treatments as they had initially undergone. The mark retention and survival of these double marked scallops were monitored over 64 days (detailed in Section 4.2). The effects of OTC concentration and buffer type on mark brightness, shell growth and survival were tested using ANOVA in the program GenStat (ed. 9, VSN International Ltd.).

4.2 Improving survival and growth of juvenile scallops in a land-based system

Presently, the commercial use for hatchery-reared scallops is focussed on a strategy which releases spat onto seabed leases when they reach 2-4 mm in shell height (around two months post-hatch). In this project, we have been required to grow scallops for a minimum of eight months to test mark longevity. Rearing methods were therefore developed and improved throughout the project to enable this evaluation of fluorescence longevity in marked shells, but the systems so developed may also be useful in future efforts where scallops may need to be reared for longer periods in land-based systems.

In an attempt to meet budgetary constraints that were placed on the project, initial methods used unfiltered seawater, prawn pond effluent, or a combination of these two water sources to rear the scallops. However, these methods were unsuccessful, and therefore more controlled though labour-intensive and costly systems were developed for Experiments 4 and 6. The aim was to facilitate higher long-term survival, as well as faster growth which more closely reflected conditions in the wild.

In all grow-out experiments, screens associated with treatments and controls were randomly arranged within the purpose built indoor raceway tanks (1840 mm long x 660 mm wide x 23 mm deep). These raceways were arranged under opaque roofing panels so as to provide natural light and daylength, and one layer of 90% shade cloth stretched over each raceway reduced the light inside to low to moderate levels (approx 270 lux at midday measured with a photometric sensor (LI-COR Inc., Nebraska, U.S.A.)). Given that very low light levels are expected to prevail in the scallop's natural habitat at 20 m depth, the low to moderate levels were considered a reasonable approximation that the scallops would become accustomed to, and which would also sufficiently challenge any light sensitivities of shell marks.

In Experiment 4, the microalgae feeding system consisted of drip feeding from outdoor bag-cultures. At least two species were used in this work, namely *Isochrysis galbana* and *Chaetoceros muelleri.* Where outdoor temperatures and light allowed, a third species, *Pavlova lutheri,* was added to this mix. These microalgae were continuously fed into a header tank where they were mixed in approximately equal proportions with fresh flowing seawater to a final concentration of 30,000 cells mL^{-1}.

To reduce silt loads, as well as pathogen introduction, seawater was filtered through a 20 µm sand filter, then through 5 µm and 1 µm in-line cartridge filters. Water quality was monitored regularly throughout the growth of these scallops. Every weekday at around mid-day, pH, salinity and dissolved oxygen levels were recorded using a YSI 556 Multi Probe System (YSI Incorporated). Diurnal temperature range was also recorded using max/min thermometers. Three times per week, algal concentration was measured using a haemocytometer. Drip rate and water flow were subsequently measured and adjusted according to cell densities to maintain constant supply rates. Scallops were transferred to clean mesh screens in a clean tank every week. Used tanks and screens were scrubbed with fresh water, washed with a chlorine solution (100 ppm) and air dried for one week before being used again. Airlifts, algal supply lines and cartridge filters were also replaced and cleaned weekly.

Survival, mark brightness and shell height were evaluated at 7, 37, 82, 127, 180, 250 and 302 days after marking. Shell height measurements were initially performed using a stage micrometer, and when the scallops were larger than 6.5 mm, a background ruler was used. Mark assessments were performed as described in Section 4.1.4. Despite the specified tank hygiene practices and more labour intensive approach using cultured microalgae, the majority of scallops in Experiment 4 had died by 210 days post immersion mark, At that stage, surviving scallops were moved into a new tank system, where they were fed water and microalgae from other experiments using the modified main system described below.

A second round of rearing system improvements was then made to further improve survival and growth for Experiment 6. These system improvements were directed at managing risks which had been identified, improving the environment for healthier scallops, and changing several operational practices that were cumbersome and impractical. It was at this stage that a sand substrate was re-implemented to provide a more realistic simulation of the natural environment on the sea floor. The potential abrasion of shell material by sand had previously been suggested as a complication for the development of shell marking technologies. Its use had previously been removed from the rearing protocol due to long-term hygiene concerns, so this was addressed by weekly rotations of screens between sand beds that were flushed with freshwater and dried on a weekly basis. This prevented the buildup of significant sulphides in the beds, as well as significant populations of predatory or problematic organisms such as copepods and stalked ciliates.

Several other factors were addressed to improve the artificial rearing environment. These were:
1) <u>Temperature fluctuation</u>. The temperature of seawater in the raceway tanks was particularly susceptible to fluctuations in air temperature, which caused diurnal fluctuations of up to 5°C, much greater than could be expected on the sea floor at 20 m depth. To address this issue, four 300 W quartz glass submersible heaters (AquaWorld) were added to each tank. Furthermore, air-lifts that were previously introducing large amounts of cold-night air into the raceway system were replaced by a 100 W pump (J & T Industry co. Ltd.) which circulated water around the tanks and into each screen individually. This allowed for much more effective insulation within the tanks. It also reduced the level of turbulence in the screens caused by the pulsing action of airlifts, which may have further reduced stressors acting on the scallops.

2) Number of microalgae species. The number of species was increased from two to five, to include *Isochrysis galbana*, *Chaetoceros muelleri*, *Pavlova salina*, *Pavlova lutheri* and *Proteomonas sulcata*. This selection was based on the most amenable species to culture conditions that were available at the BIARC facilities, and was aimed at providing a more balanced nutritional profile.

3) Reliability of algal culture. Outdoor bag cultures were susceptible to daily light and temperature fluctuations, which resulted in unacceptably variable quality and quantity. To alleviate this problem, microalgae were instead produced at high concentration in the indoor microalgal lab at BIARC. These were equally combined and diluted in seawater to provide a final feeding concentration of 30,000 cells mL^{-1}. Fresh batches of algal dilutions were made 3 times per week and this was continuously pumped into each of the scallop rearing tanks at 1.5 L min^{-1}.

4) Variable water quality. The 'batch' method of microalgae production and seawater mixing allowed us to incorporate much greater control over water qualitiy. Four tanks were used in rotation, each holding 5,000 L of seawater. A >24 hr period of settlement allowed for the heaviest of silt particles in the 20 µm filtered seawater to fall out of suspension and also allowed for the seawater quality to be inspected before being mixed with concentrated microalgae stocks and pumped through the scallop raceways. A back-up tank holding 40,000 L of settled seawater was available for use during extended periods of poor intake water quality.

5) Screen Design

A new screen design was implemented to facilitate holding the scallops in sand, allowing for 2 cm head pressure inside the screens to encourage water to slowly pass through the sand base (Figure 2). This prevented anoxic conditions from developing in the sand beds within the screen enclosures. Prior to gently washing scallops into clean screens as part of the weekly schedule of rotations, each new screen was pushed into the freshly prepared sand bed so that the bottom mesh became completely covered with sand. This screen design also conveniently allowed scallops to be collectively removed from the sand by gently lifting screens out of the sand and washing scallops to one side so that they were ready for transfer.

Figure 2. Mesh screens used in Experiment 6. Showing water supply hole (W) for 4 mm tubing, internal water height (I), external water height (E), sand level (S) and the level of the 2 mm mesh (M), which ran parallel to the floor of the tank to contain the scallops within the screen

In Experiment 6, scallops were grown without sand for the first week after marking for consistency with previous work, and then sand was added to the system. They were moved into a clean tank with clean screens weekly as in Experiment 4. Sand beds were cleaned by blasting with fresh water and mixing by hand to encourage the less dense material to rise to the surface, and then tanks were tilted and lightly sprayed with fresh water to wash off remaining debris. The tanks and sand were allowed to dry for 6 d before being refilled with seawater and microalgae 24 h before use to allow temperature to stabilise.

Improvements were also made to the methods applied to measure shell heights. These were designed to improve labour efficiencies and reduce handling stresses that were likely contributing to progressive mortalities. Shell heights were recorded using photographs of the scallops whilst they were in screens, using a fragment of a ruler to calibrate measurements (Figure 3). The images were printed and digital callipers were used to measure shell height, before converting the measurements to actual size based on measurements of the ruler fragment. This was conducted before the first chemical immersion, then 10, 27, 37 and 64 days later, after which the experiment was terminated. Mark assessments (described in Section 4.1.4) were performed on days 10, 37 and 64. The later two time points were also used to assess the second mark at 10 days and 37 days post chemical immersion. Survival was assessed with the assistance of microscopes one day prior to the commencement of the experiment and during mark assessments. Survival data were analysed in GenStat (2007) by a generalized linear model of proportions (McCullagh and Nelder 1989), using the Binomial distribution with the logit link. Growth was

analysed in the same program using ANOVA. After the termination of the experiment at BIARC, scallops were transferred to QSS Ltd for ongoing monitoring of their collective growth, survival and mark retention.

Figure 3. An image used to measure scallop shells on the base of screens

When half of the replicates in the experiment were terminated due to heater malfunction, the second tank was used to house ‘spare’ scallops, which had been retained at high density in a different tank. These scallops were maintained in the same way as the other scallops in Experiment 6. When they were first introduced to the tank, the shells of these ‘spare’ scallops were heavily fouled with stalked ciliates, and we observed the progress of these scallops over one month to examine the effect of the sand substrate on shell fouling.

4.3 Fluorescent shell marking of the pearl oyster *Pinctada imbricata*

The pearl oyster *Pinctada imbricata* was used to test marking methods and rearing facilities on an alternative bivalve. This species produces Akoya pearls and is currently being cultured commercially in Queensland on a small scale with potential for expanding into a major growth industry. Shell marking could be relevant in this species for identification of hatchery-reared stock for a variety of purposes including the tracking of animals through markets, identifying specific ownership in the event of illegally obtained stock, and generally in stock controls and management.

Spat were produced at QSS Ltd in 2007 and were reared at high density at BIARC for several months before being sorted for size to eliminate very large and very small individuals. Selected oysters were then pooled together and randomly distributed between mesh screens as described in previous experiments at a density of 20 individuals per 250 mm diameter screen. The oysters were acclimatised in rearing

tanks with no sand for one week before marking commenced in July 2007. Oysters were photographed for size measurements using the procedure described for scallops in section 4.2 prior to chemical immersion. Printed images were measured using digital callipers, using the longest possible 'diameter' to indicate shell height, and data was calibrated based on measurements of the ruler fragment in each image. Because the orientation of the oyster differed depending on the longest extension of the shell for measurement, the term 'shell height' is not entirely appropriate, but will be used for consistency with scallop data and the lack of a better term.

Chemical exposures for this experiment were identical to those used in Experiment 6 (Section 4.1.6); OTC at 100, 200 or 300 mg L^{-1}, each using bicarb or Tris to adjust/buffer pH, plus a control which did not undergo any chemical immersion or handling stress associated with chemical immersion. Each of the six experimental treatments plus the control group was replicated four times with 20 individuals per replicate. Equipment and procedures used for this marking were identical to those used in Experiment 6. After chemical immersion, oysters were placed in tanks with filtered (20 µm) seawater flowing at 750 mL min^{-1} supplied with five species of algae at 30,000 cells mL^{-1} as described in Section 4.1.6 for one week. After this period mortality and mark assessments were performed using the procedures described for scallops previously, and photographs were taken to monitor shell growth.

To determine the effect of rearing environment on mark retention, as well as oyster growth and survival, the experiment was divided into two tanks, providing 'sand' and 'no sand' environments, with each tank holding two replicates of each treatment (14 mesh screens in each tank). After one week, the chlorophyll *a* content of the water inside screens was measured to test the effect of sand on suspended microalgal abundance. Single samples from each tank were taken from the surface of the water inside screens and analysed off-site (Queensland Health Scientific Services). Assessments for mark brightness, growth and survival were performed at 31 and 59 days after the beginning of the experiment, after which the experiment was terminated. The significance of the effect of OTC concentration, buffer type and environment on scallop growth, survival and mark brightness were tested using ANOVA in the program GenStat (ed. 9, VSN International Ltd.).

5. RESULTS AND DISCUSSION

5.1 Developing a shell marking system for hatchery reared saucer scallops

During the course of this study, live scallops were successfully labelled with marks that were identifiable several months later. Whilst an epifluorescence microscope was routinely used in this work, alternative sources of UV light were trialled with OTC marks because less expensive alternatives for mark detection will need to be developed for commercial application. A hand-held 4.5V UV torch with five LEDs was capable of detecting strong marks, however the light from the microscope was much more intense and capable of providing the necessary light to see marks without magnification of the field of view (Figure 4). It was also necessary for confidently detecting much weaker fluorescence.

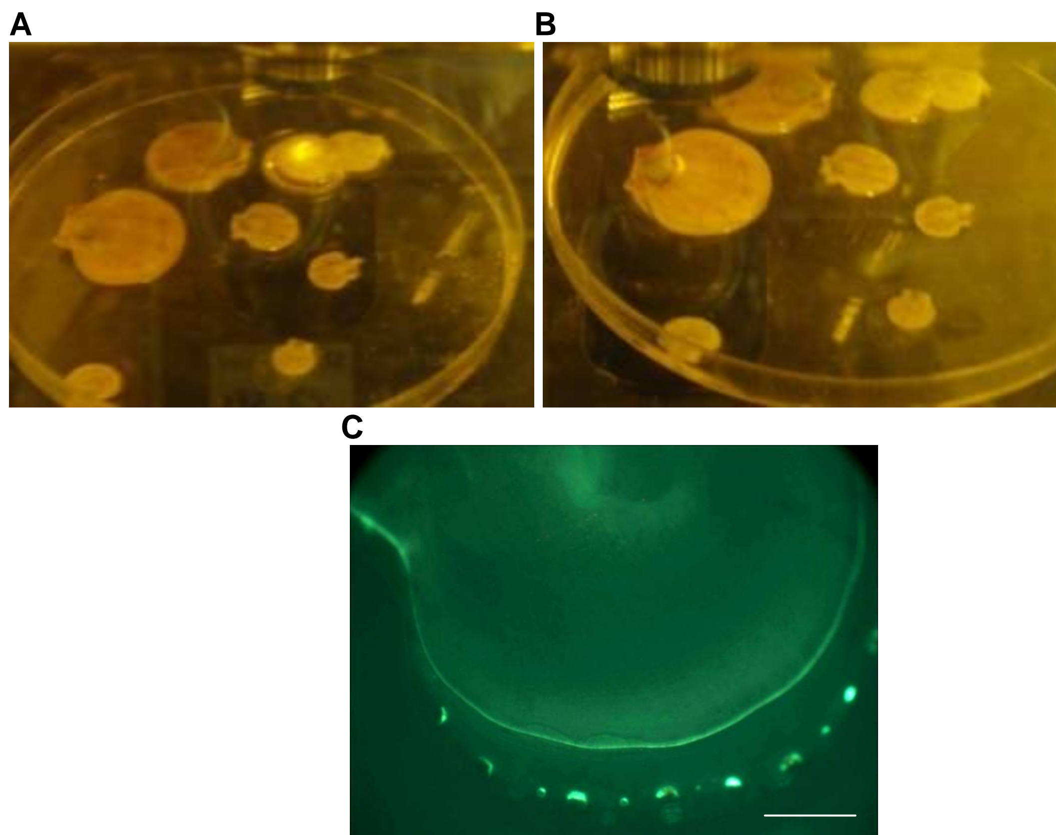

Figure 4. Observing marked scallops under UV light
Light was emitted from the 10X objective on the epifluorescence microscope. Observing scallops marked with 300 mg L^{-1} OTC on the microscope platform, through an orange plastic viewing shield used to safely inspect scallops and locate fluorescent marks. Shown are examples of very bright (A) and medium-bright (B) marks. (C) demonstrates the view of a 300 mg L^{-1} OTC-marked shell through the microscope (scale bar = 1mm).

A more cost-effective method for quickly, safely and reliably detecting marks should be relatively easy to develop in the future. Live marked scallops are currently with QSS Ltd for further development of broader scale mark detection and/or assessment techniques.

Regarding broad scale mark detection, extreme caution should be exercised when viewing marks and using strong UV light emitters. Eye damage can easily occur without the correct eye protection in the presence of strong UV light. Incidental UV

light generated by broad scale methods will be an important work place health and safety consideration in industrial applications.

5.1.1 Experiment 1 - Using OTC, calcein and alizarin red S to mark large and small juvenile scallops

Exposure to alizarin red S at 20 mg L^{-1} for four days resulted in 80 % mortality in both large and small scallops. Calcein at 50 mg L^{-1} and OTC at 500 mg L^{-1} were far more successful, resulting in only one fatality (a small specimen in the calcein treatment). Marking was successful in all treatments, with clear incorporation of all three chemicals into new shell growth, although the relative brightness of these marks was not quantified.

5.1.2 Experiment 2 - Testing 2 concentrations of OTC, calcein and alizarin red S using 5 mm spat

After two days of treatment with different chemicals and seven days on-growing in sand-filtered seawater, most scallops showed clear marks very close to the edge of the shells. New unmarked shell was observed outside the marks, which represented seven days of post marking growth. Figure 5 shows comparative images of scallops marked with the different chemicals. Except for the eyespots at the edge of mantles, and the hinge area of the shell, which were auto-fluorescent, no fluorescence was detected on the shells in any of the control specimens, making detection of marked individuals a straight-forward process.

All scallops treated with calcein (25 mg L^{-1} and 50 mg L^{-1}) and OTC (500 mg L^{-1} and 750 mg L^{-1}) were reliably marked. However, marks were only detected in an average (± s.e.) of 67 ± 0 % and 83 ± 11 % of scallops treated with 10 and 20 mg L^{-1} alizarin red S, respectively (Figure 6).

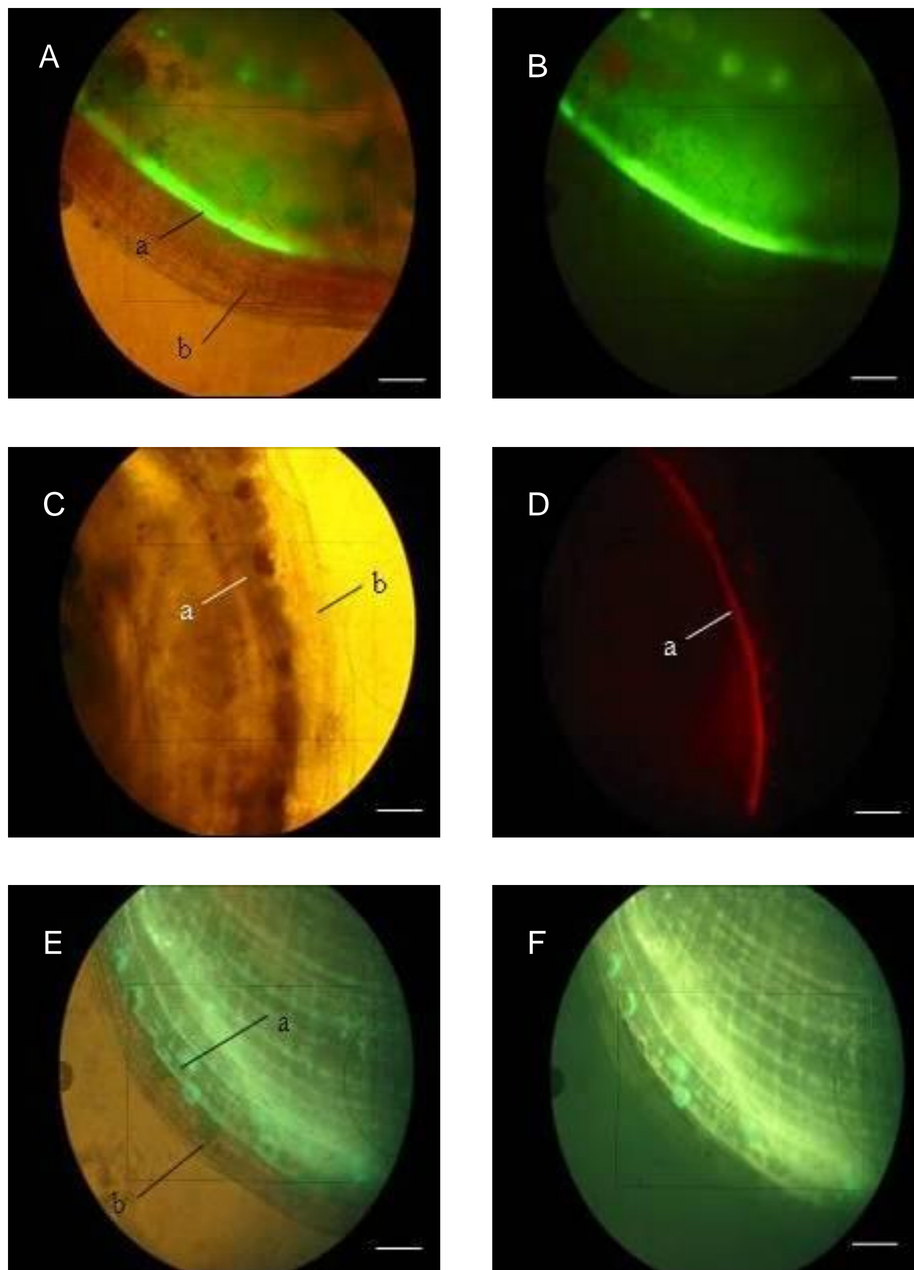

Figure 5. Marks produced in the shells of *Amusium balloti* spat using each chemical; calcein at 50 mg L^{-1} (A and B), alizarin red S at 20 mg L^{-1} (C and D) and OTC at 750 mg L^{-1} (E and F). Scallops were treated for 48 h, and then on-grown for 7 d. Specimens were observed and photos were taken under an epiflorescent microscope with specific light filters for each dye, and added natural light for A, C and E to show shell growth incorporating marks (a), and post-mark shell growth (b). Scale bars = 200 µm

In all cases including the control, some mortality occurred during the week following immersion marking. Most treatments suffered somewhat higher mortalities than the control (Figure 7), although the low replication prevented thorough comparisons in this regard. Nevertheless, it was apparent that the highest alizarin red S concentration (20 mg L^{-1}) caused the highest mortalities in the experiment. As in Experiment 1, the level of mortality for this chemical and concentration was unacceptable. This certainly justified the testing of alizarin red S at the lower 10 mg L^{-1} concentration, but the practical significance of the lower level of mortality that resulted from that treatment was countered by its substantially lower marking success.

Unfortunately, one month after moving these marked animals into the raceway system, complete mortality was realised. Water quality data that had been collected on a weekly basis from the raceway could not identify causative conditions for this loss, although concerns were raised about the suitability of the culture system and possible improvements that could be made to reduce the attrition that was occurring (discussed later in Section 4.2). Although all animals had died, the chemical marks were still detectable on the marked shells.

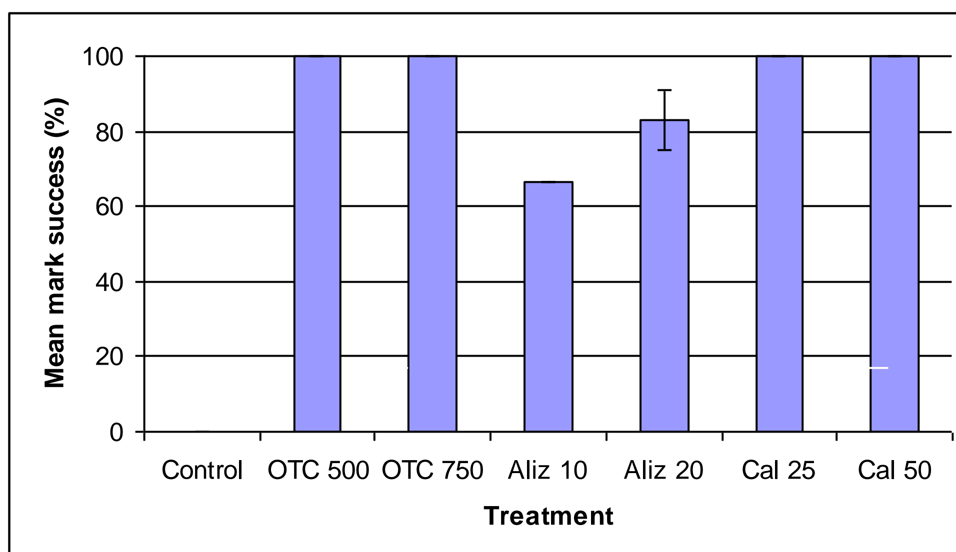

Figure 6. Mean percentage of scallops (n = 2, ± s.e.) that were successfully marked in Experiment 2, seven days after treatment. Note: Identical scores in both replicates resulted in no error bar for most treatments

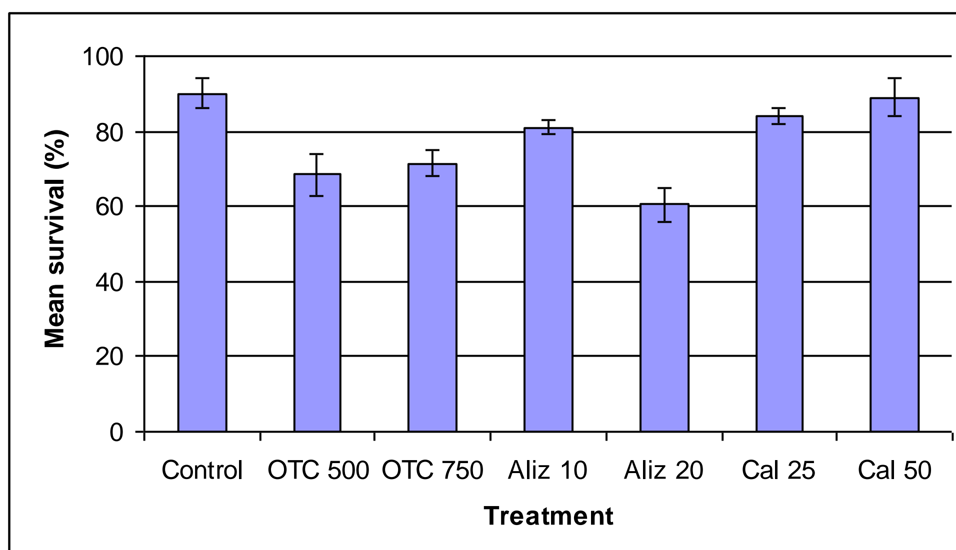

Figure 7. Mean percentage survival of scallops in different treatments (n = 2, ± s.e.) in Experiment 2, seven days after treatment

5.1.3 Experiment 3 - Double-cross marking with OTC and calcein

After chemical immersion, all scallops again showed clear marks close to the edges of their shells. Double-cross marks were clearly visible under the fluorescent microscope (Figures 8 and 9). One week after marking, all control animals (not marked previously, but marked with either OTC or calcein in this experiment) were

still alive. Most mortality occurred in the 4-6 mm calcein treatments (four died), as well as one individual from each size class of OTC treatment (Table 6). Due to the low numbers in this experiment, this data was used as a guide only.

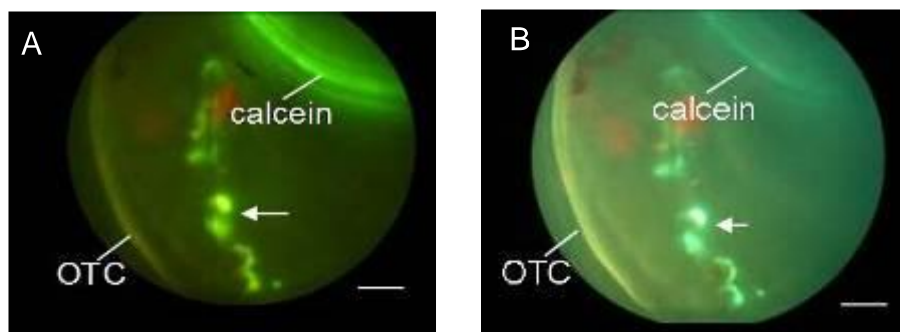

Figure 8. Scallop spat double-marked with calcein, then OTC. Viewed under epifluorescent microscope with a DM510 filter for calcein detection (A) or a DM455 filter for OTC (B). Arrows indicate autofluorescence from retracted mantle and eyespots. Scale bars = 200 µm

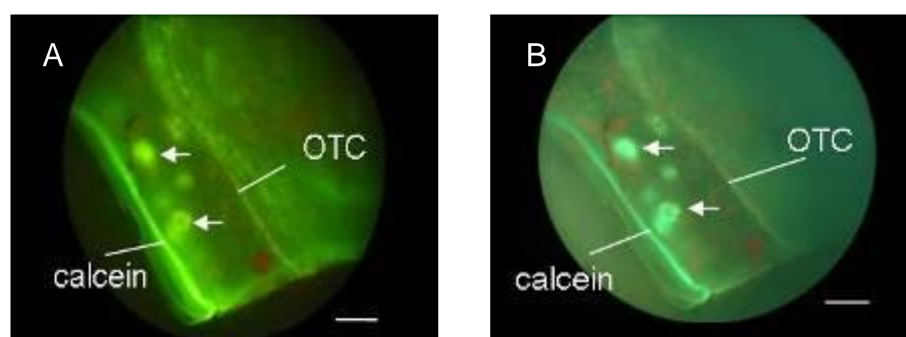

Figure 9. Scallop spat double-marked with OTC and then calcein. Viewed under epifluorescent microscope with a DM510 filter for calcein detection (A) or a DM455 filter for OTC (B). Arrows indicate autofluorescence from eyespots. Scale bars = 200 µm

Table 6. Experimental design and survival from Experiment 3

Chemicals used in Experiment 1	OTC		Calcein		Control	
Chemical used in Experiment 3	Calcein		OTC		Calcein	OTC
Size (mm)	4-6	14-25	4-6	14-25	14-25	
Number alive at start of Experiment 3	8	5	7	5	12	12
Number alive at end of Experiment 3	4	5	6	4	12	12
Percentage surviving	50 %	100 %	86 %	80 %	100 %	100 %

5.1.4 Experiment 4 - Marking spat using OTC and calcein with various concentrations and exposure times

Mark brightness scores were particularly consistent within treatments tested in this expanded fourth experiment. Since particular attention was paid to the randomised blind testing of treatments, these results can be considered robust in their assessments of these seven treatments. All concentrations of calcein resulted in lower mark brightness than the OTC treatments (Figure 10). Survival associated with calcein treatments was comparable to that of controls, whereas all OTC

treatments caused unacceptably high mortalities, especially in the seven day exposures (Figure 11).

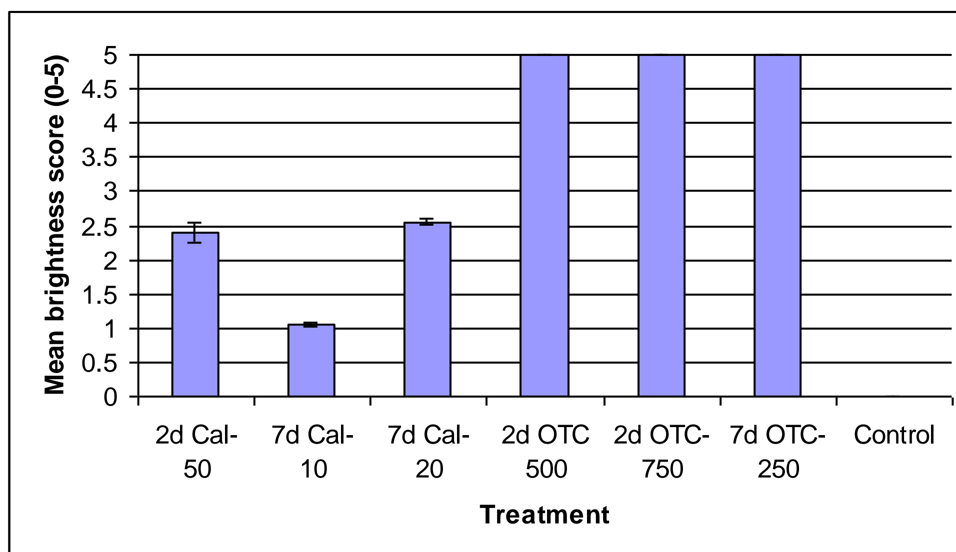

Figure 10. Mean (n = 2, ± s.e) assigned mark brightness of scallops 1 week after marking treatments in Experiment 4

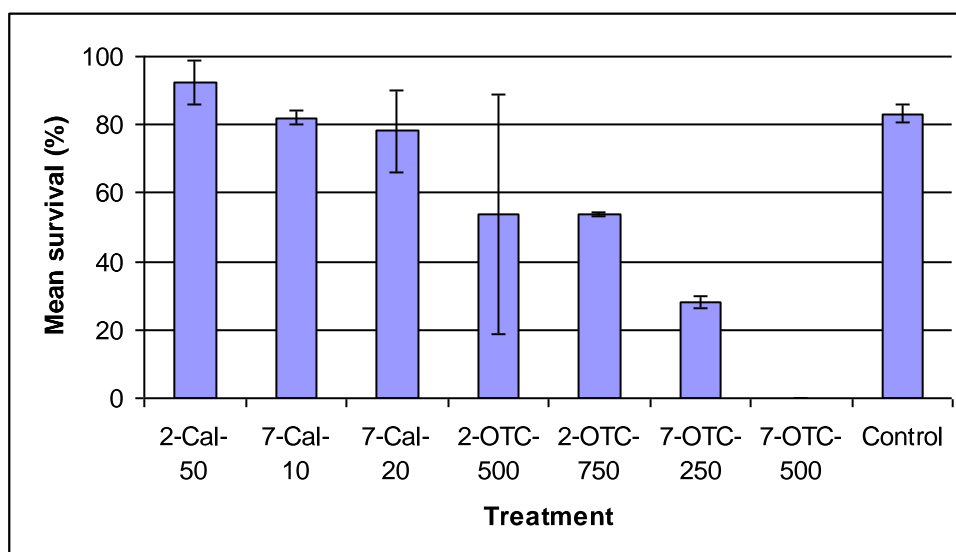

Figure 11. Mean (n=2, ± s.e) survival of scallops 1 week after marking treatments in Experiment 4

5.1.5 Experiment 5 - Testing Tris and bicarb to stabilise pH during OTC exposure

Using bicarb as a buffer

The series of short term trials conducted in 2007 provided evidence for the practical limitations of using bicarb to stabilise the pH of OTC solutions. In general it appeared that as pH increased, the effect of bicarb on pH decreased such that pH 7.7 was an approximate upper limit to the solutions regardless of bicarb concentration (Figure 12).

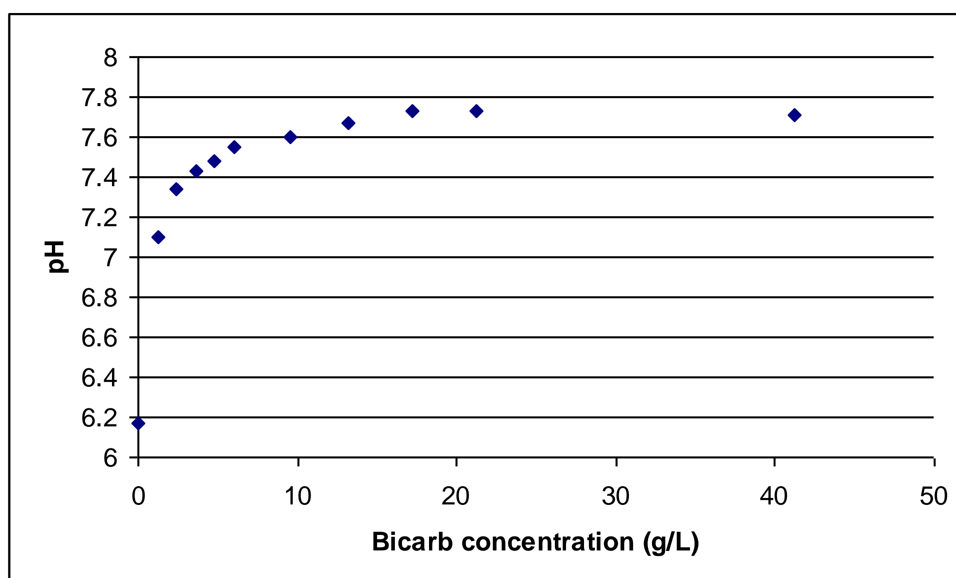

Figure 12. Adjusting pH using bicarb in 250 mL seawater with 300 mg L^{-1} OTC

When a large amount of bicarb was added in an attempt to reach pH 8 (close to an assumed optimal seawater pH), the resultant pH after 24 h of overnight aeration was excessive at 9.4. An optimal level was identified in the order of 1.14 g L^{-1} bicarb, which after 16 h gave an approximate pH of 8 - 8.2 in 100, 200 and 300 mg L^{-1} OTC (Figure 13).

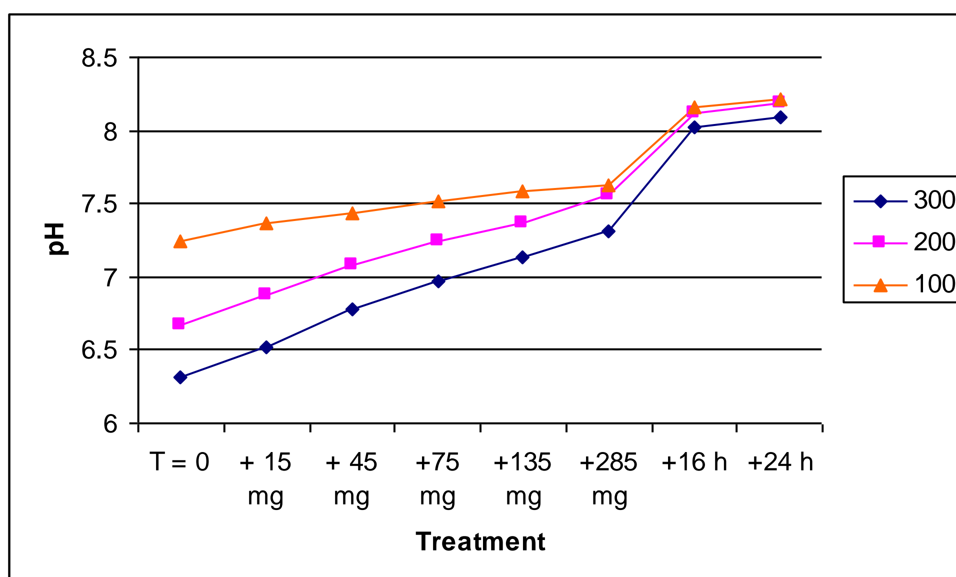

Figure 13.The effect of bicarb, then time on pH of OTC solutions. The legend indicates OTC concentrations in mg L^{-1}. Note bicarb quantities along the x-axis are cumulative masses progressively added to 250 mL of seawater, which should be multiplied by four for concentration in mg L^{-1}

<u>Using Tris as a buffer</u>
Tris had an immediate stabilising effect on the pH at around pH 8 for all OTC concentrations tested (Figure 14).

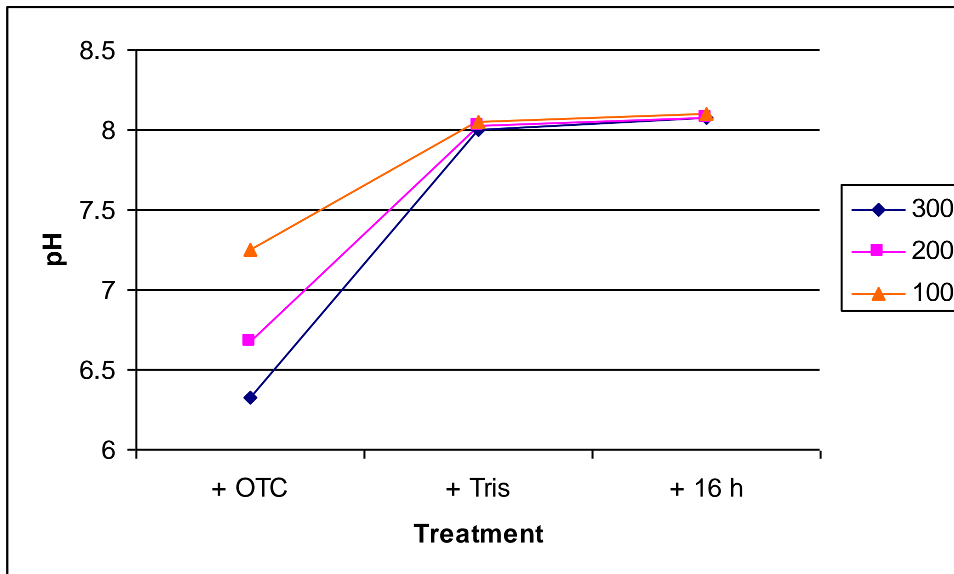

Figure 14. The effect of Tris and then time on OTC solutions. Tris was added at 0.6 times the concentration of OTC. The legend shows OTC concentration in mg L^{-1}

As OTC solutions aged their pH increased. This effect was buffered by Tris, thereby preventing such change over time, whereas the effect of bicarb was to provide an immediate adjustment but no such longer-term stability (Figure 15).

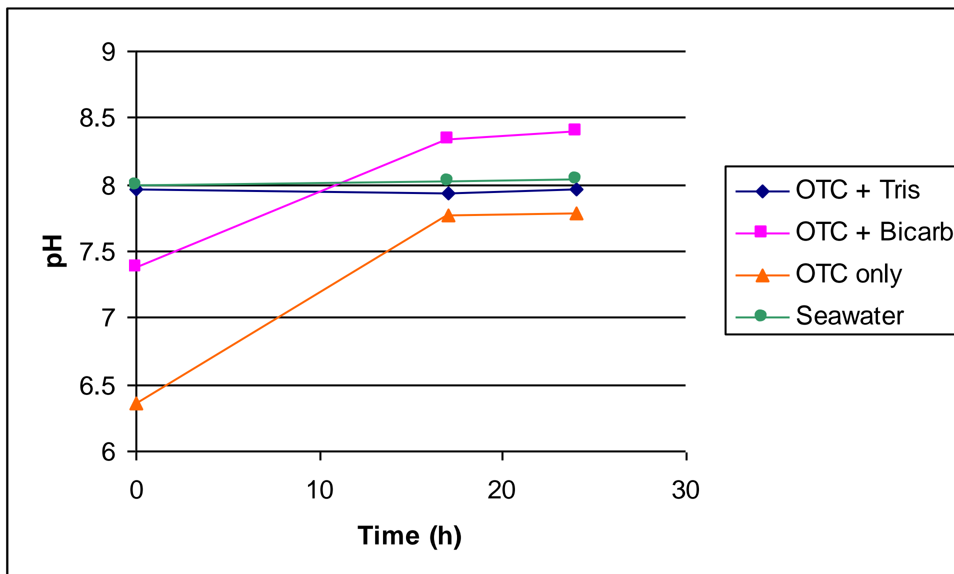

Figure 15. Comparing Tris and bicarb as buffers for OTC at 300 mg L^{-1} over 24 h. Using un-buffered OTC at 300 mg L^{-1} and plain seawater as controls

<u>The effect of buffer and pH on precipitation</u>

There were marked differences between these two forms of pH control in terms of the amounts of foam produced in aerated containers and the formation of precipitates (Figures 16 and 17).

Figure 16. Froth in four solutions after 17 h aeration. Clockwise from top left: 300 mg L^{-1} OTC (no buffer), seawater, 300 mg L^{-1} OTC plus 600 mg L^{-1} bicarb, 300 mg L^{-1} OTC plus 180 mg L^{-1} Tris buffer

After 17 h of aeration, the highest precipitation was found in the bicarb-buffered solution (Figure 17). This was also the solution with the highest pH (see Figure 15), which may explain the extra precipitation. It was noted that when bicarb was initially added (and pH was lower), precipitation was not evident, however as the solution aged and pH increased, precipitation became evident. In Tris-buffered solution, a small amount of precipitation was evident throughout the duration of the trial, matching the stable moderate pH of the Tris-buffered solution.

Figure 17. Precipitation using bicarb or Tris after 24 h. 300 mgL^{-1} OTC with Tris 180 mgL^{-1} (left) or bicarb 600 mg L^{-1} (right)

5.1.6 Experiment 6 - Double-marking spat using OTC at three concentrations and buffered with Tris or bicarb

The more robust factorial design of this sixth experiment permitted more rigorous analyses for the effect on scallop survival and brightness of marks of the range of concentrations and buffers tested. The longer term effects of treatments on growth and survival are presented with other mark longevity data in Sections 5.2 and 5.3.

The three day chemical immersion procedure incorporating solution replacement each day resulted in rapid and substantial shifts in pH in the bicarb-buffered treatments, whilst the Tris-buffered treatments maintained a comparatively steady pH that was similar to the seawater controls (Figure 18). Despite these changes in pH in some treatments, survival was high in all experimental units and no significant differences ($P > 0.05$) were detected between treatments and the control (Figure 19). Precipitation and frothing appeared to increase with OTC concentration and higher pH in both Tris and bicarb treatments, however it did not appear to present a problem in any of the treatments.

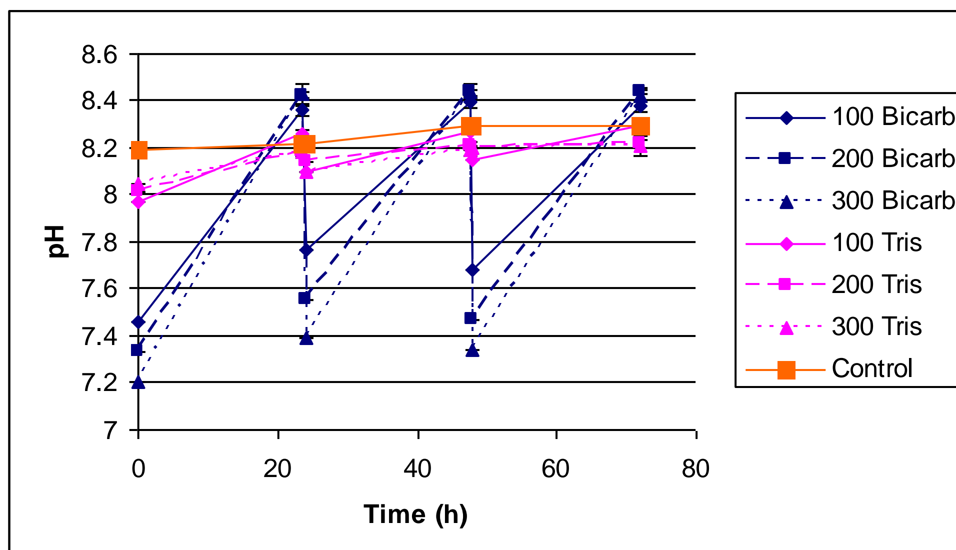

Figure 18. Changes in pH during chemical immersions in Experiment 6. Data shown are means ± s.e. (error mostly not visible)

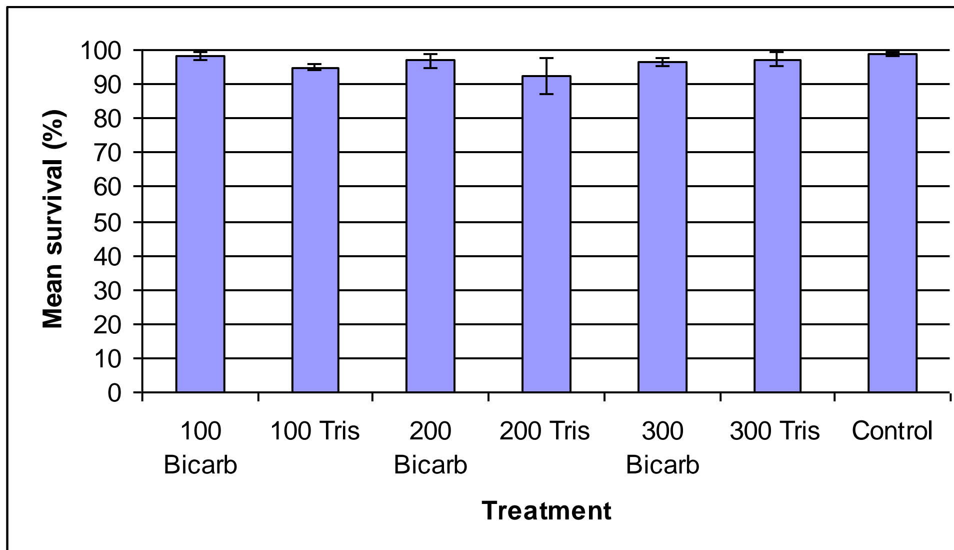

Figure 19. Mean survival (*n* = 4, ± s.e.) of scallops one week after treatment in Experiment 6

Although buffer type did contribute to a significant interaction between treatments, overall, mark brightness scores were equally rated in both bicarb and Tris treatments. There was however an observed difference in the quality of marks. Bicarb sometimes resulted in 'banding' patterns on the surface of shells as well as mark incorporation into new shell growth, whereas Tris-buffered treatments resulted in a single band only where new shell growth had occurred (Figure 20).

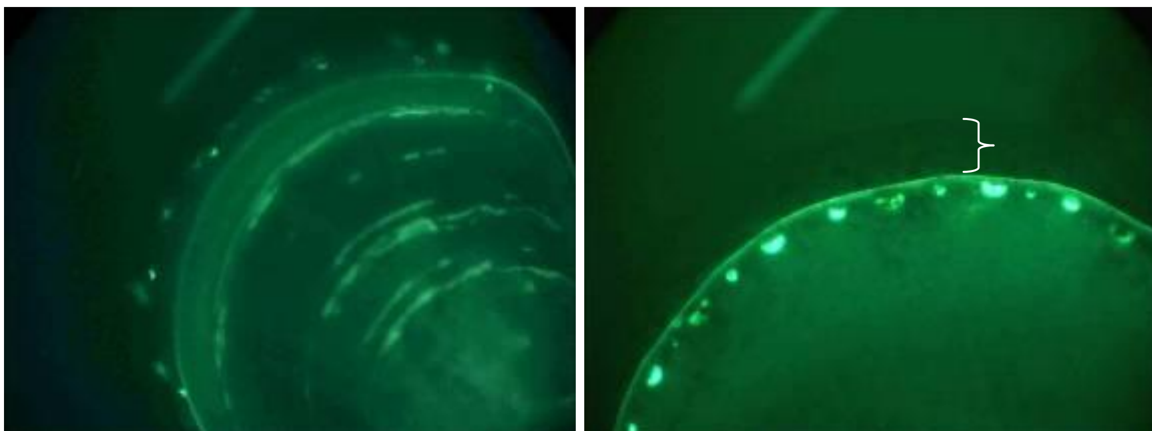

Figure 20. Marked scallop shells using OTC with bicarb (left) or Tris (right). Both images were captured using x 40 magnification on an epifluorescent microscope using a DM455 filter (Nikon). The bracket indicates new growth without fluorescence, deposited after chemical immersion

When viewed under higher magnification (100 x), specific growth rings within marks were identified in some specimens from Tris-buffered treatments (Figure 21). The OTC concentration had an obvious affect on mark brightness (Figure 22), with 100 mg L^{-1} being less bright than the other treatments.

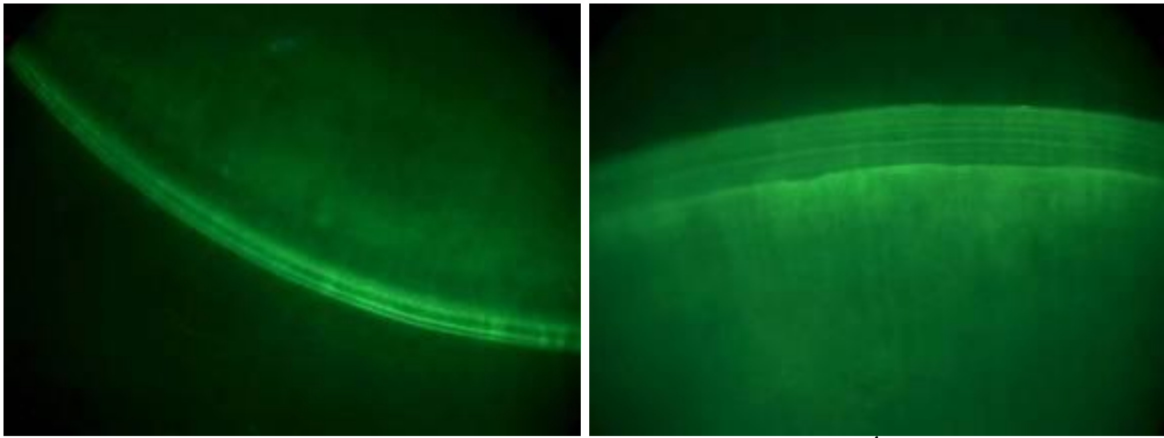

Figure 21. Detailed view of single marks generated by 300 mg L^{-1} OTC with Tris buffer. Both images were captured using x 100 magnification on an epifluorescent microscope using a DM455 filter (Nikon)

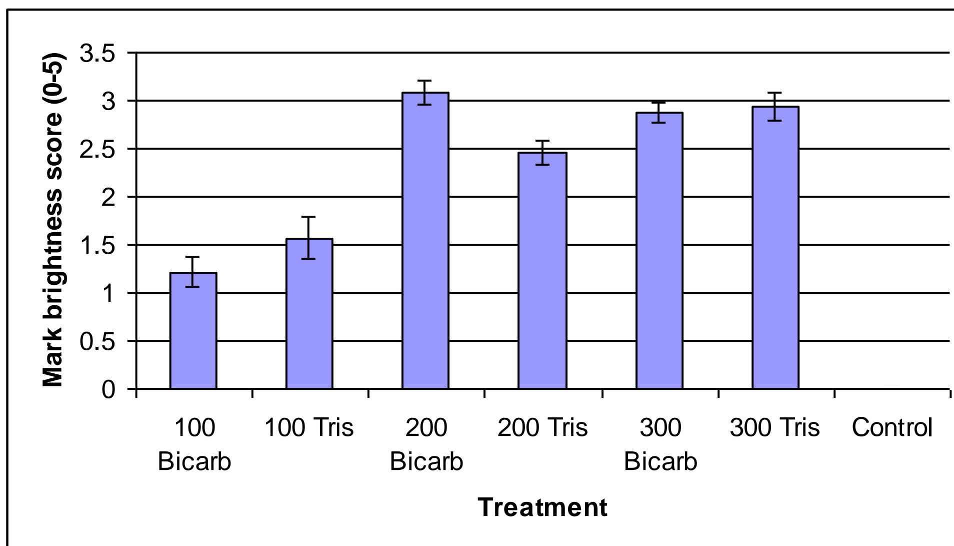

Figure 22. Mean (n = 4, ± s.e) mark brightness scores from Experiment 6 one week after treatment. Treatment labels along x-axis indicate OTC concentration in mg L^{-1} and buffer type used

Due to heater malfunction in one raceway, half of the scallop replicates in this experiment were compromised by sub-optimal conditions (water temperatures over 40ºC) and were therefore discontinued. This forced the follow up double-marking procedure to only be applied to two replicates per treatment. Again survival was high soon (7 d) after the second chemical immersion (Figure 23). This indicates that the scallops had probably recovered from any stress which may have been caused by the first chemical treatment, which occurred 28 days prior to the second chemical immersion.

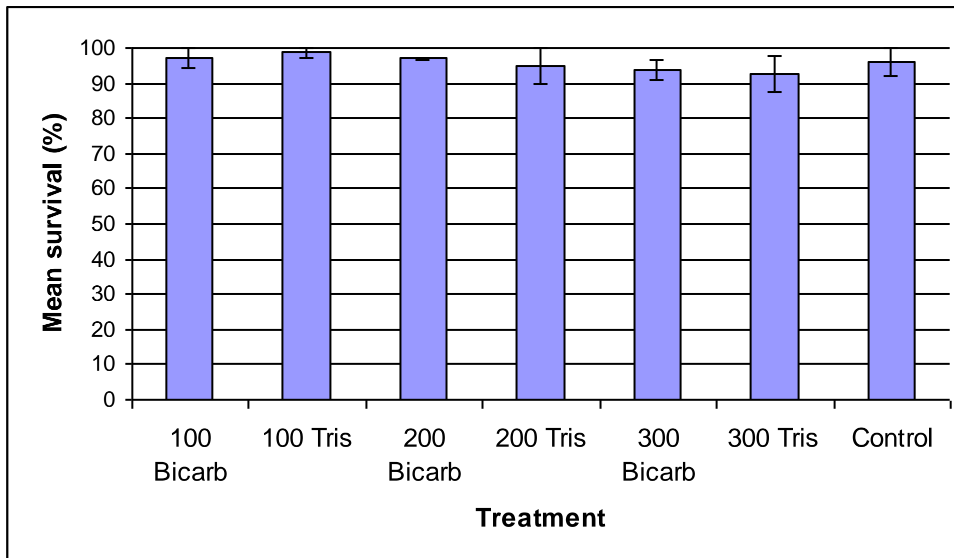

Figure 23. Mean (*n* = 2, ± s.e.) survival 1 week after chemical treatment for mark 2 in Experiment 6

Brightness scores for this second mark (Figure 24) had a similar pattern of results to those detected for the first mark (shown in Figure 22 above). Buffer type again did not greatly impact on mark brightness or survival, and the mark brightness was again lower in the 100 mg L^{-1} treatments than in the other higher OTC concentrations.

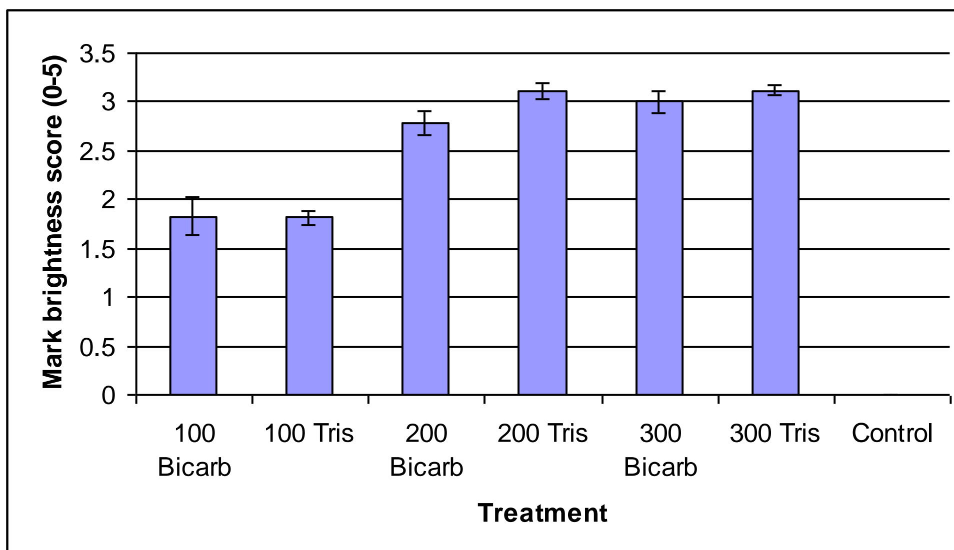

Figure 24. Mean (*n* = 2, ± s.e.) mark brightness scores for the second mark in Experiment 6, 7 d after treatment

ANOVA for mark brightness showed an interaction between assessment date (marks were assessed on 3 separate occasions), OTC concentration and pH buffer (bicarb or Tris). This is summarised in Table 7. The highest scores were from the 200 and 300 mg L^{-1} Tris treatments in the third evaluation, which were significantly higher than all other treatments except for the 200 mg L^{-1} bicarb, also in the third evaluation. An apparent overall trend for brighter marks as time progressed can probably be attributed to our greater confidence in distinguishing between dull and bright marks with experience, despite efforts to keep methods standardised throughout the experiment.

Table 7. Table of mean mark brightness scores from Experiment 6 showing interaction between buffer, OTC concentration and assessment date. Cells with matching letters (in superscript) are not significantly different ($P > 0.05$)

Assessment Date	OTC concentration pH adjuster	100 mg L^{-1}		200 mg L^{-1}		300 mg L^{-1}	
		Bicarb	Tris	Bicarb	Tris	Bicarb	Tris
1		1.663^{a}	1.840^{ab}	3.070^{d}	2.638^{c}	2.931^{cd}	2.949^{cd}
2		2.127^{b}	2.002^{ab}	2.880^{cd}	3.155^{d}	2.809^{cd}	3.256^{d}
3		2.630^{c}	2.084^{b}	3.440^{de}	3.771^{e}	2.946^{cd}	3.787^{e}

In order to assess the overall effect of buffer type and OTC concentration without the potentially complicating effects of different assessment dates, the data from different dates were pooled together. When this was done there was a significant interaction effect between OTC concentration and mark number (mark 1 or mark 2 from the first and second chemical immersions, respectively). This data is shown in Table 8. In both marks the 100 mg L^{-1} treatment resulted in significantly lower mark brightness than the other concentrations. In mark 1, there was no difference between 200 and 300 mg L^{-1} OTC, whereas for mark 2, 200 mg L^{-1} OTC resulted in significantly higher brightness than 300 mg L^{-1} OTC. This could be explained by lower toxicity and resultant faster growth during the medium-concentration treatment, which could result in greater overall uptake of fluorescence into the calcium carbonate shell matrix.

Table 8. Table of mean mark brightness scores from Experiment 6 showing interaction between OTC concentration and mark number. Cells with matching letters (in superscript) are not significantly different ($P > 0.05$). Note that different LSD values are used within and between columns

OTC Concentration	Mark Number	
	Mark 1	**Mark 2**
100 mg L^{-1}	1.730^{c}	2.713^{a}
200 mg L^{-1}	3.025^{de}	3.426^{bf}
300 mg L^{-1}	3.148^{dfg}	3.043^{aeg}

5.2 Improving survival and growth of juvenile scallops in a land-based system

<u>Survival of scallops in experimental systems</u>

The initial attempts to grow saucer scallops of various sizes using prawn pond effluent were unsuccessful; resulting in 100 % mortality after several days is three separate trials (no data collected). Efforts in this regard also included providing a blend of pond water and unfiltered seawater so that algal densities we much reduced. However the variable nature of pond phytoplankton blooms and variable levels of silt which periodically occurred in the unfiltered seawater supplies at that time caused a series of uncontrolled events which made this style of watering system fraught with unmanageable and compounding risks.

When the system was improved to provide scallops with filtered water and cultured microalgae survival improved, such that a small number of scallops survived for over 200 days in the system. Despite the improvements, consistent mortalities were observed in all treatments (Figure 25). In Experiment 6, survival was initially comparatively high in all treatments, however, mortalities invariably increased towards the end of the experiment (Figure 26).

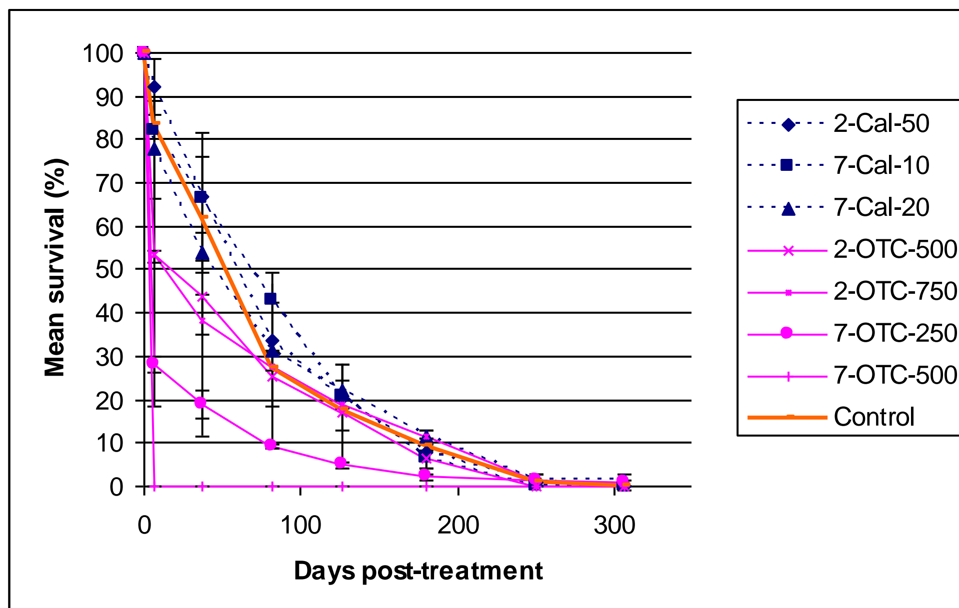

Figure 25. Mean survival (± s.e) of treatments from Experiment 4. At the beginning of the experiment there were two replicates per treatment, each containing 60 individuals. The legend shows abbreviations for treatments, indicating the length of chemical immersion, then the marking chemical used (calcein or OTC), then the concentration in mg L^{-1}

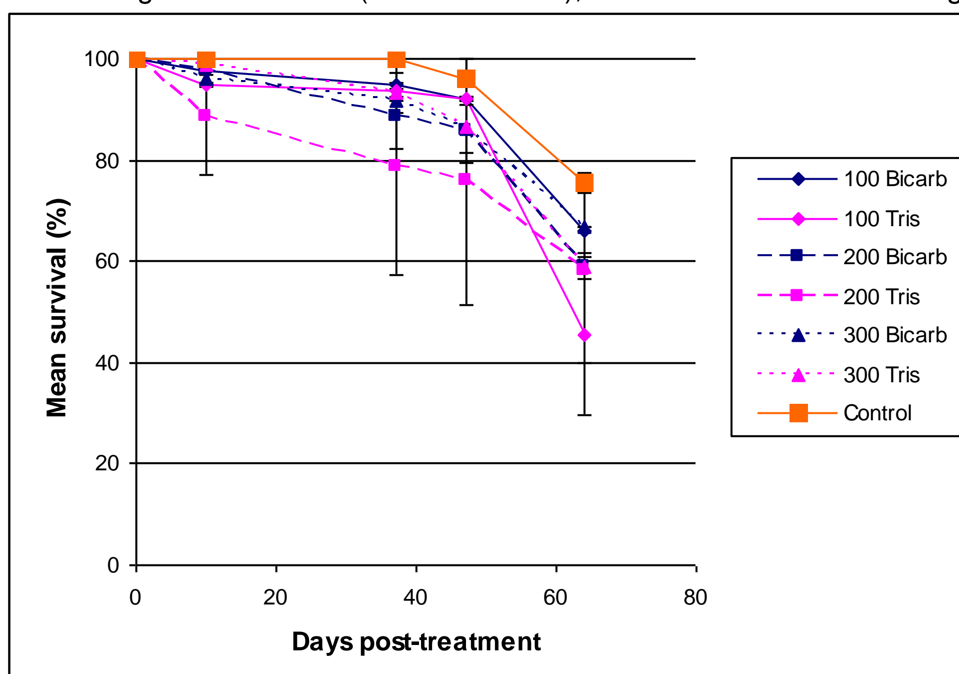

Figure 26. Mean survival (± s.e.) of treatments from Experiment 6

In both Experiments 4 and 6, mean survival rates were generally higher in the untreated control groups than in the combined treatments (Figure 27), although the effect of treatment on survival was not significant in Experiment 6 ($P = 0.204$). Furthermore, neither buffer nor OTC concentration affected survival in Experiment 6 ($P > 0.05$). Nevertheless the general trend observed in both Experiments 4 and 6 suggests that scallops marked prior to release could be expected to have slightly higher mortality levels than unmarked scallops. The comparisons shown in Figure 27 also demonstrate the better overall survival results achieved in the sixth and final experiment, suggesting that improvements in the artificial rearing environment had in fact been achieved.

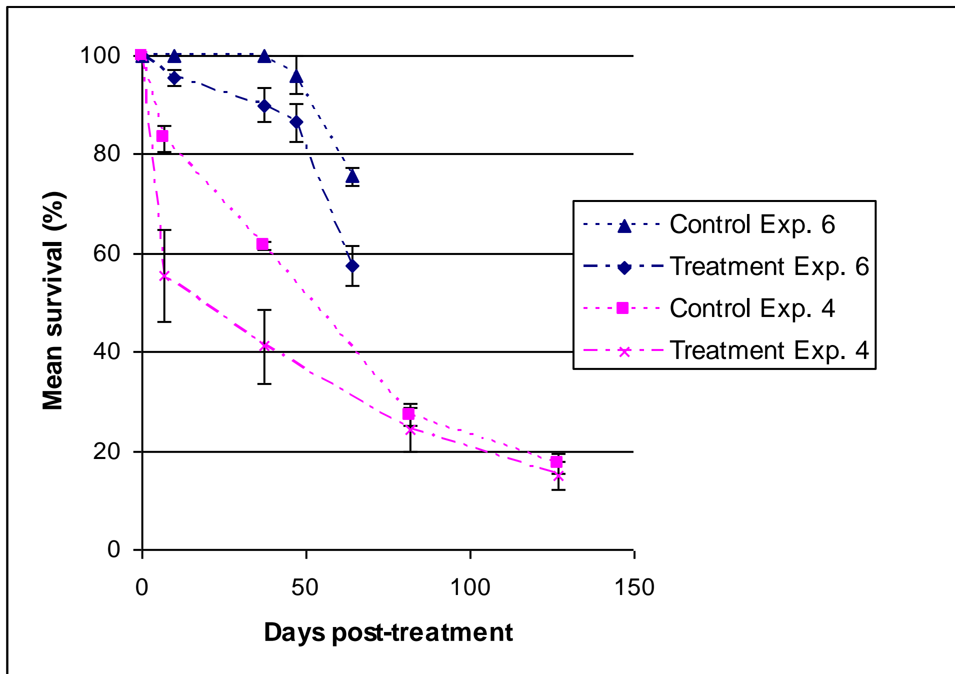

Figure 27. Average (± s.e.) survival of combined treatments (All Exp.) and control groups (Cont. Exp.) between Experiments 4 and 6

Growth rate

Using the microalgae drip-feeding system in Experiment 4, growth rates were slow throughout the experiment. A modest improvement in growth rate was evident towards the end of the experiment (see Figure 28). Although the systems changes made on day 210 were the most obvious causative factors for this, several other factors such as greatly reduced densities, water temperature and selection for the healthiest individuals were also likely to have played a part.

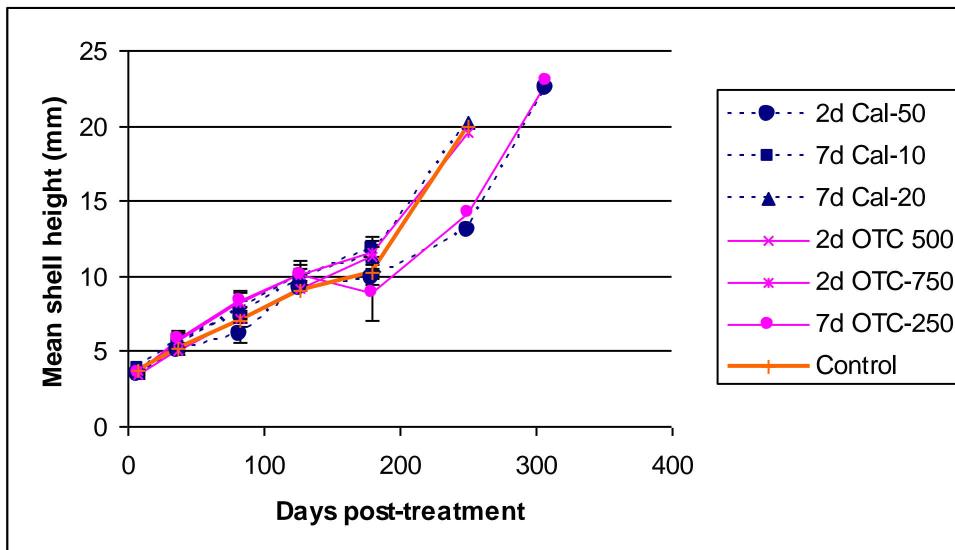

Figure 28. Mean (± s.e.) shell growth of scallops in Experiment 4. Note that the rearing environment changed at day 210, when batch-feeding was introduced. Also note that due to high mortality, the total number of scallops measured in the 250 day and 307 day time points were eight and three, respectively. The legend shows abbreviations for treatments, indicating the length of chemical immersion, then the marking chemical used (calcein or OTC), then the concentration in mg L^{-1}

In Experiment 6, the design improvements made to the raceway system again appeared to facilitate faster growth rates, together with improved survival (discussed above). Figure 29 provides the growth data for scallops in this final experiment. When ANOVA was performed on this growth data with initial size as a co-variate, we found that initial size had a significant effect on the growth data ($P < 0.01$). The data was adjusted to allow for this effect in GenStat, and ANOVA was repeated. In this analysis there was a significant interaction between OTC concentration and buffer ($P = 0.028$) in the 37 day time point, but there were no significant effects of treatments in the 10 or 64 day time points. In the 37 day time point, the Tris-treated scallops grew significantly faster than the bicarb-treated scallops from the 200 mg L^{-1} OTC treatment (Table 9). The slower growth in bicarb may be a consequence of mild pH stress, although this was not seen at any other OTC concentrations.

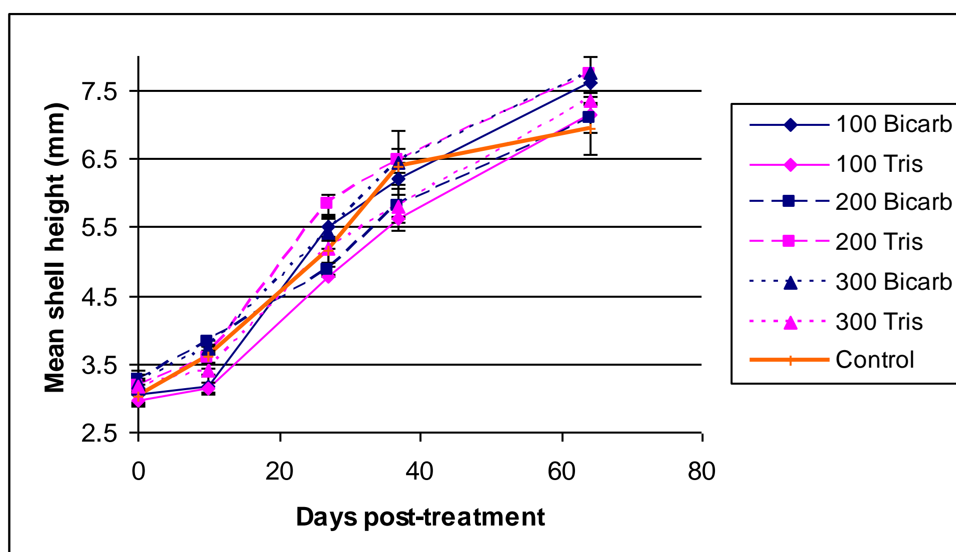

Figure 29. Mean (± s.e.) shell growth of scallops in Experiment 6. The legend describes the treatments, indicating OTC concentration in mg L^{-1} and the buffer used

Table 9. Table of mean growth (mm) from Experiment 6 showing interaction between OTC concentration and buffer. Cells with matching letters (in superscript) are not significantly different ($P > 0.05$)

OTC concentration	Buffer	
	Bicarb	Tris
100 mg L^{-1}	3.162^{ab}	2.680^{ab}
200 mg L^{-1}	2.535^{a}	3.343^{b}
200 mg L^{-1}	3.148^{ab}	2.594^{ab}

A slowing of the growth trajectory was evident in Experiment 6 during the last growth period, particularly in the control treatments (see Figure 30). We attribute this to density dependant factors associated with the microalgal feed supply and the waste products of the growing biomass contained in the screens. The scallops in control screens had generally higher average survival than other treatments (noted above and see Figure 27), and the resultant density differences between screens was not adjusted during the experiment. Observations suggest that over-crowding may have been a factor as the scallops grew large enough to potentially compete for food and produce significant levels of faeces (Figure 31). When the scallops were smaller, a build-up of uneaten algae could be observed on the surface of the sand, and faecal matter was barely noticeable after one week. This build up of microalgae on the surface of sand within the screens provided some evidence that the head-pressure inside the screens was functioning as planned, slowly pushing water through the sand to help prevent the development of anoxic conditions in the sand beds. It is very likely that this “sand filter” effect also helped to make the microalgae more available for the scallops, since it caused a concentration of microalgae in their close proximity. *A. balloti* are not known deposit feeders, however they do move regularly by clamping their valves together, which stirs up sediment (pers. obs.), making the ‘benthic’ algae available for filter-feeding.

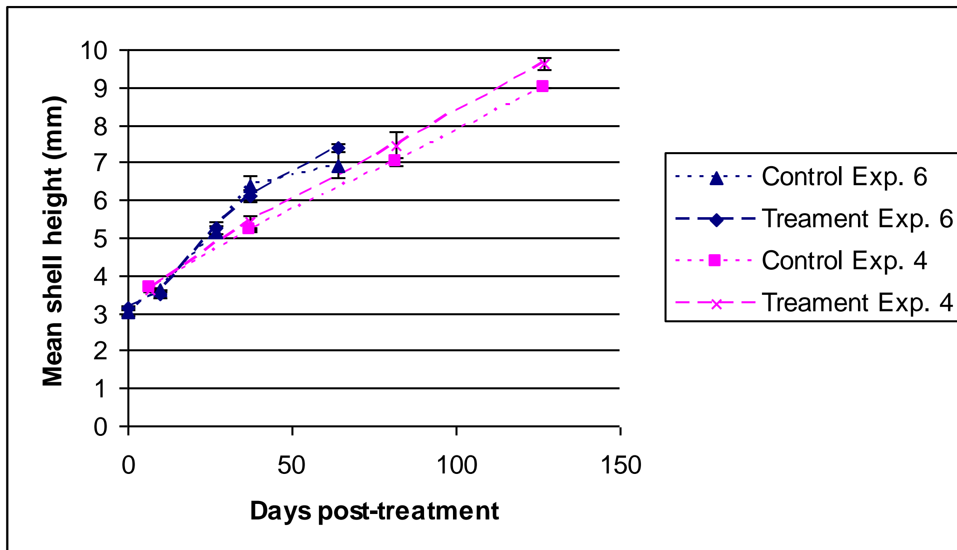

Figure 30. Comparisons between the average (± s.e.) growth of combined treatments (All Exp.) and control (Cont. Exp.) groups between Experiments 4 and 6

Figure 31. Comparing seabeds after one week of use with (left) or without scallops. This comparison was made towards the end of Experiment 6 when growth rates began to slow and mortality increased. Note the relatively clean sand in-between the screens, that the scallop-free screen has microalgae concentrated on the top of the sand (brownish tinge), and the screen with scallops has no apparent microalgae deposited on the surface but plenty of faecal matter

Does sand improve scallop health?

While the first stage of Experiment 6 was in progress, excess scallops were kept in a spare tank without sand, supplied with water which overflowed from the experimental tanks. These scallops were not regularly maintained and were kept at high density. Some of these scallops became overgrown with stalked protozoans from the family Ciliophora, class Oligohymenophora, subclass Peritricha (Ruppert and Barnes, 1994). Although positive identification was not achieved, they were similar in appearance to the common crustacean parasite *Epistylis* sp (Hudson and Lester, 1994). These epibionts have been described previously on the shells of molluscs (Dias *et al*., 2006). Although they are not usually directly harmful to the host, they can cause stress and other problems for the host when at high density (Puckett and Carman, 2002). Observations under light microscope indicate that scallops that were heavily fouled by these ciliates were sluggish in their movement and usually had retracted mantles, indicating poor general health. Figure 32 shows the typical sand covered habit of a healthy feeding scallop and one that is covered in stalked ciliates that appears to be in a non-feeding state where the outer margins of the shell are not exposed.

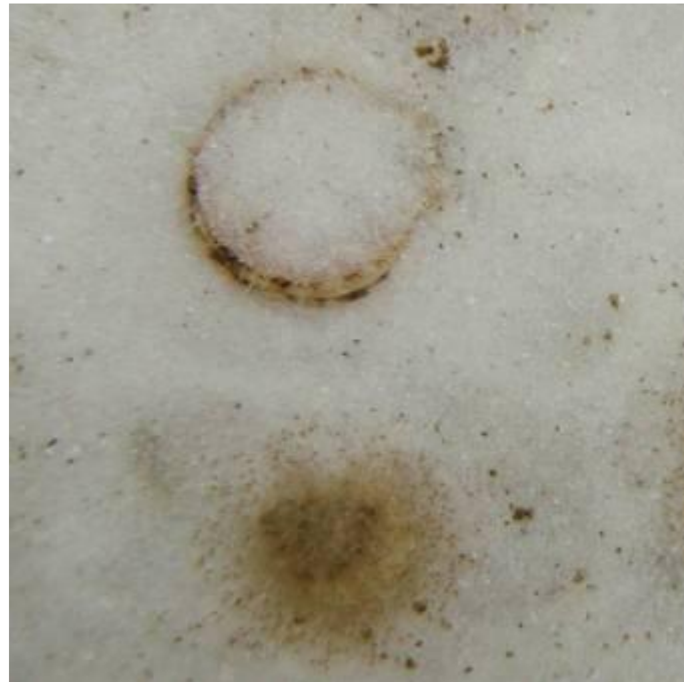

Figure 32. A healthy scallop covered in sand (top) and below it, a scallop covered in stalked ciliates

Although this study was not designed to identify husbandry related difficulties faced by terrestrial based rearing systems for scallops, the re-introduction of well managed sand beds certainly appeared to improve the culture conditions. The presence of sand over the upper surface of the shell is likely to protect the scallops from this type of infestation. *A. balloti* is not generally known for any form of external fouling on their shells in the wild, and this may be due to the use of sand as a protective cover in the wild. Our incidental observations of the recovery of fouled scallops when introduced into healthy sand beds (see Figure 33) provide strong evidence for the important anti-fouling role of sand for juvenile *A. balloti*.

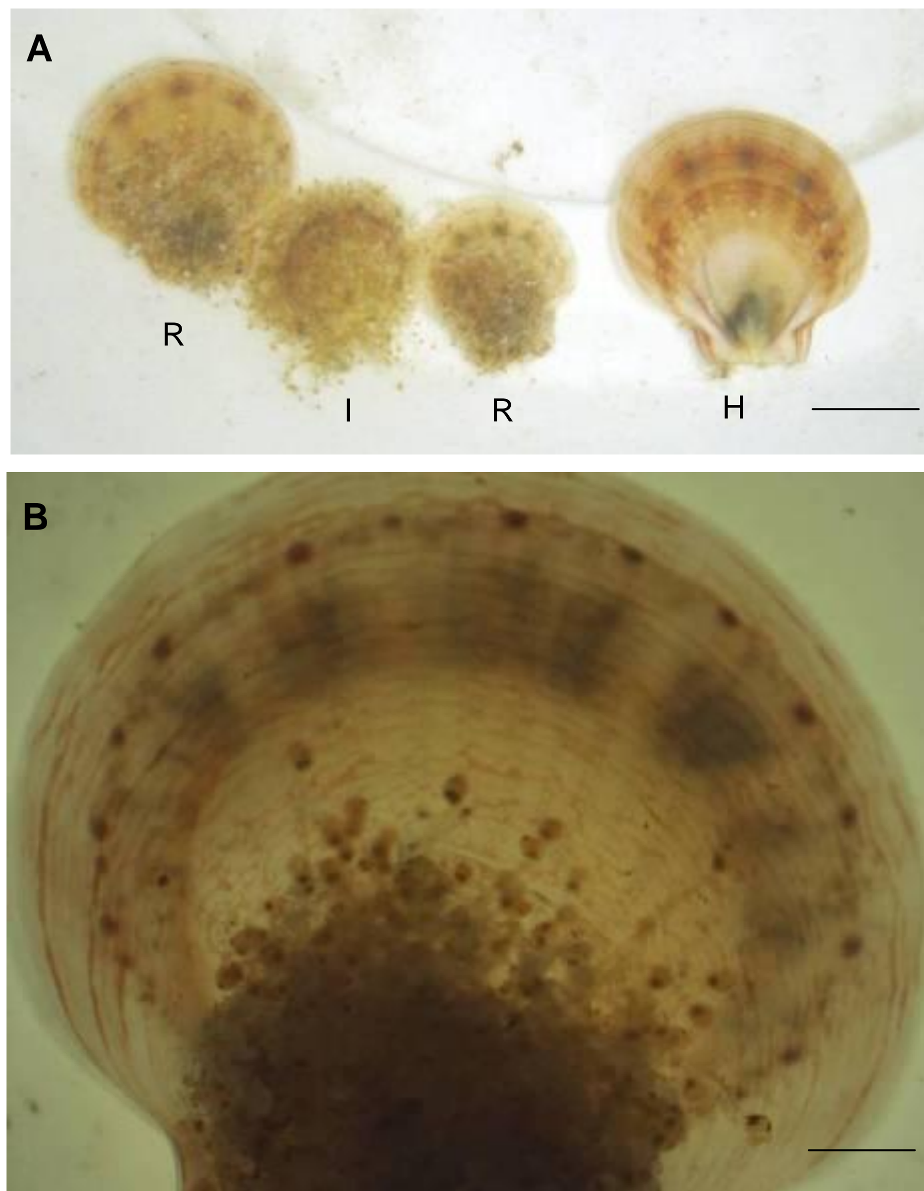

Figure 33. Scallops fouled with stalked ciliates after 1 month in a seabed simulation system. (A) A range of scallops from healthy (H) to infested (I) and two individuals that show signs of recovery (R), scale bar = 10 mm. (B) Using a dissection microscope we can clearly see the dense coverage of stalked ciliates and the new un-fouled shell growth that has occurred since introduction to the seabed simulation system, scale bar = 2.5 mm

5.3 Retention of fluorescent shell marks over time in the saucer scallop *Amusium balloti*

In Experiment 4, we observed the mark brightness of living scallops over more than 10 months. Although OTC marks were initially much brighter than calcein marks, they both appeared to fade at a similar rate (Figure 34). This work did not investigate whether higher concentrations of calcein could provide brighter and longer-lasting marks. However it did show that OTC marks can last for over 10 months in a land based system. Fluorescence in both OTC and calcein is known to fade with exposure to light (Choate, 1964). Although translucent plastic sheeting and shade cloth were provided for the scallop rearing facilities during this experiment, it is likely that light levels on the ocean floor would be lower. The lack of sand in this experiment also may have contributed to fading of the mark, since

scallops naturally protect their shells from light exposure with a thin layer of sand (discussed in previous Section 5.2).

The bright UV light used for mark detection throughout Experiments 4 and 6 would have also caused extra fading that would not be encountered in the wild. This was accounted for in the experiments by ensuring similar UV light exposure levels for each replicate and treatment were applied when collecting the data that was necessary to adequately track the mark quality through time. Exposure times during testing were also minimised.

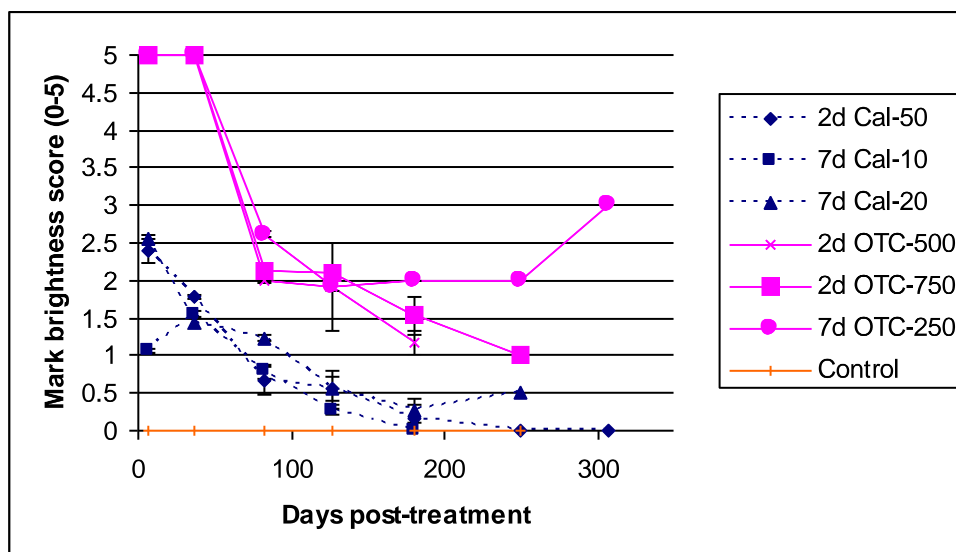

Figure 34. Mean (± s.e.) mark brightness scores from Experiment 4. The legend shows abbreviations for treatments, indicating the length of chemical immersion, then the marking chemical used (calcein or OTC), then the concentration in mg L^{-1}

Other difficulties noted with mark brightness assessments included the subjective nature of ratings. This was addressed by using one person to collect the scallops from their labelled containers and deliver them in a random unlabelled order to the brightness evaluator, which eliminated potential for bias based on treatment labels. This made comparisons within a single time-point valid, however, there was no way of fully calibrating mark brightness scores through time.

Double-marking of scallops in Experiment 6 partially solved this problem by making it possible to view marks of a different age in a single time-point. In this case, the first mark was produced one month earlier than the second, yet there were few significant ($P > 0.05$) difference between the brightness of the marks after 7 d or 37 d of further growth (Figures 35 and 36, respectively). Overall comparison of mark 1 and mark 2 shows that mark 2 was significantly brighter at both 100 and 200 mg L^{-1} OTC concentrations ($P < 0.05$; see Table 8, section 5.1.6). This makes sense since the second mark was more recent (less faded), and also because the thickness of shell deposition would have been greater for mark 2 because the scallops were larger. Mark brightness is thought to be determined by the rate of calcification (Pirker and Schiel, 1993). The overall pattern of greater brightness in mark 2 for 200 mg L^{-1} treatments is not evident in single-time-point comparisons shown in Figures 35 and 36. This is probably caused by relatively low ratings in the first mark assessment, in which there was no second mark for comparison. This final experiment showed that the best marks were achieved in solutions containing 200 and 300 mg L^{-1} OTC, and

that these were retained by scallops in a simulated sea-bed environment for at least two months with no evidence of fading.

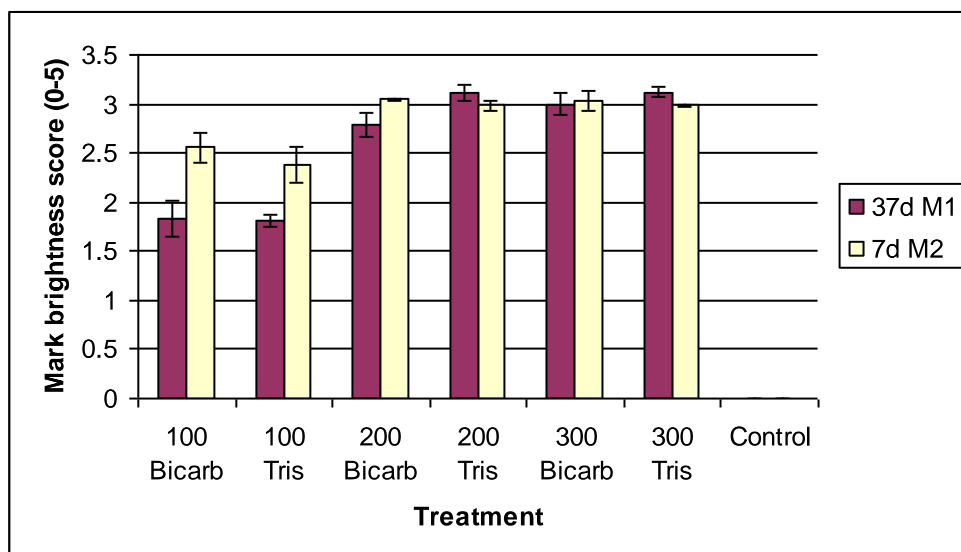

Figure 35. First comparison of the brightness of the first and second marks in Experiment 6. Mean mark brightness scores (± se) for mark 1 (37 days post-treatment) and mark 2 (7 days post-treatment)

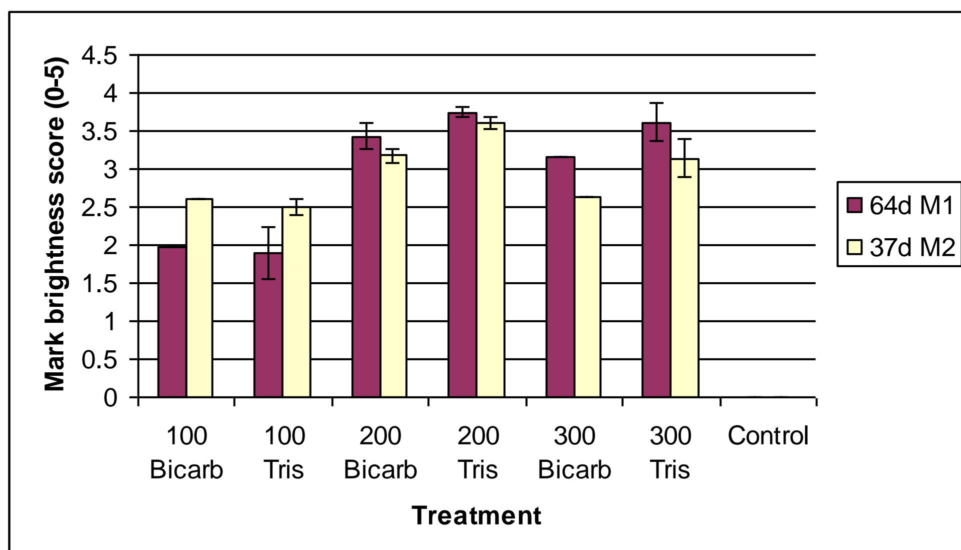

Figure 36. Second comparison of both marks in Experiment 6. Mean mark brightness scores (± se) for mark 1 (64 days post-treatment and mark 2 (34 days post-treatment) Treatments with 100 and 300 Mg L-1 OTC with bicarb buffer do not show error bars due to lack of replication

5.4 Fluorescent shell marking of the pearl oyster *Pinctada imbricata*

This supplemental experiment with pearl oysters successfully tested the procedures and systems invoved in the shell marking protocol that had been re-designed (from previous experiments) for scallops. Mortalities were negligible throughout the experiment.

At the beginning of the experiment oysters were measured using the longest possible measurement of shell to indicate shell ‘height’. Average (± s.e.) oyster shell height was 21.0 ± 3.4 mm. Growth was very rapid over the two months in the system

(Figure 37), with a final average shell height of 36.1 ± 0.3 mm. The OTC concentration and buffer type did not significantly effect growth or survival.

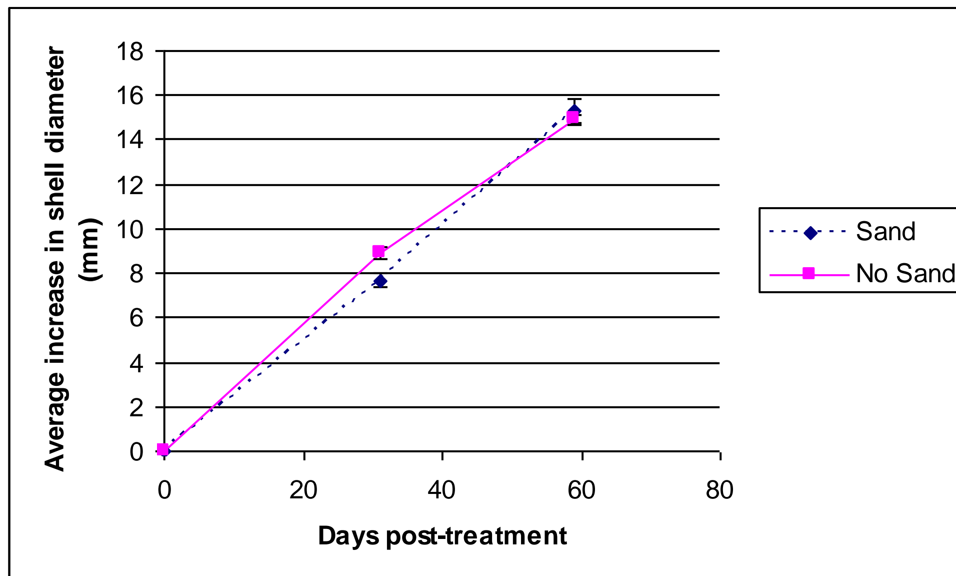

Figure 37. Increase in mean shell height of oysters (*n* = 14, ± s.e.) with or without sand on the floor of screens

Assuming there was no tank effect (note that this was reduced by alternating tanks weekly), screens within tanks were used as replicates to compare the growth and mark retention with and without a sand bottom environment. These effects were also not significant. This result in particular suggested that although microalgae was clearly filtered out of suspension by the down-welling water current through the sand (shown previously in Figure 31), this did not negatively affect food supply in the water column for the oysters in the simulated sea bed system. The availability of suspended microalgae in both systems was supported by chlorophyll *a* concentrations, which was measured at 3 µg L^{-1} in both tanks (no replication). Unlike scallops, the oysters were usually attached to the side of screens rather than sitting amongst the sand, so would be unlikely to utilise the concentrated algae on the surface of the sand as was postulated for scallops.

The large and complex shells of the oysters made mark detection extremely difficult using a microscope, due to a high level of autofluorescence and uneven depth of field. It was more practical to view oysters through the plastic shield of the microscope without magnification. Using this method combined with regular checking of potential marks under the microscope, we were able to distinguish between treatments and controls based on average mark brightness scores ($P < 0.01$). But there were no significant differences between treatment groups detected (Figure 38). A large number of false-positives were recorded to the extent that these marks would probably not be useful for commercial application. The very low mortality observed in this study indicates that concentration time of immersion time could potentially be increased to produce brighter marks that may be more easily distinguished from the autofluorescence which is present in *P. imbricata* shells. Alternatively, a different marking chemical such as calcein may be more easily distinguished from this natural autofluorescence.

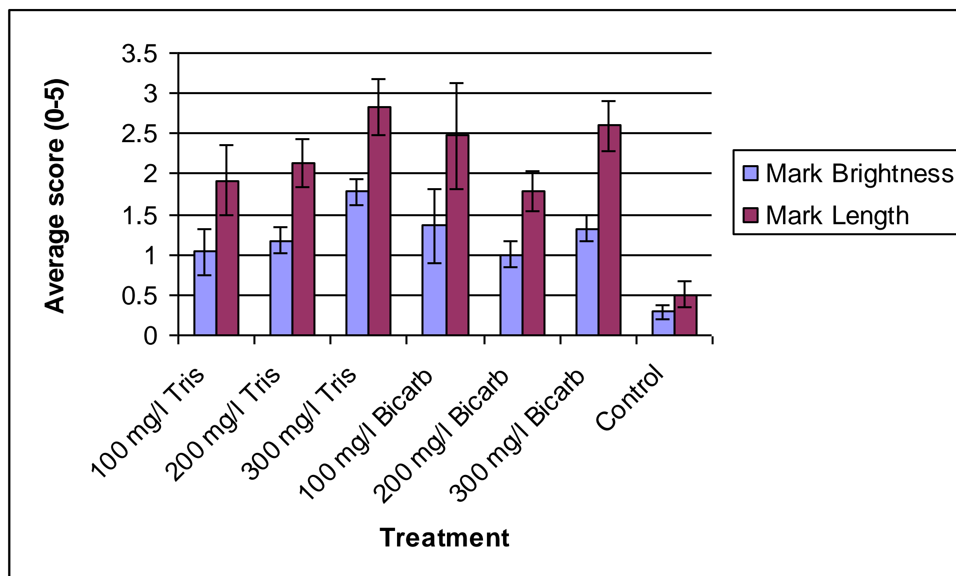

Figure 38. Mean (± s.e.) mark length and mark brightness scores (*n* = 4) among treatments in pearl oysters

5.5 General Discussion

<u>Suitability of the marking chemicals for *A. balloti*</u>

Alizarin red S has been used successfully in the past to mark fish (Blom *et al*., 1994; Eckman, 2003; Bashey, 2004; Jenkins *et al*., 2006; Baer and Rosch, 2008) as well as the clams *Mercenaria mercenaria, Mya arenia* and *Mulinina lateralis* (Hidu and Hanks, 1968) at concentrations ranging from 0.25 to 20 mg L^{-1}, and in abalone at 10-60 mg L^{-1} (Day *et al*., 1995). Strong, clearly visible marks were achieved in the present study using alizarin red S at 10 or 20 mg L^{-1}, however a lower proportion of marked individuals and higher mortality in alizarin red S treatments relative to other treatments and controls indicated that alizarin red S is not ideal for marking *A. balloti*.

Calcein has been used in the past to mark fish (Wilson *et al*., 1987; Brooks *et al*., 1994; Leips *et al,* 2001), and molluscs (Day *et al*., 1995; Kaehler and McQuaid, 1999; Moran, 2000; Moran and Marko, 2005). Our study has demonstrated that *A. balloti* can be reliably marked with little or no toxicity at doses of 50 mg L^{-1} or lower. The marks produced by calcein were not as strong as OTC marks, however concentrations used in our study did not reach a toxic level, and therefore it is likely that higher concentrations of calcein could be suitable for shell marking in *A. balloti*. A concentration of 100 mg L^{-1} was used with no negative effect in the bay scallop *Argopectens irradians* (Moran and Marko, 2005). Calcein has been used to mark freshwater mussels at 250 mg L^{-1} with negligible mortality (Eads and Layzer, 2002) and up to 640 mg L^{-1} with no mortality in the brown mussel (Kaehler and McQuaid, 1999).

The high cost of calcein (AUD$22 g^{-1}) reduces its commercial appeal (Secor *et al*., 1991). Uncertainty over the toxicity of calcein and alizarin red for release into the wild and also for human consumption (Mohler, 2003) make these two chemicals less preferable for commercial use than OTC, although the food and drugs administration in the USA has given calcein an Investigative New Animal Drug permit, whereby it can be used to mark fish smaller than 2 kg (Heneyfield *et al*., 2006). The use of calcein or OTC in scallops would be far less dangerous than in fish because there is

no risk of accidental consumption of the scallop shell, unlike the small scales and bones of fish.

OTC has been used in the past to mark a range of organisms including fish (Koenings *et al.*, 1996; Taylor *et al.*, 2004) and a range of marine molluscs (Jackson, 1990; Pirker and Schiel, 1993; Day *et al.*, 1995). In this study OTC was shown to produce strong marks with negligible mortality at concentrations of 200 or 300 mg $L^{-1.}$ Higher mortalities observed in some long-term OTC treatments indicated that the concentrations used in our study were approaching a toxic threshold for *A. balloti*. It is the only one of the three chemicals used in this study that is already approved for minor use by the APVMA. Based on this, OTC was deemed to be the most suitable chemical for ongoing use for commercial purposes.

Choosing the best buffer for OTC solutions

Although both bicarb and Tris are historically used with biological systems, the evidence presented here suggests that Tris should preferentially be used to stabilise the pH of OTC water baths instead of bicarb. Its effects are quicker, more stable over several hours, and without the potentially damaging precipitation effects of higher pH which can result from bicarb use. The relatively low concentrations of bicarb which avoid excessive chemical precipitation can not adequately adjust the pH of relatively high OTC concentrations, whilst Tris concentrations in the order of 0.6 g per 1 g OTC provide an assumed acceptable and stable pH of 8. Not withstanding the results from Experiment 6 where these buffers did not significantly affect mortality or growth, if bicarb is to be used for future trials, it may be best to age the solution to stabilise pH before exposure to the scallops. The other benefit of Tris over bicarb is that the mark produced was more defined using Tris, which would be especially important when using multiple marks to differentiate cohorts.

Multiple marking can provide more detailed feedback

During this study it has been shown that it is possible to mark the shells of scallops more than once without causing significant mortality. Multiple marks are of great interest to industry for their potential to create a ‘bar code’ system, whereby specific cohorts with specific deployment strategies can be labelled separately and their success can be tracked though time. This type of marking would enable the industry to improve spat deployment strategies in a rapid way. Multiple marking has been a priority for marking in fish otoliths (Tsukamoto *et al.*, 1989; Taylor *et al.*, 2005a), where the use of alternating chemicals has been successful to make bar-codes more easily distinguished (Secor *et al.*, 1991). Our study has shown that both OTC and calcein can be used to double-mark *A. balloti*, and it is possible that both of these chemicals could be useful for further investigation. With 27 days in-between double-marking with OTC, both marks were clearly distinguishable in the vast majority of individuals. Ideally though, marks would be closer together to minimise the time spent in the hatchery, so the use of alternate chemicals may be helpful to allow better bar-codes to be produced. Further work may be required to develop protocols for barcode production for commercial use.

Mark retention in the wild

The maximum lifespan of *A. balloti* is around 3 years in the wild (Dredge, 1988). For OTC, mark brightness is slowly diminished by exposure to light (Choate, 1964; Blom *et al.*, 1994). Light conditions in our study were not measured, although it can be expected that light penetration to the scallop’s normal habitat at 20 m depth would be relatively minimal, especially considering the tendency for scallops to cover

themselves in sand. The retention of marks in this study would likely be an underestimate, firstly because they were constantly exposed to low-level light (although this was minimised by housing them indoors with shadecloth covers), and secondly because the scallops were exposed to very intense light for assessment of marks. If mark fading were to become an issue in future experimental work, OTC can be detected at very low levels using a fluorometric technique (Koenings et al., 1986). This method could help to test our ability to detect marked shells after initial trials, however it would come at an extra financial cost to industry.

Could sand abrasion wear the mark off?

The bivalve shell is formed by the mantle, which begins by creating an initial, often pigmented layer on the extremity called the periostracum, followed by a calcite layer and then an aragonite layer on the inside (Fritz et al., 1994; Marin and Luquet 2004; Lin and Meyers, 2005). Fluorescence in marked shells is incorporated into all new shell during marking, which includes the entire thickness of the new shell. To increase thickness and strength of the shell as the animal grows, the aragonite is continuously deposited on the inside of the shell. The outer layers cannot be repaired because the mantle has no access to the exterior shell surface. In a scallop's shell, the outer shell on the left valve is pigmented, and the inside is white. If shell abrasion were to occur, we would therefore see white, smooth shell on the oldest, originally thinnest part of the shell of wild-caught adults, not pigmented periostracum from the original shell deposition. Since wild-caught adults generally retain pigmented shell on one valve showing concentric growth rings right to the shell ligament where the earliest shell was produced (Figure 39), we can have some confidence that shell marks will not be removed by abrasion in the wild.

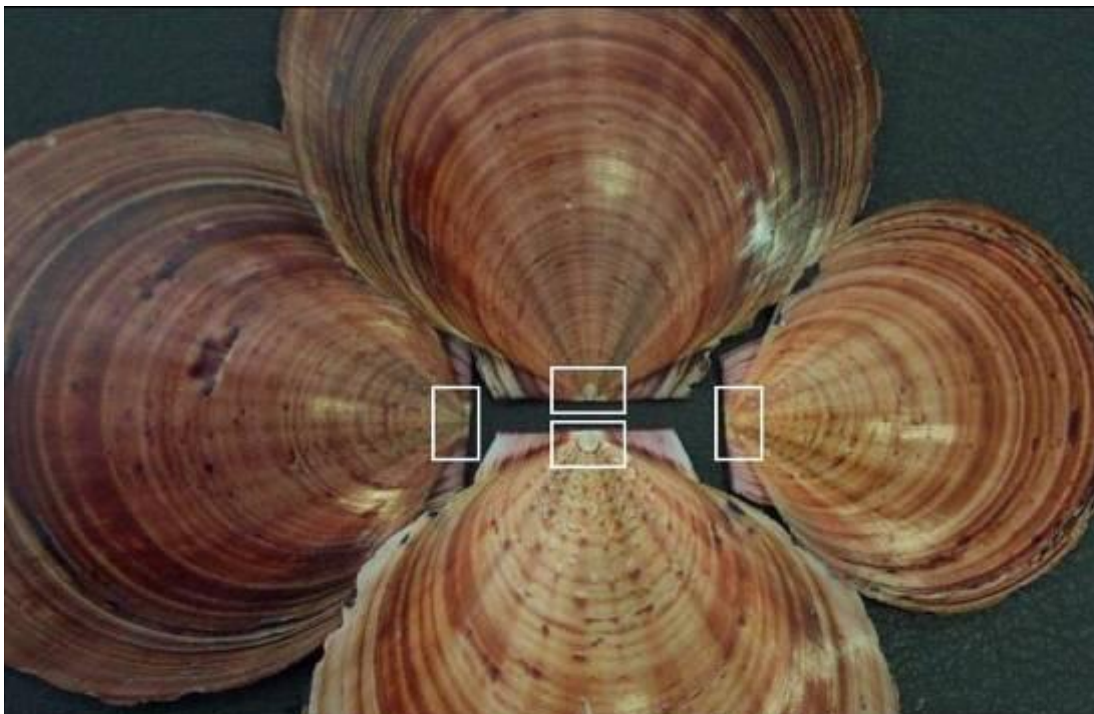

Figure 39. Adult wild-caught *Amusium balloti* shells. Boxes show the oldest part of the shell which the scallop laid down as a juvenile. Note very early shell is often less pigmented

Some individuals (top and bottom in Figure 39) show white shell in this area, however it is not clear whether this is from lighter colour in the original juvenile shell or abrasion. Light-coloured juvenile shell has been observed in hatchery-reared *A. balloti* (pers obs.). No abrasion was observed during the two month trial in this study

with scallops reared in a sand-bottomed, seabed simulation system. In a previous study where larval shells of bay scallops *Argopecten irradians* were marked with calcein, wild-caught juveniles were observed under electron microscope and their larval shells were still intact, indicating that shell abrasion is very minimal in *A. irradians* in a similar natural habitat to *A. balloti* (Moran and Marko, 2005).

6. BENEFITS AND ADOPTION

This project has demonstrated that the saucer scallop can be marked using fluorescent chemicals which are incorporated into the shells during shell formation whilst the scallops are immersed in the chemicals. Methodology has been refined to reduce stress on the scallops during chemical treatment and maximise the brightness of the mark. Longevity of marked shells has been tested in an indoor system and the marks have been shown to remain visible for at least 10 months.

The availability of this method will enable our commercial partners QSS Ltd to identify hatchery-produced scallops in trawl catches. This will enable the success of re-seeding to be evaluated, which will provide better information for investors into future re-seeding ventures (eg. Western Australia). The first marking trials have been conducted by QSS Ltd in late 2007 with advice from DPI&F staff. This type of marking has enormous potential to benefit the scallop ranching industry and the fishery because it allows reliable marking of large numbers of small individuals in a cost-effective, safe manner. Information gathered from mark/ recapture data can not only help to establish the economic benefits of ranching, but also provide ecological information regarding the survival, movement and growth of scallops in the wild. The ability to conclusively identify hatchery-produced scallops may also assist in the prosecution of those suspected of illegal trawling inside the sea-ranch areas.

We have also demonstrated that multiple marks can be produced. The ability to distinguish between different batches of spat will allow deployment strategies to be optimised, which should increase the efficiency and overall effectiveness of scallop re-seeding.

7. FURTHER DEVELOPMENT

This work forms the most recent part of a growing body of knowledge (Dredge, 1981; Rose *et al.*, 1998; Cropp, 1993; Dredge *et al.*, 2002; Jebreen *et al.*, 2003; O'Brien *et al.*, 2005; Scoones and McGowan, 2006; Wang, 2007), which contributes towards enabling the saucer scallop to be re-seeded in an effective and responsible way. In 1997, Blankenship and Leber published some standard protocols for developing, appraising and managing marine stock enhancement programs. They listed the ability to identify hatchery produced stock as a critical component of their 10-step management approach. Protocols for marking and differentiating cohorts allows quantitative measures of success to be defined, as well as the use of empirical processes to define optimal release strategies. The ability to trace the movement of scallops after release to some extent also provides the industry with the capability to generate species-based biological and ecological information such as validated life-history patterns used when optimising fishery decision-making processes. The ability to mark hatchery-reared scallops together with improved understanding of their movement patterns after deployment may also assist in prosecution of illegal fishing inside the ranch areas. This has been raised as a priority for the industry, as enforcement against suspected unlawful trawling practices has been difficult.

Taylor *et al.* (2005b) have recently reviewed responsible marine re-stocking efforts in Australia. Batch marking methods have been considered vital to the approaches of all of these programs (eg: Russell and Rimmer, 1997; Taylor *et al.*, 2005a). Chemical marking has been investigated in most of these programs, and where such methods have proved difficult (eg: OTC in Palmer *et al.*, 2000) other methods have been employed. Logically, the next step for *A. balloti* is to test the present shell marking and identification methods in the field and to develop a rapid bar-coding system that provides maximum information to the industry with low mortality. The ability to refine deployment strategies by marking various groups of released individuals resulted in a 400% increase in survival of re-stocked striped mullet in Hawaii (Leber *et al.*, 1997), and similar improvements could result from optimising the size, location and timing of release of *A. balloti*.

8. PLANNED OUTCOMES

This project provides shell marking methods which could potentially solve the key issue of determining the origins of saucer scallops recovered from sites where juvenile scallops have been released as part of ranching activities. This was the primary planned outcome from this research. This planned outcome was generated by the previous inability and future need of sea bed ranching projects to unequivocally prove the value of hatchery release programs.

The marking protocol developed in the present project was successfully tested in a replicated artificial environment. Its testing in the field was outside of the possible scope of this project, but should be considered a vital part of future research. Once validated in the natural environment, these marking and identification methods will be instrumental in allowing researchers to quantify the survival, growth and dispersion of juveniles deployed onto seabeds.

The project achieved multiple marking results which surpassed its primary goals. This will further aid in understanding the natural biology of the species, and in assessing and optimising possible release strategies. The involvement in the project of researchers from Queensland and Western Australia, as well as industry, will help ensure these results are fully available for commercial uptake.

Supplementary marking research for another molluscan species, namely the Akoya pearl oyster *Pinctada imbricata,* was also undertaken as part of this research project. This was another species identified as one whose separation of hatchery from wild recruits would help solve husbandry problems or could provide beneficial management options. This secondary activity was planned as a risk management strategy, and for pre-trialling scallop marking protocols and equipment. It was executed opportunistically using time windows created by scallop spat supply shortfalls, and provides useful background information for this and other species.

Artificial rearing system improvements for juvenile scallops were also investigated in attempts to improve the mark testing environment. Methods developed during approved project extensions were successful in significantly reducing mortalities in marked juveniles.

9. CONCLUSIONS

Fluorescent chemicals can be incorporated into the shells of *A. balloti* without causing mortality. These marks can remain visible in live scallops for at least 10 months in land-based systems, and probably much longer in the lower-light conditions of their natural seabeds. Optimal marking in this study was achieved by immersing 2-5 mm scallop spat in seawater with OTC at 200 or 300 mg L^{-1} and 120 or 180 mg L^{-1} Tris, respectively. It is possible that calcein could also be useful for shell marking for *A. balloti*; however with the concentrations used in this study the marks were not bright enough for confident identification. OTC has the advantages of being less expensive than Calcein and having approval for minor use by the APVMA. Alizarin red S was also used in this study, however it resulted in unacceptably high mortalities and poor mark quality.

The pearl oyster *Pinctada imbricata* was also tested for mark incorporation using OTC. The marking was successful, however marks were very difficult to distinguish from autofluorescence, to the point where marking with OTC would not be recommended for commercial use with *P. imbricata*.

Conditions for rearing juvenile scallops were developed during this study. Improvements in survival and growth of juvenile scallops provided further understanding of optimal nursery conditions for scallops, and this may have direct application for industry participants wishing to grow spat to larger sizes prior to release.

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11. APPENDICES

11.1 Intellectual property

No intellectual property issues arose during the research.

11.2 Staff

Dr Paul Palmer – Principal Investigator from June 2007

Dr Elizabeth O'Brien – Principal Investigator until June 2007

Dr Sizhong Wang – Fisheries Technician from late 2005

Dr Tim Lucas – Fisheries Biologist from April 2007

Dr Elizabeth Cox – Fisheries Biologist 2006-April 2007

Dr Nicholas Wade – Fisheries Biologist 2005

Mrs Jan Rose – Casual Fisheries Technician 2006-2007

Dr Kate Simpson – Fisheries Biologist 2005

Mr Provan Crump – Fisheries Technician 2005

11.3 Publications and other dissemination

The dissemination, extension and commercialisation plan for this project has been implemented, 1) to inform industry of methods developed to mark saucer scallops for later identification on recapture, and 2) to inform researchers, other interested industries and the wider public of these methods for general information and broader use. This plan has highlighted the successful use of FRDC funds and the quality of our research. The key messages have been the value of identifying hatchery stock for industry development and fisheries research, whilst stating that the marks are not harmful to animals or the consumer.

Project officers have had regular email and telephone contact with the industry partner, QSS Ltd, and have supplied them with all milestone reports with detailed follow-up discussions. The wider aquaculture and seafood industries, researchers, and the general public have been progressively informed of project results through the newsletter articles, conference presentations and general publications listed and presented (where possible) below. Several copies of the final report (when accepted by the FRDC) will be supplied to QSS Ltd and other parties who have registered specific interest in the work, and a peer reviewed scientific publication has been submitted. This final report contains a summarised manual (see Section 11.5) which details stepwise procedures for the optimal marking methods for scallop spat as determined in this research project.

The successes of this communication plan are demonstrated by the repeated requests from general media outlets for stories about the project.

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Abstract for poster to be presented at the Australasian Aquaculture Conference in Brisbane, August 2008.

FLUORESCENT MARKING OF SCALLOP SHELLS TO KEEP TRACK OF HATCHERY-PRODUCED STOCK IN THE WILD.

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The saucer scallop *Amusium Balloti* is a valuable fisheries resource for Queensland, however wild catches are known to fluctuate dramatically between seasons. Methodology has now been developed to stabilise catches by supplementing natural recruitment with hatchery-produced spat at 2-5 mm. This project aimed to help identify the hatchery-produced spat upon recapture so that the success of the re-seeding strategy could be evaluated. Furthermore, identification of hatchery-produced scallops enables feedback, which will help to optimise spat deployment strategies. The perfect marker would incorporate into the shell in a rapid, reliable way, be easily identified upon recapture 8-12 months later, be non-toxic for both the scallops and human consumption, and be approved and affordable for commercial use.

Three chemicals alizarin red S, calcein and oxytetracycline (OTC) were tested in this study for mark quality, mark retention and toxicity to scallops. Marks were identified and visually assessed for brightness using an epifluorescence microscope. Of the three chemicals, OTC was identified as producing a brighter, more persistent mark than calcein, and lower mortality than alizarin red S. Fluorescence incorporated into the scallop shells was still visible after 10 months of culture in controlled conditions.
In a separate experiment, scallops were then marked twice, one month apart with negligible mortality, and reared in a simulated sea-bed environment with a sand substrate. This demonstrated that the brightness of the initial marks was comparable to new marks, and that 'bar codes' could be created with multiple shell mark patterns. Multiple marks could be used to distinguish between cohorts or size classes of cultured spat. The sand substrate was found to prevent shell-fouling and resulted in higher survival and growth than previous experiments.

This study determined that immersing scallops for 3 days in OTC at 200 or 300 mg L^{-1} can produce reliable fluorescent shell marks with negligible mortality. Marks can persist for at least 10 months in living scallops, and this indicates that the methodology will be useful for application in the commercial hatchery in Bundaberg. Our commercial partners Queensland Sea Scallop Ltd are currently undertaking preliminary commercial trials.
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Article (in prep) for '**Aust-Asia Aquaculture**' outlining scallop marking success and general scallop rearing methods at BIARC.

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Queensland Government 'Report to Farmers' (Ross Lobegeiger and Max Wingfield, 2008) for aquaculture production in 2006/2007. p39.

Scallop marking

In Queensland and Western Australia, ranching of saucer scallops *Amusium Balloti* is being undertaken in order to reduce the variability of wild catch between seasons and increase overall scallop production. Queensland Sea Scallops (QSS Ltd), based in Bundaberg, have been able to produce large numbers of scallop spat from wild-caught broodstock in recent years. They then release 2-4 mm spat into specific seabed leases with the aim of recapturing a proportion of them the following season.

In order to evaluate the success of ranching, methods needed to be developed for discriminating hatchery-reared scallops upon recapture. Marking the hatchery reared stock would also facilitate monitoring of scallop growth and movement after release. Since 2005, a project has been underway to develop and optimise methodology for marking the shell of the saucer scallop using fluorescent chemicals. The original list of 3 possible marking chemicals has now been narrowed down to one – oxtyetracycline (OTC), which can be incorporated into the calcium carbonate of the shell during shell formation with low cost, low stress for the scallops and no residues in the meat, which is sold for human consumption.

Recent trials at the Bribie Island Aquaculture Research Centre have demonstrated that scallops can be immersed in an OTC chemical solution for 3 days, which results in a mark which is easily visible under a microscope using ultra-violet light. The scallops showed negligible mortality during or after chemical immersion. Scallops can even be treated twice over a month to produce 2 fluorescent growth rings, which will allow specific batches to be tracked in the wild. A mark retention trial has shown that the marks can remain visible for at least ten months in living scallops, and so the marks are likely to be useful for commercial purposes. QSS Ltd is currently undertaking shell marking trials for release into the wild.

This Project is supported by the FRDC and our industry partners, Queensland Sea Scallops.

For further information contact Tim Lucas (Fisheries Biologist) on (07) 3400 2019 or tim.lucas@dpi.qld.gov.au

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Three minute segment on television program 'Escape with ET'.
This story featured DPI&F research into scallop shell marking at the Bribie Island Aquaculture Research Centre, and the benefit of the FRDC funded program for industry and the public. Story went to air on 22/03/08.

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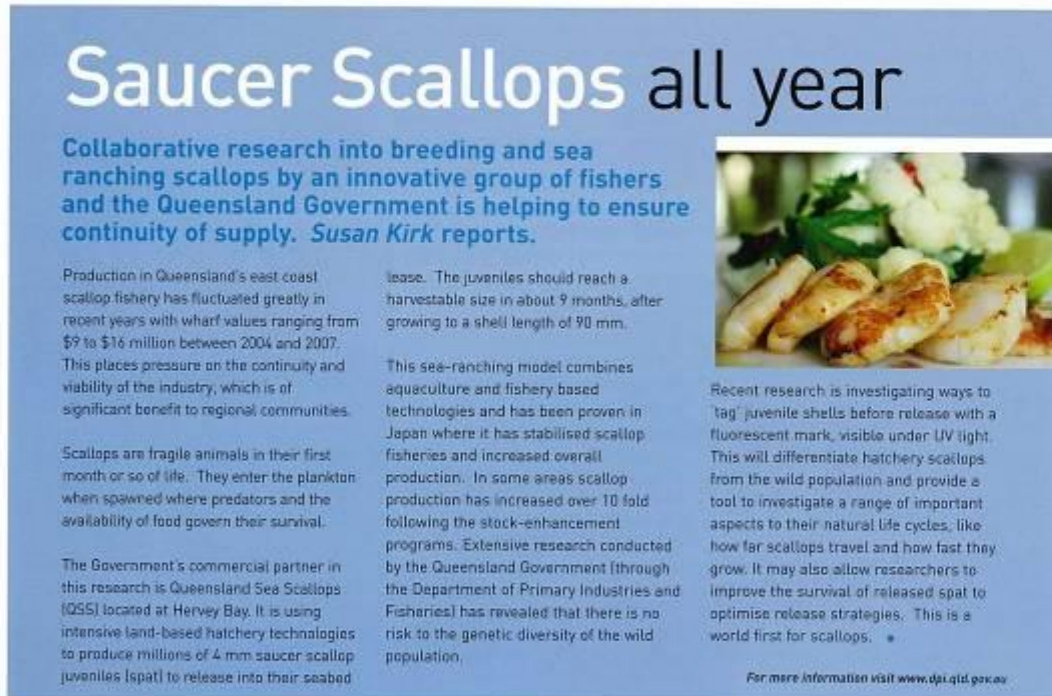
Article published in 'Taste Queensland 2008'



Saucer Scallops all year

Collaborative research into breeding and sea ranching scallops by an innovative group of fishers and the Queensland Government is helping to ensure continuity of supply. *Susan Kirk* reports.

Production in Queensland's east coast scallop fishery has fluctuated greatly in recent years with wharf values ranging from $9 to $16 million between 2004 and 2007. This places pressure on the continuity and viability of the industry, which is of significant benefit to regional communities.

Scallops are fragile animals in their first month or so of life. They enter the plankton when spawned where predators and the availability of food govern their survival.

The Government's commercial partner in this research is Queensland Sea Scallops (QSS) located at Hervey Bay. It is using intensive land-based hatchery technologies to produce millions of 4 mm saucer scallop juveniles (spat) to release into their seabed lease. The juveniles should reach a harvestable size in about 9 months, after growing to a shell length of 90 mm.

This sea-ranching model combines aquaculture and fishery based technologies and has been proven in Japan where it has stabilised scallop fisheries and increased overall production. In some areas scallop production has increased over 10 fold following the stock-enhancement programs. Extensive research conducted by the Queensland Government (through the Department of Primary Industries and Fisheries) has revealed that there is no risk to the genetic diversity of the wild population.

Recent research is investigating ways to 'tag' juvenile shells before release with a fluorescent mark, visible under UV light. This will differentiate hatchery scallops from the wild population and provide a tool to investigate a range of important aspects to their natural life cycles, like how far scallops travel and how fast they grow. It may also allow researchers to improve the survival of released spat to optimise release strategies. This is a world first for scallops. •

For more information visit www.dpi.qld.gov.au

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2007 Enterprising Women in Rural Industries Trade Show October 9 & 10. Royal on the Park Hotel in Brisbane- project profiled on the DPI&F stand demonstrating how science can contribute towards industry development, with interactive display of live scallops and posters. Liz O'Brien and Liz Cox present.

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Science in parliament 8th August 2007. Meeting held to discuss the project with Mr Jack Dempsey MP, Member for Bundaberg, Shadow Parliamentary Secretary to Shadow Minister for Police and Corrective Services. Dr Paul Palmer and Dr Liz O'Brien in attendance. Article published in the proceedings as follows.

Seafood delicacy starts life on land – new approach offers potential to rejuvenate Qld scallop industry

A revolutionary approach to grow saucer scallops on land is set to rejuvenate the Queensland scallop industry – potentially increasing productivity from $15 million to $150 million a year.

DPI&F scientists assisted in the development of culture techniques and genetic baseline studies for saucer scallops. This underpinned the development of a land-based commercial scallop hatchery at the Port of Bundaberg.

The hatchery is now growing scallop eggs to a juvenile size of about 4 mm long. These "spat" are then transported to sea beds in the Hervey Bay area where they continue to grow into healthy 90 mm scallops ready for harvest.

The beauty of this approach, if commercially successful, is that the scallop fishing industry would be stabilised with more reliable harvests, enabling long-term employment prospects. Substantial flow-on effects could be expected through the full range of support services including trawler operators, processing facilities, transport companies and many other related industries. It also means a healthier, more sustainable marine environment – with the capacity to precision harvest the sea beds in the lease area.

DPI&F scientists are continuing to support this promising restocking initiative with further technology development. Current DPI&F research is developing tools to mark the hatchery-produced scallops so that return on investment can be assessed and factors such as migration and survival studied..

The project has been a collaborative venture from the outset, with a number of past and present DPI&F researchers working closely with Queensland Sea Scallop Pty Ltd, the University of the Sunshine Coast, the University of Queensland, and researchers and industry representatives from Western Australia.

Contact Details:
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Dr Liz O'Brien
Project Leader (2001 - 2006)
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Email: liz.o'brien@dpi.qld.gov.au
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Oral presentation, 16th Intenational Pectinid Workshop
Halifax, Nova Scotia, Canada
11-18 May 2007
Scallop Aquaculture Workshop Session
Presented by Peter F. Duncan
Title: "The Ups and Downs of Scallop Aquaculture Down Under"

Abstract

Scallop aquaculture has had a relatively poor record in Australia, despite several commercial species, well-developed fisheries, domestic and export seafood markets, clean water and diverse climatic conditions. Reasons for limited aquaculture development are those typically reported in many other high-cost economy countries.

Fisheries declines over the last two decades have encouraged efforts to develop aquaculture. This paper reports on current scallop aquaculture and research activity in Australia.

Victoria

In 2002 the Commonwealth Government funded research by RMIT University to investigate hatchery production of *Pecten fumatus* for potential reseeding. However project was abandoned largely due to water-quality problems.

There is no state government-based R&D work on scallop culture in Port Phillip Bay, although in June 2006 the Victorian Government auctioned marine aquaculture leases. Leases are for 21 years, with additional 10-year option for a government-determined market-value fee. A combinatorial auction was conducted on-line over 2-3 hours, and used a weighted bidding system to maximise value for the state government. Bidders submitted expressions of interest, and a AU$5000 refundable bond.

Seventeen leases, between 2.5-27 hectares were sold, with 7 deep enough for suspended culture, 5x18ha (16-18m) and 2x27 ha leases (20-25m). Average sale price was AU$3000 per hectare. Lease conditions require site development within 3 years, i.e. at least one longline per ha, otherwise the lease is cancelled. After 3 years lease can be sold, although the auction attracted no speculators. Additional leases will be available in future (14 x 27 ha, > 30m). Leases likely to be initially for *Mytilus edulis* culture, with scallop culture later. Scallop culture in Port Phillip Bay will rely on natural settlement, although hatchery development is proposed.

There are no seabed leases and dedicated restocking is not allowed. The issue of seabed property rights has yet to be addressed.

Queensland

Queensland Sea Scallop (QSS) Ltd, established in 2002, comprises 24 local share holders, 6 full-time and 5 casual employees. The company conduct spat (hatchery) production and seabed ranching operations with *Amusium balloti* in Hervey Bay, where they have unique seabed leases totalling 72 km^2.

An 80,000 L larval-rearing capacity hatchery was constructed in 2004/05, and has mixed-species algal production capacity of 800 L /week from batch culture and 1,500 L /week via a Bayes system. Approximately 500,000, 4mm spat were seeded in 2004, 3,000,000 in 2005 and 5,000,000 in 2006 (total 8.5 million). Harvest of hatchery-produced scallops occurred in 2005, with intended expansion to densities of 1 scallop/m^2 on seabed sites. Future programme development includes multiple research projects; marking hatchery spat (see below), broodstock maturation,

modelling hydrology, algae and larvae dispersion around seeding sites and hatchery-bacteria control.

Identification of hatchery-reared animals is important for gauging stocking success, differentiation from natural settlement, ownership issues etc, but molecular markers are expensive and require specialised facilities. Several chemical dye markers are being trialled; calcein, oxytetracycline and alazarin red, all having a shell-calcium binding mechanism. Fluorescent bands (Figure) are visible under hand-held UV light for the first two chemicals, and under normal light for alizarin red S. Ongoing work is investigating tag longevity, toxicity effects and multiple applications for cohort differentiation.

Tasmania
Main Tasmanian hatchery (Shellfish Culture Ltd) is not currently producing scallop spat, since their single customer is now focussed on mussel culture. The hatchery is producing mainly oyster (*C. gigas*) spat.

Western Australia
The *Amusium balloti* hatchery and seabed culture operation in WA has finished, mainly due to variable results in hatchery production following the stocking of 12 million spat in 2003. Broodstock nutritional status may be a critical factor in variable larval quantity and quality. The hatchery has now closed and the leases are likely to lapse.

No scallop production or research activity is reported from New South Wales**,** South Australia or Northern Territory.

Peter F. Duncan, University of the Sunshine Coast, Maroochydore DC, Queensland, 4558, Australia. Phone: 0061 7 5430 2831, pduncan@usc.edu.au,

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Revolutionary Science Day- Organised by DPI&F for promotion of science as an exciting career for gifted students. Live and cooked scallops were part of an interactive display set up by Tim Lucas for the 'Innovative Aquaculture' stand, held at the Queensland Academy of Science, Mathematics and Technology, Toowong, Qld,13th April 2007.

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Queensland Government 'Report to Farmers' (Ross Lobegeiger and Max Wingfield, 2007) for aquaculture production in 2005/2006. p34
Scallop marking

The marking of scallops (*Amusium balloti*) for release and recapture is part of the FRDC supported project designed to distinguish hatchery-produced stock from wild stock.

Saucer scallop fishery is one major component of multi-species fisheries in Queensland and Western Australia. Saucer scallop is widely recognised as the

world's finest scallop meat and attracts landed prices of more than $20 per kilogram on domestic markets, and significantly more in overseas markets.

The majority of scallop production is destined for export to Asia, Europe and the US. Annual landings have fluctuated dramatically both in Queensland and Western Australia. Overseas experiences have shown that sea ranching of scallops could not only reduce the landing fluctuation, but also increase production by over 10 times. In Australia, to offset the variable catches of saucer scallops resulting from the wild fishery, sea ranching of saucer scallops is currently being undertaken by industry in Western Australia and Queensland using hatchery-produced juvenile.

One of the key issues to evaluate the success of the sea ranching venture is to be able to identify hatchery-produced stock. Managers can then determine the contribution of hatchery stock to the final harvest as well as having the ability to monitor survival and dispersal. Identification of hatchery seed is also essential to determine the optimal size of scallops and time for deployment to the seabed. However, the choice of identification method is restricted by the cost, ease of use, stability and precision.

A wide range of chemicals have been evaluated and tested for marking hatchery-produced juveniles. These chemical candidates must be harmless to the animals and safe for human consumption. Preliminary trials indicated that two of three chemicals showed very promising results. Based on these, experiments are being conducted to systematically evaluate a suitable treatment time and concentration of potential chemicals. Also, the retention time and intensity of these chemical marks on the juveniles are being assessed. The trials are also being extended to test additional mollusc species such as pearl oysters. The Australian Pesticide and Veterinary Management Authority has approved field trials of commercial numbers of scallops to test the suitability of a marker in the natural environment.

For further information contact Liz Cox (Fisheries Biologist) on (07) 3400 2019 or liz.cox@dpi.qld.gov.au

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Food Bulletin Magazine News story 30 October 2006

Department of Primary Industries and Fisheries
Queensland Government

Research serves up scallop sensations

Queensland has long been known for its quality ocean delicacies, with prawn cocktails, fish and chips, and barramundi a menu staple. But it is the Queensland Saucer Scallop that is now on centre stage with innovative aquaculture research.

Scallops are a premium product, known for their delicate taste as a stand-alone seafood that requires little other ingredients as a serving suggestion. However until recently its variable catch rate has meant poor market availability.

Researchers at the Queensland Department of Primary Industries and Fisheries are stabilising catches through new technology to culture juvenile saucer scallops in land based hatcheries before they're released to sea.

DPI&F Senior Industry Development Officer Liz O'Brien said the early stages of the saucer scallop's life are the most vulnerable, so the hatcheries give them a head start.

"We grow the larvae to 2 to 4 mm in a controlled environment and then transport them to aquaculture lease to grow free-range on the sea bed," Ms O'Brien said.

"Because they are free-ranged, they can breed with the wild scallops and increase the natural population."

But as with any breeding program, the effects on the genetic make-up of a species' population are a top priority.

"The mass release of scallop juveniles has the potential to alter the genetic structure of the existing saucer scallop population, so we instigated new research to monitor genetic diversity."

DPI&F's genetic feasibility study is a world first for scallops, established on international best practice standards to measure the reproductive interaction between populations and species diversity.

"We compared specimens from Queensland and Western Australian locations and found that they appear to reproduce in isolation – which basically means that Queensland hatchery broodstock can be obtained from any Queensland location without interfering with the genetic make-up.

"Accurately monitoring genetic diversity is essential to maintaining natural genetic variation, otherwise the scallops' capacity to adapt is lost – increasing the threat of new diseases, predators and climate change."

The success of the new scallops research is further demonstrated through tagging to track whether the majority of harvested shell actually originated in the hatchery or through wild recruitment.

"The scallop shell is marked with a fluorescent tag visible only under UV light, using dyes harmless to the scallop and subsequent consumer."

So the good news for the consumer is that the Queensland Saucer Scallop is now available as a market-ready seafood, based on sustainable production methods able to meet international demand.

"The Queensland Saucer Scallop is a significant export commodity particularly in Asia, with a gross value of $10-12 million making it a growing investment market," Ms O'Brien said.

"Its popularity as a premium food product is due to the pleasing presentation of its very white flesh when cooked, and a sweet, delicate flavour.

"The wider availability of the Queensland Saucer Scallop for processors means seafood lovers can look forward to seeing it on restaurant shopping lists."

For further information please contact:

DPI&F on 13 25 32 or (07) 3404 6999
www.dpi.qld.gov.au

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Article in 'Aust-Asia Aquaculture'
Issue: Vol 20.4 Aug-Sep '06

Mollusc team at Bribie kick goals for commercial beginning

The QDPI Bribie Island mollusc team headed by Dr Liz O'Brien is thrilled with recent achievements in saucer scallop (*Amusium balloti)* and tropical abalone (*Haliotis asinina*) research that have forged a solid beginning for a promising commercial harvest in a few years time.

Located 70 kilometres north-east of Brisbane on the ocean side of Bribie Island, the Queensland Department of Primary Industries and Fisheries (DPI&F) Bribie Island Aquaculture Research Centre (BIARC) covers an area of 15 hectares. Employing about 30 scientists the centre's research includes an impressive range of projects (see Table).

Facilities at BIARC include:

- General purpose biology laboratories;
- A comprehensive biotechnology laboratory, with three dedicated rooms and capacity to undertake a range of molecular techniques;
- Boats (1x5.7m SeaJay, 130Hp, 1 x 5.2m Sea Jay, 70Hp);
- An extensive seawater supply and filtration system, including UV sterilised water for larval rearing;
- A variety of tanks (from 40 tonnes to 100 litres) for holding and carrying out replicated experiment on live marine organisms;
- A wide range of scientific equipment for carrying out biological research;
- Offices, workshop and boatshed; and
- Ponds (4 x nursery ponds, three of those are covered, 4 x growout ponds).

An interest in culturing saucer scallops (*Amusium balloti)* was sparked following a massive drop from $23 million to $7.5 million in Queensland's wild scallop fishery (from 2001 to 2003). The BIARC was quick to respond to the industry's woes and first cultured the saucer scallop in 1999 and the 'mollusc team', as Dr Liz O'Brien affectionately calls it, was born. Consisting of just three people, Dr Peter Duncan as the team leader, Jan Rose, the algal technician, and Sizhong Wang, a PhD candidate from the University of Queensland they succeeded in a world first culturing of saucer scallops.

As part of a collaboration with the University of Queensland, Dr Duncan and Mr Wang commenced work with Dr Wayne Knibb (DPI&F) and Dr Bernie Degnan (UQ) in 1999 to develop the technology to be able to culture *A. balloti* in the hatchery. During his studies, Sizhong successfully conditioned wild broodstock and stimulated them to spawn on cue. He also determined the optimal rearing temperature and undertook preliminary nutritional trials to increase larval survival. At settlement he was able to achieve 30% survival and then begin to understand the settlement process through detailed experimentation. This included testing cues for settlement including chemical and tactile stimuli.

The research team also described the attachment process and discovered that there is a byssal attachment but it is very weak compared with other bivalves. Historically it was thought that this species were unable to attach.

Because of the promising results of Sizhong's research Fisheries Research and Development Corporation (FRDC) invested into another project to determine whether scallop restocking was feasible in Australia. The feasibility project was led by Mike Dredge. The study included biological, economic and social analysis and concluded that searanching *A. balloti* was feasible. In particular the rapid growth rate and high value of the product was an advantage.

The FRDC project '*Enhancement of Saucer Scallops (Amusium balloti) in Queensland and Western Australia – Genetic Considerations 2003/033*' was completed in 2005. Dr Duncan and Ms Rose have since left the team but Dr O'Brien, who has led the team since 2001 and expanded the research to also focus on tropical abalone, is thrilled that their work to date has played a large part in industry development.

The FRDC report listed the following outcomes achieved by the research:

- Equipped management with a resource to make more informed decisions regarding the management of scallop sea ranching ventures in Queensland and Western Australia.
- Increased industry and management awareness and understanding of the value of genetic management in aquaculture, particularly sea-ranching ventures.

- Collected baseline genetic microsatellite data for the *Amusium balloti* sea-ranching areas prior to significant restocking for post-stocking comparison over time.
- Development of polymorphic microsatellites for *Amusium balloti* that can be used in genetic management of the hatchery.
- Clarified taxonomic status of Australian *Amusium balloti.*

Following analysis of the data, Dr O'Brien explained that the distribution of genotypes was similar between the areas sampled, which paved the way for maintaining genetic diversity while reseeding. "The research aimed to understand the genetic make up of the animals in the wild so broodstock selection could be managed for responsible reseeding. The data indicates that the genetic information of saucer scallops is shared between animals within Queensland waters. In other words, there must be movement of larvae up and down the coast because the adult scallops we sampled are related based on their DNA."

Dr O'Brien said the BIARC encouraged industry to try commercial scallop aquaculture following the centre's success culturing the scallop. "It was very satisfying for us that our research has directly helped industry. It is a testament to the value of the research that it was only four years between concept and commercialisation. We maintain a very close research relationship with the company Queensland Saucer Scallop Ltd and are developing and undertaking new projects with them." (QSS Ltd is a private company formed by seafood processors and trawler owners to commercially farm scallops in Queensland.)

Broodstock and spawning

"QSS Ltd is using intensive land-based hatchery technology to produce millions of 4mm saucer scallops to release into its sea bed lease in Hervey Bay in areas known to support good scallop growth," Dr O'Brien explained. "Usually a year later the scallop are harvested by trawling, not dredging, at about a size of 90mm."

Not wanting to share QSS's techniques, Dr O'Brien was guarded about the specific details of seedstock production. However, she said the broodstock used for the research at DPI&F, about 300 per season, were collected from Harvey Bay or Gladstone before being transported back to Bribie Island in chilled sea water. Broodstock were assessed for gonad status and spawned at a 1:1 ratio.

To induce spawning the scallops were kept in cool static seawater and then given a heat shock with warmer water; a common practice to induce spawning in a bivalve. "The scallops spawn very quickly, usually within a two hour period. After fertilisation and then hatching, the free-swimming larval stage lasted for about 18 days before the minute molluscs settled. The scallops were grown in tanks for another 2 months and when they reach about 4mm they were used for experiments."

Marking method

Dr O'Brien said the next and most vital problem to overcome was to mark the scallops to differentiate the hatchery-reared scallops from the wild stocks so the farm could confidently claim the stock as its own. This tagging had to meet several criteria including:

- Ability to mark small individuals;
- Can be detectable in the adult;
- Unique to the cultured population;
- Suitability for identification of individuals or cohorts;
- Inexpensive to apply and detect;
- Harmless to the tagged animals and subsequent consumer and
- Acceptability to the public.

"Based on the criteria, chemical tags (dyes) in the shell, invisible to the naked human eye, are being trialled. The juvenile scallops, provided by industry partner QSS Ltd, were immersed in a seawater bath containing one of three dyes, which made it inexpensive to apply and suitable for small individuals. After staining for two days the bands are visible under a hand-held UV light although some may require some level of magnification, making it relatively inexpensive to detect."

Marked scallops were held in a simulated seabed system for a year to determine if the dyes would be visible in the adult. "Scallop survival is recorded to ensure the dyes were harmless and, if required, multiple application of the dye could occur."

Dr O'Brien said the dyes bind to the calcium in the shell and did not affect the animal on the inside because exposure time to the dye was limited and the animals grew significantly from 4mm to 90mm before harvest. "The process is harmless to the subsequent consumer."

The first stage of the marking research was nearly complete. "All dyes have successfully been incorporated into the juvenile shell and are detectable under UV light. The next stage is to optimise the marking methods and then document retention of the dye in the growing shells over time."

The marking project (FRDC 2005/016) was co-funded by the FRDC, QDPI&F and QSS Ltd and was collaboration between QDPI&F scientists, mollusc expert Rick Scoones from Western Australia and QSS Ltd staff.

An additional saucer scallop project involves investigating the reproductive status of broodstock throughout the season to better understand impediments to spawning and larval survival.

Tropical abalone

With the scallops research under control the mollusc team turned their attention to another interesting mollusc the tropical abalone (*Haliotis asinina*). Dr O'Brien thought that the tough little creature had an exciting commercial future. "They're amazing, they live on a coral reef where temperature can vary from 17 to 38^{0}C. The species naturally spawn in synchrony every two weeks during summer and grow to a market size of 55mm in a year."

"The University of Queensland, a source of technical information for the BIARC, has worked for the past six years on biotechnology of tropical abalone. The information gathered by the University presented a positive future for tropical abalone farming."

BIARC, together with industry partner Coral Sea Mariculture in Bundaberg, are undertaking a research project '*Aquaculture of the tropical abalone: identifying and selecting for factors promoting high settlement, survival and growth'*. Dr O'Brien said the research was funded by an Australian Research Council Linkage grant. "We plan to commence commercial trials of tropical abalone culture as well as looking at the genes involved in settlement and growth."

To complement this research Dr O'Brien is also organising a feasibility study to investigate the suitability of tropical abalone aquaculture for Queensland. This aims to provide potential investors with the background to make an informed decision about investing in tropical abalone aquaculture.

The DPI&F mollusc team are also working to develop technical expertise to assist the edible oyster (*Saccostrea commercialis*) and Akoya pearl (*Pinctada imbricata*) industry as research requirements develop for these industries.

By Emma Rudge and Dos O'Sullivan

For more information contact Dr Liz O'Brien, Department of Primary Industries and Fisheries, Bribie Island Aquaculture Research Centre, PO Box 2066, Woorim QLD 4057, Phone: 07 3400-2019, Fax: 07 3408-3535, email: liz.obrien@dpi.qld.gov.au

Table 1: Projects underway at Bribie Island Aquaculture Research Centre

- sand (*Portunus pelagicus*) and mud crab (*Scylla serrata*) culture,
- soft shell crab trials,
- selective breeding of tiger prawns (*Penaeus monodon)*, and banana prawns (*Penaeus merguiensis*)
- culture of marine prawns (*P. merguiensis*) in inland saline water,
- use of existing inland water, such as ring tanks and water used for agriculture, for aquaculture,
- production of organic prawns,
- marine biotechnology projects such as identifying genes involved in the moulting process in crabs and maturation in fish.
- developing molecular markers for aquaculture species for selective breeding (parentage identity) and aquaculture management.

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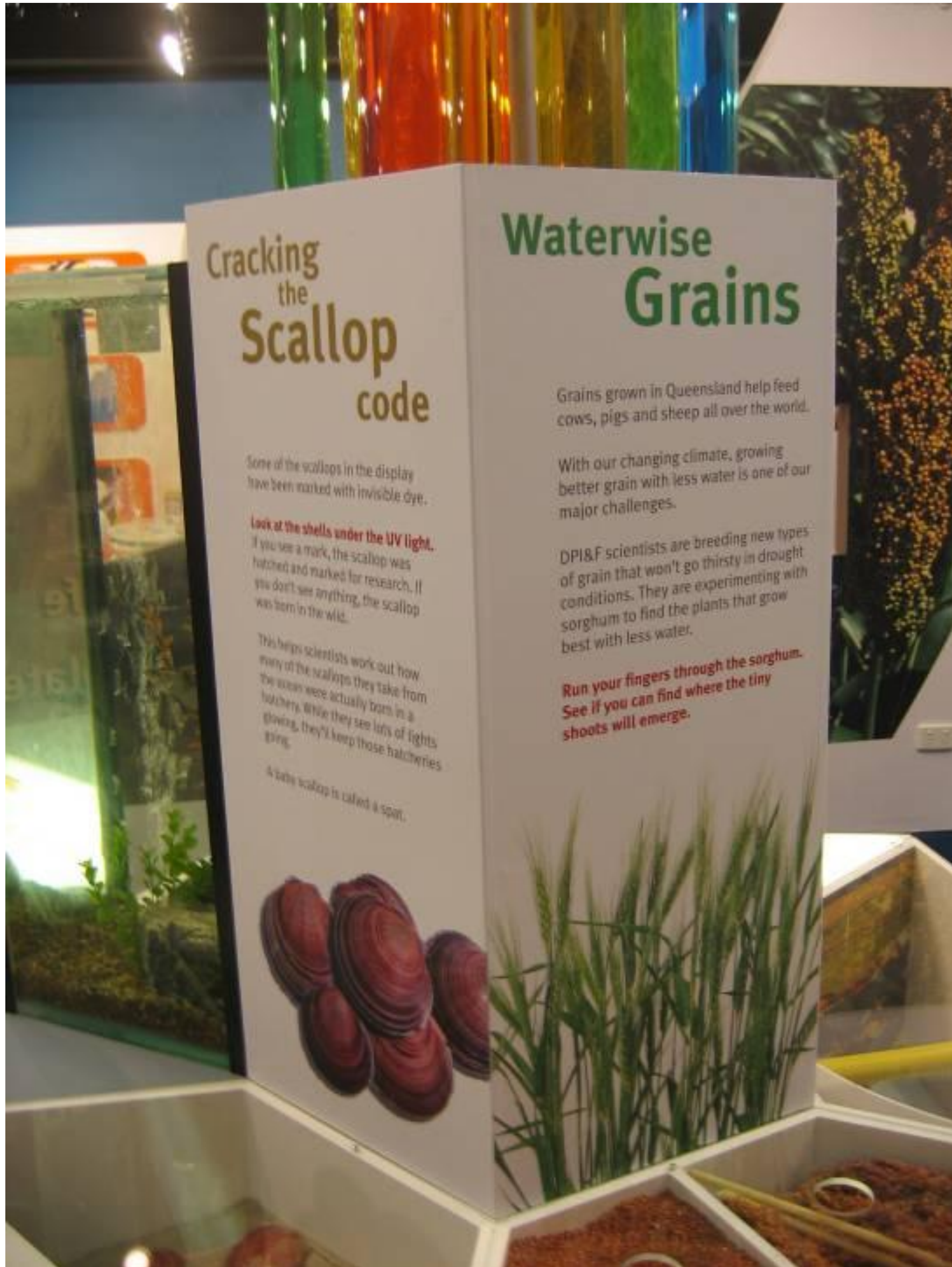
Royal Brisbane Exhibition August 2006 interactive display at the DPI&F corporate stand.



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Scallops - marked for success. Department of Primary Industries and Fisheries Aquaculture Newsletter Issue 28, July 2006

By Liz O'Brien

Juvenile saucer scallop with fluorescent mark
Hidden under the coloured bands of the beautiful saucer scallop shell (*Amusium balloti*) is another band, visible only under UV light. These shells are a result of early trials in a Fisheries Research and Development Corporation (FRDC) funded project being run at DPI&F. The aim is to mark hatchery reared scallops to differentiate them from wild stock.
Queensland Sea Scallops Ltd (QSS Ltd) are using intensive land-based hatchery technology to produce millions of 1-4mm saucer scallops to release into their sea bed lease. This approach aims to increase the reliability and availability of scallops within the lease area. These animals will be harvested in around a years time, after growing to a stunning shell length of 90mm.
However, the costs of production for hatchery stock are high and to verify reseeding success researchers need to demonstrate that a majority of the harvested shell actually originated in the hatchery and were not the result of a successful wild recruitment. One method to determine success is to count the proportion of tagged scallops in the final harvest. The ability to identify stock can also be used for other projects such as deployment method trials and growth and survival data collection.
There are several criteria a tagging method should satisfy (Rothlisberg and Preston 1992), it should: be able to mark small individuals; be detectable in the adult; be unique to the population; be suitable for identification of individuals or cohorts; be inexpensive to apply and detect; be harmless to the tagged animals and subsequent consumer and be acceptable to the public. Based on these criteria, trials of invisible chemical tags in the shell were chosen.
The dyes chosen are applied by immersion of the juvenile scallops in a saltwater bath containing one of three dyes making it inexpensive to apply and suitable for small individuals. It is anticipated that the bands should be visible under a hand held UV light, that may require some level of magnification, making it relatively inexpensive to detect. To determine if the dyes will be visible in the adult, marked scallops will be held in a simulated sea bed system for a year. Scallop survival will be recorded to ensure the dyes are harmless and, if required, multiple application of the dye can occur. This can facilitate cohort identification through unique or multiple dye banding patterns. The dyes bind to calcium in the shell not affecting the animal inside. Exposure time to the dye is limited and the animals will grow significantly from 4mm to 90mm before harvest meaning that the process will be harmless to the consumer.
The first stage of the research is nearly completed. All dyes have been incorporated into the juvenile shell and are detectable under UV light. The next stage is to optimise the marking methods and document retention of the dye in the growing shells over time.
This project (FRDC 2005/016) is co-funded by the FRDC, DPI&F and QSS Ltd and is a collaboration between DPI&F scientists, mollusc expert Rick Scoones from WA and QSS Ltd staff.
URL: http://www.dpi.qld.gov.au/cps/rde/xchg/dpi/hs.xsl/30_2789_ENA_HTML.htm

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Queensland Government 'Report to Farmers' (Ross Lobegeiger and Max Wingfield, 2006) for aquaculture production in 2004/2005. p37

Scallop marking

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One of the key issues to evaluate the success of the sea ranching venture is to be able to identify hatchery-produced stock. Managers can then determine the contribution of hatchery stock to the final harvest as well as having the ability to monitor survival and dispersal. Identification of hatchery seed is also essential to determine the optimal size of scallops and time for deployment to the seabed. However, the choice of identification method is restricted by the cost, ease of use, stability and precision.

A wide range of chemicals have been evaluated and tested for marking hatchery-produced juveniles. These chemical candidates must be harmless to the animals and safe for human consumption. Preliminary trials indicated that a number of chemicals showed very promising results. Based on these, experiments are being conducted to systematically evaluate a suitable treatment time and concentration of potential chemicals. Also, the retention time and intensity of these chemical marks on the juveniles are being assessed.

Publication

O'Brien, E., Bartlett, J., Crump, P., Dixon, B., and Duncan, P., (2005), Enhancement of Saucer Scallops *(Amusium balloti)* in Queensland and Western Australia – Genetic Considerations, Project Number 2003/033

For further information contact Liz O'brien (Fisheries Biologist) on (07) 3400 2019 or liz.obrien@dpi.qld.gov.au

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11.4 Permit for commercial use of OTC

Australian Pesticides &
Veterinary Medicines Authority

PERMIT TO ALLOW RESEARCH USE OF AN AGVET CHEMICAL PRODUCT

PERMIT NUMBER -PER9622

This permit is issued to the Permit Holder in response to an application granted by the APVMA under section 112 of the Agvet Codes of the jurisdictions set out below. This permit allows a person, as stipulated below, to use the product in the manner specified in this permit in the designated jurisdictions. This permit also allows any person to claim that the product can be used in the manner specified in this permit.

THIS PERMIT IS IN FORCE FROM 20 DECEMBER 2006 TO 31 DECEMBER 2008.

Permit Holder:
Department of Primary Industries and Fisheries
144 North St
WOORIM QLD 4507

Persons who can use the product under this permit:
Researchers of Queensland Department of Primary Industries and Fisheries, and Queensland Sea Scallop Ltd.

CONDITIONS OF USE

Product to be used:
CCD OTC (Oxytetracycline Hydrochloride Water Soluble Powder)

Directions for Use:

Animal	Procedure	Rate
Juvenile *Amusium balloti*, Queensland Saucer Scallop	Identiciation/marker - incorporation of the dye into the calcium matrix of the shell.	750 mg/L oxytetracycline in buffered seawater

Critical Use Comments:
Harvest adults scallops at a shell length of 90 mm.

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Withholding Period:
12 months after immersion in oxytetracycline/buffered seawater solution.

Jurisdiction:
QLD

Specific Location:
Hervey Bay, Queensland Sea Scallop Ltd aquaculture lease.

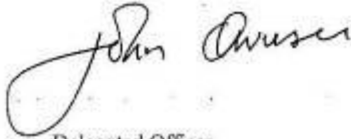
Additional Conditions:
PERSONS who wish to prepare for use and/or use the products for the purposes specified in this permit must read, or have read to them, the permit particularly the information included in DETAILS OF PERMIT and CONDITIONS OF PERMIT.

This Permit provides for the use of CCD OTC (Oxytetracycline Hydrochloride Water Soluble Powder) in a manner other than specified on the approved label.

Authorised persons are permitted to immersed juvenile scallops of shell length 2-4 mm once in a solution of 750 mg/L oxytetracycline hydrochloride in buffered seawater for seven days during the spawning season.

Maximum Amount of Scallops Treated:
Up to 10 million juvenile scallops

Issued by



Delegated Officer

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11.5 Manual for the marking of scallop spat with OTC

Marking the shell of saucer scallop spat with fluorescent dye

A manual to assist commercial development of marking techniques for *Amusium balloti*

Tim Lucas, Paul Palmer and Sizhong Wang

The Department of Primary Industries and Fisheries

July 2008

Brief background

The saucer scallop *Amusium balloti* is undergoing sea ranching in Hervey Bay, Queensland. The success of sea-ranching is yet to be determined because survival of hatchery-reared spat in the wild is not able to be assessed. Marking the hatchery-produced spat before release will enable survival to be estimated upon recapture, and hence the economic viability of the business to be evaluated. Multiple marking will allow more detailed feedback regarding survival, growth rates and movement of specific cohorts of spat. This kind of information could help to optimise release strategies and also help to understand the ecology of this species.

Incorporation of fluorescent dyes into the shell can be done cheaply, quickly, easily and reliably. Millions of spat can be marked at once with negligible mortality, and without chemical residue in the flesh. Detection of the mark in adult scallop shells should be relatively straight-forward and rapid, although this has not been tested in full-sized shells, and the mark longevity has not been tested in the wild, although in our testing, the marks were retained in living scallops for more than 10 months in an indoor system. The broad-spectrum antibiotic oxytetracycline (OTC) has been tested for this application and has been approved by the Australian Pesticides and Veterinary Medicines Authority for commercial use by Queensland Sea Scallops until the end of 2008. This manual is designed to give commercial producers a practical insight into our procedures at the Bribie Island Aquaculture Research Centre (BIARC) to facilitate rapid uptake of the shell-marking methodology on a large scale.

Acclimatising the scallops

Because the brightness of the mark is dependant on the amount of shell deposited during chemical immersion, it is important that the scallops are growing well during the chemical treatment. Scallop spat were obtained from QSS Ltd and transported in 20 L carboys with aeration, which contained around 2000 spat. We often found that there was a period of high mortality following transport, so in order to ensure that only healthy spat are involved in marking, one week should be given to transported spat before use in marking to allow recovery. We found that scallops grew well on a diet consisting of 5 species of microalgae, namely *Isochrysis galbana*, *Chaetoceros muelleri*, *Pavlova salina*, *Pavlova lutheri* and *Proteomonas sulcata* at a final concentration of 30,000 cells. This was grown at high concentrations in 2-4 L flasks before being mixed together in 5,000 L tanks which were then used to slowly supply scallops over a 48 h period. Water quality was also important. To improve water

quality at BIARC we used 20 µm sand-filtered water with additional settlement for at least 24 h to reduce fine suspended particles. Ideally, the water temperature in the marking solution should be stable, between 20-28°C and the temperature shock should be minimised by acclimatising them to this constant temperature before chemical treatment begins. All counting and handling should also be done well before marking commences.

Equipment

In order to conduct chemical marking of scallops there is an implied requirement for scallops rearing facilities, so the specific requirements for marking will obviously vary according to existing facilities. Our chemical immersion protocol involves daily renewal of chemical solutions over three days. To reduce handling stress on the scallops, we used mesh screens to hold the scallops (Figure 1), so that they could be lifted out of the old solution and into fresh solution with minimal exposure to the air and without being physically handled. Our most recent scallop marking was done on a small scale with 40 scallops in a 100 mm diameter mesh screen in buckets with 6 L seawater. This would obviously have to be scaled up for commercial use, keeping in mind that over-crowding may reduce growth rate and hence reduce mark incorporation. A room with controlled temperature at around 25°C is also ideal. Because there is no water flow and the volumes are so small, temperature fluctuation in a major potential stressor to the scallops and should be minimised. Apart from the container used to hold the marking solution and chemicals, another equally sized container should be kept in the same room to allow the water for the next water change to acclimatise. A third container is required as a reservoir for used marking solution whilst it is slowly released through carbon filtration. The room should have adequate lighting to maintain microalgae which will be at 30,000 cells mL^{-1} in the marking solution. This lighting should be switch off at night to reflect natural day length.

Figure 1. Mesh screens used to hold scallops, immersed in marking solutions. These were constructed using 250 mm PVC pipe, with 500 µm mesh around 30 mm from the bottom. Vents should be cut in the PVC underneath the mesh to allow water circulation. Each of these screens housed 60 scallops with average shell diameter 5 mm.

Chemicals

We found that there were two equally effective concentrations of OTC, 200 or 300 mg L^{-1} both work equally well. For simplicity we will use 200 mg L^{-1} in this manual. OTC has an expiry date and should not be used after that date. The biological ½-life is about 10-15 hours (Chemwatch, 2007) so should only be used freshly mixed. It is also toxic, especially when inhaled as a dust, so be careful when handling it. Also gloves, eye protection, a dust mask and lab coat should be worn to reduce risk of exposure. Because OTC creates an acidic solution in seawater, a buffer is also required. Our recommendation is to use Tris buffer at 0.6 × the OTC concentration. This will ensure that the pH remains close to that of seawater (8.1-8.2) for the duration of the treatment. Sodium bicarbonate can also be used at 2 × the OTC concentration. We found that although both Tris and sodium bicarbonate could be used with very low mortality, Tris was more effective at stabilising pH and resulted in lower precipitation and a clearer mark.

Protocol

1. Acclimatise seawater to the temperature of the room (may take 24 h)
2. Add algae to final concentration of 30,000 cells mL^{-1}
3. Weigh out appropriate volumes of OTC (200-300 mg L^{-1}) and Tris (0.6 × OTC volume). For a 200 L container that's 40 g OTC + 24 g Tris.
4. Mix well. Both OTC and Tris dissolve easily. The solution should look slightly yellow.
5. Add scallops, taking care to minimise the exposure time of the scallops to the air, and add some mild aeration. We used 4 mm tube with no air stones in 13 mm airlifts for aeration. Strong aeration can result in a lot of froth which we thought was best avoided. Frothing and precipitation occurs at higher pH (over ~8.2; common using sodium bicarbonate as buffer), but does not appear to affect scallops or the marking process.
6. Fill new tank for acclimation to room temperature
7. After 24 h mix up fresh solution of microalgae, OTC and Tris and transfer scallops. Before disposal of the old marking solution, first treat it by dripping the solution slowly through activated carbon to adsorb toxic molecules.
8. Fill new tank to acclimate
9. After a further 24 h renew solutions again and discard used solution. Leave scallops for another 24 h
10. Three days (72 hours) after scallops were first immersed in OTC solution, remove them and return them to their regular culturing environment (or release directly to seabed).

This process can be repeated to produce multiple marks, however this has only had limited testing. We know that with a gap of 27 days between chemical immersions scallops can be successfully double marked with negligible mortality. To achieve good resolution with double marks, and to be able to put the marks closer together with no confusion, it is recommended that calcein marks should be alternated with OTC marks, however the methodology and approval for this needs further work.

Mark Detection

For our experimental purposes, mark detection was done using a epifluorescence microscope (Nikon Microphot FXA) using a super-high pressure mercury lamp with an ultra-violet (UV) filter (DM455, Nikon). Marks can also be detected using small hand-held UV torches, however marks are not easily visualised in this way. It is likely

that specialised lights will need to be developed for commercial detection of the marks, incorporating portability with the light intensity required for effective discrimination of fluorescence.

Scallops only have a tiny amount of autofluorescence around the hinge, and the fluorescence of eyespots is easily distinguished from marked shell because it is not continuous. After immersion in OTC for 3 days, a ring of fluorescence will be visible around the periphery of the shell, where the majority of new calcium was being deposited during that time. A less intense fluorescence should also be detectable over the entire shell because a small amount of shell is constantly deposited to thicken existing shell (so this fluorescence is actually coming from the inside of the shell and is only visible because the shell is opaque). Figures 2 and 3 show marked shell through the viewing shield (unmagnified) and through the microscope eyepiece. Through the microscope, fluorescence is visible as bright green light, but without the microscope marked shell is a whitish colour, which appears to be much brighter than surrounding unmarked shell. In our experience marks were very easy to detect under the viewing platform without viewing through the microscope eyepieces, which would indicate that a commercial mark-viewing light can be easily developed. Note that intense UV light is dangerous and protection should be given to eyes and skin during exposure to UV light.

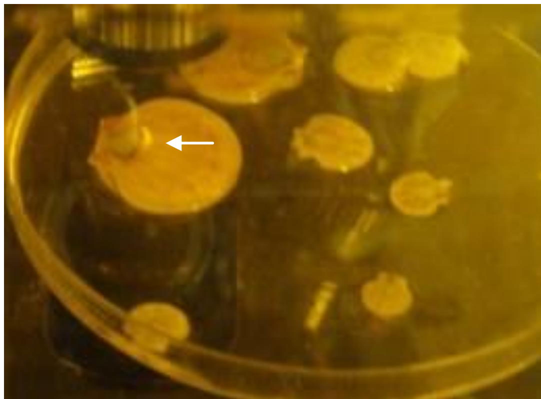

Figure 2. OTC-marked scallops viewed without magnification through protective plastic shield. White arrow indicates fluorescent mark under UV light.

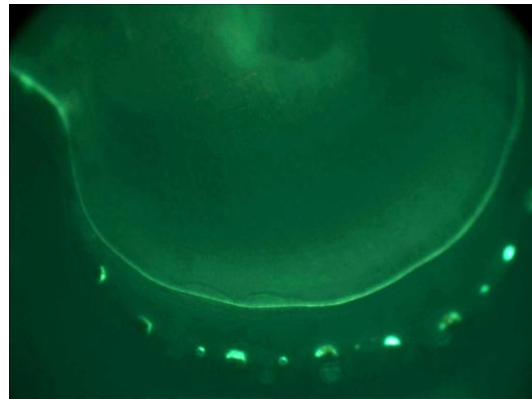

Figure 3. A typical OTC-marked scallop under 40X magnification with appropriate light filter.