Evaluation of antifoulants on overcatch, other forms of biofouling and mudworms in Sydney rock oysters

Final Report to
Fisheries Research and Development Corporation

Project No. 98 / 314

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CMBB

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Objectives

1. To evaluate the effectiveness and commercial practicality of an antifoulant coating as a means of protecting cultured oysters from overcatch of both Sydney Rock and Pacific Oyster spat and from other forms of biofouling.
2. To assess and improve the cost effectiveness and commercial practicality of these treatments.
3. To determine whether benefits attached to objectives 1 and 2 can be achieved when extended to a representative array of commercial oyster farming areas and techniques used throughout NSW, Tasmania and South Australia.
4. To assess whether successful types of antifouling coating techniques pose significant risks to the environment, to the oysters themselves or to consumers.

Non-Technical Summary

Outcomes Achieved

The outcomes of this project provide the Australian Oyster industry with an alternative method for the control of fouling directly on shell and on equipment. This will provide economic benefits through reduced cleaning costs and increased subtidal culture of rock oysters. It may also facilitate the culture of oysters in areas previously unusable due to severe fouling with prohibitive cleaning regimes. The project will benefit the environment through a decrease in waste production by the industry. The availability of an alternative fouling

control mechanism will also contribute to improved shell properties which are required for the acceptance of rock oysters in the lucrative export trade.

FRDC Project 98/314 was commissioned to develop a solution to the problem of biofouling of oysters in the NSW oyster industry. The project was a collaboration between the Centre for Marine Biofouling & Bio-Innovation (University of New South Wales), New South Wales Fisheries, the Aquaculture CRC and Wattyl Australia Pty. Ltd. The project was strongly supported by the NSW Oyster Industry and NSW Fisheries and the objectives of the project were set through a consultative process with industry.

The objective of the project was to develop an antifouling treatment that is easily applied and prevents fouling, in particular oyster spat (over-catch), on rock oysters for six months. A successful outcome would result in a significant reduction in production costs primarily through reducing labour costs for the cleaning of shell. A successful treatment would also allow increased use of sub-tidal culture methods and decrease competition for food by fouling organisms resulting in increased growth rates. Furthermore, reduced mortalities due to mudworm infestation and current antifouling practices will increase productivity.

Trials were conducted over three years with each trial building on the results of its predecessor. Between trials coating formulations were improved to increase their efficacy in preventing fouling, and improve their life span. Trials were conducted in major oyster growing regions of New South Wales, Tasmania and South Australia. Over the three year tenure of the project the efficacy of the coatings was continuously improved resulting in a final fouling deterrent coating, OysterClear, which prevents the settlement of oyster overcatch, and inhibits the settlement and growth of barnacles, ascidians, tubeworms and algae. OysterClear is a non-toxic low surface energy coating that prevents fouling through physical "non-stick" properties for six months.

The project is being continued beyond the original three-year tenure by Wattyl Australia Pty. Ltd. as part of their commercialisation process to improve efficacy against fouling by a broader spectrum of fouling organisms, and to develop a quality assured product for the rock oyster and other shellfish industries. The final outcome of the project, OysterClear, a commercially available coating that prevents fouling for the rock oyster and other shellfish industries is projected to be released in 2003.

2. Introduction

NSW Oyster Aquaculture

Oyster farming is NSW's oldest and largest aquaculture industry. Production of the Sydney rock oyster (*Saccostrea glomerata)* currently dominates NSW aquaculture production comprising 78.8% of production (tonnes) and 66% of value (O'Sullivan and Dobson, 2001). The NSW oyster industry is based on a large number of small producers, with farmers spread over 41 estuaries (Nell, 1993).

Although the most common species farmed in NSW is the Sydney rock oyster, the Pacific oyster (*Crassostrea gigas*) has been cultivated in Port Stephens since 1991 following its earlier introduction and proliferation in that area. Prior to this the Pacific oyster was considered a pest because of its higher reproductive output, and because Pacific oyster spat outcompete Sydney rock oyster spat. Pacific oysters have a significant advantage over Sydney rock oysters as they have a shorter production cycle, reaching plate size in about 2 years compared with 4 years for Sydney rock oysters. The spread of the Pacific oyster in NSW estuaries has led to increased production costs and workloads for Sydney rock oyster farmers in NSW because of the problem of overcatch (Nell, 1993). However since legal farming of the Pacific oyster in Port Stephens, this industry has grown in value to $0.93 million in 1996/97 and further increased to $1.7 million in 1999/2000 (Aquaculture Production Report, 1999/2000).

Several trials have also assessed the viability of farming Flat oysters (*Ostrea angasi*) in NSW. A range of culturing methods are proving effective however the industry is struggling with post harvest and marketing problems. These arise from factors such as a shorter shelf life of this oyster (two-three days) and variable meat quality. Some of the factors that affect meat quality are growing height and biofouling (Heasman and Lyall, 2000).

Rack and tray culture is by far the most dominant method of production in NSW (Aquaculture Production Report, 1999/2000) and growers rely on abundant natural settlement for spat collection. Sydney rock oysters are serial spawners which means they release gametes several times during a season and can release up to 25 million eggs per spawning (Holliday, 1985). The spawning and spat settlement seasons in NSW vary between estuaries but generally occur between December and May. For example, at Port Macquarie (31°25'S; 152°55'E) a small spawning event occurs around December with the largest spawning most commonly occurring around March/April (L. Lardner pers. comm.). This is when the majority of farmers put out their spat collectors. Growers are moving toward single seed culture where spat are collected on wood or plastic strips, removed and then farmed in trays or baskets. This culture method has the advantage of producing a superior cup shape which is important for the half shell market (Nell, 1993).

Most oyster culture in NSW was initially subtidal but with the appearance of mudworm infestations, farmers adopted intertidal farming methods which provide some protection against mudworm (Nell, 1993). Subtidally farmed oysters generally grow faster than intertidal oysters as they are continuously submerged and therefore feed for longer periods of time (Wisely *et al*., 1979). However, the problems of fouling and mudworm are increased using subtidal culture methods. Farmers attempt to find the trade-off between optimal growth rates and condition and the control of fouling and mudworm infestation (Handley and Berquist, 1997).

Biofouling and Oyster Culture

Cultured oysters are particularly susceptible to biofouling as they are submerged for long periods of time, often occur in nutrient rich, high fouling areas and are grown in high densities. The common fouling organisms affecting oyster farmers vary considerably among different regions. However, the greatest problem in NSW is oyster overcatch (both Pacific and Sydney rock) and if left unchecked the spat grow over the entire shell and kill the oyster. They also deform the shell effecting oyster value in the half shell market. Furthermore, there are problems with transport as overcatch is less tolerant to dessication and dies quickly resulting in unsightly and odorous oysters.

Mudworm infestations are a significant problem and are the principal reason that intertidal culture was developed (Nell, 1993). Mudworms are boring polychaete worms with the most damaging species being *Polydora websteri* (Nell, 1993) which burrows into the shell and forms a blister on the inside of the shell. The blister is unsightly, particularly for the half shell market, and is easily broken releasing sediments, faecal deposits and anaerobic by-products (Handley and Berquist, 1997). It is very difficult to determine from the outside whether an oyster is infected with mudworm so measures for controlling infestation need to be preventative.

Barnacles are another problem fouling organism in NSW and South Australia. Extreme barnacle fouling interferes with the oyster's feeding behaviour and reduces growth (Nell, 1993). In Tasmania the main fouling problems stem from barnacles, solitary ascidians (*Ciona intestinalis*) and serpulid worms. Other fouling organisms on oysters include colonial ascidians, sponges, bryozoans, tube worms and algae. These organisms are often filter feeders and compete directly with the oysters for food and consequently affect growth rates (Claereboudt *et al*., 1994). While these organisms have a less pronounced effect than barnacles or overcatch they reduce the aesthetics of the oyster, particularly those on the half shell, through odour and unsightliness.

There are a number of established methods in oyster culture to reduce the effects of biofouling. Many NSW farmers control biofouling by taking oysters out of water for a 2-5 days every 4-8 weeks over the heaviest fouling period. This kills small spat and mudworm (Nell, 1993). Immersing oysters in hot water (~80^0C) for a few seconds and intertidal culture methods which expose fouling organisms periodically to air also control fouling. All of these methods are costly as they are labour intensive and they reduce the growth and condition of the oysters (Rikard *et al*., 1997).

The NSW oyster industry is currently in decline as farmers are not able to cost effectively produce oysters due to problems such as poor water quality, QX disease, winter mortality and biofouling. O'Sullivan (1998) reported a downturn in edible oyster production in 1996/97 attributed to consumer nervousness over food safety, losses due to disease, and importantly for this project, "outdated culture practises". Further, O'Sullivan and Dobson (2002), reported a continuing negative trend in Sydney rock oyster production due to the same concerns of food safety, diseases, outdated culture practises and reduced Sydney rock spat settlement due to competition from Pacific spat.

These problems are not restricted to NSW with edible oyster farmers in Tasmania and South Australia needing to control biofouling. Decreasing production costs while increasing production has been identified as critical for the further development of the oyster industry in S.A. and Tasmania (Australian Aquaculture Yearbook, 2001).

Farmers have identified that current methods to solve biofouling problems are not sustainable or cost effective and decrease oyster growth and condition. The industry has identified a clear need for alternative biofouling control.

Previous Antifouling Trials

The Centre for Marine Biofouling and Bio-Innovation at the University of NSW is a recognised leader in antifouling research and technology development. Over a period of several years a new generation of biodegradable, broad acting natural antifoulant products have been successfully isolated from macroalgae by the CMBB of the University of NSW (CMBB/UNSW). Laboratory trials conducted collaboratively by CMBB/UNSW and NSW Fisheries in September 1996 demonstrated that settlement and metamorphosis of hatchery Sydney rock oyster larvae could be totally inhibited by coating adult oysters with a carrier solution containing low levels of antifoulant without apparent adverse effect either to the coated oysters or to larvae that settled normally on the bottoms and sides of holding vessels.

Further improvements in coating technology were incorporated in an antifouling trial funded by a NSW Oyster Industry Research and Advisory Committee (ORAC) (Grant 1997). This trial, initiated in November 1997, comprised an intertidal and subtidal tray (raft) farming component, each comprising twelve fully replicated test and control treatments. The effects of carrier and coating combinations were evaluated in relation to overcatch by both Pacific oysters and Sydney rock oysters, and other common forms of biofouling (barnacles, sponges and filamentous algae). The coatings were very effective at deterring the settlement of oyster spat and other fouling organisms.

In response to the success of these trials and to develop a commercialisation strategy the CMBB and Aquaculture CRC developed a collaborative research program with Wattyl Australia. This gave the program access to the latest coatings technology and allowed for a quick response time between trials to develop coatings. Technical criteria for a fouling deterrent product requires that the product is quick drying to reduce stress, and the time the animals are out of the water. It must be non-toxic to the oyster itself and the surrounding environment, and easy to remove before shipment to markets.

Results of early trials were used as a lead-in to this FRDC funded project which includes a representative array of aquaculture species and farming sites and trials of application methods at Port Stephens, including Port Macquarie (NSW) and Tasmania and South Australia (in collaboration with the South Australian Research and Development Institute).

This report details all laboratory and field trials showing the progression from an antifouling coating containing an active ingredient to a non-toxic coating that has modified surface properties that act as a fouling deterrent. It also addresses commercial scale application of the coating technique to meet industry requirements. The aim of this project was to develop coatings where a complete fouling deterrent response was required.

3. Need

Over the past 20 years oyster production in NSW has declined by more than 40%. Two major problems exacerbating the industry's continuing decline are:
1) escalating costs of production associated with slow growth and high mortality rates suffered by Sydney rock oysters due to QX disease ,winter mortality and mud worm infestation.
2) severe overcatch generated by both Sydney rock oysters and Pacific oysters and other biofouling problems.

The problem of combating overcatch and fouling is compounded by the protracted 3-4 year grow-out times for Sydney rock oysters. These problems are currently being addressed by periodic manual cleaning and hot water dips in conjunction with the use of elevated intertidal growing heights. The latter exposes overcatch oysters and other biofouling organisms and mudworms to lethal levels of heat stress and desiccation. The hidden costs of elevated growing height are reduced feeding and growth rates, increased heat kills in summer and the unsightliness of intertidal rack and tray culture.

Direct costs of combating overcatch and other forms of biofouling have been estimated as high as 30% of total operating costs. These have contributed considerably to the progressive decline of many formerly productive leases. This has been a major concern to both government and community based conservation groups. Derelict leases in Port Stephens alone total almost 900ha and will cost an estimated $8 million dollars to rehabilitate.

Development of a cost effective fouling deterrent coating has the potential to significantly arrest the decline of the oyster industry in NSW, and to reduce costs of production in Tasmania and South Australia. By reducing costs associated with production the industry will significantly improve its competitiveness.

4. Objectives

1. To evaluate the effectiveness and commercial practicality of one application per overcatch season of antifoulant coating as a means of protecting intertidal tray cultured oysters from overcatch of both Sydney Rock and Pacific Oyster spat and from other forms of biofouling commonly encountered in Port Stephens. This objective was amended to include Port Macquarie, and all final experiments were carried out at Port Macquarie.
2. To assess and improve the cost effectiveness and commercial practicality of these treatments: a) in relation to other forms of farming single seed oysters (intertidal basket and subtidal raft farming) and b) to enable a lowering of the usual growing height of oysters thereby accelerating their growth without jeopardising survival or market quality.
3. To determine whether benefits attached to objectives 1 and 2 can be achieved when extended to a representative array of commercial oyster farming areas and techniques used throughout NSW, Tasmania and South Australia.
4. To assess whether successful types of antifouling coating techniques pose significant risks to the environment, to the oysters themselves or to consumers.

The objective of providing an antifouling coating for edible oysters prompted development of a coating that relied on non-toxic physical surface characteristics to deter fouling (OysterClear), rather than conventional means of toxicity.

Figure 1.

PROGRAM SEQUENCE

Section 5. Initial Field Trials
Tested at Port Stephens and Port Macquarie
October 1998 – Feb 1999

↓

Section 6. OysterClear
Original, Standard, Superthin
and waterbased *Coating 16* and *18*

6.1	**6.2**	**6.3**	**6.4**
Lab trials	Walsh Bay, Sydney October 1999 – December 2000	St Helen's, Tasmania September 1999 – October 2000	Port Macquarie, NSW December 1999 – May 2000

↓

Section 7 & 8. OysterClear
Original, Coating 1 and *2*

Section 7. Pre-Commercial Trial at Port Macquarie
August 2000 – January 2001

↓

Section 8.1 Tasmania
Field trial
February 2001
Pacific Oysters

Section 8.2 South Australia
Field trial
December 2000 – January 2001
Pacific oysters

Section 9. Commercial Trial at Port Macquarie
OysterClear *Original* and *Coating 2*
April 2001 – November 2001

Section 10. Wattyl Trial at Port Macquarie
OysterClear *Coating 3*
November 2001 – April 2002

Commercial Release of OysterClear™
December 2002

Figure 2. Map of sites of field experiments in New South Wales, South Australia and Tasmania.

5. Field Trials
5.1 Field Trial 1 - Port Stephens

5.1.1 Introduction

The first trial of antifouling coatings was conducted subtidally at two sites within Port Stephens, Honey Creek and Oakey Island. The trial tested two carriers (1 and 2) in combination with four active ingredients (AI) (AI1, a commercial biocide and AI 2a, b & c which are organic antifouling compounds). A six month length of activity was aimed for as set in consultation with ORAC. The coatings were tested using Pacific oysters (*Crassostrea gigas*).

5.1.2 Materials & Methods

All coatings were formulated at the Centre for Marine Biofouling and Bio-Innovation using paint industry standards. Active ingredients were loaded at 10-15% of the dry weight of the film. Coatings were applied by dipping individual oysters for a few seconds into the formulations. Oysters were individually labelled and 10 per treatment were coated for each site. Coated oysters were placed within two larger trays and these were deployed subtidally, one at each location.

Table 5.1.1. Combinations of carriers and active ingredients tested on Pacific Oysters at Port Stephens, October 1998.

Treatments	Honey Creek No. of oysters	Oakey Island No. of oysters	Total
Uncoated control	10	10	20
Carrier 1 control (no AI)	10	10	20
Carrier 1 + AI 1 10%	10	10	20
Carrier 1 + AI 2a 10%	10	10	20
Carrier 1 + AI 2c 10%	10	10	20
Carrier 2 control (no AI)	10	10	20
Carrier 2 + AI 2a 10%	10	10	20
Carrier 2 + AI 2b 10%	10	10	20
Carrier 2 + AI 2c 10%	10	10	20
	90	90	180

Fouling cover was monitored approximately every four weeks after deployment and the trial ran for 15 weeks at Honey Creek and for 21 weeks at Oakey Island.

Coated oysters were photographed in the field using a Kodak 260 Digital Camera and fouling cover measured in the lab using techniques modified from Hodson et al, 1995. Percent cover of overcatch was determined, and coating integrity was monitored for the duration of the trial.

There was a very low percent fouling in several of the treatments so they were not compared statistically as they did not meet the assumptions of homogeneity of variances.

5.1.3 Results

There was large variation in the degree of fouling between sites at Port Stephens and also between depths within each site. However, at both sites the most common fouling organism was oyster overcatch. At Honey Creek the most successful treatments (AI 1 in Carrier 1 and AI 2c in Carrier 2) strongly inhibited the settlement of overcatch for 15 weeks with a mean fouling cover of 8 and 10% respectively. Control oysters showed a 30% cover (Figure 5.1.1). The coatings began to fail after 15 weeks with fouling on all treatments. At Oakey Island the most effective treatments (AI 1 in Carrier 1 and AI 2c in Carrier 2) reduced overcatch settlement to 3% when compared with uncoated controls at 17% after 21 weeks.

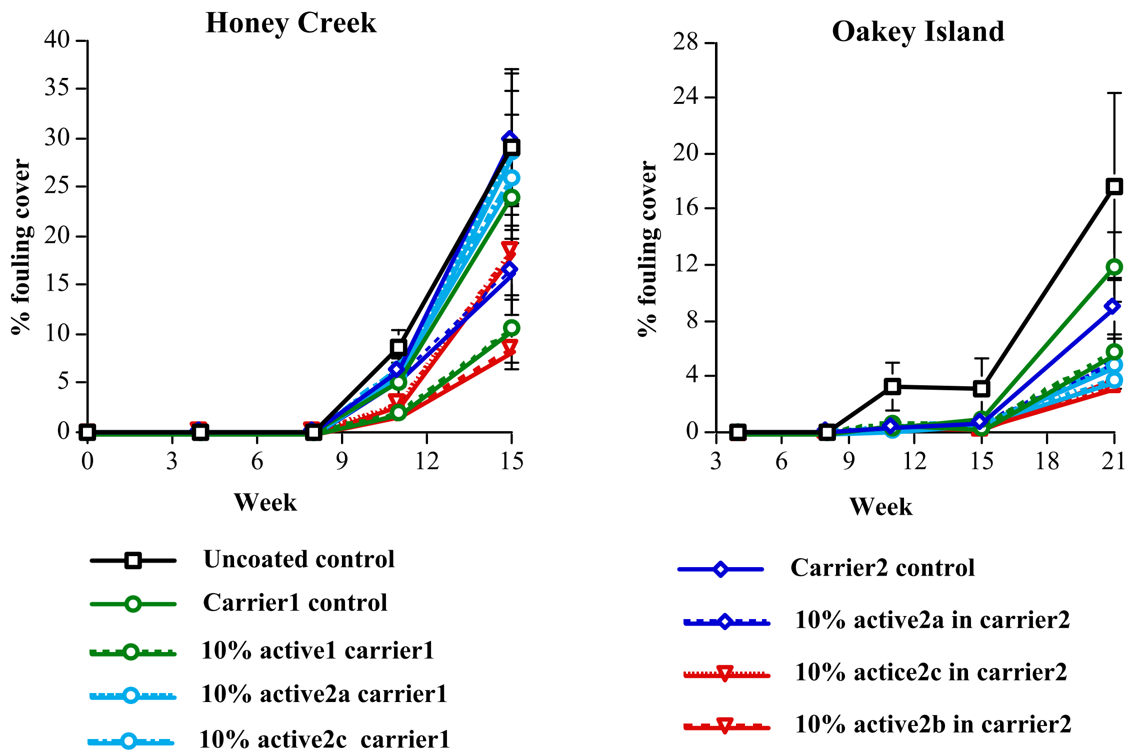

Figure 5.1.1. Percent cover of overcatch on control and coated Pacific oysters at Honey Creek and Oakey Island, Port Stephens. Data are means ± SE. n=10.

5.1.4 Summary

- AI 1 and AI 2c are effective antifoulants.
- Carrier 1 and 2 are not robust enough to last under farming conditions and both types of carriers were effective only with the addition of an AI.
- Carrier 2 performed better than Carrier 1 with respect to adhesion and longevity.
- Further developments of carriers were required.

5.2 Trial 2 - Port Stephens and Port Macquarie

5.2.1 Introduction

Coatings development done concurrently with Trial 1 led to new, improved formulations of Carrier 1 and 2. Reformulation of Carrier 1 resulted in a thicker coating and therefore a potentially longer lifetime. The reformulation of Carrier 2 improved the stability of the coating. Trials were expanded to include a site at Port Macquarie and subtidal and intertidal zones were tested within each location. The Port Stephens trial was expanded to include Sydney rock (*Saccostrea glomerata*), Flat oysters (*Ostrea angasi*) and Pacific oysters (*Crassostrea gigas*). The Port Macquarie trial tested coatings on Sydney rock and Flat oysters.

5.2.2 Materials & Methods

Table 5.2.1. Formulations tested at Port Stephens and Port Macquarie, October 1998.

Treatments
Uncoated control
Carrier 1 control
Carrier 1 + 15% AI1
Carrier 1 thick control
Carrier 1 thick + 10% AI1
Carrier 2 control
Carrier 2 + 10% AI1

All oysters were individually tagged before coating and all formulations were applied via dipping. Ten oysters per treatment were coated for each site, depth and oyster type (see design below).

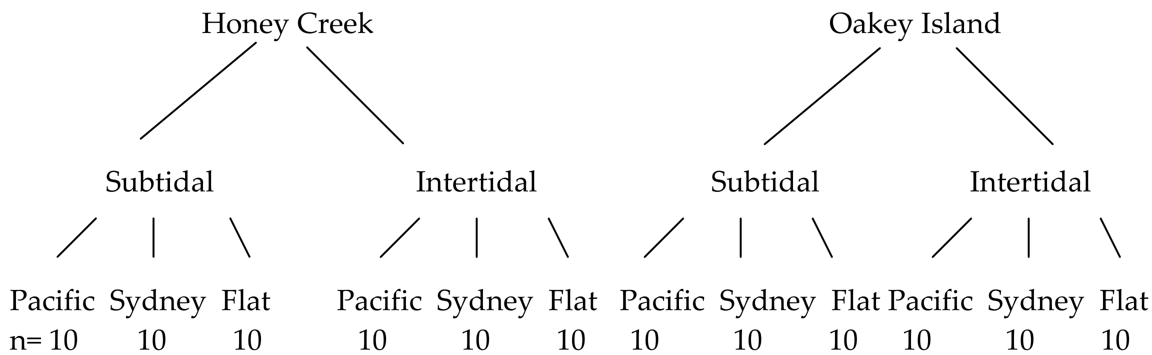

Figure 5.2.1. Experimental design at Port Stephens.

The experimental design was also reproduced at Port Macquarie at the Bay and Stormy Point sites and Sydney Rock and Flat oysters were trialed.

Oysters were randomly placed in 100 x 200 x 3 cm, six celled trays. At Port Stephens, ten trays were deployed - five trays (three subtidal and two intertidal) were positioned at Honey Creek (Lease 8478) and five at Oakey Island (Lease 37114). The trays were placed horizontally over wooden rails. The trial ran for 15 weeks at Honey Creek and for 21 weeks at Oakey Island.

At Port Macquarie, six trays were deployed at Limeburners Creek. Subtidal trays were suspended from both the Bay and Stormy Point pontoon, while the intertidal trays were positioned at the Bay and Stormy Point shallows. This trial ran for 21 weeks.

Fouling cover was monitored every four weeks as described above and performance of the coatings was also monitored. No statistical analyses were performed due to the large numbers of zeros in the data sets which meant that the assumptions of homogeneity of variances were not met.

5.2.3 Results

While the most effective coatings initially appeared to inhibit fouling on the Flat oyster, the oyster surface 'flaked' easily and consequently the coating integrity was disrupted leading to premature failure of the coating. Therefore results are not presented for Flat oysters at either site.

5.2.3.1 Port Stephens

The two major fouling species, oyster overcatch and barnacles made up 98% of the fouling community with overcatch contributing approximately 80-90% of fouling. This is consistent with the regions past role as a major wild spat collection and production area. Only data for oyster overcatch fouling is presented.

The most intensive fouling at Port Stephens occurred in the intertidal zone at Honey Creek where fouling on control Sydney rock and Pacific oysters reached 85% and 65% cover respectively after 15 weeks (Figure 5.2.2). Fouling cover at intertidal sites was two to three times subtidal levels for both species.

The most effective active-carrier combination was Carrier 2 + 10% AI1 which strongly inhibited fouling at all sites regardless of depth. At the Honey Creek intertidal site, where fouling was the most intense, this formulation reduced oyster overcatch to a mean fouling cover less than 10% for Pacific oysters and 30% for Sydney rock oysters (Figure 5.2.2). Fouling on controls exceeded 65% and 85% respectively. At the other sites this formulation reduced fouling to less than 5% with controls exceeding 20%.

A low level of mortality (< 3%) was seen across all treatments including controls. None of the coatings with or without active ingredients had any effect on oyster mortality when compared with controls (Appendix 4, Table 1).

At the end of the trial, coating cover for Carrier 2 varied between 30-80% coating cover and Carrier 1 was almost completely removed from the shell. The coatings began to fail after 15 weeks, due to a reduction in coating integrity which allowed settlement on and subsequent overgrowth of exposed oyster shell by overcatch.

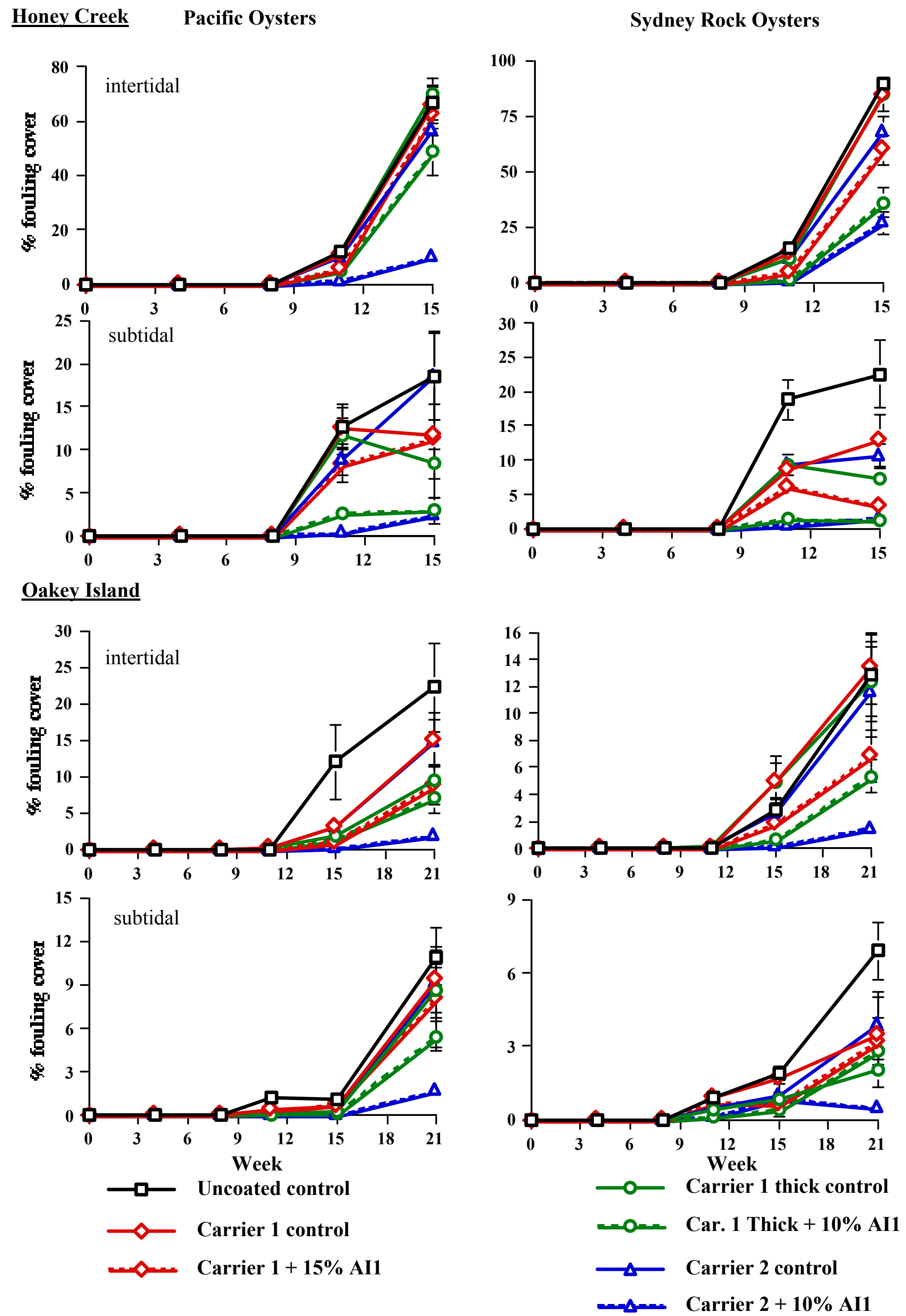

Figure 5.2.2. Percent cover of overcatch on control and coated Pacific and Sydney Rock oysters. Data from intertidal and subtidal sites at the Honey Creek and Oakey Is. locations. Data are means +/- SE. n=10

5.2.3.2 Port Macquarie

Fouling at both Port Macquarie sites was exclusively oyster overcatch. Both the intertidal and subtidal sites at the Bay location experienced the highest level of fouling with controls having 25% cover, while at Stormy Point the fouling ranged from ~6% at the intertidal site and 15% at subtidal (Figure 5.2.3). Antifouling activity was improved by the addition of AI for all coating types with Carrier 2 + AI1 performing the best, inhibiting fouling to less than 5% across the range of depths and sites.

The two most effective coatings, Carrier 2 + 10% AI1 and Carrier 1 thick + 10% AI1 had 0-2% mortality (depending on site/locality), the same as controls. All treatments had less than 5% mortality (Appendix 4 Table 2).

All Carrier 1 coatings suffered abrasion resulting from oyster movement within the tray. This was most predominant at the peak and the shell edges. Continual immersion increased the brittleness of the coating resulting in greater erosion. Carrier 2 performed well and maintained its integrity and cover for the 21 week trial period.

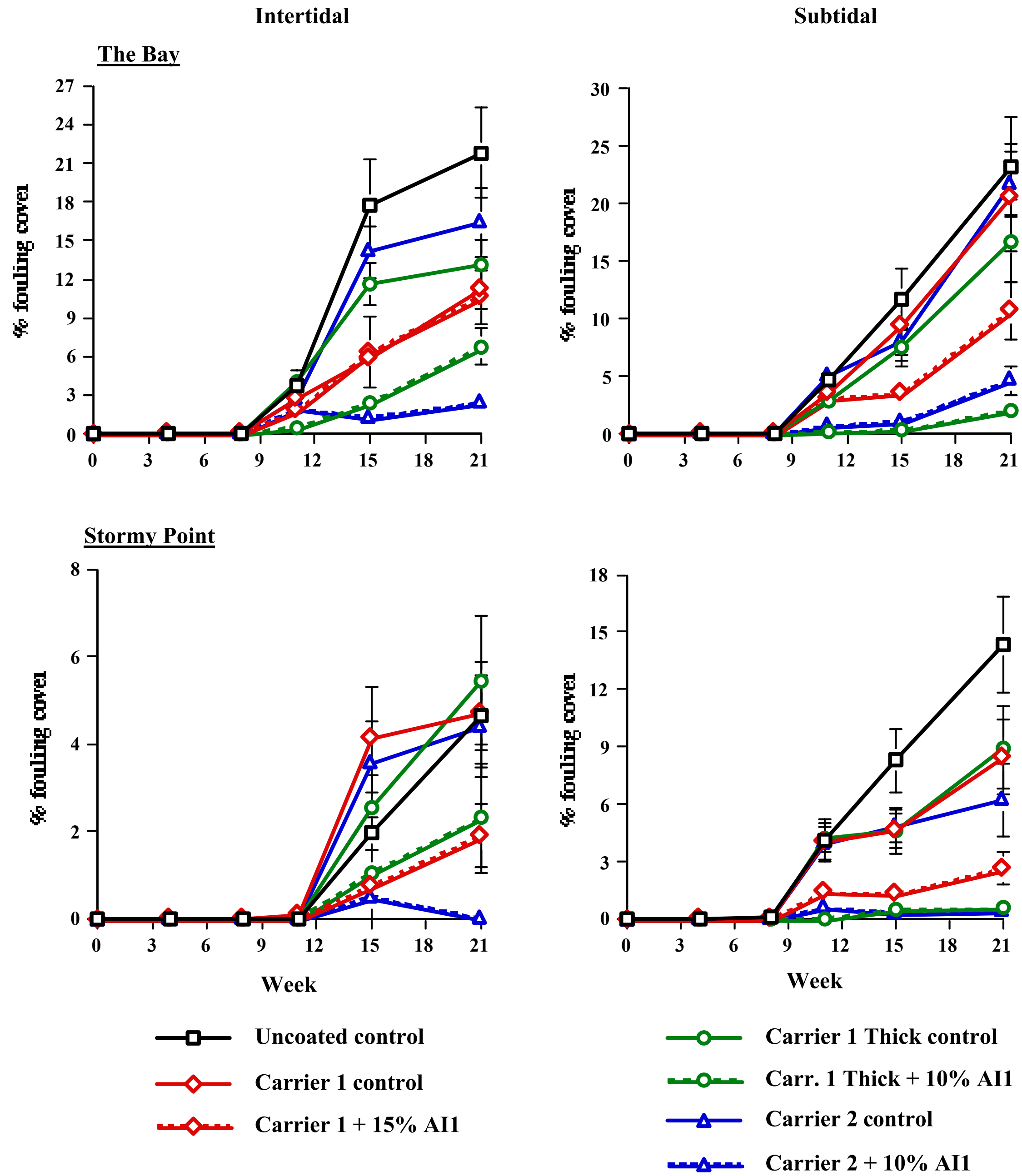

Figure 5.2.3. Percent cover of overcatch on control and coated Sydney rock oysters after 21 weeks. Tested at intertidal and subtidal depths at the Bay and Stormy Point sites. Data are means ± SE, n=10.

5.2.4 Summary

- The new formulations were effective at inhibiting fouling for 15-21 weeks.
- Carrier 2 coatings had better integrity than Carrier 1 coatings
- The efficacy of coatings needs to be extended to six months.

5.4 Overall summary

- Neither Carrier 1 or 2 were robust enough to deal with farming conditions for a six month period.
- AI 2c (a natural antifoulant) performed similarly to commercial antifoulants.
- Reprioritisation to focus on a coating whose activity is based on deterrent surface properties (*ie*. a 'slippery' coating) due to food and environmental issues and general consumer perception about edible oyster safety

6. OysterClear

6.1 Laboratory trials

6.1.1 Introduction

The first trials involving antifouling coatings were limited in their success by the integrity and longevity of the selected coatings. To overcome these problems we undertook to develop new coating formulations and conduct a series of trials to evaluate permutations of formulation factors to achieve improved adhesion to shell, improved flexibility, increased lifespan, and improved resistance to wear.

Furthermore, we developed a new coating, the activity of which was based on modified surface properties rather than the addition of an active ingredient. This was named **OysterClear** and is a hot melt, quick drying, ductile and opaque coating. Many of its parameters such as melt point temperature, ductility, thickness, 'slipperyness', and hardness can be modified by additives. This resulted in several versions of OysterClear named **Original**, **Standard** and **Superthin** which have melt points varying from 50-85^{0}C.

Carrier 2, a water based emulsion from the previous trial, was improved through the addition of pigment which stabilised the coating and lead to quick drying times (within 1 hour). This resulted in two white coatings; without an active ingredient (**Coating 16**) and with active (AI1) (**Coating 18**) loaded at 10% of the dry weight of the film (AI1 was used as this was the most effective active from the previous trial).

The application method for both coating types also significantly affects adhesion, integrity and antifouling activity. Therefore application methods were first tested in the laboratory before field trials. Based on the laboratory trials the coatings were tested for antifouling activity at a local test site at **Walsh Bay, Sydney** and then at oyster growing regions in **Tasmania**, and at **Port Macquarie**, **New South Wales**.

6.1.2 Materials & Methods

The optimal film thickness and adhesion for each type of coating was established by testing application methods suitable for regular use in the oyster industry. The variations of Oyster Clear were applied to oysters by quickly immersing the oyster into the coating to cover the oyster completely. The time the oyster was in the coating was kept to a minimum, ~1 sec, and the coating temperatures varied from 60-85^{0}C. This is in an acceptable heat range for oysters based on current overcatch control methods in which oysters are dipped in 80-90^{0}C water for a few seconds.

Further, oysters were dipped in the presence of normal (18-20^{0}C) and hot (50^{0}C) ambient air temperature as this affects the resulting coating thickness on the shell. Coating 16 and 18 (containing AI1) were applied to the shell with a Wagner® handheld spray gun and both were applied hot (50^{0}C) as this results in a very quick drying coating. Coated oysters were left to dry until the film was hard and relatively non-tacky. The oysters were then immersed in a flowing seawater system for 24 - 72 hours to monitor oyster mortality and coating integrity. Coatings were applied to Sydney rock oysters and 20 oysters were coated per treatment.

Oysters were also coated by dipping either individually or together within trays (30x20x3cm untreated wooden and plastic mesh trays) to establish whether this had any effects on oyster mortality. The ability of the coating to adhere to trays gives us useful information about the possibility of use of antifouling coatings for farming equipment.

6.1.3 Results

Oysters coated at the high ambient air temperature (50^{0}C) resulted in a reduced coating thickness and oysters opened quickly after immersion. No mortalities were recorded. However, oysters coated at ambient conditions (28^{0}C) had a thick coating and experienced higher mortality. This suggested that a thinner coating of OysterClear is important to increase survivorship. A hot melt coating spray gun was especially designed for FRDC Project No. 2000/254 which allows a thinner but smooth and even coat of OysterClear and this application method was included in the field trials.

Oysters that were dipped within the trays also suffered higher mortality suggesting that factors such as the latent heat retained by the tray and longer dipping time affect oyster survival.

Coating 16 adhered well to the oyster shell and the wood and plastic of the racks. At room temperature the coating dried within one hour and was smooth, hard and non-tacky. The oysters opened almost immediately after immersion, without tearing the coating and no mortality was recorded. However, after 12 hours immersion the coating became slightly brittle and was easily scratched off the shell.

Coating 18 had a thicker application on shell than 16, and was rough and tacky to touch after one hour drying. The coated oysters were immersed in aerated seawater tanks, but remained tacky 24 hours after coating. Less than 15% of the oysters opened after immersion. Mortality was ascribed to coating thickness and the viscosity of coating 18 was decreased for application by hot spraying in field trials.

6.1.4 Summary

- Application temperature greatly affects the coating thickness for OysterClear.
- A thinner coating of OysterClear is an important factor in oyster survival.
- The addition of an active ingredient adversely alters the properties of Coating 18.

6. OysterClear Field Trials

6.2 Walsh Bay, Sydney

6.2.1 Introduction

Results from the laboratory trial resulted in improved coatings and these were tested at Walsh Bay, Sydney. The application temperature of Coating 18 was raised to 70^{0}C resulting in a thinner and very quick drying coating. OysterClear treatments were also applied at higher temperatures (100-120^{0}C) to achieve a thinner coating.

6.2.2 Materials & Methods

Table 6.2.1. Coatings and application methods trialed at Walsh Bay, October 1999.

Coatings	Application method
Uncoated Control	-
OysterClear Original	dip
OysterClear Standard	dip
OysterClear Super Thin	dip
Coating 16	cold spray
Coating 18	hot spray

Three replicate trays, each containing twelve oysters were allocated for each of the six treatments (36 oysters per treatment). The oysters and trays were thoroughly cleaned and dried before coating and oysters were coated individually. The racks that the oysters were deployed in were also coated in the same treatments.

Coated oysters and trays were deployed subtidally at the Walsh Bay wharf, Sydney in October, 1999. The trial was monitored every four weeks for 12 weeks. At this time the experiment was vandalised and terminated. Barnacle and bryozoan counts, and an estimation of tube worm cover were measured and digital camera images recorded. Oyster mortality and coating integrity were assessed.

Due to the large number of zeros in the data sets, normality and homogeneity of variances could not be achieved thereby precluding formal analysis.

6.2.3 Results

After 12 weeks all coatings had deterred barnacle settlement (Figure 6.2.1). The best performing coatings were OysterClear Superthin and Coating 18 with an average of 2-3 barnacles per oyster. This was compared with a mean of 17 barnacles per control oyster. None of the coatings deterred the settlement and growth of the marine worm *Hyroides elegans* or bryozoans after 12 weeks (Figures 6.2.2 & 6.2.3).

A subjective analysis of barnacle and tubeworm adhesion suggested that they were more easily removed from the OysterClear coatings than Coating 16 or 18.

No mortality was recorded and coating integrity was good for all coating types.

6.2.4 Summary

- OysterClear Superthin and the waterbased Coating 18 successfully deterred the settlement of barnacles.

- The coatings were not effective against *Hyroides elegans* or bryozoans.

- There was no mortality associated with the methods of dipping or spraying.

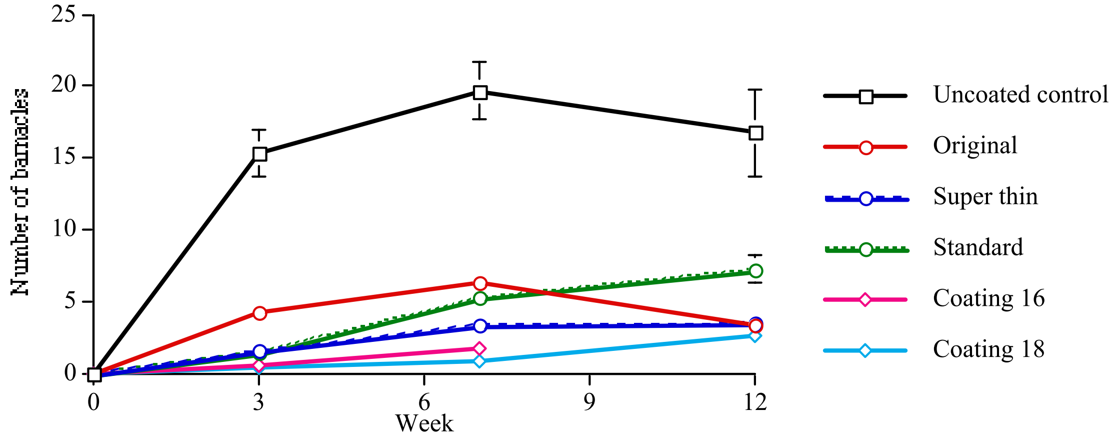

Figure 6.2.1. Number (mean +/- SE) of barnacles on control and coated Sydney rock oysters after 12 weeks

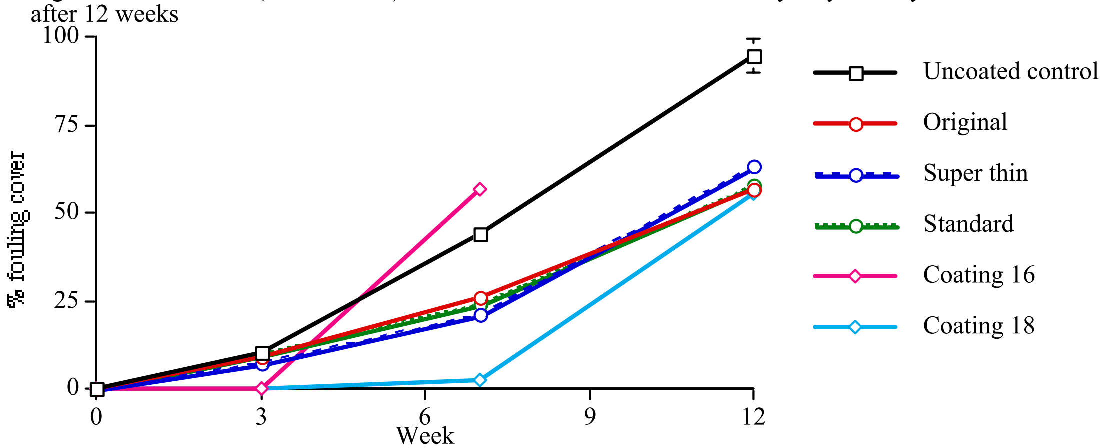

Figure 6.2.2. Percent fouling cover (mean +/- SE) of the marine worm *Hydroides elegans* on control and coated Sydney rock oysters after 12 weeks

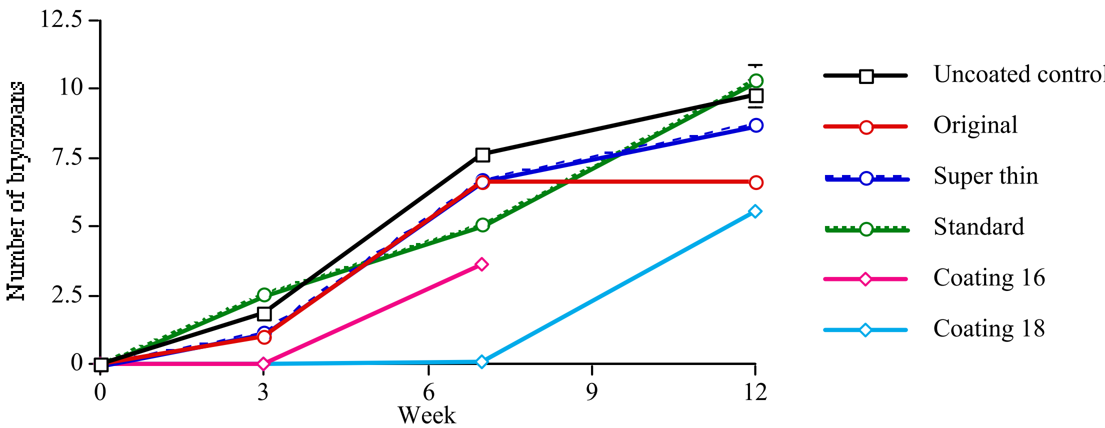

Figure 6.2.3. Number (mean +/- SE) of bryozoans on control and coated Sydney rock oysters after 12 weeks

6.3 Tasmania

6.3.1 Introduction

This trial tested the antifouling efficacy of OysterClear Standard and the water based emulsion (Coating 16 - no active) on the solitary ascidian *Ciona intestinalis* which causes significant fouling problems for the oyster industry in this region. Jumbo Pacific oysters were coated and deployed at the St Helen's Oysters Pty lease at St Helens, Tasmania, in September 1999.

6.3.2 Materials & Methods

Table 6.3.1. Coatings and application methods trialed in Tasmania, September 1999.

Coatings	Application method
Uncoated Control	-
OysterClear Standard	dip
Coating 16	cold spray

The oysters were cleaned and dried before coating. Single trays containing 30 oysters coated with either OysterClear Standard or Coating 16, or 30 control oysters were placed in subtidal culture for 10 weeks. The oysters were undisturbed for this period after which the number of ascidians adhering to each shell was counted.

6.3.3 Results

After 10 weeks OysterClear Standard had a strong effect on the settlement of *Ciona intestinalis* with 50% of the shells having no fouling by *Ciona* (or fouling of any other sort). The remaining 50% of OysterClear treated shell had a mean of two *Ciona* per shell. Control oysters and those coated with Coating 16 had a mean of six *Ciona* per shell (Figure 10). There was 100% survival in all treatments.

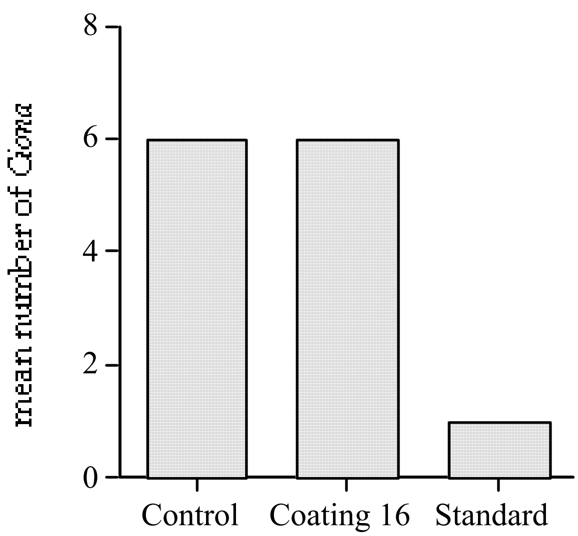

Figure 6.3.1. Mean number of *Ciona intestinalis* on control and coated Pacific oysters after 10 weeks. Data are mean. n=30.

6.3.4 Summary

- OysterClear Standard was very effective at deterring the settlement of *Ciona intestinalis*.

- Coating 16 was not effective with the same degree of fouling as controls.

- There was no mortality associated with the coatings or the coating process.

6.4 Port Macquarie

6.4.1 Introduction

This trial tested variations of OysterClear as fouling deterrents at Port Macquarie. Sydney rock oysters were coated and deployed at subtidal and intertidal locations in December 1999. In this trial the application temperature of OysterClear Superthin and Original was elevated to facilitate a thinner coating. The spray gun commissioned for FRDC Project No. 2000/254 was used to compare the fouling deterrent efficacy between a sprayed and dipped coating of the same formulation (Superthin). Coating 16 and 18 were also included to compare waterbased coatings.

In this trial the post harvest technique of 'rumbling' oysters to remove fouling and coating was also tested. Rumbling is a common practise within the industry to remove mud and a small amount of fouling immediately before sale (J. Zappa pers. comm.). A commercial rumbler was made available by Sydney City Oysters. Large numbers of oysters (~ 1000) are placed in a stainless steel drum and as the drum spins and pressurised water is applied the oysters knock against each other and the chamber wall and fouling and mud are removed (see photographs in Appendix 5). All the mud and fouling that is removed is captured by a series of filters and strainers. Often farmers will use cement mixers for the same purpose but the water used for rinsing is not refreshed and the method is not as effective (J. Zappa pers. comm.).

In the case of these trials it was useful to determine how much fouling and coating could be removed by this end stage process. It was observed in previous trials that if fouling occurred on coatings it was generally restricted to the surface of the coating (did not adhere to the shell). Therefore rumbling may facilitate the loss of a high proportion of fouling relatively easily and also have a secondary but important role in removing the coating. At this stage it was important to establish methods for the easy removal of coating at the end of a trial as industry perception was that consumers would be nervous about an edible product with coating on the shell. Further, there would be issues of cooking coated oysters etc.

6.4.2 Materials & Methods

Table 6.4.1. Coating and application methods trialed at Port Macquarie, December 1999

Coatings	Application method
Uncoated Control	-
OysterClear Original	Dip (~140°C)
OysterClear Standard	Spray (hot spray gun)
OysterClear Super Thin	Dip (~140°C)
OysterClear Super Thin	Spray (hot spray gun)
Coating 16	cold spray (Wagner®)
Coating 18	hot spray 70°C (heated in microwave, Wagner®)

Five replicate trays (30cm X 20cm), each containing 10 oysters were used for each treatment at intertidal and subtidal sites (50 oysters per treatment per site). The oysters were cleaned and dried before coating and sprayed /dipped individually. Within 20 minutes of coating the oysters were submersed in seawater and were deployed either subtidally or intertidally within 12 hours.

Fouling cover and oyster mortality were recorded every four to six weeks and the trial ran for 26 weeks. Percentage fouling cover and mortality were determined in the field and digital photos were taken to determine the composition of the fouling community. Coating adhesion and integrity (percent cover of coating remaining) were also recorded.

As well as the above measurements, percentage marketability was determined at the conclusion of the trial. Percentage marketability is an estimate of those oysters that would go directly to market without further processing (*ie* manual cleaning of fouling) and high marketability is attributable to the low presence of oyster spat on the surface of the shell. Percentage marketability was determined by the manager and the owner of the Holiday Coast Oyster lease by visual inspection. The oysters were divided into marketable/non marketable oysters within each treatment and for each site (subtidal/intertidal). All oysters were pooled and results presented as a proportion of the total oysters.

The oysters were then transported to Sydney and ‘rumbled’. Treatments were rumbled separately, n=50. After rumbling, the percentage marketability was reassessed as well as the percent of coating lost.

For fouling and coating remaining data homogeneity of variances, even after transformation, could not be achieved so no formal analyses were performed. No analyses were performed on marketability data as all replicates were pooled and marketability assessed for the total number of oysters and presented as percent marketable.

6.4.3 Results

6.4.3.1 Fouling

Intertidal

Fouling cover on intertidal oysters during the 11 to 26 week immersion period consisted mainly of the settlement and growth of overcatch (Figure 6.4.1). Of the OysterClear coatings tested the most successful were Superthin (spray) and Original (dip) with an approximate 70% reduction in fouling cover compared to the uncoated control after 26 weeks (Figure 6.4.1). This value was increased to approximately 85% for both treatments after rumbling (Figure 6.4.1).

Coating 18 (containing active ingredient) strongly inhibited oyster overcatch settlement with a 60 and 75% reduction in fouling cover compared to Coating 16 (no active) and the uncoated control respectively (Figure 6.4.1). Rumbling of the oysters reduced the fouling cover to varying degrees on all the treatments including controls (Figure 6.4.1). Barnacle settlement did not occur on any intertidal treatments or controls.

Subtidal

Fouling cover on the subtidal oysters consisted of an initial barnacle settlement event followed by oyster overcatch at 11 weeks. The most effective OysterClear coatings against overcatch, were Superthin (dip and spray) and Original with a 65-80% reduction in fouling compared with controls before rumbling and a 75-90% reduction after rumbling (Figure 6.4.2a). Coating 18 also showed strong inhibition of oyster overcatch settlement.

The most successful coatings against the settlement of barnacles in the subtidal zone were OysterClear Original (dip) and Coating 16 with a 50% reduction in fouling cover compared to the uncoated controls (Figure 6.4.2b). Rumbling of the oysters removed the majority of barnacle fouling from all treatments (Figure 6.4.2b).

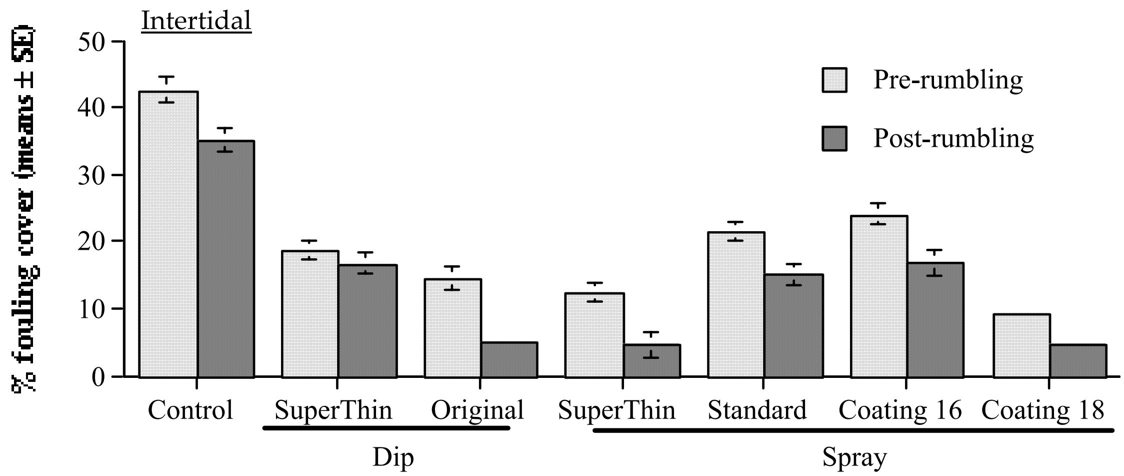

Figure 6.4.1. Percent fouling cover of oyster overcatch on control and coated intertidal oysters pre and post-rumbling at 26 weeks. Data are mean ± SE. n=50.

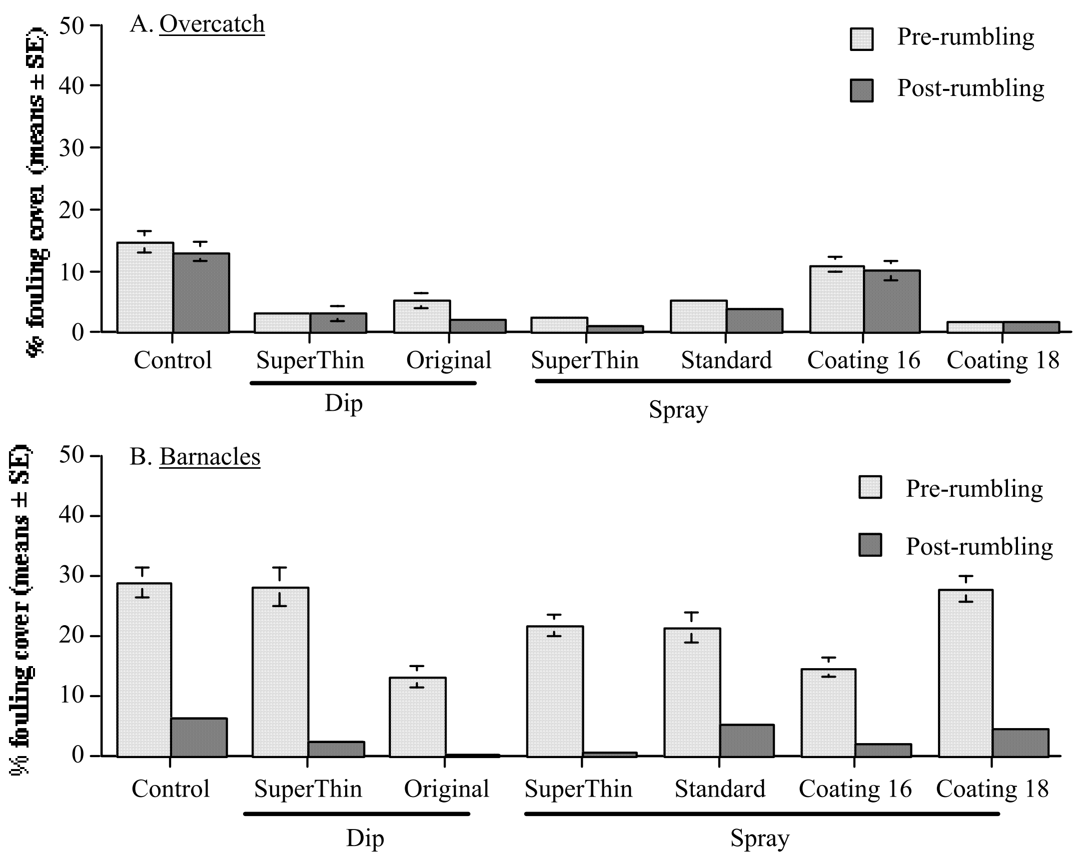

Figure 6.4.2. Percent fouling cover of oyster overcatch (A) and barnacles (B) on control and coated subtidal Sydney rock oysters pre and post-rumbling. Data are means ± SE. n=50.

6.4.3.2 Marketability

Marketability is influenced by the number and size of fouling organisms, particularly overcatch, on the oyster shell and was determined subjectively by oyster growers. In the intertidal site where the rate of spat settlement was higher, the marketability of oysters was greater for all coated treatments compared to controls (Figure 6.4.3a). None of the control oysters were marketable while the best performing OysterClear coatings, Superthin (spray) and Original (dip), increased marketability to 65 and 60% respectively before rumbling (Figure 6.4.3a). Coating 18 (active) produced 84% of marketable oysters compared to its control, Coating 16 (no active) with less than 7% (Figure 6.4.3a).

Oysters from the subtidal region produced higher numbers of marketable oysters across all treatments including controls (Figure 6.4.3b). All of the OysterClear coatings produced over 80% of marketable oysters compared to the uncoated control with 60% suitable for sale (Figure 6.4.3b). Coating 18 (with active) also produced 84% of marketable oysters compared to the uncoated control and Coating 16 (no active) with less than 40% of marketable oysters (Figure 6.4.3b). The higher marketability seen in the subtidal is due to the settlement trend where an initial settlement of barnacles was followed by oyster overcatch ~ 11 weeks later which settled on and over the barnacles. If barnacle settlement occurs early in the spat-collecting period it may reduce spat numbers (Nell, 1993) which in turn results in improved marketability, however the mode of action of this inhibition is not clear. Barnacle fouling does not significantly influence an estimation of marketability as they do not contribute greatly to an odorous or unsightly oyster.

'Rumbling' of the oysters improved the marketability for all treatments, with the exception of the uncoated controls in both the intertidal and subtidal regions (Figure 6.4.3a & b). The OysterClear coatings facilitated the easy removal of settled overcatch as they were restricted to the surface of the coating, while the overcatch on the control oysters was difficult to remove. Rumbling greatly improved marketability for Coating 16 from both the subtidal and intertidal sites.

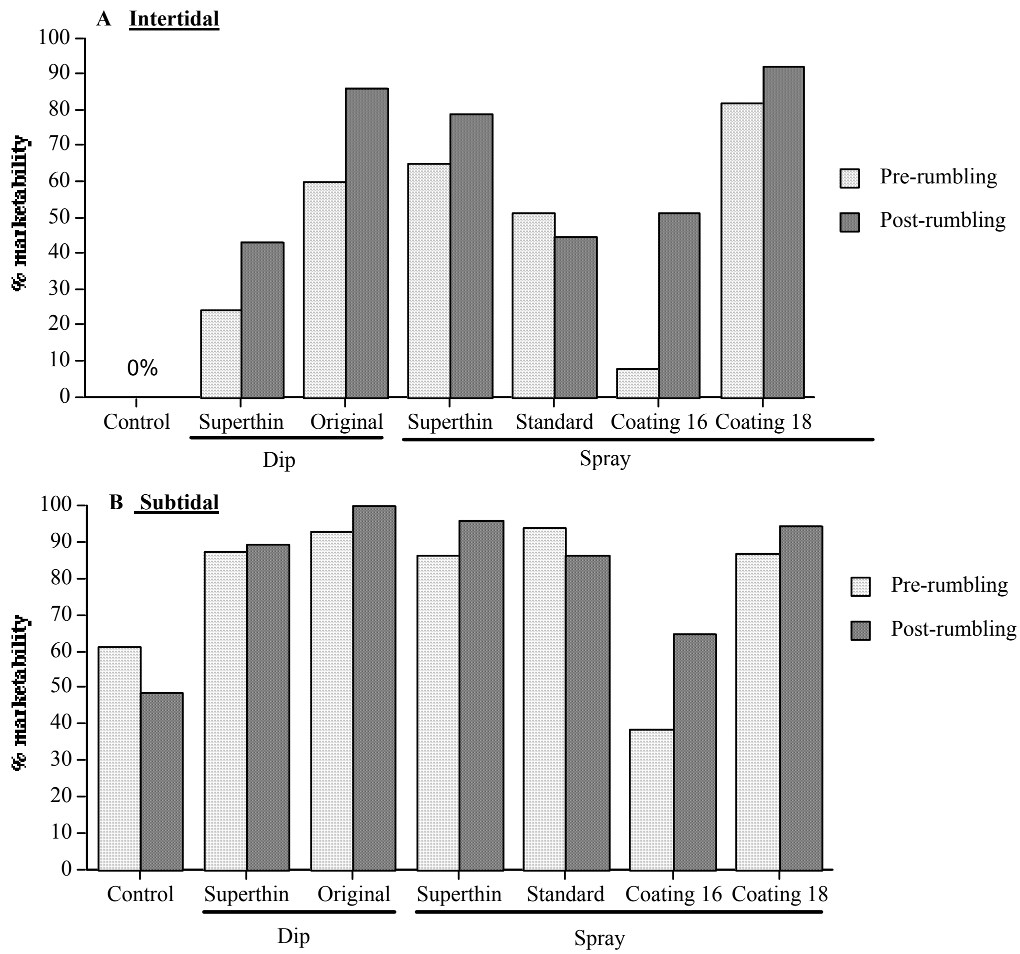

Figure 6.4.3. Percent marketability of control and coated Sydney rock oysters after 26 weeks at intertidal (A) and subtidal (B) sites. n=50.

6.4.3.3 Coating integrity

Data for coating remaining on the shell was only collected after rumbling. There was greater coating loss on all treatments on subtidally grown oysters (Figure 6.4.4) compared with intertidal oysters (Figure 6.4.5). Patterns of coating loss differed between subtidal and intertidal treatments for all coating types.

After rumbling, OysterClear Original had the greatest coating loss with only 20% of coating remaining (Figure 6.4.4a & 6.4.5a). Coating 16 and 18 showed the most resistance to rumbling, resulting in the least amount of coating removed by 'rumbling', with up to 80% cover of Coating 18 remaining on the shell (Figure 6.4.4f & 6.4.5f). The different application methods of dip and spray did not influence the final coating cover remaining for Superthin (Figure 6.4.4c,d & 4.5.5c,d).

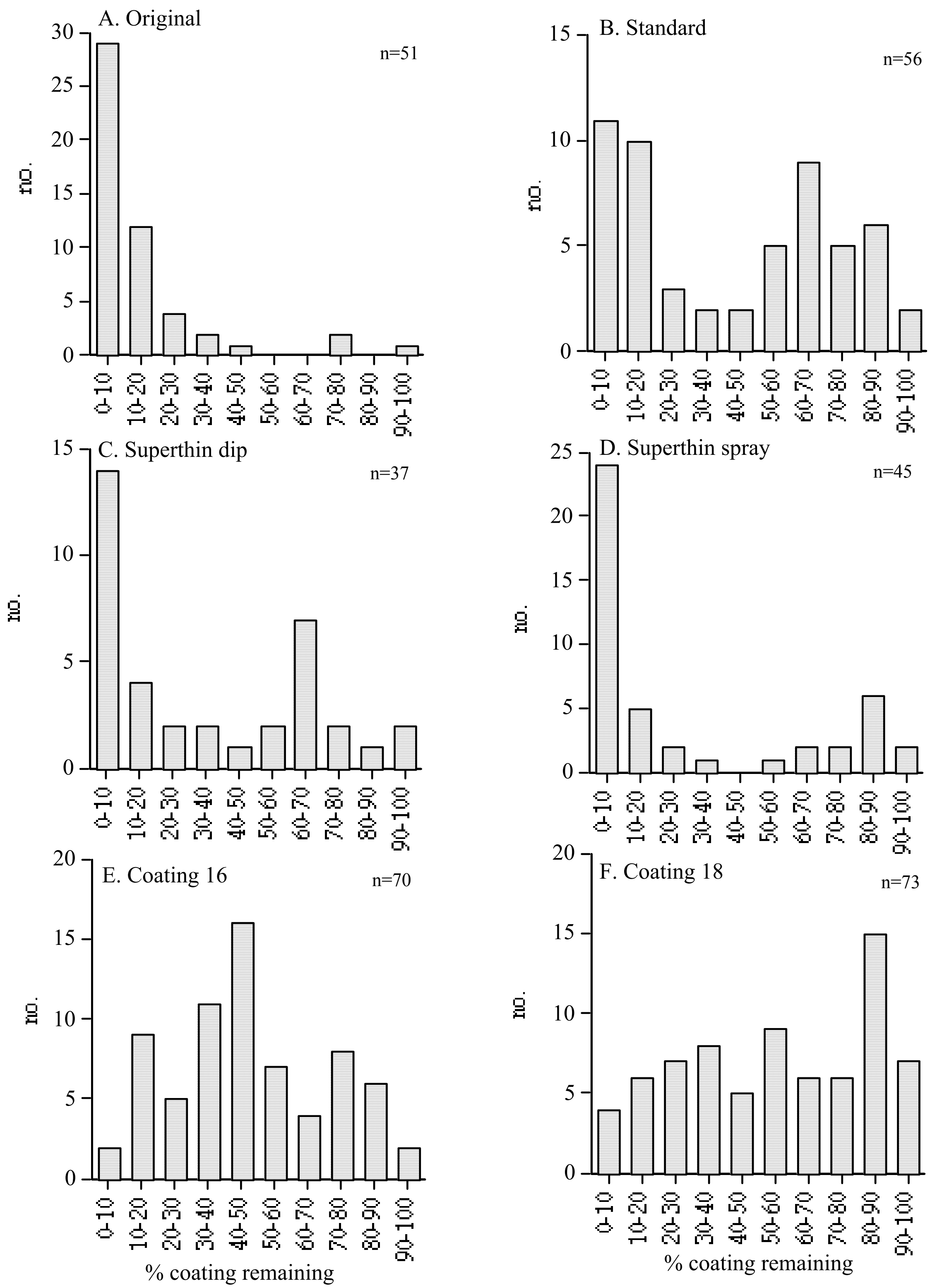

Figure 6.4.4. Frequency histograms of the percentage of coating remaining on shells after rumbling,from the <u>subtidal region</u>. A=Original, B=Standard, C=Superthin Dip, D=Superthin Spray, E=Coating 16, F=Coating 18.

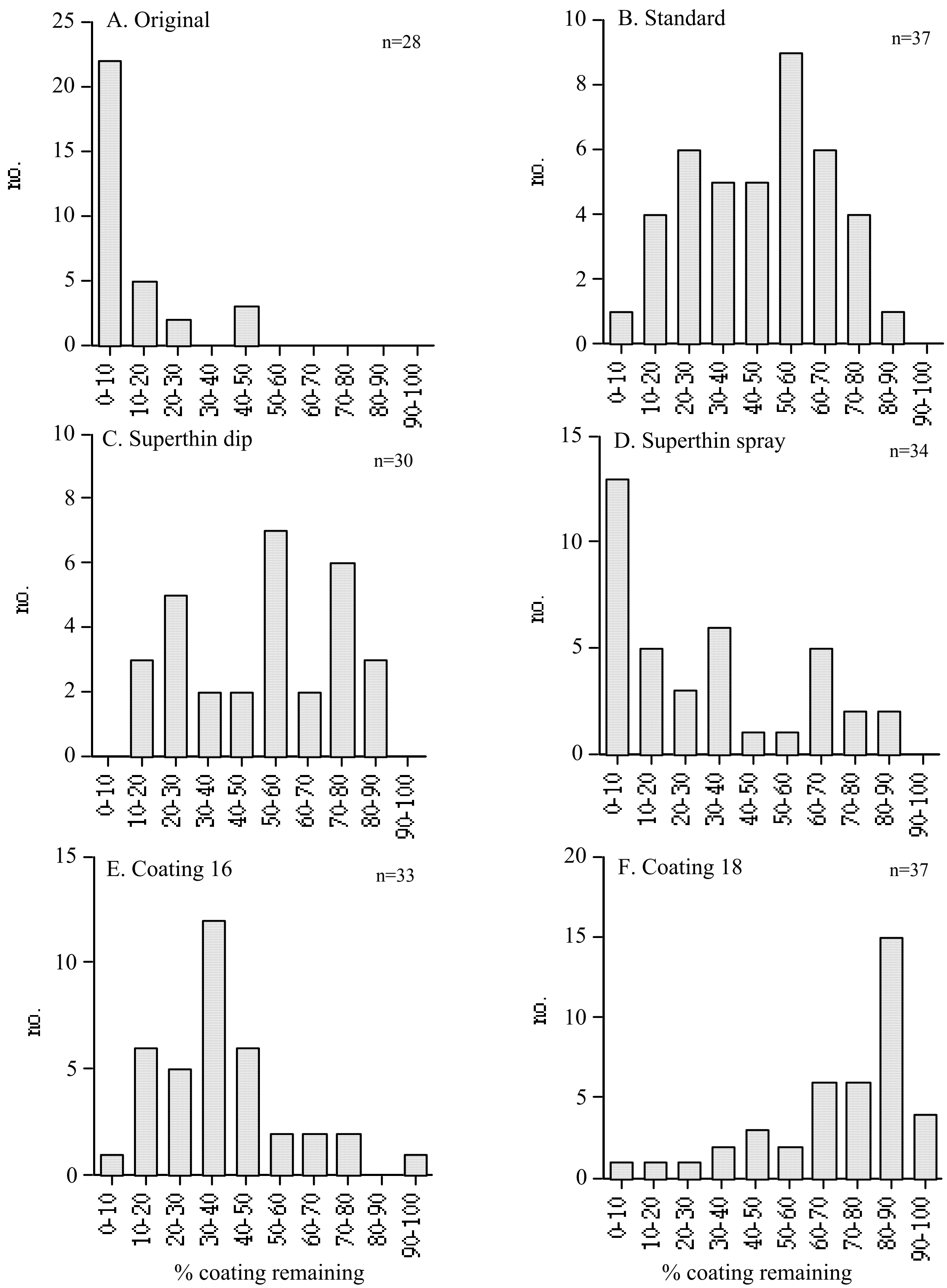

Figure 6.4.5. Frequency histograms of the percentage of coating remaining on shells, after rumbling, from the intertidal region. A=Original, B=Standard, C=Superthin Dip, D=Superthin Spray, E=Coating 16, F=Coating 18.

6.4.3.4 Mortality
In intertidal treatments OysterClear Original and Coating 18 had the highest mortality at 12% compared to the control with 4% mortality, measured at 12 weeks. All the remaining treatments in the intertidal had mortalities ranging from 4-8%. Mortality of control subtidal oysters was 8% and coated treatments were not different from controls after 12 weeks.

6.4.4 Summary

- OysterClear coatings were successful at inhibiting fouling for a 6 month period (26 weeks).

- Rumbling of the oysters facilitated the removal of a high proportion of coating for OysterClear variations.

- OysterClear Original (dip application) and Superthin (spray application) were the best coatings inhibiting the settlement of oyster overcatch and barnacles. These coatings gave the best overall inhibition of fouling and the greatest coating integrity during the period of immersion combined with the highest percentage marketability at the conclusion of the trial.

- Coating 18 also inhibited fouling well but the presence of the AI, the white coating and the difficulty in removing the coating makes it unsuitable for this application.

- The use of high application temperature coatings did not affect survivorship.

- Superthin spray had marginally better efficacy and resulting marketability that the dip application but both performed similarly with respect to coating integrity. It was concluded that the mechanism of action of these coatings needed to be better understood. Investigation of surface characteristics using microscopy was undertaken and is included in Appendix 6.

- The remainder of the trials focus on OysterClear variations as they use fouling deterrent solutions and are more readily removed from the shell before transport to market.

7. Pre-commercial Trial at Port Macquarie, NSW

7.1 Introduction

At this stage of the project several coatings successfully prevented fouling for a six month period. However it was deemed important to upgrade the trials to industrial scales and adapt the coating technology to meet scale-up requirements following consultation with stakeholders.

Coating development led to two improved OysterClear coatings **Coating 1** and **2**. While these coatings were new technical developments, they are based on the Superthin formulation and incorporate the best coating features of adhesion and longevity while improving the fouling deterrent characteristics of these coatings. The coatings are hotmelt products with an application temperature of ~90^{0}C. Oysterclear Original was also tested.

To meet industry requirements where oysters are generally handled in bulk, two coating methods (dipping oysters separately and dipping oysters together in larger numbers in trays) were compared to measure their effects on survivorship.

7.2 Materials & Methods

Table 7.1. Coating treatments, temperature and design tested at Port Macquarie August 2000.

Treatments	Dipping Temp. ^{0}C	Method of dipping	Replicate trays	Oysters per tray
Uncoated Control	-	together	6	40
	-	separate	6	40
OysterClear Original	60	together	6	40
	60	separate	6	40
Coating 1	90	together	6	40
	90	separate	6	40
Coating 2	90	together	6	40
	90	separate	6	40

Oysters were transported from the test site at Port Macquarie to Sydney for coating as larger scale coating equipment was only available in Sydney at the time of the trial. The period that oysters were out of water was five days (Sydney rock oysters can cope with this time out of water as a common method of fouling control is to leave oysters out of the water for sometimes up to 10 days (L. Lardner pers. comm.)). The oysters were cleaned and dried before coating and housed in timber frame trays (30cm x 20cm) covered with standard plastic oyster tray mesh. Oysters coated 'separately' were packed in these trays immediately after coating, and oysters 'dipped together' were dipped into the coating in the tray at a density of 40 oysters per tray. Immediately after coating oysters were placed in water and then deployed at the test sites within 24 hours.

At the commencement of the trial in August 2000, there were six replicate trays per treatment intended for the subtidal site. At the start of the trial each tray contained 40 oysters (=240 oysters per treatment). However, growth was rapid after six weeks and cramping was evident in the trays therefore half of the oysters were removed and placed into new racks and deployed intertidally. Therefore, after six weeks there were six replicate trays containing 20 oysters (=120 oysters per treatment) at both subtidal and intertidal sites.

The trial was monitored every six to eight weeks and oyster spat and barnacle fouling measured as percentage cover of shell on a subset of 60 oysters per treatment. Coating cover (percent remaining) and integrity were also recorded. At 10 weeks the effect on mortality of coating separately or together was measured. After 10 weeks this factor was removed from analysis. Digital images were taken for future reference.

The trial was concluded after 24 weeks.

Percentage marketability of all the oysters was determined by the manager and the owner of Oyster Technologies oyster lease by visual inspection. The treatment replicates were combined (n=120) and 'rumbled', as described previously, to facilitate removal of coating and fouling and the percentage marketability reassessed.

Percent fouling data was transformed using arcsin-sqrt transformation to normalise the data and outliers were removed (only 1-2 data points per treatment were removed as they were three standard deviations from the mean). The effect of coating on percent cover of barnacles was compared by separate one-way ANOVAs for intertidal and subtidal data. For oyster overcatch data percent cover was very low in both intertidal and subtidal treatments and therefore were not compared statistically as they did not meet the assumptions of homogeneity of variance.

7.3 Results

7.3.1 Fouling

Intertidal

In the intertidal treatments two spat falls that occurred during the 24 weeks of the trial were both minor, and subsequently numbers of spat were very low (5% on controls, Figure 7.1a). However, despite the low overall settlement there was very little (<1%) or no spat on coated shell. Both Coating 1 and 2 significantly deterred barnacle settlement but Original was not significantly different from controls (one-way ANOVA, Tukey's test, $\alpha = 0.05$; Figure 7.2a, Appendix 7).

Subtidal

The results from the subtidal trial followed the same trend but fouling by barnacles was marginally greater, giving a more reliable indication of coating effectiveness. All coatings, including Original significantly deterred barnacle settlement (one-way ANOVA, Tukey's test, $\alpha = 0.05$; Figure 7.2b, Appendix 7). Spat numbers were low and all coatings inhibited spat settlement with coated treatments limiting fouling to < 1% cover compared with controls that were 7% fouled (Figure 7.1b).

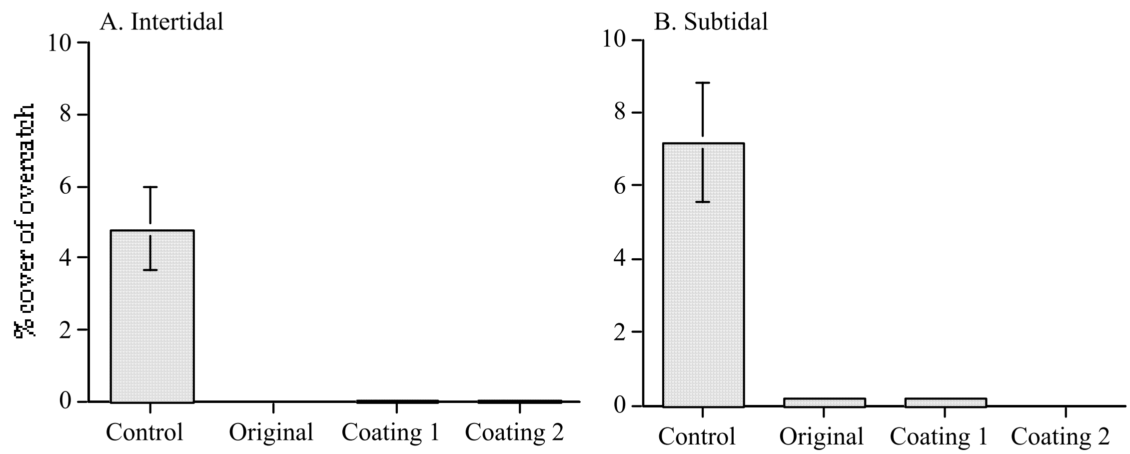

Figure 7.1. Percent overcatch cover after 24 weeks on control and coated oysters from intertidal (A) and subtidal (B) treatments (Data are mean ± SE, n=60).

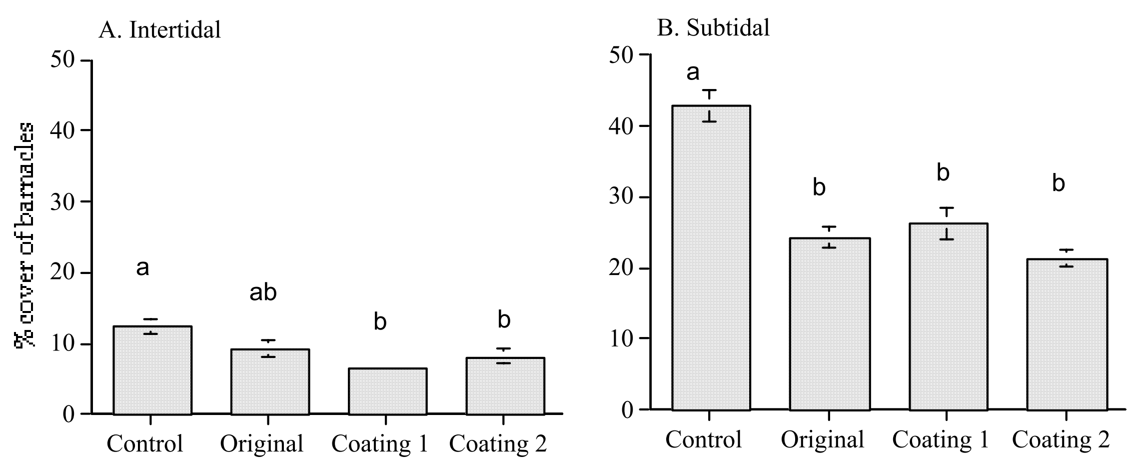

Figure 7.2. Percent cover of barnacles after 24 weeks on control and coated oysters from intertidal (A) and subtidal (B) treatments (Data are mean ± SE, n=59; for each graph bars sharing the same letter are not significantly different from each other as determined by Tukey's post-hoc tests).

7.3.2 Marketability

At the end of 24 weeks the results for percent marketability for all coated oysters were excellent (Figure 7.3). 100% of oysters from all coated treatments were marketable from both intertidal and subtidal locations, compared to 91% and 83% of controls respectively. These figures did not change after rumbling.

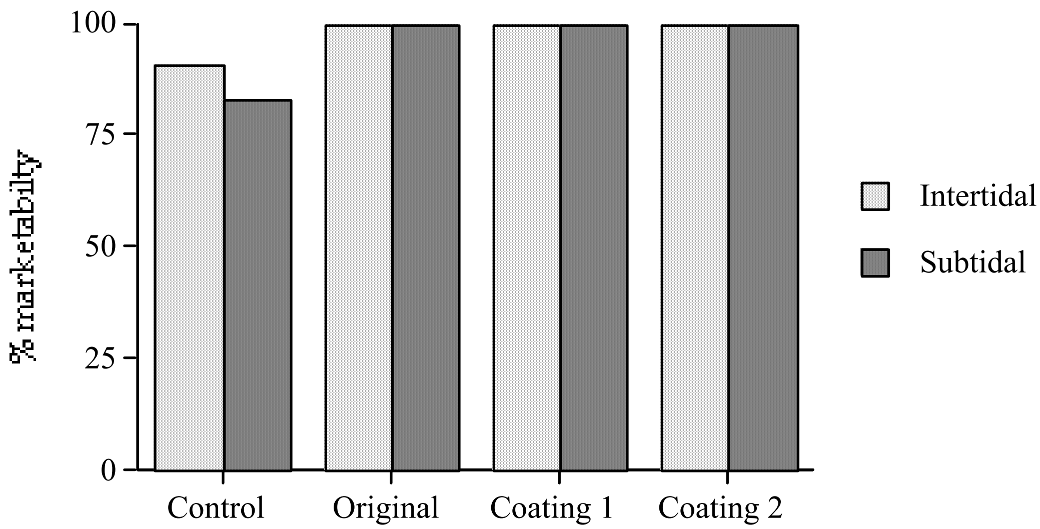

Figure 7.3. Percent marketability of control and coated oysters from intertidal and subtidal treatments before rumbling (n=120).

7.3.3 Coating integrity

After 24 weeks Coating 2 had the best coating cover and coating integrity with many oysters retaining over 90% cover of coating (mean 75% ± 2SE), and the coating was ductile and firm (Figure 7.4e & 7.5e). The few oyster spat that had settled on the coating were easily removed. Similarly, small barnacles were restricted to the surface of the coatings, facilitating easy removal, with only the largest barnacles growing through the coating. In the cases where they had grown through, the surrounding coating had not cracked and remained in good condition. Coating 1 performed similarly to Coating 2 although did not maintain cover as well (Figure 7.4c & 7.5c). Original had the lowest cover of coating remaining at the end of the trial (< 50%) (Figure 7.4a & 7.5a).

At the completion of the trial oysters were 'rumbled'. Coating loss through rumbling was the most effective for oysters coated with Original (compare Figures 7.4a & b and 7.5a & b). This correlates with its suboptimal adhesion in the trial. Rumbling was also more effective for subtidal treatments with a further 55% of Original lost (Figure 7.5b) and 60% and 30% for Coatings 1 and 2 respectively (Figure 7.5d & f). In the intertidal treatments coating loss was lower with a further 40% removed from oysters coated with Original (Figure 7.4b) but only 5 and 15% for oysters coated with Coating 1 and 2 respectively (Figure 7.4d & 7.4f).

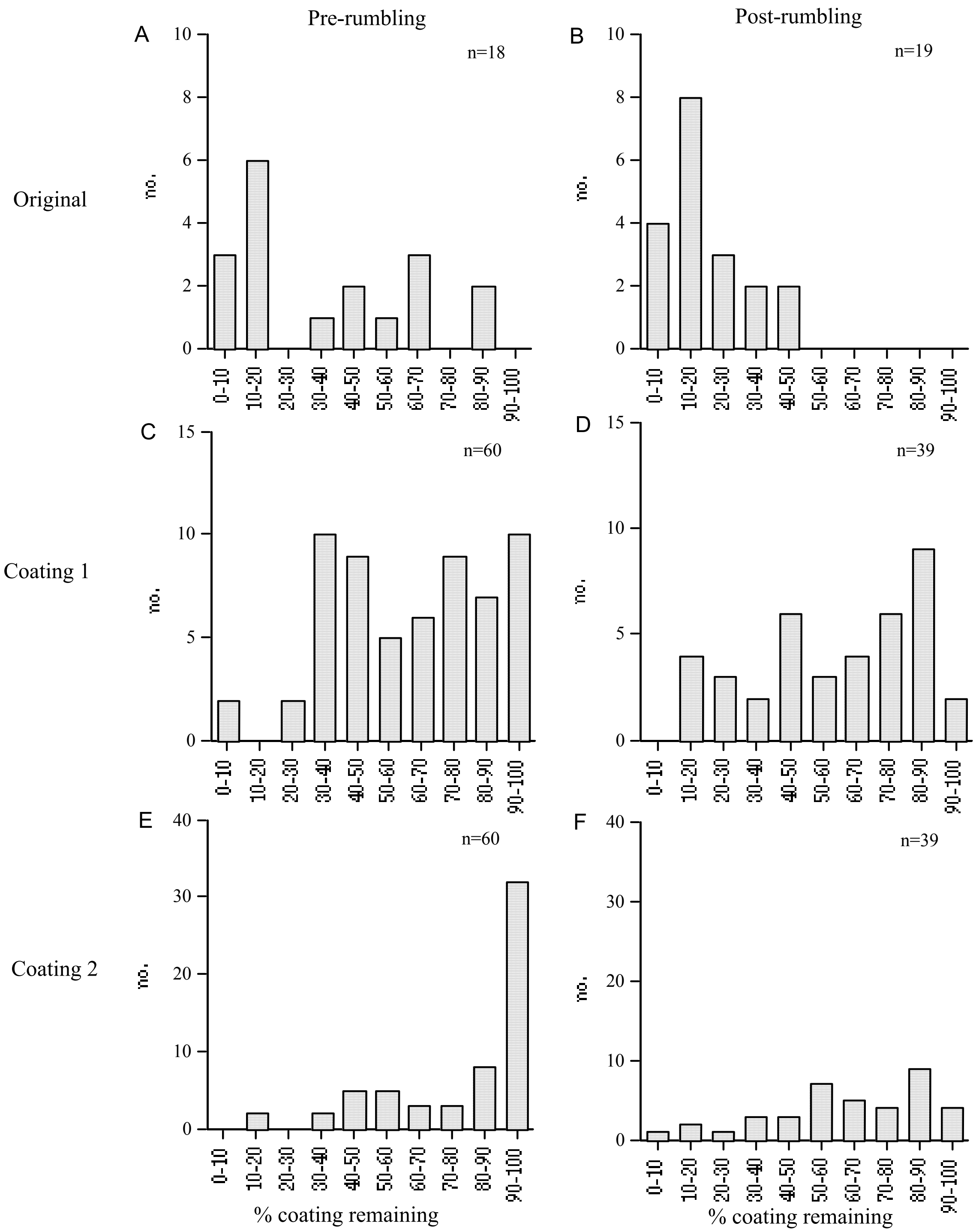

Figure 7.4. Frequency histograms of the distribution of coating remaining on oysters pre and post-rumbling from the intertidal site at Port Macquarie. Pre-rumbling of Original (A), Coating 1(C), and Coating 2 (E). Post-rumbling of Original (B), Coating 1(D), and Coating 2 (F).

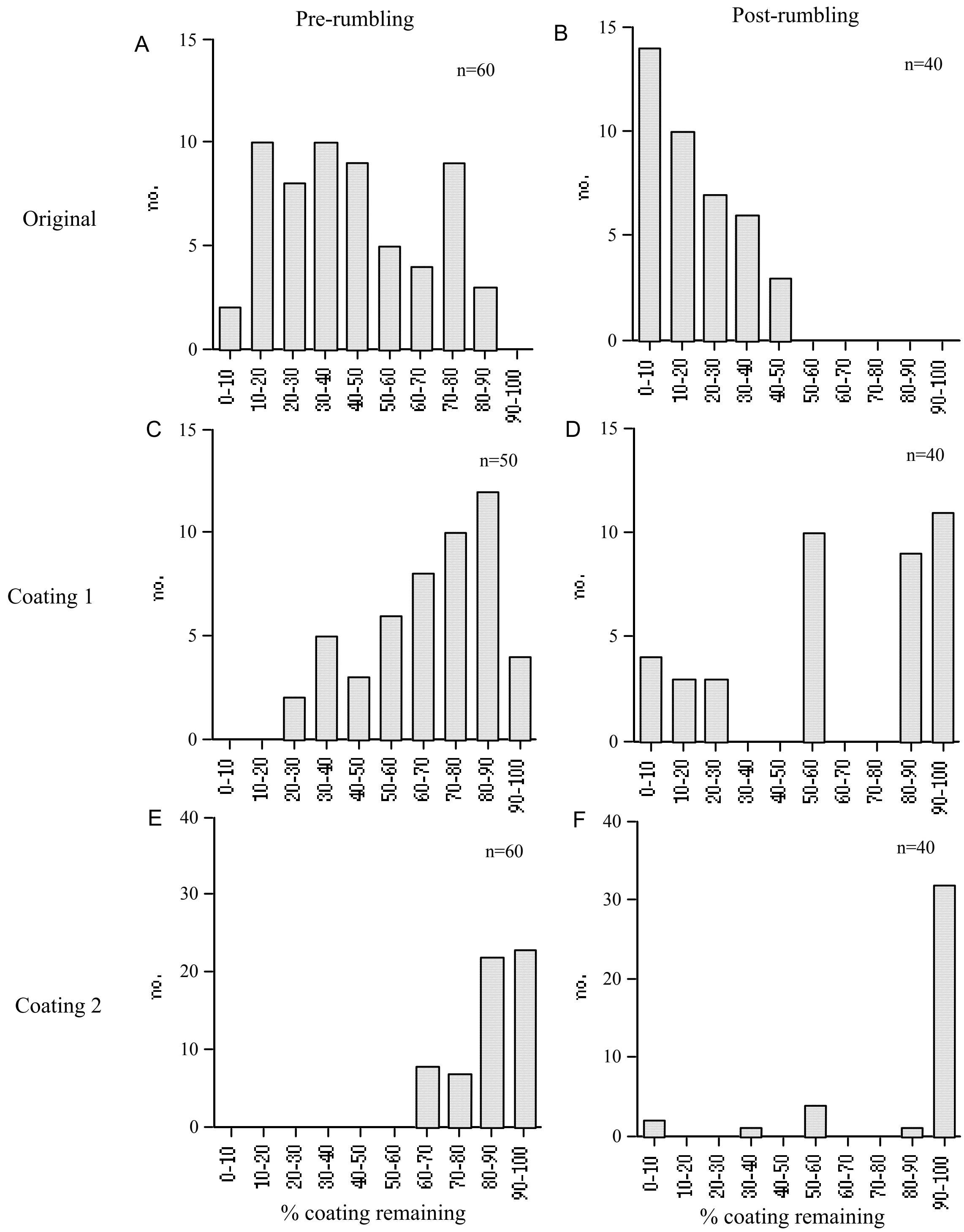

Figure 7.5. Frequency histograms of the distribution of coating remaining on oysters pre and post-rumbling from the subtidal site at Port Macquarie. Pre-rumbling of Original (A), Coating 1(C), and Coating 2 (E). Post-rumbling of Original (B), Coating 1(D), and Coating 2 (F).

7.3.4 Mortality

Mortality occurred in the first 10 weeks after coating and was higher, across all coating treatments, for the oysters dipped together than those dipped separately. Oysters coated with Original suffered the highest mortality (42% in dipped together). Coating 1 and 2 had similar levels of mortality (~22%) and these were much higher than for controls which had a mortality of 4.2 and 5% in the separate and together dipped treatments respectively.

These are the highest mortality figures in any trials to date with up to 60% of oysters never opening. The oysters used in this trial had been transported from Port Macquarie, coated in Sydney, and then returned to the water in Port Macquarie with a turn around time of five days. It is possible that the oysters were in a suboptimal state before coating. Previous trials using Original have shown no difference in mortality between control and coated oysters. In later trial oysters were coated on site to minimise mortalities.

7.4 Summary

- Original, Coating 1 and 2 all strongly inhibited overcatch and barnacle fouling.

- Overcatch was very low but all coated treatments showed inhibition.

- Coating 2 adhered to the shell extremely well but was also the most difficult to remove with rumbling. Original was the easiest to remove and had the lowest adhesion and integrity.

- Marketability was similar across all coating types and is related to the low level of fouling.

- The high level of mortality on oysters coated with Original was unexpected. The condition of the oysters prior to treatment may have had a significant impact on mortality.

8.1 Field Trial in Tasmania

8.1.1 Introduction

An efficacy trial commenced at Camerons of Tasmania Pty Ltd, in February 2001. This trial evaluated the effectiveness of OysterClear Original and Coating 2 on Pacific oysters at a site in southern Tasmania. Fouling at this location differs significantly from that typical to Tasmania's East coast, with serpulid worms being the major fouling problem. This trial aided the project by providing valuable data on antifouling differences attributable to geographic and biological variation.

8.1.2 Materials & Methods

A total of 180 oysters were used for the trial. Two groups of 60 oysters used for coating and a group of 60 oysters used as a control. Oysters were dried for 5 hours before coating, and the coating applied by dipping each oyster. Each oyster was immersed into a bucket of seawater within 2-5 seconds of coating application.

Each group of 60 oysters was divided into triplicate sets of 20 shells, and each set placed into an individual tray for the trial. Control oysters were dried with the treatment oysters prior to placement into triplicate trays. The trays were immersed in a temporary holding area (flow-through seawater) within 2 hours of coating application, before being transported to the lease site the following day.

All trays were fixed into a single stack (of nine trays). The triplicates were located in a blocked design so each treatment (Control, Original and Coating 2) was located throughout the stack to account for variations in fouling due to depth.

Mortality was checked after six weeks and at twelve weeks the number of serpulid worms settled on each shell was counted and the trial was ended.

Data were log(1+x) transformed to achieve homogeneity of variances tested by Levene's test and a nested ANOVA comparing treatment and trays nested within treatment was performed. The number of worms on each treatment was then compared using Tukey's post hoc tests.

8.1.3 Results

Oysters coated with Original were significantly less fouled than the controls but were not significantly different from oysters coated with Coating 2 (nested ANOVA, p<0.001, Figure 8.1.1). Original limited the number of worms per oyster to five as compared with controls which had an average of 16 worms. Results with Coating 2 were not significantly different from the controls but this coating showed a deterrent effect limiting settlement to an average of seven worms per shell. A significant interaction occurred with the numbers of serpulid worms and height of the trays within the block design (Appendix 8). There was less fouling on higher trays and fouling increased with depth.

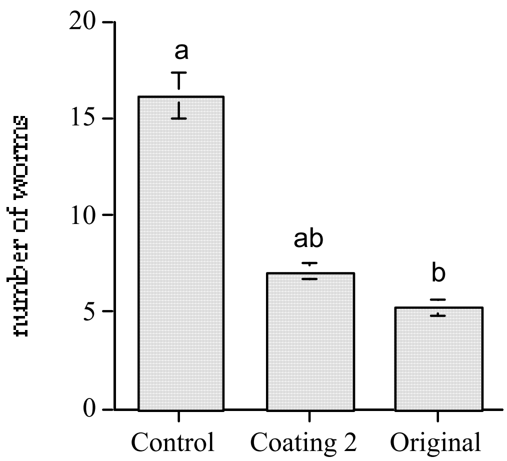

Figure 8.1.1. Number of worms settled on control and coated Pacific oysters after 12 weeks. (Data are mean ± SE. n=60. Bars sharing the same letter are not significantly different at the 0.05 level).

There was no mortality in control and Coating 2 treatments however there was 20% mortality in the Original treatment.

8.1.4 Summary

- OysterClear coatings were effective deterrents of serpulid worm settlement.
- Original was the most effective coating but both performed well compared with controls.
- There was no mortality in oysters coated with Coating 2 however there was 20% in oysters coated with Original.

8.2 Field Trial in South Australia

8.2.1 Introduction

In December 2000 a trial was established in collaboration with the South Australian Research and Development Institute (SARDI) and P. Williams (Stirling University, Scotland) to test two antifouling coatings on bivalve species farmed in South Australia. The species coated were Pacific oysters (*Crassostrea gigas*), Doughboy scallops (*Mimachlamys asperrima*) and Queen scallops (*Equichlamys bifrons*). The coatings tested were OysterClear Original and Coating 2.

8.2.2 Materials & Methods

8.2.2.1 Pacific Oysters

1200 oysters were taken from two farms in South Australia: Stansbury on the Yorke Peninsula and Cowell on the Eyre Peninsula, and transported to the SARDI South Australian Aquatic Science Centre (SAASC) in Adelaide where coating took place. Oysters were cleaned to remove any prior fouling and dried for two hours before coating. Original and Coating 2 were heated to 55^0C and 70^0C respectively using hotplates and an electric wok. Oysters were dipped into the coatings using a sieve and remained in the coating for approximately one second. They were immediately placed into cool water to reduce any possible heat shock. After coating, the oysters were placed back into tanks at SARDI for two days and then returned to their respective leases and deployed subtidally at Stansbury and intertidally at Cowell.

Table 8.2.1. Coatings tested and design of trial in South Australia, Dec. 2000.

	Cowell	**Stansbury**	
Control	40 oysters/basket = 200 oysters	40 oysters/basket = 200 oysters	= 400
Original	40 oysters/basket = 200 oysters	40 oysters/basket = 200 oysters	= 400
Coating 2	40 oysters/basket = 200 oysters	40 oysters/basket = 200 oysters	= 400
	= 600	= 600	1200

The oysters were sent to SAASC at 4, 8 and 12 weeks when total percent fouling was measured. At the end of the trial oysters were measured and weighed (wet weight after all fouling was removed). At 12 weeks oysters were rumbled to establish how much fouling and coating could be removed. Due to the lack of a commercial machine, oysters were placed in 60cm tall plastic drum with a 55cm circumference and the drum was manually rotated for a set period of time. The drum also contained dead oyster valves to act as an abrasive (Williams, 2001).

Fouling data (%) was transformed using sqrt-arcsine transformation to normalise the data. A one-way ANOVA was used to test the effect of treatment on fouling cover for the 12 week sample only. Separate one-way ANOVA were performed to test the effect of coating on the height and weight of oysters after 12 weeks (Williams, 2001).

8.2.2.2 Scallops
Twenty individuals of each species, Queens and Doughboys, were coated initially as it was important to determine if the coating and/or coating process had any adverse affects on the scallop. The scallops were coated using the same method as for the oysters. After coating, the scallops were returned immediately to a flowing seawater system and checked for mortality after 24 hours.

8.2.3 Results

8.2.3.1 Fouling
Both Original and Coating 2 significantly inhibited fouling at Cowell after three months reducing cover by 50% compared with controls (One-way ANOVA, $p<0.01$; Figure 8.2.1). The fouling assemblages varied considerably between the two sites with fouling at Cowell almost completely dominated by barnacles (98-99% of the total cover) and algae dominated the assemblage at Stansbury (~ 98%). No boring polychaete worm scars were found on coated oysters at either site. Fouling at Stansbury was variable and after three months coated and control oysters had a similar covering of algae (Figure 8.2.2) (Williams, 2001).

Rumbling removed fouling to varying extents from all treatments including controls (Figure 8.2.3). At Cowell rumbling removed a further 50% of fouling on control oysters and oysters coated with Original and 25% from oysters coated with Coating 2 (Figure 8.2.3a). Further, barnacles that had settled on the coated shell were far easier to remove compared to those on the uncoated surface. Original and Coating 2 performed similarly with regards to fouling deterrence however coating integrity over the length of the trial was noted to be better for Original. The percent cover of coating remaining at the end of the trial and after rumbling was not quantified (Williams, 2001).

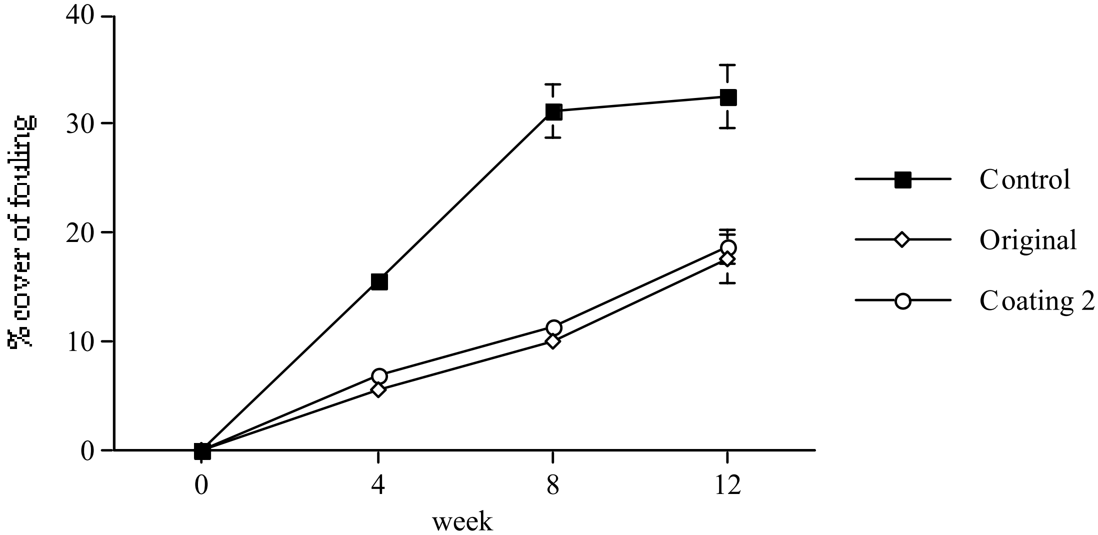

Figure 8.2.1. Percent cover of fouling (all fouling types combined) on control and coated oysters over three months at Cowell S.A. (mean ± SE. n=40) (Williams, 2001).

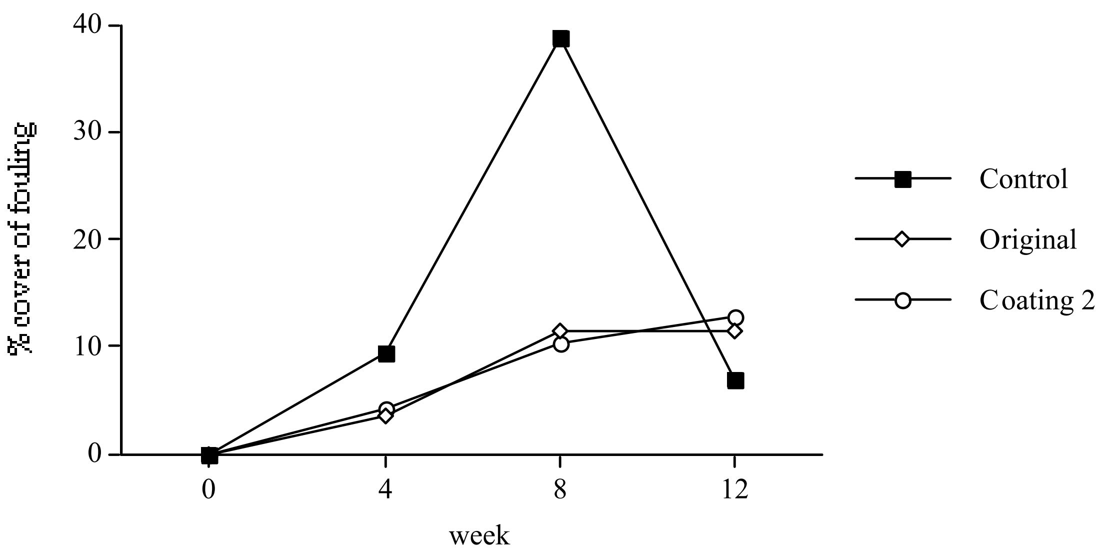

Figure 8.2.2. Percent cover of fouling (all fouling types combined) on control and coated oysters over three months at Stansbury, S.A. (mean ± SE. n=40) (Williams, 2001).

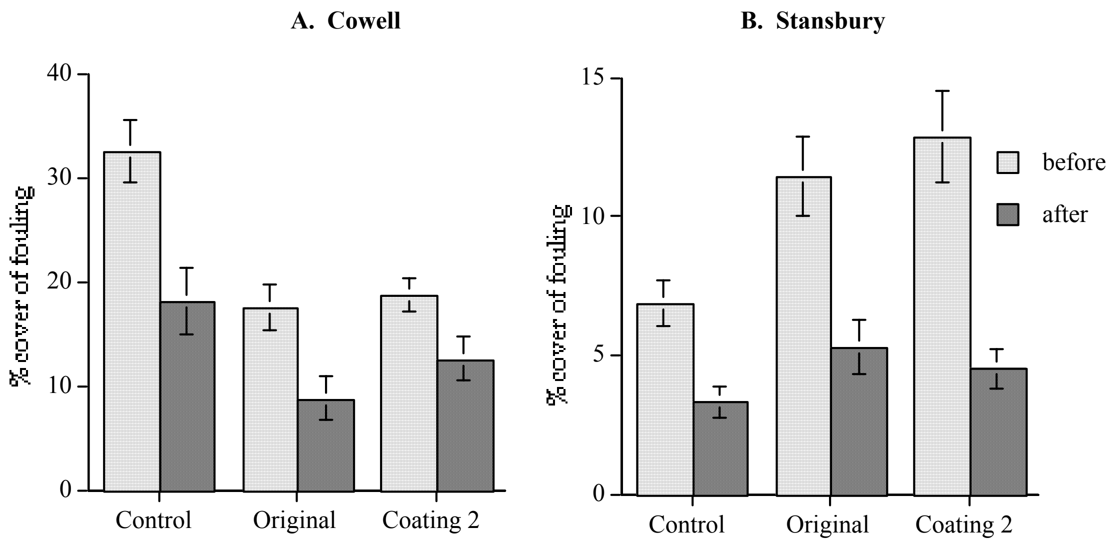

Figure 8.2.3. Effect of rumbling on percent cover of fouling on control and coated oysters after three months at Cowell (A) and Stansbury (B) (Data are mean ± SE. n=40). (modified from Williams, 2001).

8.2.3.2 Weight and height of oysters

All oysters survived the coating application and there was very low mortality over the duration of the trial, on both coated (2 dead) and control oysters (4 dead). After three months there was no long term effects of the coating on oyster growth as neither oyster weight or height at Cowell or Stansbury, was significantly affected by coating when compared with controls (One-way ANOVA, p>0.05 for weight and height, Figure 8.2.4 a & b).

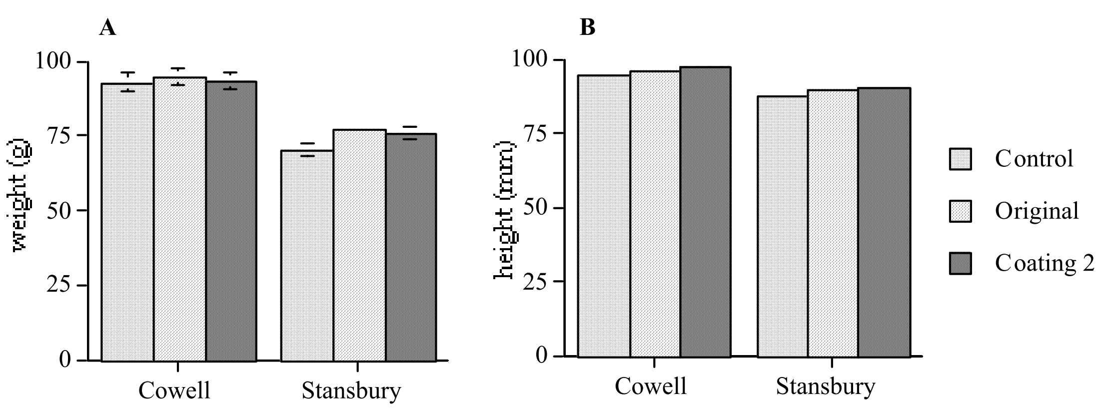

Figure 8.2.4. Effect of coating on weight (A) and height (B) of control and coated Pacific oysters after three months. (Data are means ± SE; n=30 weight; n=60 length). (modified from Williams, 2001).

8.2.3.3 Scallops

Queen scallops were amenable to coating with a survival equal to that of the uncoated controls (88%). However only a small percentage of Doughboys survived the coating process (<5%). This is because Doughboys do not close completely and the coating leaks inside the shell. The technology cannot be adapted to overcome this problem and as Doughboys were the prime target species, trials on scallops were not continued past the initial lab experiments. However, wild *Mimachlamys asperrima* have been found to have an association with epizoic sponges which protects them from predation by starfish and may protect them from fouling (Pitcher and Butler, 1987).

8.2.4 Summary

- Original and Coating 2 reduced fouling on Pacific oysters by ~ 50% when compared with controls at Cowell after 3 months. The coating was effective against both barnacles and algae.
- The coatings adhered well to the shell for the length of the trial.
- Removal of coating was not measured.
- The technique of rumbling was successful at removing fouling.
- The coating process has no effect on oyster mortality, height and weight of Pacific oysters.
- The technology is also suitable for Queen scallops.
- The technology is unsuitable for Doughboy scallops.

9. Commercial Trial at Port Macquarie, NSW

9.1 Introduction

This trial aimed to test the coating application process on a commercial scale by treating large numbers of oysters in a method that would be appropriate and easy to incorporate into management practices for small and large farms. The trial was conducted at Holiday Coast Oyster Technologies at Port Macquarie. A 1.44m^3 bath was custom built to accommodate this commercial scale application. The bath was constructed from marine-grade aluminium, with a removable tray insert (2.4m x 1.2m x 0.5m). This is housed in a gas-heated water jacket, allowing coatings to be evenly and quickly heated to a range of temperatures. The cost of the bath construction was additional to FRDC funding and was incurred by the Centre for Marine Biofouling and Bio-Innovation, UNSW. The bath was designed to be portable for transport between farms. The bath was designed to facilitate coating of oysters using different culturing methods including trays and baskets. In the pre-commercial trial Coating 1 and 2 performed similarly with respect to fouling deterrence but Coating 2 was chosen to continue with in the commercial trial on a cost basis.

9.2 Materials & Methods

OysterClear Original and Coating 2 were tested, based upon the results of the pre-commercial trial at Port Macquarie. Original was included as a control coating *ie* one that has been used across the whole range of trials and importantly because it is to date still the easiest coating to remove at the end of a trial. The coatings were trialed on Sydney rock oysters and the trial commenced in April 2001. See Table 9.1 for design.

Table 9.1. Design for Commercial trial at Port Macquarie, April 2001

	Control	Original (70^0C)	Coating 2 (85^0C)	
Intertidal	**3 trays** 384 oysters/tray = 1152 oysters	**3 trays** 384 oysters/tray = 1152 oysters	**3 trays** 384 oysters/tray = 1152 oysters	= 3456
Subtidal	**3 trays** 384 oysters/tray = 1152 oysters	**3 trays** 384 oysters/tray = 1152 oysters	**3 trays** 384 oysters/tray = 1152 oysters	= 3456
	= 2304	= 2304	= 2304	= 6912

Oysters were placed into large trays (at the densities shown above), dipped into the coating in the custom built bath for ~ 2 s and then immediately immersed in cool water. The oysters were then transferred to new trays and these were deployed in both the intertidal and subtidal sites at Port Macquarie within six hours of coating. Appendix 9 shows the application methods.

The trial was monitored at 7, 14 and 31 weeks and percent fouling cover, oyster mortality and coating performance were assessed on a subset of oysters (n=50 for each treatment). At 14 weeks, after measurements were taken, half of the treated and control oysters (*ie* 576 oysters per treatment for both subtidal and intertidal) were transported back to Sydney and 'rumbled' to determine the amount of coating and fouling removed by this process. These oysters were then returned to the growers to be returned to the normal production cycle. The remaining oysters were left in the water, and the fouling estimated and coating re-evaluated at 31 weeks when the trial was terminated and all the oysters brought to Sydney for rumbling.

Due to the large number of zeros in the data sets no formal statistical analyses were performed as they did not meet the assumptions of normality or homogeneity of variances.

9.3 Results

9.3.1 Fouling

Original and Coating 2 were highly successful at inhibiting oyster overcatch for seven months under intense fouling conditions. They reduced cover of fouling to less than 11% intertidally and less than 5% subtidally (Figure 9.1a & b). Control oysters had a cover of 75% at both the intertidal and subtidal sites.

Barnacles were more abundant in the subtidal zone (Figure 9.3b) with 25-30% cover on coated shell and 10% on control shell after 31 weeks. More barnacles were counted on coated shell than controls. This may be due to the overcatch on the control shell outcompeting the barnacles.

Due to the overall low numbers of overcatch on coated shell, rumbling did not significantly reduce overcatch cover (Figure 9.2a & b). Rumbling removed more than 50% of barnacles from coated shell in the subtidal treatment and had no effect on control shell (Figure 9.3b).

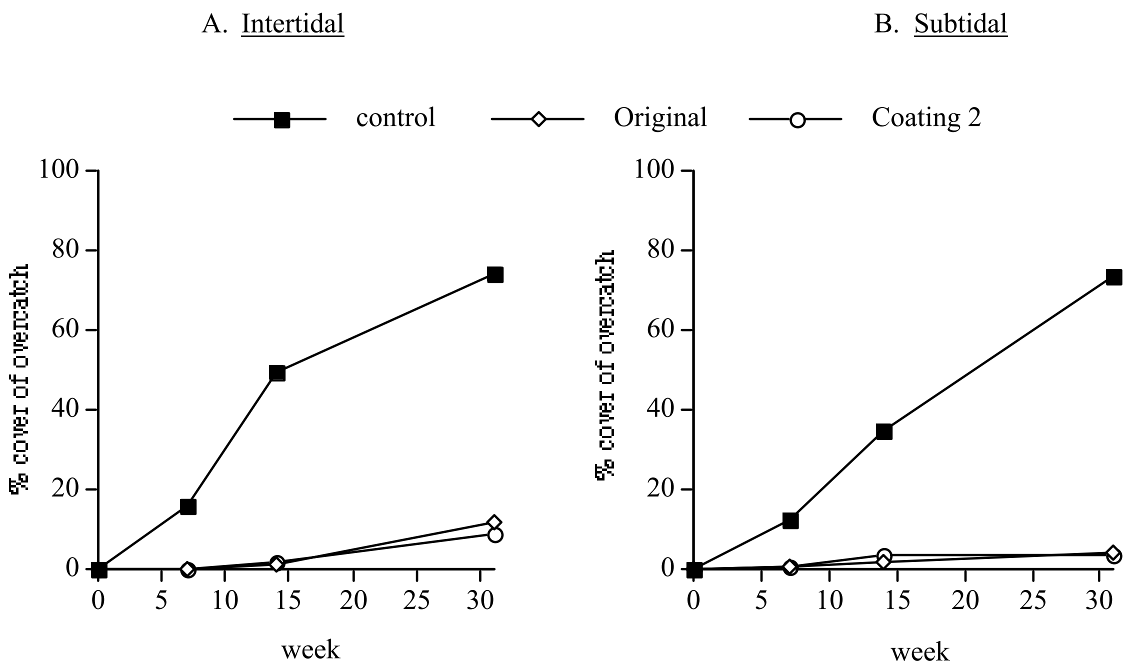

Figure 9.1. Percent overcatch cover on control and coated Sydney rock oysters over 31 weeks grown intertidally (A) and subtidally (B) at Port Macquarie. (Data are mean. n=47).

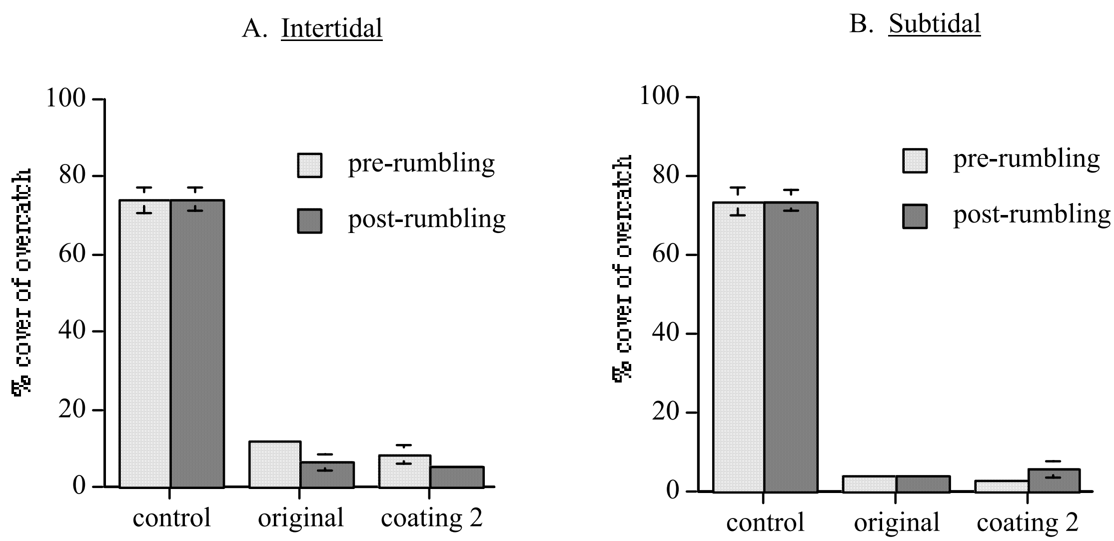

Figure 9.2. Comparison of percent overcatch cover on control and coated shell pre and post-rumbling for intertidal (A) and subtidal (B) sites after 31 weeks at Port Macquarie (Mean ± SE, n=47).

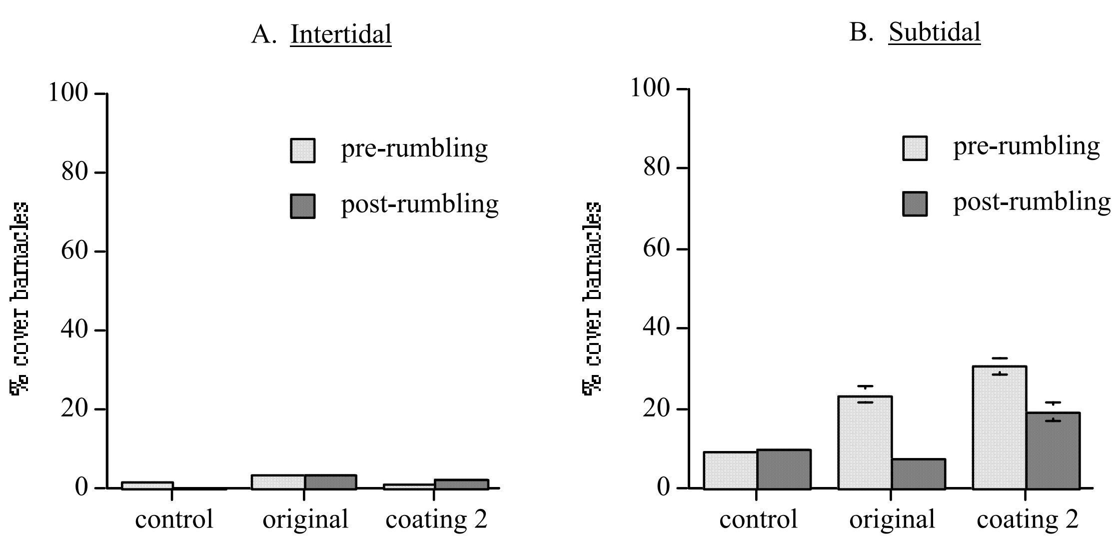

Figure 9.3. Comparison of percent cover of barnacles on control and coated shell pre and post-rumbling for intertidal (A) and subtidal (B) sites after 31 weeks at Port Macquarie (Mean ± SE, n=47).

9.3.2 Coating integrity

Coating 2 had significantly better adhesion and integrity over the length of the trial. After 14 weeks Coating 2 maintained 100% coverage both intertidally and subtidally whilst Original had 75% and 60% respectively (Figure 9.4a & b). Rumbling did not remove Coating 2, however, the process of rumbling removed the majority of Original leaving only 10% cover (Figure 9.4a & b).

After 31 weeks the cover of Original had been further reduced and only ~ 30% of the coating remained prior to rumbling (Figure 9.5a). Rumbling removed the remaining coating. Oysters coated with Original in the subtidal zone followed a similar trend but cover was only 10% before rumbling and this was reduced to 2% after rumbling (Figure 9.5b). Coating 2 had superior adhesion and integrity after 31 weeks with 100% cover remaining on intertidal oysters and 80% on subtidal oysters (Figure 9.5a & b). It was difficult to remove this coating from the shell using the rumbling technique.

The coating integrity of Original and Coating 2 performed differently between the subtidal and intertidal treatments. There was generally more coating loss in subtidal treatments which may be due to continual immersion which erodes the coating.

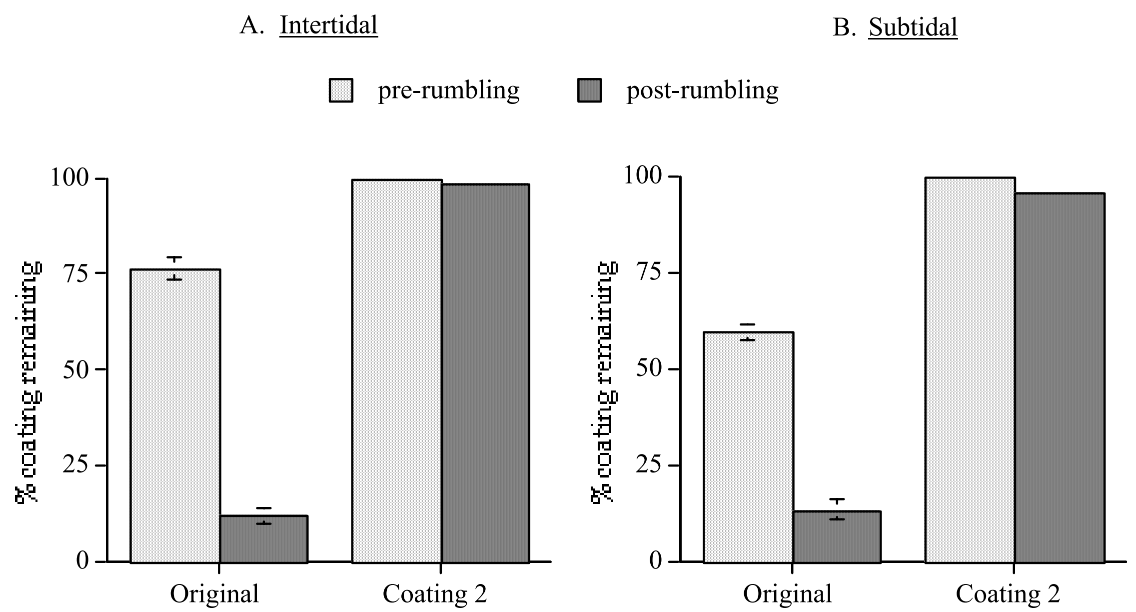

Figure 9.4. Percent coating remaining after 14 weeks on intertidal (A) and subtidal (B) oysters at Port Macquarie. (Data are mean ± SE, n=576).

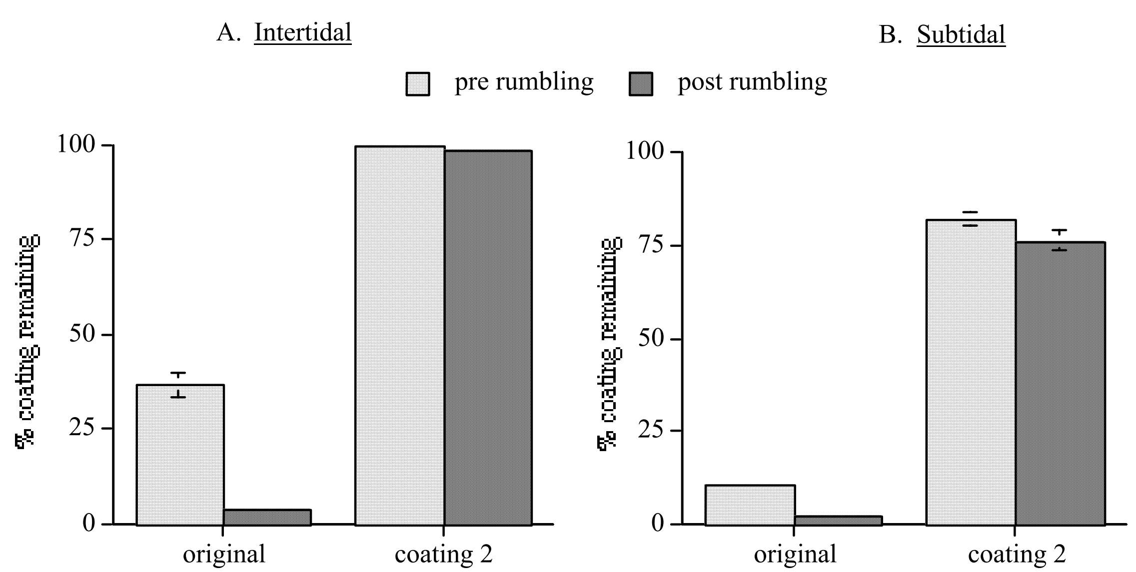

Figure 9.5. Percent coating remaining after 31 weeks on intertidal (A) and subtidal (B) oysters at Port Macquarie. (Data are mean ± SE, n=200).

9.3.3 Mortality

When the trial was first monitored at seven weeks unusually high mortalities across both coated treatments were recorded. The highest mortalities were in Coating 2 with 60% mortality in the intertidal region and 50% subtidally. Original had fewer mortalities (26% and 6% in the intertidal and subtidal locations respectively) but these were significantly higher than controls at 1.2% in the intertidal and 3% in the subtidal. Possible explanations for such a high mortality include adverse weather conditions prior to the trial and new application methods.

Continual rain had delayed the trial by seven weeks because growers were concerned about the condition of the oysters as they had been exposed to an extended period of fresh water. When there is a large fresh water influx, oysters tend to feed less and are more prone to disease and mortality. Growers modify some farm practices to lessen the effects of freshwater on the oysters by transporting them to a different estuary to recover (L. Lardner pers. comm.). Oysters can remain closed and not feeding for up to 10 days when exposed to salinities less than $15g.kg^{-1}$(Nell & Gibbs, 1986). If heavy rainfall reduces salinity to $10g.kg^{-1}$ or less for more than two weeks, then mortalities occur (Nell & Gibbs, 1986). In line with rigorous health regulations for farmers it has been recommended that farmers do not harvest oysters after heavy rainfall (Nell, 1993).

Due to time constraints and the importance of exposing coated oysters to the largest overcatch settlement event of the year in April, the trial was commenced one week after the rainfall period. The growers expected that one week would be sufficient time to condition the oysters and reduce stress associated with low salinity. While this time may be adequate for routine handling and farm procedures, coating at this stage may have caused high mortality.

Another factor new to this trial was coating in large trays with a high density of oysters. The trays are large and relatively awkward to handle, needing two people to lift them, and as a consequence the time that the oysters were immersed in the coating may have been longer. Furthermore, coating a larger volume of oysters means that the heat from the coating is retained around the oysters.

9.4 Summary

- OysterClear Original and Coating 2 strongly deterred overcatch reducing cover to less than 11% compared with 75% on the controls after 31 weeks.

- There was high mortality in oysters coated with both Original and Coating 2.

- Poor oyster condition prior to coating impacts survivorship.

- Oysters in subtidal treatments survived better than their intertidal counterparts.

- 100% of Coating 2 remained on the shell after seven months and was very difficult to remove from the shell by rumbling.

- Original lacked the same adhesion properties of Coating 2 and less than 2% remained on the shell after rumbling.

10. Wattyl Trial

10.1 Introduction

The Aquaculture Division of Wattyl Australia Pty. Ltd. is continuing the work undertaken during this FRDC project. Coatings development thus far has resulted in a coating that is highly effective at deterring overcatch. Coating improvement resulted in a new coating – **Coating 3**. This trial focused principally on the effects of OysterClear Coating 3 on the growth and survival of treated oysters.

10.2 Materials & Methods

Table 10.1. Design for Wattyl trial at Port Macquarie, November 2001

Treatment	Bag no.	No. of oysters	Total no. oysters per treatment
Control	1	56	
	2	52	108
Coating 3	1	95	
	2	92	187

The trial was conducted at Port Macquarie. A coating application bath similar to that used in the commercial trial (Section 9) was built and the costs incurred by Wattyl Australia. The bath was designed to be smaller and more portable than its predecessor and held 200L of coating. The dipping chamber is housed within a gas-heated water jacket. Oysters were coated with OysterClear Coating 3. Control oysters were handled in the same way but without dipping and placed into two baskets. Survival was assessed four weeks after coating, and growth five months after coating. Height and length were measured using vernier calipers.

There was no significant difference between baskets therefore data for height and length were pooled. Data were normally distributed and Levene's test for homogeneity of variances was not significant so separate T-tests for differences in height and width between control and coated oysters were performed.

10.3 Results

The level of mortality in both control and coated oysters was very low (2%).

The height of coated oysters was significantly larger (6.25 ± 0.03 cm) than control oysters (6.11 ± 0.04 cm) (unpaired *t-test*, t=2.635, $p < 0.05$, Figure 10.1a). This occurred despite the fact that oysters in coated treatments were packed at higher densities. There was no significant difference in lengths of control and coated oysters (unpaired *t-test*, t=0.814, $p > 0.05$, Figure 10.2b).

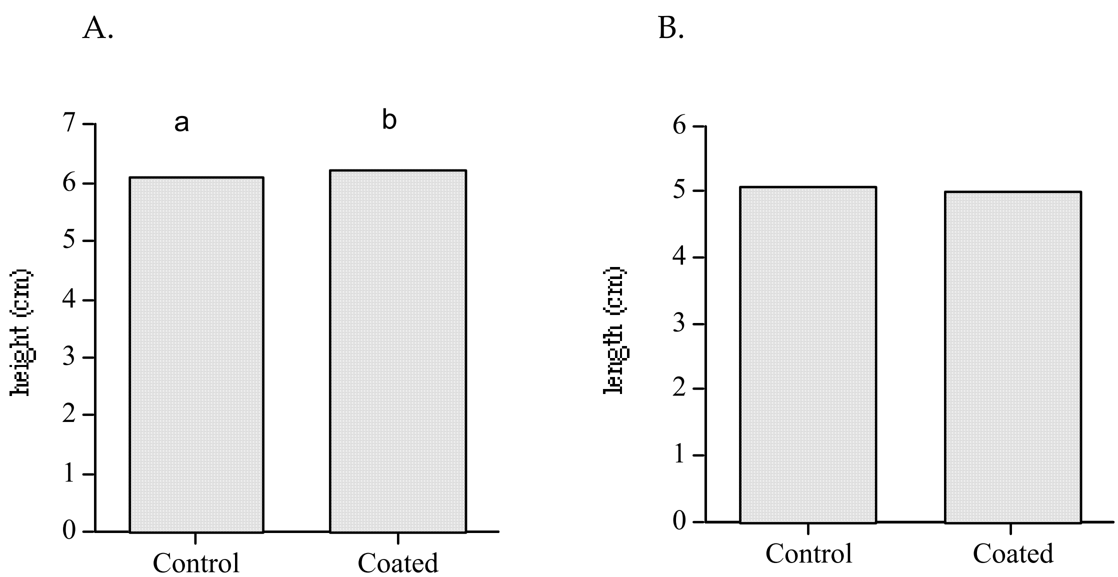

Figure 10.1. Height (A) and length (B) of control and coated oysters after 5 months (Data are mean ± SE. n=108 for control; n=187 for coated; bars sharing the same letter are not significantly different at the 0.05 level).

10.4 Summary

- The height of coated oysters was significantly greater when compared with control oysters after five months.

- There was no additional mortality associated with Coating 3 or the coating process.

11. Project Review

Fouling deterrent coatings suitable for commercial use on edible oysters were successfully developed through an extensive series of lab and field trials (Program sequence, Figure 1). OysterClear coatings are effective at deterring settlement of oyster overcatch for a seven month period under intense fouling conditions. Furthermore, the coating can be removed at the end stage before market and this also facilitates further removal of fouling. The coating has no toxic or sublethal effects on the oysters.

The first coatings tested were chosen for their ability as carriers for a new class of novel compounds. The coatings were non-toxic, being either used with food products or as a non-toxic waterbased coating. While these, with the addition of an active ingredient, proved effective antifoulants they were not ideal. They demonstrated activity for four months but lacked inherent antifouling activity. The need for an active ingredient raises issues with regard to the environment and requires registration by the National Registration Authority (NRA). Therefore, OysterClear was developed with the clear goal of providing a coating to the edible oyster industry that relied on foul-release surface properties rather than chemical deterrence.

These coatings are highly effective at inhibiting settlement of oyster overcatch, reducing it by up to 90% compared with controls in most trials. Other types of fouling such as algae, bryozoans and sponges were also inhibited by the coatings but occurred at such low levels that they were not quantified. Furthermore, these additional fouling organisms are not regarded as a problem by the industry from a marketing perspective.

The coatings deterred fouling by a wider range of fouling organisms as shown by the trials in South Australia and Tasmania. The coatings were effective against solitary ascidians, barnacles and tube-building worms.

The coatings effectiveness against mud worm was not assessed as the incidence of this parasite was low over the period of trials and at the main test site, Port Macquarie.

The series of trials proved that OysterClear can be applied safely and effectively to most edible oyster species including Sydney rock and Pacific oysters. The coating and coating process is also suitable for the scallops *Pecten fumatus* and

Equichlamys bifrons (Queen scallops) but not for *Mimachlamys asperrima* (Doughboys). Early work on coatings on the scallop *Pecten fumatus* found high survivorship of coated scallops and inhibition of a wide range of fouling organisms (Heasman *et al.*, 1998).

The coatings did not inhibit growth of Sydney rock or Pacific oysters. There was no significant difference in height or weight between control and coated Pacific oysters in the trial in South Australia (Section 8.2). In a recent small scale Wattyl trial at Port Macquarie (Section 10) the height of coated oysters was significantly greater than the height of control oysters. There was no significant difference in length of oysters. Coated oysters in most trials were easily able to grow a new frill through the coating and commercial farmers commented that coated oysters appeared as healthy as controls (L. Lardner pers. comm.).

The effect of the coating and the coating process on oyster mortality was variable and appears to be linked with the condition of the oysters prior to coating. In the majority of trials the level of mortality was not different from controls. In the two trials with higher mortality, both had external factors of freshwater influx, or protracted time out of the water. All other experiments including those in Tasmania and South Australia showed clearly that coating application by dipping was well within the tolerance range of these organisms.

Application of the coating via spraying was trialled, to reduce the temperature at which the coating would cover the shell. However, dipping is the preferred method and the use of high application temperatures was not detrimental to oyster health. Nel *et al*. (1996) found a linear response of the internal core temperature of Pacific oysters with ambient water temperature. They immersed oysters for up to 45 seconds and only found high mortality after this time and concluded that when exposure was limited to 40s then the internal temperature of the oyster was not raised above 24^0C. Furthermore, dipping oysters in 70^0C seawater for 40s was very effective at reducing infestation by the mudworm, *Polydora*.

All these trials maintained the time of immersion in the coating to less than a few seconds, and no substantial rise in internal temperature was measured when oysters were dipped up to 5 secs, even up to application temperatures between 80-100^0C.

Dipping was also found to be the most practical application method to incorporate into standard management practices. The construction of the portable aluminium bath with surrounding water jacket meant that coatings could be quickly heated to the correct temperature, large numbers of oysters coated and a range of culture techniques accommodated (eg. trays, baskets). In order to minimise the handling required *ie* transferring batches of oysters from tray to tray, oysters can be coated within baskets or trays and these are then deployed.

Integrated farming practices

Subtidal oyster culture results in higher growth rates compared with intertidal culture due to longer feeding times (Wisely et al., 1979). Intertidal farming was first introduced to overcome the problems of mudworm infestation and biofouling. Coating with OysterClear means that oysters can be farmed subtidally maximising growth rates while reducing the problems associated with fouling. Potential therefore exists to significantly reduce farming periods for the Sydney rock oyster below the current duration of 3 to 4 years.

The coating is designed to be used twice during the production cycle providing 6-7 months of biofouling protection over the period when overcatch fouling is the most intense. This period is seasonal and varies across estuaries. Coatings are recommended for oysters greater than 18 months old. This is compatible with current practise as oysters up to 18 months old are regularly dried and/or cleaned during standard farm practises of grading, sorting and retraying (L. Lardner pers. comm.).

The post-harvest process of rumbling was shown to be important for the removal of fouling and coating. The coating is designed to be removed at the end of the season in one sheet rather than shed pieces over the length of activity. Rumbling of oysters to remove coating and fouling can be easily incorporated into farm management practices as many farmers already use cement mixers to remove mud. Likewise oyster wholesalers such as Sydney City Oysters already ‘rumble’ all the oysters that they handle (J. Zappa pers. comm.).

12. Benefits

Direct Benefits and Beneficiaries

The immediate benefits and beneficiaries will be NSW oyster growers. Other beneficiaries are the major oyster growing states of South Australia and Tasmania. Growers will benefit through decreased labour costs associated with the removal of fouling, and the production of an export quality product. There will also be benefits through the increased ability to conduct subtidal culture resulting in improved growth rates. The benefits described here extend beyond the scope of the initial proposal due to the positive response of the South Australian and Tasmanian oyster industries in adopting new technologies, and their participation in trials to finalise product development.

Flow of Benefits

Fisheries managed by	Commercial Sector	Recreational Sector	Traditional Fishing
NSW	40 %		
QLD			
SA	30 %		
TAS	30 %		
VIC			
WA			
AFMA			
Total	100 %		
Non-Fisheries Beneficiaries			
Summary Flow of Benefits			
Sub Total Commercial Sector	100 %		
Sub Total Recreational Sector			
Sub Total Traditional Sector			
Sub Total Non-Fisheries Beneficiaries			
Summary Flow of Benefits	100 %		

13. Further Development

The development of OysterClear has continued through a research program undertaken by Wattyl Australia Pty Ltd.

The work to date has described trials for both intertidal and subtidal culture methods. This difference in culture technique has several implications for coating development. Intertidal systems are primarily fouled by overcatch, with other fouling types controlled through drying. Subtidal systems are prone to heavy fouling by both overcatch and barnacles, with significant silt accumulation in some waterways. Consequently, coating systems designed for subtidal culture necessitates a broader-range of fouling deterrence. Moreover, variation in culture technique has several implications for coating adhesion; coatings immersed in subtidal systems will require suitable adhesion to overcome continued water immersion, but these systems are not prone to the continued 'rumbling' from wave action in intertidal cages. Consequently, it is likely different versions of the coating will be required for these two culture techniques.

Through recent formulation and field trials undertaken by Wattyl, it has been shown that coating performance may be increased to deter both barnacles and overcatch. Further, trials have identified several strategies for adhesion control and for modifying the cohesive strength of the film. Whilst OysterClear development is therefore nearing completion, commercialisation of the technology must proceed under two caveats:

(1) The coatings are fouling-deterrent, non-toxic systems that prevent the majority of overcatch. Whilst deterrence rates of up to 95% have been repeatedly observed, it is unlikely that systems will deter 100% of overcatch. Nonetheless, any overcatch that does settle is restricted to the coating surface and therefore removed during the process of coating removal.

(2) OysterClear will be released with specific adhesion characteristics that require a minimum level abrasion to remove the film prior to product sale. Given the high variation in 'rumbling' techniques used by processors, it may become more practical for a high-pressure washing during harvesting so the coating is removed prior to packing (either before or after purification).

14. Planned Outcomes

The output of this project is the pre-commercialisation product OysterClear. The program for the further development of the product(s) is clear defined in 'Further Development'. A commercial release of OysterClear by Wattyl Australia is planned for 2003 following final development trials. OysterClear was designed to prevent fouling over the six month "overcatch season" for NSW which extends from December to April. This is the period when Pacific and Sydney rock oysters spawn and larvae settle onto adult shell incurring commercial costs in husbandry to prevent and remove fouling. The initial target market for OysterClear was the intertidal culture of Sydney rock oysters, however, due to product development it has also been applied to subtidally cultured oyster systems, and to Pacific oysters cultured by rack and basket systems in Tasmania and South Australia. In most cases the use of OysterClear has been targeted to the fouling season that occurs over the summer period (December – April). Further to its use directly on shellfish, OysterClear has also been successfully applied to oyster growing equipment in South Australia, and it is envisaged that further product development will take place specifically for this use. This will result in a range of OysterClear products for applications across a broad geographic range and applications. OysterClear will provide financial benefits as the cost of cleaning and loss of growth is greater than the cost of the product and application. OysterClear will also provide environmental benefits through reduced loads of waste from farms, lower levels of fouling organisms as a source of further larval supply, and the prevention of translocation of fouling organisms (in particular overcatch) between sites (estuaries) due to transport of oysters for grow-out.

15. Conclusion

The work described through the current report clearly demonstrates the potential for non-toxic coating systems to prevent overcatch. Rather than preventing fouling through conventional means of toxicity, it has been shown that a fouling-deterrent coating can be constructed through modification of surface conditions (through an interaction of surface smoothness, hydrophobicity, and chemical structure). Moreover, several formulations developed in recent months are based primarily on food-grade materials that meet NRA regulations for direct food contact.

The work has necessarily focused on hot-melt formulations that allow rapid drying of the coating. These systems provide an inexpensive means of providing the specific surface chemistry needed for fouling deterrence, and may be applied in a single coat. Whilst non-toxic coatings are commercially available for high-speed shipping, based on silicone elastomers to control surface chemistry, these are highly expensive multi-coat systems and therefore inappropriate for oyster culture.

Current research has concentrated on alternative hot-melt polymers, and toward the inclusion of other polymers that affect coating adhesion and coating strength. The focus is to provide a system that adheres to shell over a 6-month period (the December to April window of peak overcatch settlement), but looses adhesion strength thereafter. Moreover, this loss of adhesion strength (to allow ease of coating removal during processing) is coupled with cohesive strength of the film so it is removed as a complete sheet rather than as small fragments.

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The Aquaculture CRC
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The Centre for Marine Biofouling & Bio-Innovation, University of New South Wales.

Appendices

Appendix 1 – Intellectual Property

The Intellectual Property for the project and ownership of intellectual property was assigned under Part 3 of the Project Agreement.

The Project was considered a verification trial of existing antifoulants and consistent with this (Part 3 FRDC 98/314) the agreement does not give the FRDC equity in the antifoulant products themselves.

The intellectual property developed through the project was the property of the Aquaculture CRC. The intellectual was protected by patent (PCT/AU 98 00508. *Antifouling of shellfish and aquaculture apparatus*. de Nys, Steinberg, Charlton, Christov) and upon completion of the CRC program this was assigned to Unisearch Pty. Ltd. The manufacturing rights for coatings are licenced by Unisearch to Wattyl Australia Pty. Ltd.

Appendix 2 – Staff

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Appendix 3 – Project Outputs

Industry Presentations

- Workshop held on 3 March 2000 at the Sydney Fish Markets: Problems of producing and marketing the Flat oyster *Ostrea angasi* in NSW. Novel technologies for the reduction of biofouling in shellfish aquaculture.
- NSW Fisheries Workshop - R&D Strategy Meeting. Port Macquarie, July 2000.
- NSW Fisheries Workshop - 2nd Flat oyster (*angasi*) workshop. Bateman's Bay, 20 March 2001. .
- NSW Fisheries Workshop – Alternative materials in the oyster industry. Port Stephens, April 2001.

Conference Presentations

- de Nys, R., Ison, O., McCloy, S., Hodson, S. and Steinberg, P. (1999) Biofouling in Aquaculture – novel methods of control. World Aquaculture '99. 26 April – 2 May 1999.
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- de Nys, R., Ison, O., McCloy, S., Hodson, S. and Steinberg, P. (2001) Preventing biofouling shellfish aquaculture. AquaTas
- de Nys, R., Ison, O., McCloy, S., Hodson, S. and Steinberg, P. (2001) Biofouling in Aquaculture: A Tropical-Temperate Comparison. Australian Marine Sciences Association Annual Conference. Townsville , 3-6 July 2001.

Other

Updates of research in:

- NSW Fisheries 2001. Oyster Industry Report. Status report 2000/2001
- NSW Fisheries 2002. Oyster Industry Report. Status report 2001/2002

Appendix 4 (from Section 5.2)

Table 1. Percent mortality of control and coated oysters in Trial 2 at Port Stephens (Section 5.2.3.1)

	Honey Creek		Oakey Island	
Treatments	Intertidal % mort.	Subtidal % mort.	Intertidal % mort.	Subtidal % mort.
Uncoated control	0	1	0	0
Carrier 1 control	0	0	3	0
Carrier 1 + 15% AI1	1	2	2	1
Carrier 1 thick control	2	2	1	0
Carrier 1 thick + 10% AI1	0	2	1	1
Carrier 2 control	0	2	2	1
Carrier 2 + 10% AI1	2	0	1	0

Table 2. Percent mortality of control and coated oysters in Trial 2 at Port Macquarie (Section 5.2.3.2)

	The Bay		Stormy Point	
Treatments	Intertidal % mort.	Subtidal % mort.	Intertidal % mort.	Subtidal % mort.
Uncoated control	0	1	2	1
Carrier 1 control	0	0	0	0
Carrier 1 + 15% AI1	1	5	0	4
Carrier 1 thick control	0	1	0	0
Carrier 1 thick + 10% AI1	1	1	1	1
Carrier 2 control	1	1	0	3
Carrier 2 + 10% AI1	0	0	1	2

Appendix 5 – Rumbling process

Figure 1. Stainless steel oyster rumbling machine at Sydney City Oysters.

Figure 2. Oysters being loaded into rumbler

Figure 3. Inside of rumbler showing high pressure water jets.

Appendix 6 - Microscopic investigation of the surface of OysterClear

Introduction

Coating application method and the thickness of the applied coats, particularly for this type of coating, has a major effect on adhesion and subsequent efficacy. For example, Superthin dip and spray coatings performed marginally differently with regards to fouling deterrence in the trial at Port Macquarie (Section 6.4) suggesting that there was perhaps different surface characteristics between a spray and dip applied coating. This experiment investigated the surface properties of the coatings using microscopy to better understand the mode of action of deterrence.

Active ingredient 1 (AI1) was included to see its effect on film characteristics. Coating thickness was also hypothesised to be an important factor determining the length of activity of deterrence and the presence of holes in the coating.

Materials & Methods

OysterClear was applied to 8.5cm diameter plastic petri dishes using two application methods, spray and dip. Coating characteristics were further modified by different film thicknesses and types of film. (see Table 1).

Table 1. OysterClear treatments tested

Application	**Coating Thickness (µm)**	**Temp (°C)**
Uncoated control	-	-
Spray - standard film	300	150
Spray – standard film	900	150
Spray – with active	300	150
Spray – with active	900	150
Spray – snowy film	300	70
Spray – snowy film	900	70
Dip	single	70
Dip	double	70

Description of coating types in above table:

Standard coating - The film was sprayed at 150°C. Three passes of the spray nozzle over the dishes gave a film depth of 300µm, while 9 passes gave a film depth of 900µm. The film was applied using the hot spray gun (FRDC 2000/254).

Active coating- Active ingredient 1 was added to the standard Oyster Clear at a 10% loading (by weight). The film types applied were a standard film of 300 and 900μm as above.
Snowy coating - The temperature of OysterClear was lowered to ~70°C and sprayed at a 60° angle. This treatment represents a coating modelled from a successful result when trialled with pearl oysters (FRDC Project No 2000/54)
Dip coating - The single dip treatment was achieved by dipping the petri dishes into ~ 70-80°C Oyster Clear. The double dip treatment was prepared as for the single dip, then refrigerated until cool, before a second application.

Six replicate petri dishes were allocated per treatment. The above treatments were examined for surface appearance and homogeneity, with the frequency and size of airbubbles measured for each treatment. Measurements were taken for each replicate dish in each treatment, with six random fields of view (n=36). Measurements for each field of view at 40 and 100X magnification (Olympus BX50) included the total number of holes and the diameter of each hole. Digital images were taken at 40 and 100X magnification for each treatment (NIH Imaging).

Due to the high number of zeroes in some treatments the data did not meet the assumptions of homogeneity of variances and therefore were not formally analysed.

Results

The OysterClear single and double dip coatings were visually the smoothest and most homogenous coatings with good surface adhesion (Figure 1). The sprayed standard film (300μm) (Figure 2) and active (300μm & 900μm) films (not shown) also had excellent film characteristics and homogeneity. The standard 900μm film (Figure 2) and the snowy coating, 300μm & 900μm, showed an uneven, globular film (Figure 3).

The sprayed standard film (300μm) had dramatically higher numbers of holes (seen in Figure 2) than all other coatings with a mean of 211 (± 34 SE) holes per cm^2 (Figure 4). The 900μm standard film, the 300 and 900μm active film and the dipped film had means of 39, 31, 25 and 6 holes per cm^2 of coating respectively. The double dip and snowy coatings had no holes (Figure 4).

The mean size of holes for each coating differed between treatments (Figure 5). The mean diameter for the 300μm standard film was 28μm (± 1.7 SE) while the other treatments with fewer numbers of holes had values ranging from a mean of 35 to 51μm. The mean for the single dip coating was not included due to a limited number of holes (n=2).

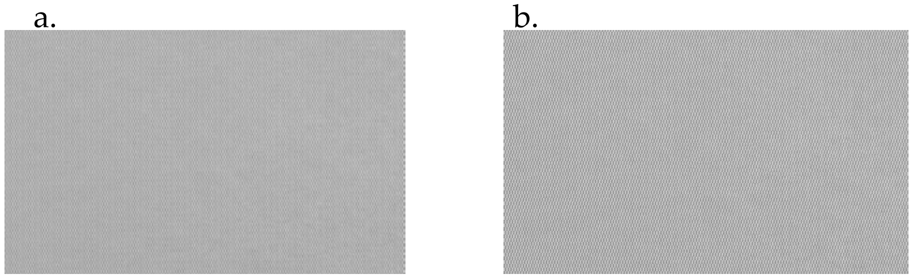

Figure 1. Standard dipped film under 100X magnification. Single (a) and double (b) dipped.

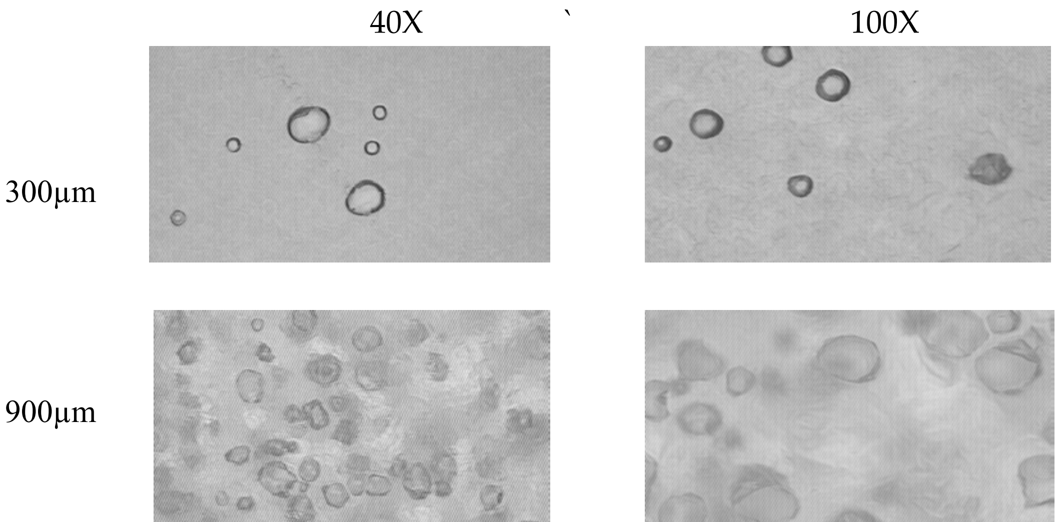

Figure 2. Standard film coatings at 40X (left) and 100X (right) magnification at 300µm and 900µm film thickness

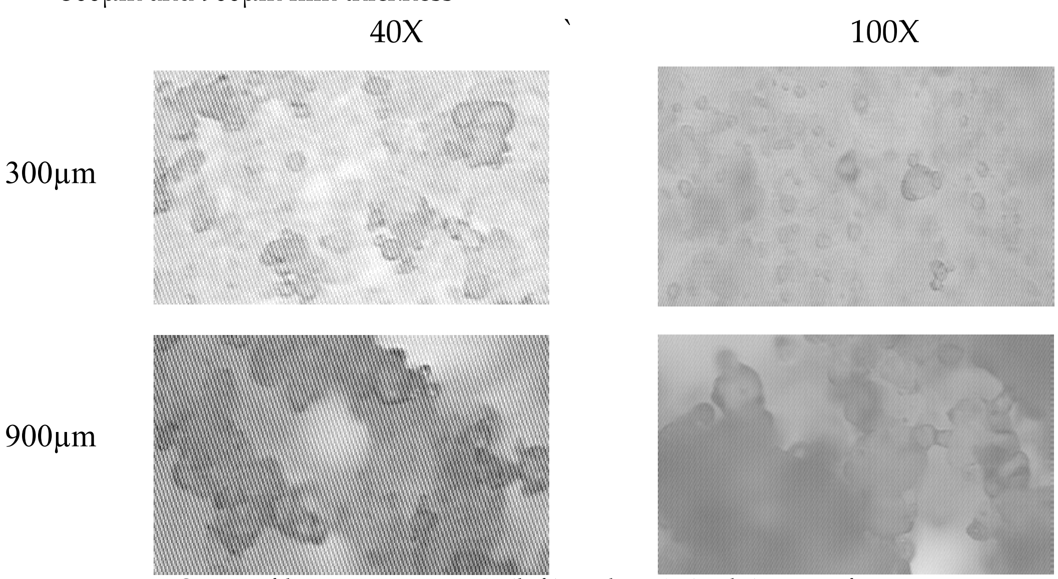

Figure 3. Snowy film coatings at 40X (left) and 100X (right) magnification at 300µm and 900µm film thickness.

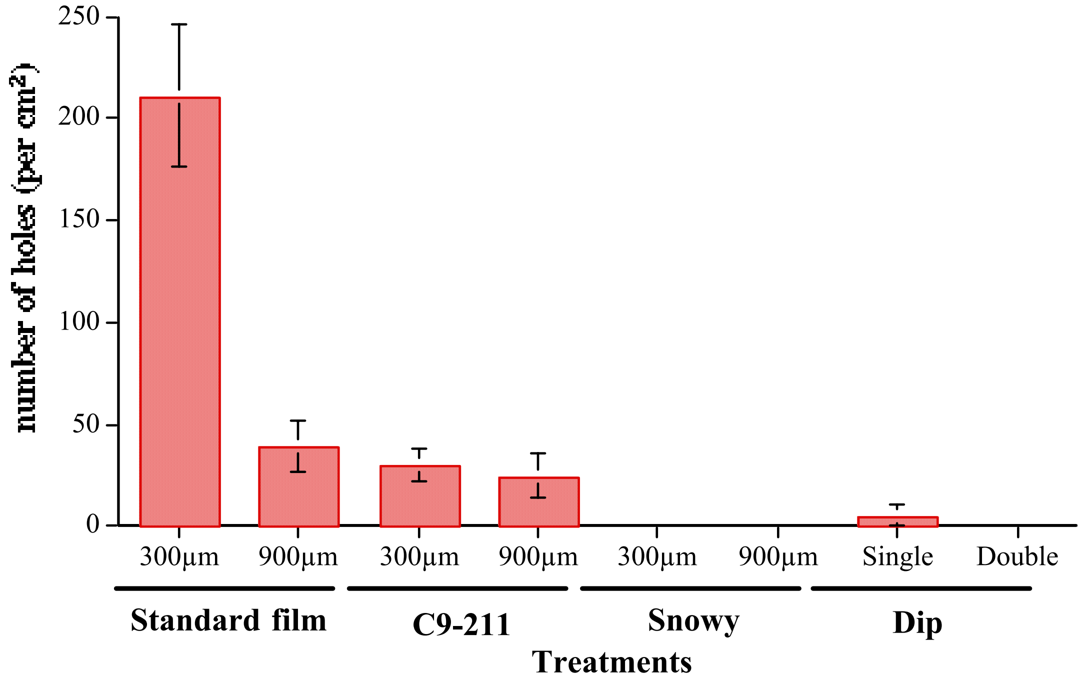

Figure 4. Total number (mean ± SE) of holes per cm² for each coating type.

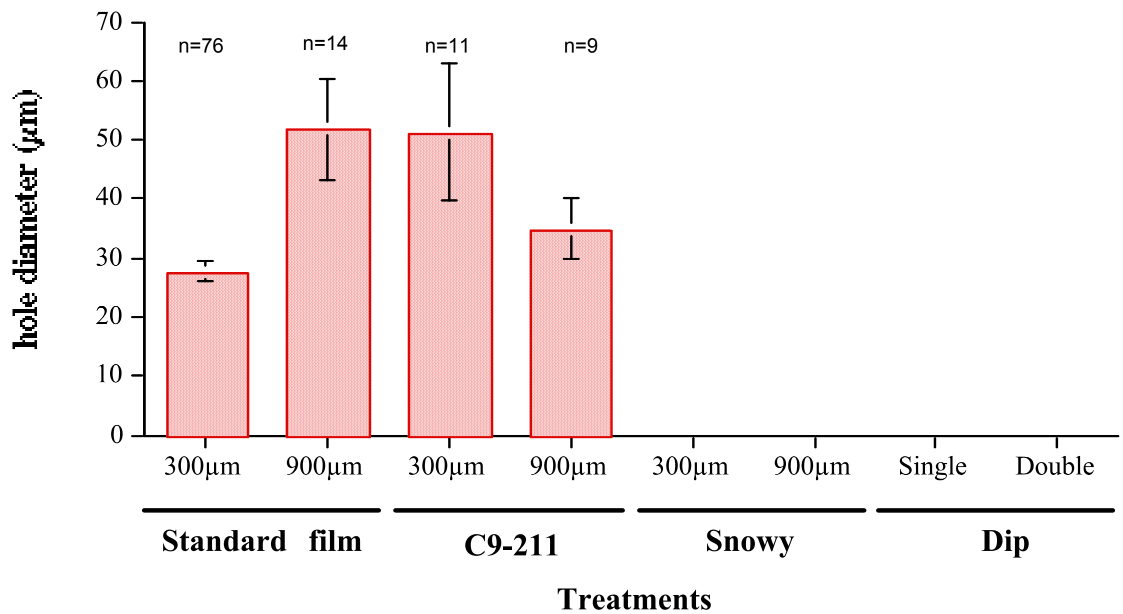

Figure 5. Mean diameter of holes in µm (mean ± SE) for each coating. N is shown for each coating.

Summary
Coatings applied via dipping had a far more homogenous surface and no holes. The thickness of the coating decreased the number of holes for the standard film.

Given the variable response of barnacle larvae to surfaces there are many possible factors that affect recruitment. These include larval behaviour, hydrodynamic forces and surface properties. Within surface properties there are many factors such as topography or roughness, surface free energy and biofilms which influence settlement.

Given the speed at which the coatings were evolving it was decided to leave the characterisation of the surface properties of these coatings until there was a final product. Coatings would then be subjected to a more extensive range of tests such as a measure of surface free energy, microtexture estimation, adhesion strength of attached organisms and barnacle behaviour when exposed to these coatings.

Asfar *et. al*. (in press) have found that barnacle behaviour is modified in the presence of OysterClear when compared with other surfaces. The barnacles are unable to explore the surface, which is vital for their settlement behaviour, and consequently turn upside down.

Appendix 7 (from Section 7.3.1)

Table 1. One-way analysis of variance (ANOVA) comparing barnacle settlement on control and coated shell from intertidal (A) and subtidal (B) sites at Port Macquarie. Data were arcsin-sqrt transformed prior to analysis.

A. Intertidal	Sum of squares	df	Mean square	F	p
Between groups	992.808	3	330.936	6.515	0.000
Within groups	9345.761	184	50.792		
Total	10338.569	187			
B. Subtidal					
Between groups	1.945	3	0.648	41.527	0.000
Within groups	3.606	231	1.561E-02		
Total	5.551	234			

Appendix 8 (from Section 8.1.3)

Table 1. Nested ANOVA comparing settlement of worms on control and coated shell. Data were transformed using log(1+x) prior to analysis.

Effect	SS	d.f.	MS	F	p
Treatment	5.799	2	2.9	7.286	<0.001
Tray within treatment	2.387	6	0.398	9.324	<0.001
Error	7.126	167	4.267E-02		

Appendix 9 – Commercial Trial

Figure 1. The coating application process in the Commercial trial at Port Macquarie. Adding OysterClear Original to the stainless steel bath housed in a gas-heated water jacket (A); Melting Original (B); Melting blocks of OysterClear coating 2 (C); mixing Coating 2 with a high speed disperser mounted on a fork lift (D); dipping oysters in a tray into Original; transferring coated oysters into cool water (F).