Australian Government
Fisheries Research and Development Corporation

JAMES COOK UNIVERSITY

Evaluation of antifouling products developed for the Australian pearl industry

FRDC Project No. 2000/254

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Evaluation of antifouling products developed for the Australian pearling industry.
Rocky de Nys and Odette Ison 2004.
FRDC Project No. 2000/254

Published by James Cook University 2004

ISBN 0 86443 717 X

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1. Non-Technical Summary

2000/254	**Evaluation of antifouling products developed for the Australian pearl industry.**

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Objectives
The initial objectives:

1. Production of efficacious antifouling coatings suitable for application to pearl oysters and pearl culture equipment.
2. Testing of novel antifouling coatings across the geographic range of the pearling industry, with assessment of variation in fouling communities during the peak fouling seasons.
3. Development of methods for routine application of antifouling coatings, to both shells and equipment, that can be successfully employed at pearling farms.
4. Identification of any effects to oyster growth, oyster health and the quality and production of pearls that results from the use of efficacious antifouling products.

Outcomes Achieved
The project had three significant outcomes. The first of these was the successful development and commercialisation of PearlSafe, a coating to remove and prevent infestation by boring sponges. Secondly the project demonstrated that an effective antifouling technology for the pearl oyster industry is technically viable. Finally several key performance indicators were identified for the further development of antifouling coatings.

FRDC Project 2000/254 was commissioned to develop an effective antifouling coating to protect pearl oysters from the settlement and growth of fouling organisms. The growth of fouling organisms results in decreased growth of oysters and mortality due to boring organisms destroying shell or other fouling organisms preventing closure of the shell resulting in predation by fishes. Fouling also increases the weight of equipment resulting in increased drag and equipment damage. The end-result is that most farms undertake extensive and expensive programs to clean shell and equipment to produce quality pearls. Therefore this project aimed to develop an effective coating to prevent the fouling of pearl oysters and pearl culture equipment. It also aimed to develop routine methods of application to shell and equipment and identify any negative effects of the coating on pearl oyster growth and pearl quality.

The project was collaboration between James Cook University, the University of New South Wales, the Pearl Producers Association (PPA) and Wattyl Australia. The project was strongly supported by Paspaley Pearls, Kailis Pearls and Maxima Pearling. The objectives of the project were set by industry and the majority of experiments conducted at industry sites with their support.

The project had three significant outcomes. The first of these was the successful development and commercialisation of PearlSafe, a coating to remove and prevent the infestation of pearl shell by boring sponges. Boring sponges settle and grow on the hinge area of mature shell and subsequently bore into the shell until it becomes porous and disintegrates. This results in the loss of both oysters and pearls, and has a significant impact as the most affected shell are the older shell that carry the most valuable pearls. The coating effectively smothers and kills boring sponges and prevents regrowth of the sponge. This results in the increased supply of large mature shell for seeding of pearls and prevents the loss of seeded shell. The coating is available from Wattyl Australia and is used by the pearl industry in Australia and Indonesia.

The second significant outcome was the demonstration that an effective antifouling technology for the pearl oyster industry is technically viable. The project developed a coating that deterred fouling in the WA pearling region for six months over the dry season (April – November) and three months over the wet season (December – March). The coating was less effective in the Northern Territory region where fouling was only deterred for three months over the dry and wet (6 weeks) seasons. However, while the coating was effective in some pearling regions the method of coating was clearly not suitable.

The third outcome of the project was the identification of key performance indicators for the development of effective antifouling coatings and the development of a new coating platform (water curing epoxy-resin based coatings) to meet these indicators. To be acceptable to the industry a coating must be an effective water-curing antifouling coating that is applied through an automated process and protects shell from fouling for six months. The development of a coating that can be applied to wet shell in an automated process on-site was initiated within the later period of the project. An epoxy based coating was developed that can be successfully applied to damp shell and it extremely hardy. This key outcome is now the basis for the further development of a coating under a new project framework 2003/206 Antifouling solutions for the Australian pearling industry: coatings for shell and equipment.

Additional outcomes include the development of an internationally identifiable research and development expertise in biofouling in both industry and academia through direct collaboration between the pearling and coatings industries and universities. However, the project would have benefited from greater consultation and communication with industry and the establishment of a strong steering committee with industry leadership is a key feature of the new program to further develop antifouling technologies for the pearling industry in Australia.

Keywords: Pearl oysters, biofouling, boring sponges, antifouling coatings, aquaculture

2. Introduction

Introduction to the Australian pearl industry

Pearling in Australia has operated since the 1880's after large natural stocks of the silver lipped oyster, *Pinctada maxima* were found off the north west coast of Australia (Aquilina and Reed, 1997). Originally the industry provided mother of pearl for button making and inlay work, however after the Second World War and the development of plastics, the Australian industry declined significantly. In the 1950's, based on the success of the Japanese in culturing Akoya pearls (*P. fucata*), the cultured pearl industry in Australia was revived and the first cultured pearl farm in Australia was established in 1956 in Western Australia (Aquilina and Reed, 1997). Cultured pearls have now replaced natural pearls worldwide.

Current Status

Pearl oyster aquaculture is now Australia's second most valuable aquaculture industry valued at $216.5 million for 2000/2001 (O'Sullivan and Dobson, 2002). It has only recently been surpassed by the southern bluefin tuna industry and until then dominated by value of production. The production of pearls contributes 95% and 92% to the value of W.A. and N.T. aquaculture industries, respectively (O'Sullivan and Dobson, 2002). Queensland has a small industry worth approximately $0.5 million p.a. (O'Sullivan and Dobson, 2002).

Despite the enormous value of the pearl industry, figures have shown a downward trend from the previous year when production was valued at $245.5 million (O'Sullivan and Dobson, 2001). The Australian pearl industry has recently faced several challenges, experiencing a drop in pearl prices which have been influenced by factors such as economic downturn, increased competition from the Asian market, particularly Indonesia, and an oversupply of low to medium quality pearls. The value of Australian pearl exports fell in 1998 to 19.6% of world exports, significantly down from 30.7% in 1997 (Tisdell and Poirine, 2000).

While Australian pearl producers have seen an increase in competition from overseas markets, the demand for a high quality product is sustaining the industry. The oversupply of low to medium quality pearls has forced Australian farmers to concentrate on high pearl quality production. Australia has the reputation for production of the highest quality 'south-sea' pearls in the world (O'Sullivan and Dobson, 2002) and produces 1% by volume of the world's pearls, but strikingly this corresponds to 25% by value (Quick, 2000).

There are over 30 operations farming oysters for pearls across Australia with the majority in Western Australia. In W.A. there are 16 licences (State of the Fisheries Report, 2002) covering a range from the Exmouth Gulf on the southern edge to the Northern Territory border, with the majority of operations centred around Broome.

The main farming regions in the Northern Territory are situated around the Cobourg peninsular and in Queensland around the Torres Strait and Gulf regions.

Pearl oysters

Pearls are cultured principally from oysters within the genera *Pinctada* and *Pteria* (Class Bivalvia). In Australia, the industry is dominated by *Pinctada maxima* production which produces the largest (nuclei vary from 9-20mm) and characteristically silver to golden coloured 'south sea' pearls which attract the highest prices (Fassler, 1995; Tisdell and Poirine, 2000). Black pearl production from *P. margaritifera* is increasing, particularly in W.A., where 10 leases have recently been approved bringing the total to 28 with an estimated worth of AUD$10-20 million in the next few years (ACWA News, 1999). There is also increasing interest in the culture of *P. fucata* (Akoya pearls) and two *Pteria* species, *Pt. albina* and *Pt. penguin*.

Other by-products of pearl culture such as oyster meat and mother of pearl are retained but do not contribute significantly to the value of production [prices of ~ $50/kg have been recorded for dried pearl meat (Sullivan and Dobson, 2002)].

Oyster stocks used for culturing pearls in Australia are sourced from wild caught, spat collections and hatchery production. The industry is regulated by a quota system involving a total allowable catch for each license to reduce over-exploitation of wild stock populations. The quota system is co-operatively managed by Government and industry and is reviewed yearly in response to factors influencing wild stock numbers. In W.A. the total combined quota was 534, 700 for the 2001/2002 period (State of the Fisheries Report, 2002) with individual company quotas ranging from 15, 000 to 330, 000 oysters. Importantly, advances in hatchery techniques have allowed the industry to undergo expansion without pressure on natural stocks.

Biofouling

There are several issues facing the pearl industry (O'Sullivan and Dobson, 2002; Fassler, 1995; Tisdell and Poirine, 2000) including biofouling which is a significant and costly problem (Taylor *et al*., 1997). Biofouling is estimated to cost between 25-30% of the operating costs of a pearl farm. This includes the removal and prevention of fouling on both pearl shell and equipment. These levels are comparative with other bivalve culturing industries such as scallops and edible oysters (Claereboudt *et al*., 1994; Arakawa, 1990; Enright, 1993).

The process of culturing pearls presents many attractive settlement surfaces for fouling and boring organisms and this can significantly impact upon pearl oyster condition and pearl production in a number of ways. Biofouling can compete with the pearl oyster for resources reducing growth, cause shell deformities, disrupt the valve opening and closing, cause stress to the oyster, disrupt nacre production or lead to the rejection of the nucleus and in the worst case, result in the death of the oyster. In addition, biofouling colonises culturing equipment such as panels, nets, ropes and floats increasing drag on the longline (Dharmaraj and Chellam, 1983) (making lines more susceptible to storm damage) and increasing maintenance on equipment (Paul and Davies, 1986).

The types of fouling common to pearl farms can be characterised into two groups, organisms that are generally restricted to the shell surface and to equipment, and those organisms that bore through or erode the shell.

Fouling organisms

Surface fouling communities are generally dominated by invertebrates such as barnacles, spat from other bivalves, bryozoans and tubiculous polychaetes (Taylor *et al*., 1997; Doroudi, 1996; Dharmaraj *et al*., 1987; Alagarswami and Chellam, 1976). Ascidians, hydroids and algae also foul shell surfaces but usually have a less significant impact.

Many studies have demonstrated that biofouling has a negative impact on the survival and growth of pearl oysters (Taylor *et al*., 1997; Alagarswami and Chellam, 1976), as well as several other commercially important aquaculture species including edible oysters (Arakawa, 1990; Minchin & Duggan 1989; Hidu *et al*., 1981) and scallops (Claereboudt *et al*., 1994; Minchin & Duggan 1989). Barnacles and bivalves, in particular, can interfere with the opening and closing of pearl oyster valves making them more susceptible to predation (Doroudi, 1996) or completely inhibiting them from opening resulting in death (Taylor *et al*., 1997; Dharmaraj *et al*., 1987). The occurrence of fouling can deform shell growth and even after removal of fouling the oyster lip growth becomes uneven (sometimes referred to as 'double back') making this area more susceptible to infestation by boring organisms such as sponges and polychaetes (Taylor *et al*., 1997). Fouling can also cause the recession of shell growth (Dharmaraj *et al*., 1987). High levels of mortality were also associated with high levels of barnacle fouling in *Pinctada fucata* farmed in the Gulf of Mannar, India (Alagarswami and Chellam, 1976).

Taylor *et al*. (1997) found no effect of fouling on mortality for *Pinctada maxima* in Indonesia, however they found a significant relationship between the removal of fouling and growth. Shell that were cleaned more regularly at 2 or 4 weeks compared with 8 and 16 weeks had significantly greater wet weight, shell height and length. Similarly, an inverse correlation between growth and fouling diversity was found for *P. fucata* cultured in the Arabian Gulf (Mohammad, 1976). The relationship was found whether the oysters were bottom farmed or suspended from a longline.

Several studies have also shown that fouling and predation can be significant in the early stages of production, impacting juvenile oyster growth and survival, influencing culture practices such as the choice of spat settlement substrate and thereby increasing labour costs (Pit and Southgate, 2003; Southgate and Beer, 2000; Dharmaraj *et al*., 1987). Optimising conditions during the hatchery and juvenile stages (Southgate and Beer, 1997) has become important, particularly with the greater emphasis on quotas supplied *via* hatchery techniques. The effect of several nursery culture methods on the growth of *Pinctada margaritifera* was tested and it was found that the success of the culture method was heavily influenced by fouling (Southgate and Beer, 2000). The poorest growth occurred in oysters that were grown in meshed panels as these meshed panels provided an attractive substrate for settlement by the bivalve *Crassostrea*. Heavy settlement resulted in competition for space and resources and a reduction in water flow to the juvenile oysters.

In a similar study to Taylor *et al*. (1997), Pit and Southgate (2003), found a significant effect of fouling removal on the growth of juvenile *Pinctada margaritifera*. Oysters cleaned every 4 or 8 weeks were significantly larger (16 – 25% larger) than those that were not cleaned for 16 weeks. In addition, the removal of predators on a monthly basis also resulted in significantly greater growth.

Fouling and boring organisms on pearl oysters in culture often differ from the organisms found on oysters in their natural environment (Doroudi, 1996; Dharmaraj *et al*, 1987; Dharmaraj and Chellam, 1983). Doroudi (1996) found fouling on natural bed oysters (*Pinctada radiata*) in the Persian Gulf primarily consisted of sponges, algae and ascidians which contrasted with the dominant fouling organisms on cultured oysters, such as barnacles, spat from other bivalves and polychaete worms. Historically, the Japanese classified fishing grounds based on the predominant animal attached to the pearl shells when collecting in the area extending from the north coast of Australia to Thursday Is. and the Arua Is. in the 1950's (Takemura and Okutani, 1955). Takemura and Okutani (1955) found a very diverse assemblage of fouling organisms across the whole region with the ascidian *Microcosmus halleri* being the most dominant species. The barnacles *Balanus amaryllis* and *B. cepa* were also abundant in the regions studied and this study recorded the species *Balanus cirratus*.

In addition to shell surface fouling organisms, there is very often a fine layer of 'silt' covering the pearl oyster shell for much of the year (P. Moase, pers comm). Silt, defined by Swain and Schultz (1996) as "absorbed organic and inorganic chemicals, trapped silt and detritus and un-identified slimes" is an important step in the fouling community succession and may harbour or promote other larger fouling organisms or predators (Littlewood and Marsbe, 1990).

Boring organisms

Boring organisms range from micro-organisms such as cyanobacteria, fungi, and green algae to macroborers such as sponges, mussels and polychaetes (Mao Che *et al*., 1996; Dharmaraj *et al*., 1987; Thomas, 1981; Alagarswami and Chellam, 1976; Mohammad, 1972). Boring or eroding organisms cause serious damage to bivalves including hinge instability, disruption of the formation of the shell resulting in fragility, blisters, brittleness and a loss of thickness (Doroudi, 1996; Mao Che *et al*., 1996; Rosell *et al*., 1999; Dharmaraj *et al*., 1987; Thomas, 1981). As the boring organisms penetrate into the shell, blisters are formed underneath the nacreous layer disrupting pearl production and mother of pearl (Mao Che *et al*., 1996) and in some bivalves these blisters may burst resulting in death (*Crassostrea gigas:* Handley and Berquist, 1997).

The pearl oyster has to deposit more nacre to combat boring infestations (Blake and Evans, 1973) and therefore fewer resources go into coating the nucleus and consequently pearls from heavily infested oysters can display discolouration and imperfections (Moase *et al*., 1999; Dharmaraj *et al*., 1987; Alagarswami and Chellam, 1976).

Of the boring organisms, polychaetes and sponges, in particular clionid sponges, contribute the most extensive damage to bivalves. Clionid sponges within the Family Clionidae (Gray) are unique in that all the genera within the family bore into calcareous substratum during their lifecycle, including the most destructive and widespread genus *Cliona* (Thomas, 1981). Clionid sponges or excavating sponges have been documented from a wide range of commercially important bivalves (see table in Rosell *et al*., 1999; Wesche *et al*., 1997).

Furthermore, rates of infestation of boring sponges in bivalve populations can be significant. In *Pinctada fucata* in India, 20% and 78% of shell were infected by boring sponges and boring polychaetes respectively (Alagarswami and Chellam, 1976). In Australia, clionid sponges were found in 51% of a commercial stock of the Sydney rock oyster, *Saccostrea glomerata* (Wesche *et al*., 1997). While there is no published estimate of infection rates in pearl oysters in Australia, anecdotal evidence suggests that up to 10% of cultured shell suffer from infestation by clionid sponges.

Infestations by boring sponges generally begin around the hinge or apex region of the shell (Mao Che *et al*., 1996) and the incidence of infestation increases with the age of the shell (Doroudi, 1996; Mohammad, 1972; Barthel *et al*., 1994). These factors are due to erosion of the periostracum, a proteinaceous external layer linked with antifouling and antiboring activity in several bivalve species including the pearl oyster, *Pinctada margaritifera* (Mao Che *et al*., 1996), and the mussel *Mytilus edulis* (Harper and Skelton, 1993; Scardino *et al*., 2003). In *P. margaritifera,* up to 8 times more damage occurs around the apex area than in the mid region of cultured shell and in 3 year old oysters as much as 32% of the shell volume was removed from the hinge area (Mao Che *et al*., 1996).

Modern culturing techniques appear to have a significant impact on the surface characteristics of pearl oyster shell making them more susceptible to bio-eroding organisms. Rates of erosion were 36 times higher in one year old *Pinctada margaritifera* hatchery shell than in natural populations, suggesting that the periostracum is worn away more quickly through fouling removal practices (Mao Che *et al*., 1996). Takemura and Okutani (1955) also reported that young shell (< 16.5cm in height) in natural populations had little or no fouling on the shell surface and Dharmaraj and Chellam (1983) found very few blisters from boring polychaetes in younger natural bed shell of *P. fucata* while they were present in cultured shell.

Current biofouling management strategies

Biofouling is a significant and wide-ranging problem facing the Australian pearl industry. Many methods for biofouling removal and prevention have been trialled for commercial bivalve species including exposure to air, freshwater dips, hypersaline dips, high temperature (Nell, 1993; Alagarswami and Chellam, 1976; Arakawa, 1990; Nel *et al*., 1996), biological control (Hidu *et al*., 1981; Minchin and Duggan, 1989; Cigarria *et al*., 1998; Enright *et al*., 1983) and mechanical and high pressure water cleaning (Wada, 1991; Taylor *et al*., 1997; Arakawa, 1990). In one study the direct application of antifouling coatings to shell surfaces was trialled (Oakes *et al*., 1995). Coating the abalone, *Haliotis rufescens,* with a wax type coating combated a parasitic sabellid polychaete with a 91-99% success rate and also resulted in a significant increase in shell length and respiratory pore formation in the abalone (Oakes *et al*., 1995)

Clionid sponge infestations (commonly called red arse) represent a major problem for pearl growers as they target older shell which have entered the operation stage and consequently cost the industry millions of dollars each year in discoloured and deformed pearls (Moase *et al*., 1999). Moreover, they are not easily combated with standard fouling removal practices. The most common strategy for dealing with boring sponge infestations involves culling infected oysters. Other forms of clionid sponge control have included treatment with formalin, brine solutions, fresh water, desiccation, liming (Dharmaraj *et al*., 1987; Velayudhan, 1983; Thomas, 1981) and the prevention of infestation such as coating the hinge area with cement, with very limited success.

The feasibility of antifouling protection for associated equipment such as panels and trays has also been investigated (His *et al*., 1996). These coatings include those using heavy metals such as copper and nickel as an active ingredient (Lee, 1992; Huguenin and Huguenin, 1982) which negatively impact cultured shellfish (Claisse and Alzieu, 1993; Paul and Davies, 1986; His and Robert, 1987) and also negatively impact on organisms in the surrounding environment (Gibson and Wilson, 2003; Callow, 1999).

In Australia, the pearl culture industry principally relies on regular cleaning using high pressure water (Taylor *et al*., 1997). The majority of farms operate specifically designed cleaning boats which carry 2-4 crew to clean shell on a rotating cycle.

Biofouling is seasonal in most pearl oyster growing regions (Lodeiros *et al*., 2002; Taylor *et al*., 1997; Dharmaraj *et al*., 1987; Doroudi, 1996; Knuckey, 1995; Gervis and Sims, 1992; Dharmaraj and Chellam, 1983) and is correlated with monsoonal wet seasons. In northern Australian and Indonesian waters growers experience very high levels of fouling, particularly barnacles, corresponding to the wet season between December and April (Taylor *et al*., 1997). The variation of biofouling over the year means that farmers adapt and modify management practices to control fouling. When fouling is the most intense farmers may clean shell on an 8 –10 day cycle while in the dry season this can be extended to a 14 - 28 day cycle (M. Doroudi pers comm; Aquilina and Reed, 1997). In addition, cleaning regimes vary with bottom or longline culture, and across the geographic range of farming.

The problem of biofouling also influences the success of culturing techniques (Southgate and Beer, 2000), the potential for commercial development of new species (Lodeiros *et al*., 2002), and management decisions such as areas suitable for culture. There is however very little information regarding the effect of regular cleaning and handling of pearl oysters on pearl quality (Otu and Shimizu, 1961) and for other bivalves increased handling can contribute substantially to mortality (Parsons and Dadswell, 1992) and reduced growth (Lodeiros and Himmelman, 1996).

Background to trials

A program for the development of novel antifouling technologies at the Centre for Marine Biofouling and Bio-Innovation at the University of New South Wales has included the development of coatings for direct application onto bivalves. These coatings have been successful in preventing fouling in the edible oyster industry (de Nys *et al*., 2003). Coating Sydney rock (*Saccostrea glomerata*) and Pacific oysters (*Crassostrea gigas*) with OysterClear, significantly inhibited oyster spat fouling to less than 10% cover compared with controls at 75% cover. This resulted in 100% of oysters being acceptable for the lucrative half shell market without any need for manual cleaning. In addition, OysterClear is easy to apply to large numbers of oysters and the coating is removed easily, prior to market, by rumbling (de Nys *et al*., 2003). OysterClear is a significant innovation for the edible oyster industry as it reduces the costs associated with cleaning.

In the Australian pearl industry, antifouling trials conducted during 1998 and 1999, at the Maxima Pearling Company in Cone Bay W.A., led to the development of shellfish coatings that deter settlement of biofouling organisms. These coatings inhibited barnacle and ascidian (squirt) settlement, algal growth and the accumulation of silt on shells. Coatings were formulated to meet specific criteria for pearl culture, with the major requirement being a rapid dry coating with good abrasion resistance.

Coatings for shells were formulated to deter fouling by providing surface properties (surface chemistry and texture) that limit the settlement and attachment of marine organisms. The coatings were non-toxic.

This report summarises the trials that have been conducted from October 1999 to March 2003 which have been built on the initial trials at Maxima. Testing commenced in October and November 1999 at Knocker Bay (NT), Cone Bay (WA) and Exmouth (WA) with coatings applied to pearl shells and mesh panels. Due to the extensive testing that has been undertaken and subsequent coating development this report has been divided into ten sections that document the development of the coatings.

3. Need

Biofouling on pearl oysters, and the equipment used in the culture of these oysters, is a major cost to the pearl industry throughout Australia. Rapid biofouling necessitates frequent cleaning, a process that is labour and capital intensive. The cost of cleaning, as estimated by the Pearl Producers Association, is approximately $15 to $20 per shell per year. The cost to the industry as a whole is likely to exceed $20 million/annum. Indirect costs include losses due to shell mortality, reduced growth rates and reduced pearl production from effects to nacre deposition.

Biofouling of oysters and equipment presents several problems if left unattended. Invertebrates dominate fouling communities common to the pearl industry and these compete directly with pearl oysters for food. Several organisms, such as the sponge *Cliona* (red arse) and boring shellfish, directly attack the pearl oysters, resulting in damage or mortality. Excessive fouling of underwater surfaces increases drag and increases the susceptibility of lines to storm damage. The resultant frequent cleaning is also problematic since evidence suggests that this type of handling reduces shell growth.

4. Objectives

The initial objectives:

1. Production of efficacious antifouling coatings suitable for application to pearl oysters and pearl culture equipment.
2. Testing of novel antifouling coatings across the geographic range of the pearling industry, with assessment of variation in fouling communities during the peak fouling seasons.
3. Development of methods for routine application of antifouling coatings, to both shells and equipment, that can be successfully employed at pearling farms.
4. Identification of any effects to oyster growth, oyster health and the quality and production of pearls that results from the use of efficacious antifouling products.

5. Program sequence

Section 7. PearlSafe Trials
Oct – Nov 1999
Cone Bay (WA)

↓

Section 8. Field trial 1 - Antifouling applied to shells and panels
Oct 1999 – Feb 2000
Conducted at Knocker Bay (NT), Cone Bay (WA) and Exmouth Gulf (WA)

↓

Section 9. Surface analysis of coatings
SEM and light microscopy analysis

↓

Section 10. Field trial 2 - Antifouling applied to test panels
Feb – Mar 2000
Knocker Bay (NT)

↓

Section 11. Laboratory bioassay with barnacle larvae

↓

Section 12. Field trial 3 - Antifouling trials of new formulations and application techniques
April 2000 – June 2000
Conducted at Cone Bay (WA) and Bynoe Harbour (NT)

↓

Section 13. Field trial 4 - Formulations from Trial 3 were improved and antifouling trials conducted
June 2000 – December 2000
Conducted at Bynoe Harbour (NT)

↓

Section 14. Field trial 5 - Antifouling trials of coatings from Trial 4 tested over summer
November 2000 – February 2001
Conducted at Bynoe Harbour (NT) and Cone Bay (WA)

↓

New Generation Coatings

Section 15. Field trial 6 - New coatings tested at
Orpheus Is. Research Station (QLD)
July 2002 – March 2003

↓

Section 16. Field trial 7 - Coatings tested on panels
Ross River (QLD)

6. General Methods

Study Sites

Field trials of antifouling coatings were performed across the geographic range of pearl farming in Australia. The majority of trials were conducted at Cone Bay, W.A. (16° 28', 123° 32') and Bynoe Harbour, N.T. (12° 40', 130° 33'). Trials at Knocker Bay on the Cobourg Peninsular, N.T. (11° 16', 131° 54'), Exmouth Gulf, W.A. (22° 07', 114° 17'), Orpheus Is., QLD (18° 37', 146° 30') and Ross River, QLD (19° 16', 146° 50') were also conducted (Figure 1).

Study Organisms

The coatings were trialled on the silver-lipped oyster *Pinctada maxima*, except at Orpheus Is. where *P. margaritifera* were used. In the PearlSafe trials 4 year old shell with an average DVL (dorsoventral length) of 173mm were used. For antifouling coating trials shell were on average 3 years old with a DVL of ~ 160-170mm. *P. margaritifera* used in the OIRS trial were 3 years old and were ~ 120mm (DVL).

Equipment

Control and coated oysters were suspended from longlines in 6 pocket panels for the majority of trials (Figure 2).

Statistical Methods

All statistical analyses were performed on SPSS Ver. 10 (SPSS Inc., Illinois, USA). For all analyses across all the trials the assumptions of ANOVA, of normality and homogeneity of variances, were checked by histograms of residuals and plots of residuals *vs* means, respectively. Data were transformed (described in each section) when they did not conform.

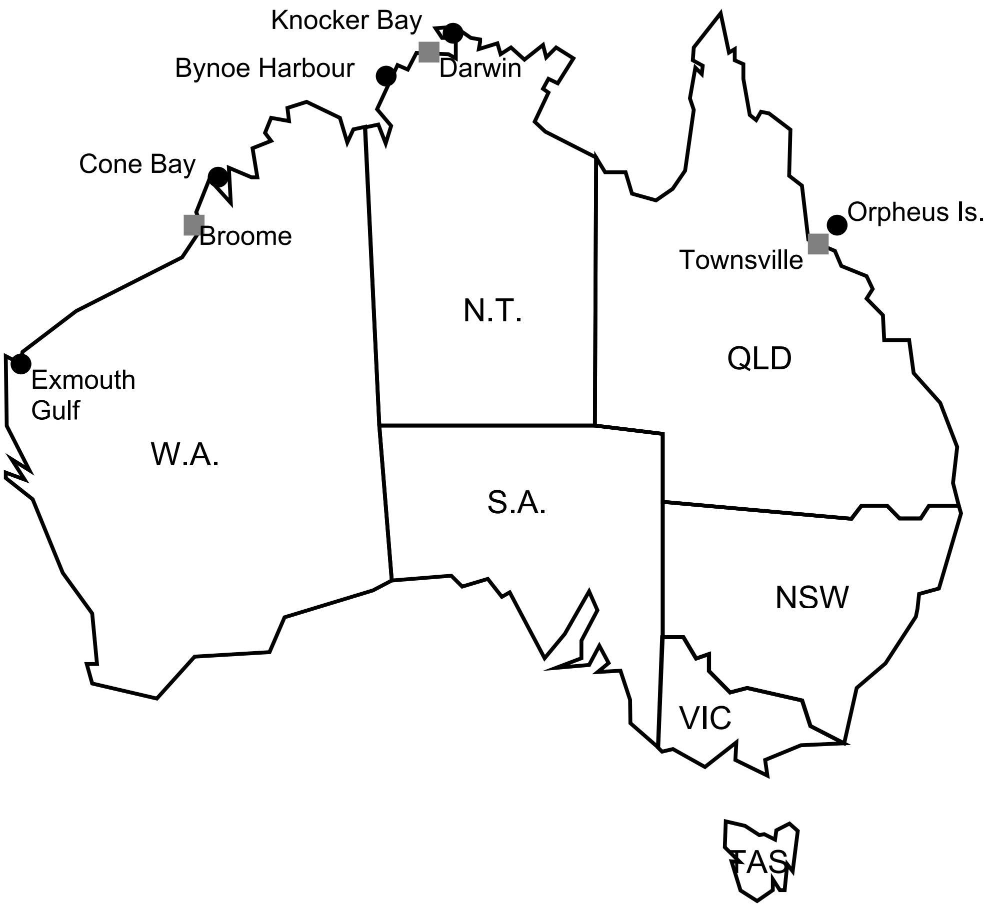


Figure 1. Locations of the study sites.

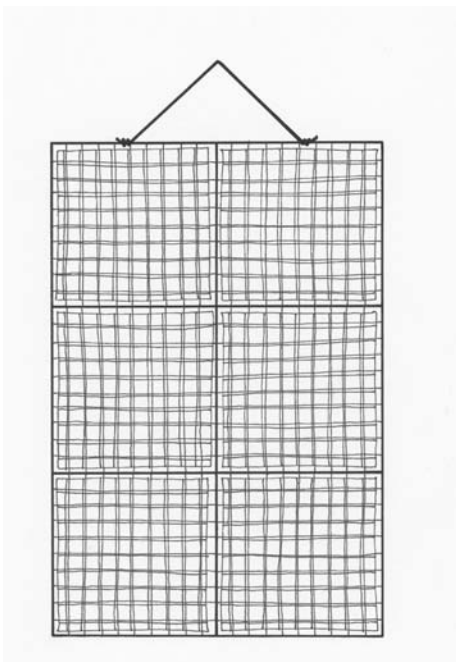


Figure 2. Six-pocket panel net.

7. PearlSafe Trials

7.1 Introduction

As part of the program to provide novel solutions for biofouling problems in the pearl industry a new coating (**PearlSafe**) was trialled to treat infestations by boring clionid sponges. These organisms are not easily removed by physical cleaning, the current method for biofouling removal, and can infect up to 10% of farmed shell. Typically the industry culls infected shell which represents a significant financial cost as it is often the more valuable shell that are affected. Infestations generally occur on the hinge region of the shell where the periostracum has been worn away (Mao Che *et al*., 1996).

Originally two wax based coatings were trialled as antifouling coatings but their potential to treat clionid sponge infestations was recognised. The coatings were termed **PearlSafe** and **Coating 3** and as both had very similar components, PearlSafe was chosen to test in the application trial as it was the better cost alternative.

7.2 Part A – Application Trial

7.2.1 Materials and Methods

All clionid sponge trials were conducted at Cone Bay, W.A. using *Pinctada maxima*. All treatment oysters were infected with one of two clionid sponges which were recognised by a deep orange to red colouration (these infestations are often called 'red arse'). Of these, one was more common and was identified in the field by a darker red colouration. Samples of sponges were collected prior to commencement of the trials for identification by the Queensland Museum and voucher specimens recorded as: Common species – *Pione* sp. 2781, Uncommon species – *Pione* sp. 2782.

Treated and control oysters had an average DVL of 172.3mm and 40 oysters were used per treatment (n=40). Un-infected oysters of the same size were used as controls. The infected oysters had a light to medium infestation of *Pione* (the level of infestation is difficult to estimate without destroying the shell however light to medium shell were categorised by 0-50% cover of sponge on the hinge area and no deep excavations or hinge instability).

Three application methods were trialed for PearlSafe, a wax based coating containing no antifouling activity: immersing the whole shell in the coating (full dipped), immersing just the hinge or infected area (half dipped) and painting the hinge area using a paint brush (half painted). For the dip application, immersion was 1-2 seconds. All shell, including controls, were air-dried for 10 mins prior to coating to remove most of the moisture and application time including coating drying time was 15 mins for the dip applications and 40 mins for the painted application. Controls were handled similarly to the dip application treatments.

Mortality was monitored and the success of the coating treatments were measured as the number of shell that showed no signs of infestation after four weeks. Coating success was analysed using χ^2 test with Monte Carlo simulations.

7.2.2 Results

The sponges were identified as *Pione* spp. based on the presence of tylostyles/subtylostyles and acanthoxes as megascleres, and spiked microrhabds as microscleres (J. Kennedy, pers comm.).

Coating infected shell with PearlSafe resulted in a significant eradication of both species of the boring sponge *Pione* (χ^2=73.416, df=3, $p<0.001$). Half dipping was the most successful treatment with *Pione* sp. killed in 92.5% of shell (Table 7.1). Full immersion was the next most effective (82.5% of shell clear of infestation) and painting was the least effective (65%). *Pione* sp. was eradicated in three control shell (7.5%). This may be due to desiccation of the sponge during the drying time.

Table 7.1. Mortality and success of coating treatments on *Pione* infested pearl shell.

	mortalities	% mortality	Number successfully treated	% success (n=40)
Control	1	2.5	3	7.5
Half dipped	1	2.5	37	92.5
Full dipped	2	5	33	82.5
Half painted	1	2.5	26	65

Mortality was low (Table 7.1) and did not differ across the treatments including controls. New lip growth was observed in all treatments but was not quantified.

PearlSafe is considered to work by creating an impervious layer over the top of the sponge, resulting in loss of water supply and the death of the sponge. Essentially the sponge is 'suffocated' by PearlSafe. In cases where infestations were still evident, it was noted that partial death of the sponge did occur and a second treatment of PearlSafe was successful in eradicating the sponge (A. Wilmot, pers comm.).

All coatings maintained cover over the 4 weeks of the trial, however dip applied coatings appeared to have better coating integrity than the painted treatment with a thicker cover and less flaking. Further, while full and half dipping showed similar levels of success, almost 50% more coating was required for full immersion. Therefore based on performance and cost factors, coating the hinge area of the infected shell by dipping was considered adequate treatment.

7.3 Part B – Treatment of heavily infested shell

7.3.1 Materials and Methods

In the previous trial oysters had a medium to light infestation. In this trial PearlSafe was tested on shell that were heavily infested and had already been relegated either for half pearl seeding or for culling. Heavily infested shell had a greater than 50% cover of the sponge and/or deep excavations of the shell and hinge instability due to brittleness (levels of infestation were qualitative).

60 shell were treated with PearlSafe and 422 shell with Coating 3. Controls were not included in this trial as the previous trial had shown that PearlSafe had a very high success rate and control shell did not need to be removed from the normal production cycle. Both coatings were applied only to the hinge area *via* dipping as described above.

7.3.2 Results

The trial found that PearlSafe and Coating 3 were highly effective at eradicating heavy infestations of sponge with 83% and 72% success, respectively (Table 7.2).

Table 7.2. Mortalities and success of coating treatments on heavily infested pearl shell.

	No. shell treated	Mortality	No. successfully treated
PearlSafe	60	5 (8.3%)	50 (83%)
Coating 3	422	60 (14.2%)	304 (72%)

Mortalities were higher in this trial (Table 7.2), however many of the shell were considered to be sufficiently infested that mortality was considered inevitable (A. Wilmot pers comm.).

7.4 Summary

- PearlSafe is highly effective at eradicating infestations by the boring sponge species *Pione*, with up to 92% of light to moderate infected shell treated successfully.
- PearlSafe is effective at treating heavily infected pearl shell with an 83% success rate.
- The application method of dipping the hinge or infected area was shown to be successful and is adequate to ensure a high percentage of death.
- PearlSafe is a coating that does not have to be heated, can be applied to damp shell and has a very short drying time.
- Using PearlSafe represents a benefit to the industry as shell that may otherwise be culled can now be retained for seeding the largest pearl sizes.

8. Antifouling Field Trial 1

8.1 Introduction

Antifouling trials conducted during 1998 and 1999, at Maxima Pearling in Cone Bay WA, prior to the initiation of this FRDC funded project, led to the development of several coatings that deter the settlement of biofouling organisms. These coatings inhibited barnacle and ascidian (squirt) settlement, algal growth and accumulation of silt on shells. The trials demonstrated the feasibility and efficacy of coatings applied directly to the shell surface of pearl oysters to prevent biofouling.

Therefore the aim of Field Trial 1 was to build on these early trials by testing improved coatings on shell, culture equipment and in addition to test the coatings performance across the geographic range of pearl culture. Testing commenced at **Knocker Bay** (N.T.) on October 27th, at **Cone Bay** (W.A.) on November 2nd and in **Exmouth Gulf** (W.A.) on November 7th, 1999.

The coatings tested were collectively called **PearlClear** and are 'foul-release' type coatings with modified surface properties to deter fouling. Manipulation of components within the coating resulted in three variations called PearlClear **Original**, **Standard** and **SuperThin**. **Coating 16** and **18** contained biocides as active ingredients and were included to provide a contrasting type of coating (water-based) and mode of action of deterrence.

8.2 Materials and Methods

Table 8.1. Coating types and application methods tested in Field Trial 1

Treatment	Coating type	Coating application method
PearlClear Original	Hot-melt	Hot-spray system
PearlClear Standard	Hot-melt	Hot-spray system
PearlClear SuperThin	Hot-melt	Hot-spray system
Coating '18'	Water-based coating[1]	Wagner spray system
Coating '16'	Water-based coating[1]	Wagner spray system
Control (uncoated) shell	N/A	N/A

[1] the coating was heated prior to application to decrease drying time.

Thirty replicate shells, divided into five panels (six pocket panels) were used for each coating and controls at each location (=180 oysters per location). Prior to coating, the shells were air dried for 20-30 mins to remove moisture from the shell surface. A dry surface is essential for effective adhesion of these types of coatings. Three of the coatings were hot-melt products (PearlClear Original, Standard and SuperThin) which were melted prior to application, and were coated onto the shells and panels with a hot-spray system (Table 8.1). The hot spray system was commissioned for this application and includes a heated line and heated spray tip, each of which has separate temperature control to optimise the coating quality. Coating 18 and 16, were water-based formulations that were also heated prior to use and applied with a conventional paint spray gun (Wagner© W300). Both types of coatings dry immediately so coated shell were placed back into water within 5 mins of spraying and returned to the longline within 6 hrs of coating. Panels were also coated with the corresponding antifouling treatment as the shell they contained. Therefore coating treatments were not randomised within panels but panels were randomly placed back onto the longline.

The main fouling species, barnacles, oyster spat and ascidians were counted at 6 weeks at Cone Bay. Only photos were taken for Knocker Bay and Exmouth Gulf trials at 6 weeks and no direct counts were made. Due to the overall low level of fouling at the Exmouth Gulf site the trial was extended until 12 weeks. The trials at Knocker Bay and Cone Bay were ended after 6 weeks.

8.3 Results

At Cone Bay after 6 weeks, Superthin, Standard and Coating 16 inhibited barnacle settlement to 6-7 barnacles per shell compared with controls at 11 per shell (Figure 8.1a, Appendix 4.1). Original and Coating 18 did not deter barnacles. After 6 weeks Coating 18 had the highest cover of silt (twice that of controls) (Figure 8.1c) and all coatings inhibited the settlement of ascidians when compared with controls (Figure 8.1b).

After 6 weeks at Knocker Bay there was very heavy barnacle fouling (Appendix 4.2), but limited fouling in Exmouth Gulf (Appendix 4.3). Whilst a few antifouled shell showed reduced barnacle settlement at Knocker Bay, the majority did not differ greatly from controls and had heavy barnacle settlement.

Sampling was extended to 9 and 12 weeks for Exmouth Gulf (Appendix 4.4), with significant seaweed fouling, but no discernable difference between control and antifouled shell.

A low level of mortality was recorded in some of the coating treatments at Knocker Bay. Mortalities from the same coatings did not occur at either Cone Bay or Exmouth Gulf. The mortality is likely to reflect the length of time each shell was dried before coating, and the resulting temperature increasing inside shells, rather than an effect from the coatings themselves.

Coating integrity varied across all of the coatings. Standard showed the best integrity but in general performance was substandard. Furthermore, the coatings did not persist on the panels and crumbled off after a few weeks.

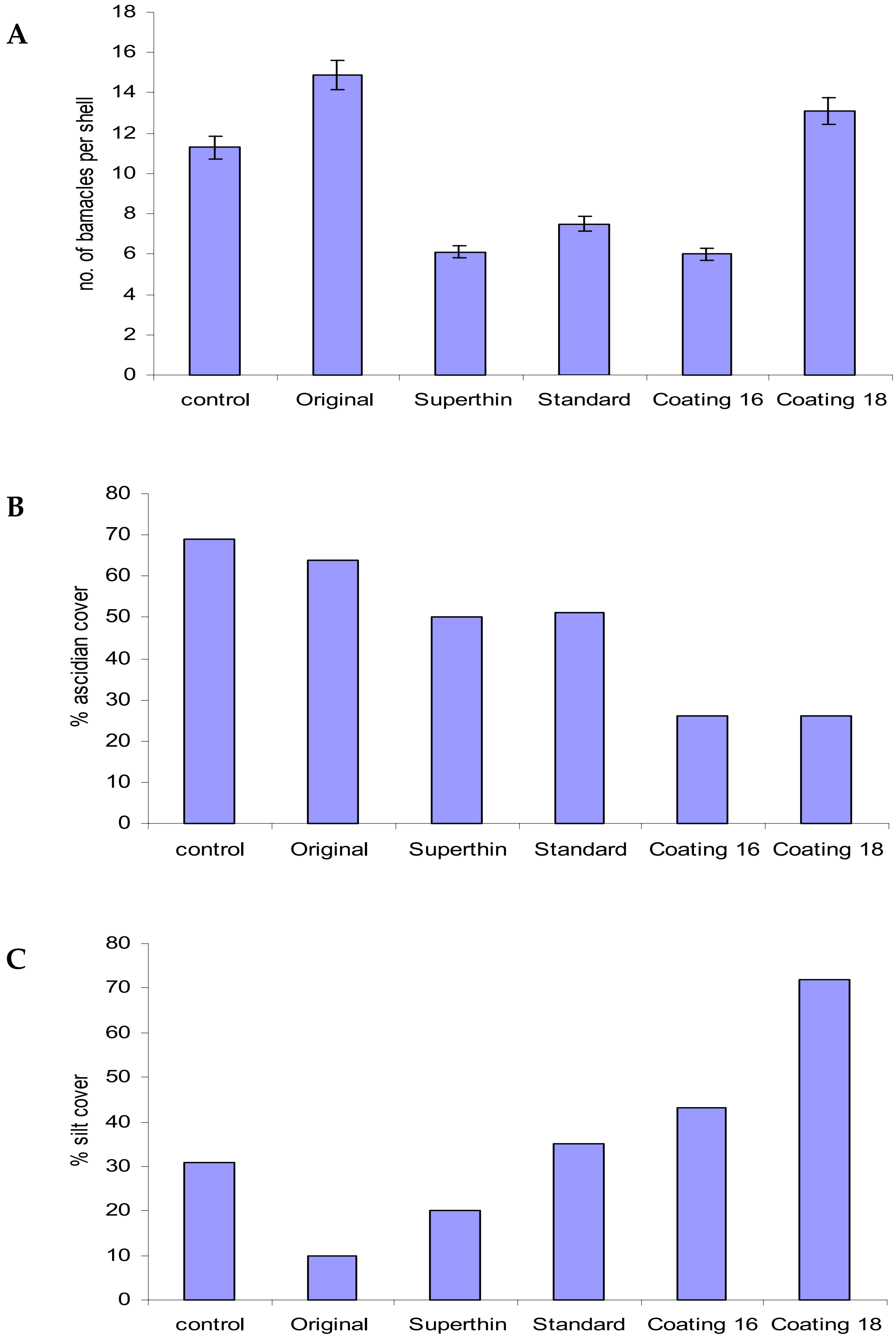


Figure 8.1. The efficacy of control and coated treatments against (A) barnacles, (B) ascidians and (C) silt at Cone Bay after 6 weeks. (Data are mean, n=30).

8.4 Summary

- Fouling varied greatly across the three sites. Barnacle fouling was the heaviest at Knocker Bay, reduced at Cone Bay and algal fouling was dominant at Exmouth Gulf.
- Superthin, Standard and Coating 16 deterred barnacles at Cone Bay compared with controls.
- All coatings deterred the settlement of ascidians.
- Coating performance and integrity was not as good as that seen in the early trials at Maxima. Possible reasons for this include the application technique.

9. Surface analysis of coatings

9.1 Introduction

Throughout Field Trial 1 and earlier trials it appeared that the efficacy of antifouling coatings was affected by the application method, as this influences both the quality and adhesion of the coating film. The variable fouling inhibition observed during Field Trial 1 was assumed to partly reflect coating characteristics (these factors were also examined in de Nys *et al*., 2003). Consequently, a range of coatings and application techniques (Table 9.1) were tested and the resulting surface features were measured by scanning electron microscopy (SEM) and light microscopy.

9.2 Materials and Methods

PearlClear **Standard** was used as the base coating as it performed well against barnacles at Cone Bay (Section 8) and also has good coating properties (de Nys *et al*., 2003). The application method of Standard was modified in several ways resulting in a range of different surfaces (Table 9.1). Reduced temperature spraying produced a rough surface effect, hereafter described as 'snowy'. Standard was also applied *via* dipping and both single and double dip applications were tested. An active ingredient treatment (incorporated at 10% of the dry weight of the film) was also included and termed 'plasticised'. Two film depths, 300 and 900µm were tested for the majority of the coatings.

Coatings were applied to 9cm^2 area polystyrene petri dishes. Six replicate dishes were used for each coating. Three replicates were analysed by SEM to visualise any microtopography present and the number of holes in the film was determined by light microscopy for the remaining three replicates.

Table 9.1. Coating types and application methods for analysis of coating topography.

Treatment	Application method	Coating thickness	Application temperature[1]
PearlClear Standard	Hot-spray	300µm	140°C
PearlClear Standard	Hot-spray	900µm	140°C
Snowy (Standard)	Reduced-temp spray	300µm	110°C
Snowy (Standard)	Reduced-temp spray	900µm	110°C
PearlClear Standard	Dip – single	n.d.[2]	70°C
PearlClear Standard	Dip – double	n.d.	70°C
PearlClear plasticised	Hot-spray	300µm	140°C
PearlClear plasticised	Hot-spray	900µm	140°C
Coating 16	Wagner spray gun	n.d.	room temp.
Coating 18	Wagner spray gun	n.d.	room temp.

[1] Application temperature for the hot spray system shows the temperature inside the coating reservoir, the lines and gun tip. Coating temperature once it reaches the shell surface is approximately 60°C.

[2] n.d. = not determined.

9.3 Results

Coating application method was found to markedly affect both coating topography (in terms of roughness) (Figure 9.1) and the prevalence of small holes in the coating surface (Figure 9.2). SEM images revealed that dip applied coatings (both single and double) were extremely homogenous and smooth, lacking all microtopography (Figure 9.1b). In contrast, snowy at both thicknesses presented a very rough and complex surface (Figure 9.1c & d).

Importantly, the experiment showed that application of coatings with a hot-spray system must be conducted with sufficient film thickness because if the coatings are applied too thinly numerous small holes occur across the surface (Figure 9.2). For example, a 300µm coating of Standard had four times as many holes than at 900µm coating depth.

The addition of an active ingredient did not appear to modify surface features.

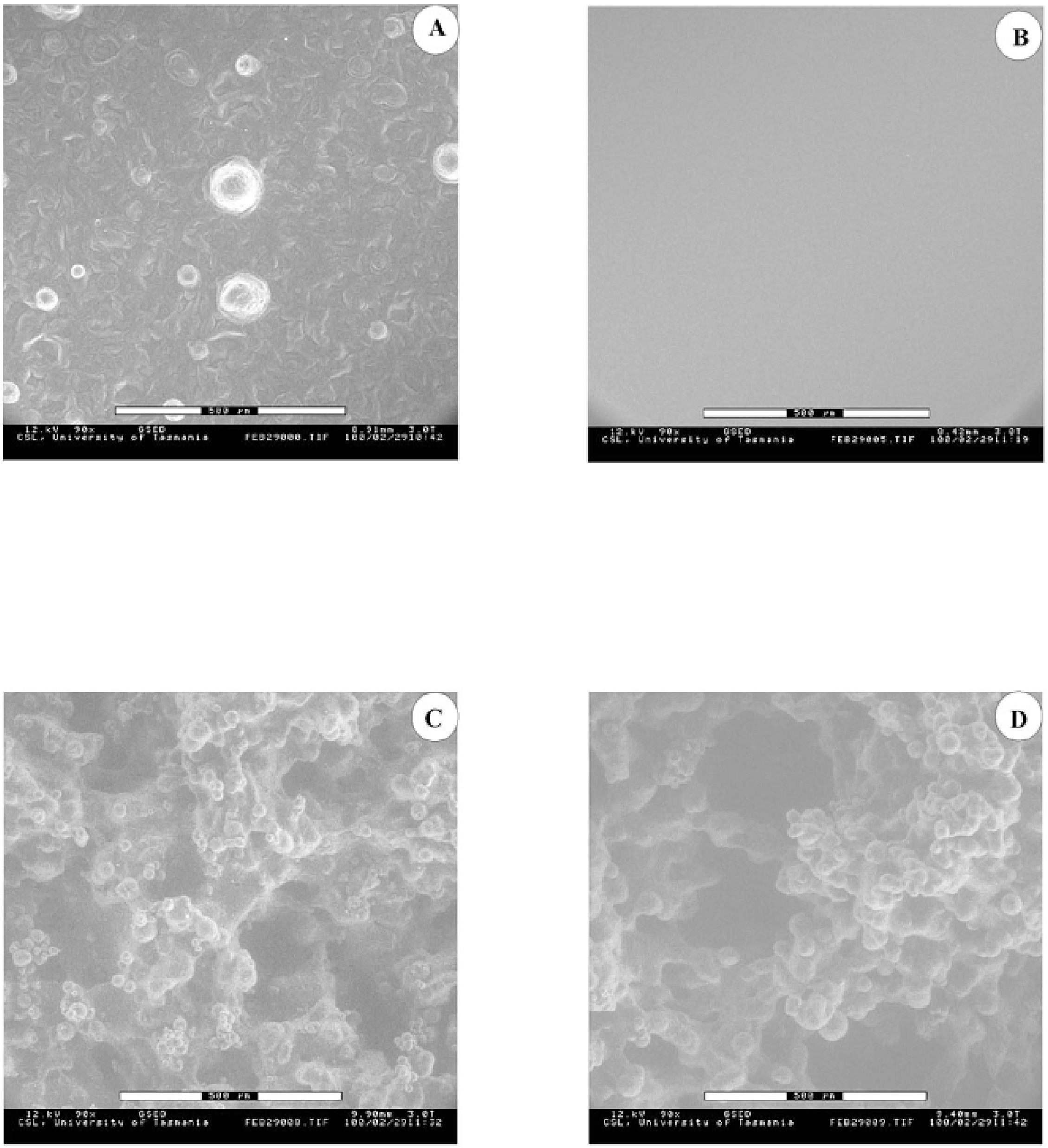


Figure 9.1. SEM images of PearlClear Standard coated to plates with four different application techniques. (A) standard hot spray, (B) dip application, (C) 300µm thick snowy film and (D) 900µm thick snowy film.

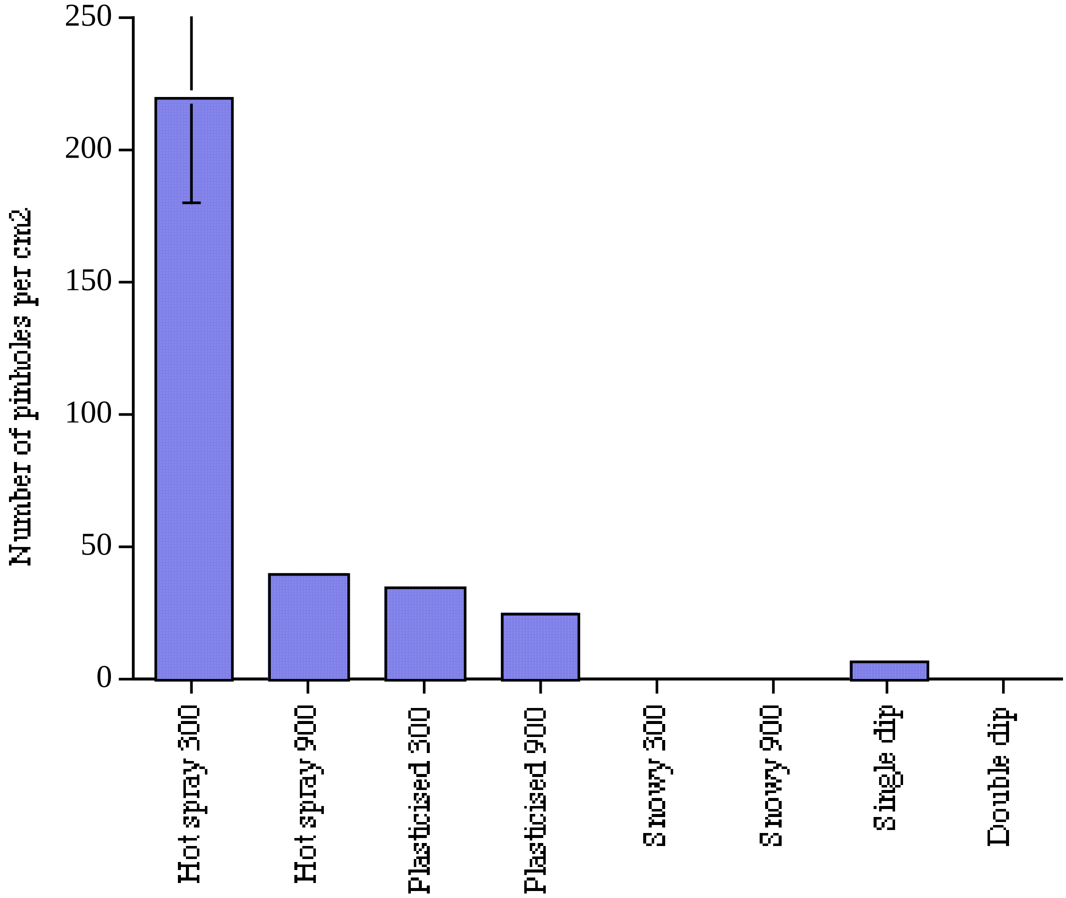


Figure 9.2. Mean number of pinholes observed in PearlClear coatings applied using different application techniques (300 and 900 refer to film depth in µm, n=3).

9.4 Summary

- Visual inspection of the coatings by SEM and light microscopy revealed that application technique significantly affects the resulting coating topography.
- The presence of holes (30-50µm diam.) was found for several of the coatings, particularly hot-spray Standard.
- The number of holes decreased with coating thickness.
- Strict adherence to a minimum coating thickness is necessary to ensure consistency in the coating surface.
- To determine the effect of coating topography on biofouling deterrence each of the above treatments was applied to replicate perspex tiles for field testing at Knocker Bay (Section 10, Tile Trial).

10. Tile trial at Knocker Bay, N.T.

10.1 Introduction

The aim of this experiment was to relate the surface characteristics observed in the laboratory (Section 9) with antifouling efficacy in the field. Therefore, each of the coatings and application techniques trialed in Section 9 were used to coat perspex tiles for field testing at Knocker Bay, N.T., a site of high barnacle fouling. Tiles were placed in the water in February 2000, in the season generally associated with increased barnacle fouling in this region (Taylor *et al*., 1997).

10.2 Materials and Methods

Table 10.1. Coating types and application methods for analysis of coating quality.

Treatment	Application method	Coating thickness	Application temperature[1]
Standard	Hot-spray	300µm	140°C
Standard	Hot-spray	900µm	140°C
Pinholed (Standard)	Hot-spray	300µm	140°C
Pinholed (Standard)	Hot-spray	300µm	140°C
Snowy (Standard)	Reduced-temp spray	300µm	110°C
Snowy (Standard)	Reduced-temp spray	900µm	110°C
PearlClear Standard	Dip – single	n.d.[2]	70°C
PearlClear Standard	Dip – double	n.d.	70°C
PearlClear plasticised	Hot-spray	300µm	140°C
PearlClear plasticised	Hot-spray	900µm	140°C
Coating 16	Wagner spray gun	n.d.	room temp.
Coating 18	Wagner spray gun	n.d.	room temp.

[1] Application temperature for the hot spray system shows the temperature inside the coating reservoir, the lines and gun tip. Coating temperature once it reaches the shell surface is approximately 60°C.
[2] n.d. = not determined.

Coatings were applied as per Table 10.1 to 8x8cm perspex tiles. An additional treatment was included to determine if holes in the coating surface increased barnacle settlement. This was achieved by applying a standard sprayed film at 300µm and 900µm and then imprinting an easily-recognisable pattern of pinholes across the coating surface (= pinholed treatment).

Three replicate tiles per treatment were used. For simplification, a complete set of replicated tiles was tied together to form an array (one for each sample period) and these were suspended along a longline at Knocker Bay. After 7 and 21 days immersion an array was retrieved and sent to the University of New South Wales where the number of barnacles per tile was counted and coating integrity assessed.

For 21 day data heterogeneity of variances was improved by sqrt transformation prior to a one-way ANOVA followed by Tukey's *post hoc* tests.

10.3 Results

No fouling was present after 7 days on either the control or coated tiles. It is expected that this was due to very high rainfall in the area at this time (D. Mills, pers comm.).

After 21 days the fouling community was completely dominated by barnacles and settlement was highly variable across the different coating surface topographies (Figure 10.1).

The occurrence of holes in the coating surface (of approximately 50µm diam.) did not influence barnacle settlement (pinholed 300 and 900µm), rather the settlement of barnacles was dependent on surface roughness, however the differences were not always significant (Figure 10.1, one-way ANOVA, $F_{12,26}$=3.926, p=0.002, Tukey's test, α=0.05).

Surfaces that were very smooth (single dip) or very rough (snowy 900µm) had no barnacles, whereas surfaces of intermediate roughness had the most barnacles (6-7 per tile). These results are consistent with recent ecological literature on the settlement of barnacle larvae (Crisp, 1974; Andersson *et al*., 1999; Berntsson *et al*., 2000a, b). Several studies have found that microtopography ranging from 1 – 500 µm (Kohler *et al*., 1999; Andersson *et al*., 1999; Berntsson *et al*., 2000a, b) to mm's (Hills and Thomason, 1998) deters the settlement of barnacles. In addition Afsar *et al*. (2003) found that when barnacle larvae were exposed to PearlClear coatings their behaviour was modified and they were unable to settle.

The efficacy of the active ingredient ('plasticised') treatment was related to film thickness as the 900µm film completely inhibited settlement while some settlement (2 barnacles per tile) was seen on the 300µm treatment, however neither treatment was significantly different from each other or controls (Figure 10.1).

The very different level of fouling between single (0 barnacles per tile) and double (17 barnacles) dipped coatings is unexpected as the surface analysis (Section 9) showed that both coatings presented very similar surfaces.

Coating 16 and 18 performed variably, in fact coating 18 was significantly more fouled than some of the treatments but not from controls (Figure 10.1). Therefore further development of this type of coating was not continued.

The majority of coatings maintained their integrity and adhesion to the tiles over the 21 days. Snowy 300µm tended to flake easily and decreased in thickness over time suggesting that for this type of coating a minimum thickness is required in order to maintain efficacy. The single and double dipped treatments were judged to have the best adhesion.

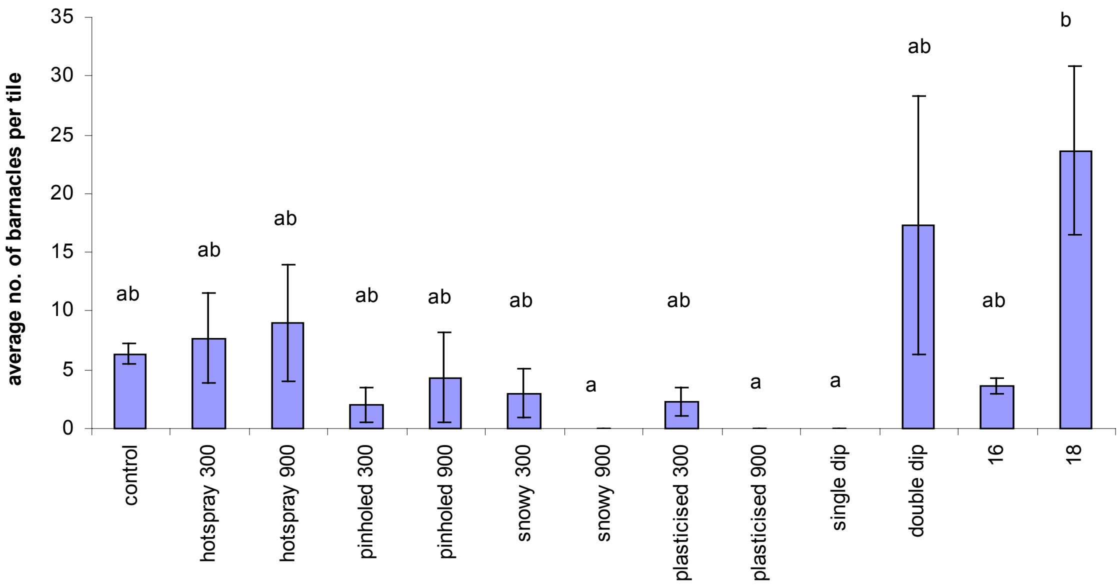


Figure 10.1. Average number of barnacles on coated and control panels after 21 days immersion at Knocker Bay, NT. (Data are means ± S.E., n=3, 300 and 900 refer to film depth in µm).

10.4 Summary

- Surfaces presenting very rough (snowy) or very smooth (single dipped) topography deterred barnacle settlement.
- The trial revealed that more information was needed to understand the interactions between barnacle settlement and coating type and application method.
- Coating 16 and 18 were were not tested further as they had limited antifouling activity and poor coating characteristics.

11. Laboratory bioassay

11.1 Introduction

The field trials and tile trial demonstrated the importance of coating characteristics in deterring barnacle settlement. In order to better understand the mechanisms of deterrence a series of formulations was exposed to barnacle larvae in laboratory assays. Laboratory assays focus on the early stages of settlement and provide useful information on coating performance and barnacle behaviour (Asfar *et al.*, 2003). A modification of Standard, **HB2,** not previously evaluated in field trials was included in the laboratory trial. HB2 was formulated with constituents which cause the coating surface to have a low surface energy and consequently inhibit or modify larval settlement behaviour.

11.2 Materials and Methods

All coatings were applied to $9cm^2$ area petri dishes, with uncoated dishes used as a control surface. Coatings were applied as per Table 10.1 (Section 10) with hot-spray, snowy, dipped and HB2 treatments tested. Approximately 50 barnacle cyprid larvae (*Balanus amphitrite*) were placed in each petri dish (six replicate dishes per treatment) with 5mLs of filtered seawater (0.20μm filter). The dishes were held in a controlled environment of 15:9 hour light/dark cycle at 24-26°C. The percent settlement was determined after 48 hours under a dissecting microscope (for more detailed methods see de Nys *et al.*, 1995).

HB2 strongly inhibited the settlement of cyprid larvae and as this treatment included almost all zeros, thus disproportionately influencing the assumptions of ANOVA, it was excluded and not compared statistically. A one-way ANOVA followed by Tukey's *post hoc* tests was performed on the remaining data to determine the effect of coating type on cyprid settlement.

11.3 Results

Consistent with the findings from the field trial, surfaces of high roughness (snowy 300 and 900μm) and very smooth surfaces (single and double dipped) significantly inhibited cyprid settlement compared with controls (Figure 11.1, one-way ANOVA, $F_{6,31}=7.265$, $p<0.001$, Tukey's test, $\alpha=0.05$). All other coatings performed similarly to each other with decreased settlement compared with controls.

The new formulation, HB2, almost completely inhibited all barnacle settlement with less than 2% settlement compared with controls with 56% settlement (Figure 11.1). Furthermore, the results for the single and double dipped treatments are consistent with information from the surface analysis as they were not significantly different from each other (Figure 11.1).

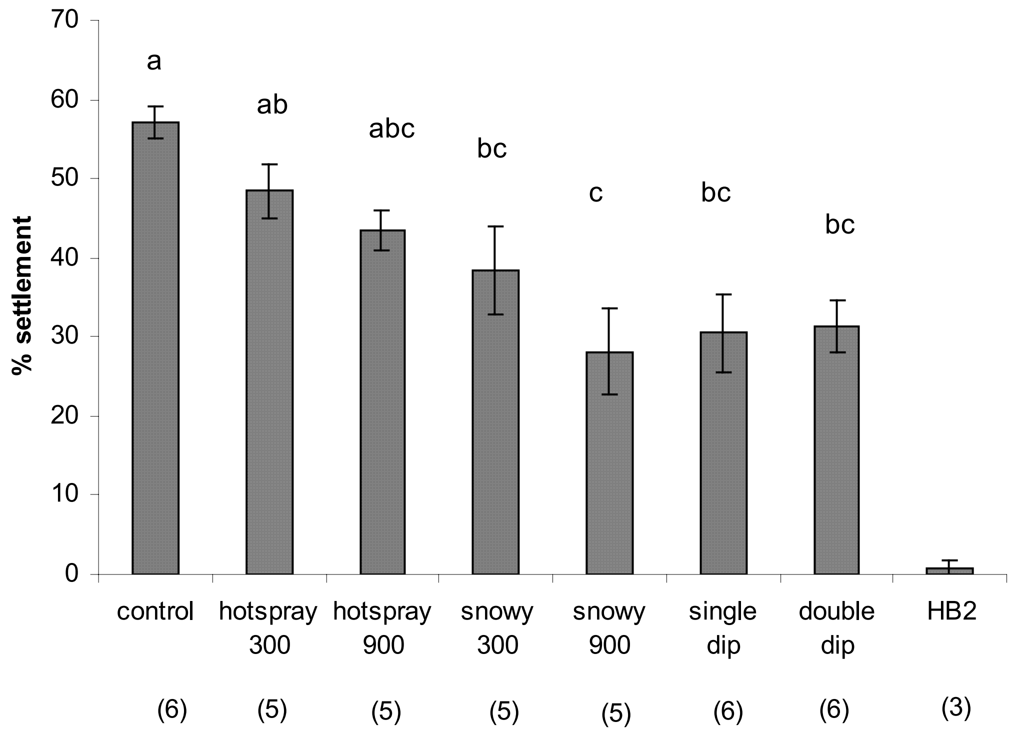


Figure 11.1. Percentage of barnacle larvae settled on test dishes coated with antifouling treatments. All coatings, other than HB2, are PearlClear Standard. (Data are mean ± S.E., numbers in brackets refer to replicates, 300 and 900 refer to film depth in μm, bars sharing the same letter are not significantly different at α=0.05 with HB2 excluded from analysis).

11.4 Summary

- HB2 was clearly the most effective coating and therefore was chosen as the basis for further coating development.
- Snowy and dipped coatings had significantly less settlement than controls.
- The results are consistent with findings from other studies that have shown that surfaces of high smoothness and high roughness deter larval settlement (Crisp, 1974; Hills and Thomason, 1998; Andersson *et al*., 1999; Berntsson *et al*., 2000a, b; Callow *et al*., 2002).

12. Field Trial 3 - Improved formulations tested at Cone Bay and Bynoe Harbour

12.1 Introduction

Based on the results from the tile trial and the laboratory assay a series of formulations was tested at Cone Bay, W.A. and Bynoe Harbour, N.T. Eleven coatings (A1-A11) were trialed on live shell at Cone Bay, and nine coatings at Bynoe Harbour, in April 2000. Four of the coatings (A7-A10) were based on HB2 and included new constituents designed to modify surface texture and increase antifouling efficacy while the remaining coatings (A1-A6) compared the efficacy of different surface topographies against biofouling.

12.2 Materials and Methods

Table 12.1. Trial design at Cone Bay and Bynoe Harbour, April 2000

Treatment name	Base formulation	Low surface energy	Application method	Cone Bay N=	Bynoe Harbour N=
coating A1	Original	-	dip	18	18
coating A2	Original	-	spray	18	18
coating A3	Original	-	snowy	24	15
coating A4	Standard	-	dip	18	18
coating A5	Standard	-	spray	18	18
coating A6	Standard	-	snowy	24	18
coating A7	HB2	A50	dip	18	18
coating A8	HB2	A1000	dip	18	18
coating A9	HB2	B50	dip	18	18
coating A10	HB2	A350	dip	18	
Control			uncoated	18	18

The coating types and application methods are shown in Table 12.1. Replicate shell were divided into six pocket panels. Six of the coatings were dipped and the remainder sprayed onto the shell using the custom designed spraying equipment. Prior to coating, the shell were air-dried in the shade for 20-30 mins and immediately returned to the water after coating. The trials were conducted for 12 weeks at Cone Bay and 9 weeks at Bynoe Harbour in April and June, 2000.

During the application stage at Bynoe Harbour all the coatings became contaminated with water. This markedly changes the characteristics of the surface and influences the coating integrity and adhesion so it was decided to repeat the experiment as the results would be unreliable and not indicative of true coatings. When the experiment was repeated at Bynoe Harbour, there was very high mortality in many of the coated treatments. However, the design and method were re-evaluated and coatings were re-tested at Bynoe Harbour in July 2000 (see Section 13).

The number of barnacles was counted at Cone Bay and Bynoe Harbour at 6 weeks. At this time it was noted that coating integrity was deteriorating rapidly and some replicate shell had lost coating so barnacle settlement was not measured after 6 weeks at Bynoe Harbour and 12 weeks at Cone Bay. The trial at Bynoe Harbour was photographed after 9 weeks. Data were not analysed formally due to the large number of zeroes in many of the treatments.

Mortality data from the Bynoe Harbour trial was analysed by omitting controls (this treatment contained many zeroes and thus lead to violations of the assumptions of ANOVA) and for the remaining coating treatments a one-way ANOVA followed by Tukey's *post hoc* tested the effect of application method on survivorship.

12.3 Results

12.3.1 Fouling

After 6 weeks at Cone Bay, many of the coatings showed inhibition of barnacle settlement compared with controls (Figure 12.1). Coating A9 completely inhibited all fouling and coatings A1 – A3 limited barnacle settlement to less than 1 per shell compared with controls which had 5 barnacles per shell.

After 12 weeks at Cone Bay, coatings A9, A3 and A1 were the best performing, limiting fouling to less than 8 barnacles per shell compared with controls at 16 per shell (Figure 12.2, Appendix 4.5). The remaining coatings showed limited inhibition and were fouled similarly to controls.

After 6 weeks at Bynoe Harbour control shell had a mean of 4 barnacles per shell (Figure 12.3). The Original (A1-A2) and Standard (A4-A6) formulations had an antifouling efficacy similar to controls (Figure 12.3). Of the HB2 series, A7 and A8 had twice as many barnacles as controls. However, coating A9 performed well, limiting settlement to less than one barnacle per shell (pictures after 9 weeks, Appendix 4.6).

The influence of modified surface topography on antifouling activity was variable. Coating A3 and A6 performed well as these 'snowy' coatings simulated a very rough surface texture, however the smooth coatings (A1-A2, A4-A5) did not show antifouling activity. The low surface energy constituent also showed variable activity. Coating A9, containing B50, had the best activity while the other HB2 coatings performed similarly to controls.

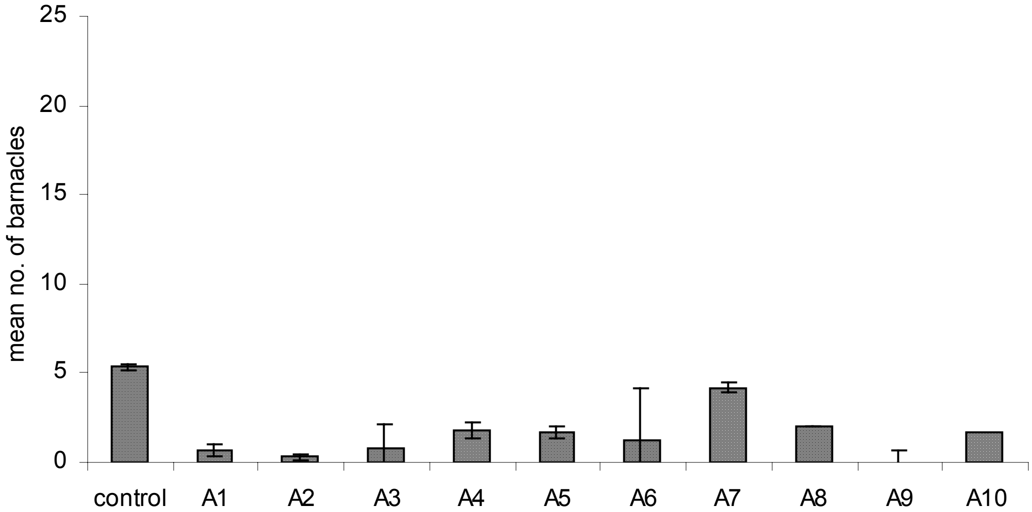


Figure 12.1. Number of barnacles on control and A series coated shell after 6 weeks at Cone Bay. (Mean ± S.E.).

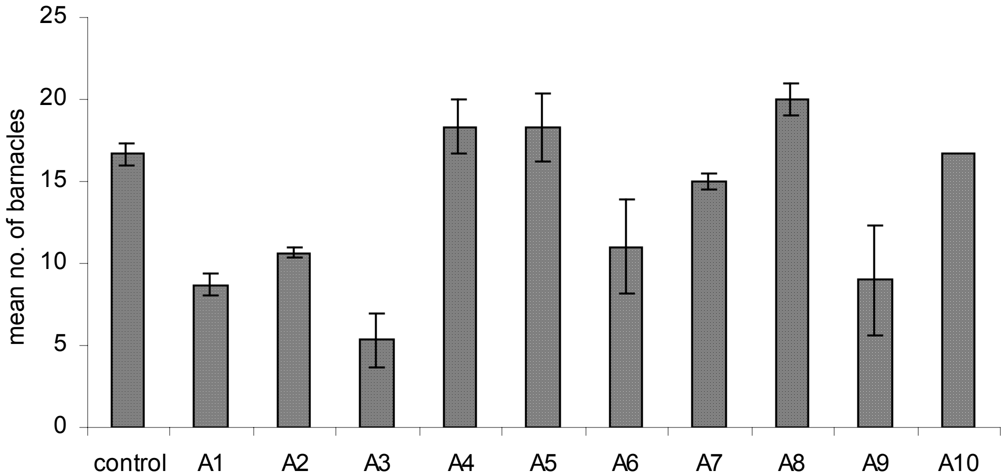


Figure 12.2. Number of barnacles on control and A series coated shell after 12 weeks at Cone Bay. (Mean ± S.E.).

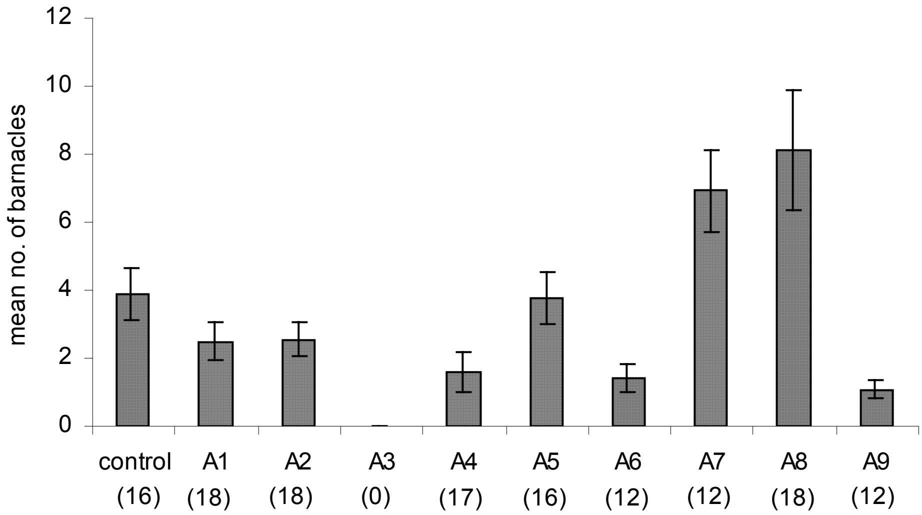


Figure 12.3. Number of barnacles on control and A series coated shell after 6 weeks at Bynoe Harbour. (Mean ± S.E., numbers in brackets refer to replicates).

<u>12.3.2 Coating integrity</u>

Application method had a strong influence on coating integrity. The 'snowy' coatings, A3 and A6, had very poor adhesion and the coating was lost from many shells. Coatings A7 - A10 (variations of HB2) performed well, maintaining adhesion and integrity over the 12 weeks at Cone Bay (Appendix 4.5).

<u>12.3.3 Mortality</u>

A high level of mortality was observed in all coated treatments at Bynoe Harbour compared with controls (Figure 12.4). However, there was no significant effect of the method of application on mortality (one-way ANOVA, $F_{8,18}$=1.282, p=0.312, controls were excluded from analysis). This might be expected if coating application was the major cause of mortality as, for example, immersion into the hot coating as happens for the dip treatment may stress oysters more than the spray application. It is likely then that mortality was a result of handling and shell condition prior to application and the application process increased stress. The same coatings and application methods were trialed at Cone Bay with very few mortalities (0-1 per treatment) and there were no mortalities in the first experiment where these coatings were tested (water damaged).

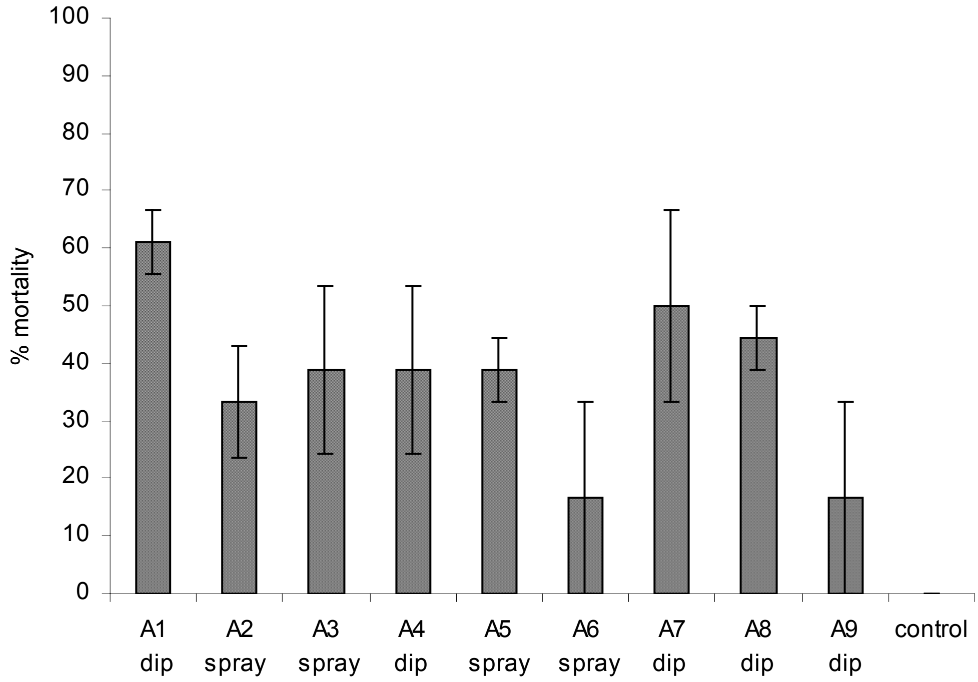


Figure 12.4. Percent mortality of coated and control shell at Bynoe Harbour, June 2000. (Data are means ± S.E., n=3).

12.4 Summary

- Coating A9 performed the best at both sites with respect to fouling and coating integrity and therefore was used as the basis for a further series of modified formulations in Trial 4 (Section 13).
- 'Snowy' applied coatings (A3 and A6) had good antifouling efficacy, however they had very poor adhesion to the shell.
- The trial at Bynoe Harbour revealed that oyster condition prior to coating may impact on the survivorship of oysters.

13. Field Trial 4 - Testing at Bynoe Harbour

13.1 Introduction

Despite the problems encountered in Trial 3, coating A9 was found to perform well and was therefore modified further and a more comprehensive matrix (**B1 - B10**) tested at one site, Bynoe Harbour, N.T., beginning in June, 2000. This trial also endeavored to minimise problems associated with handling and application.

13.2 Materials and Methods

Several formulations were tested using two base coating as well as three different additives (Table 13.1). Low surface energy (1) and active ingredient (3) are designed to improve antifouling efficacy and additive 2 influences coating properties such as hardness and adhesion.

Table 13.1. Coatings tested at Bynoe Harbour, NT, June 2000

Coating	Base formulation	Low surface energy (1)	Coating hardness (2)	AI (3)	N=
coating B1	Standard	B50 (10%)	0.75%		18
coating B2	Standard	A50 (10%)	0.75%		18
coating B3	Standard	A1000 (10%)	0.75%		18
coating B4	Standard	A50 (5%)	0.75%		18
coating B5	Standard	A1000 (10%)	-		18
coating B6	Standard	C (10%)	-		18
coating B7	Original	A1000 (10%)	0.75%		18
coating B8	Original	-	-		18
coating B9	Standard	B50 (9%)	0.75%	3%	18
coating B10	Standard	-	-		18
Control half shell					18
Control live shell					18

All coatings were applied to half shell *via* dipping. Eighteen half shell per coating were used, divided between three, six pocket panels. Before coating all shell were air-dried in the shade for 20-30 mins and returned to water immediately after coating. Live controls were also included and these were handled in a similar manner to the half shell. Coating performance was assessed by counting the number of barnacles and oyster spat that had settled after 7, 12, 19 and 24 weeks. At the trials conclusion at 24 weeks, representative panels of shell were put through a high pressure water cleaning machine. This was qualitative at this point to determine if cleaning could be used in conjunction with these coatings to improve antifouling efficacy.

No formal statistical analyses were performed on the barnacle fouling data due to the large number of zeros across all treatments. Only 12 week oyster spat data was compared statistically as there was substantial loss of replicates at week 19 and 24 due to two longlines tangling together. The effect of coating on the settlement of oyster spat was compared using a one-way ANOVA followed by Tukey's *post hoc* tests on 12 week data. Spat data was not homogenous after transformation, however there were large sample sizes and ANOVA is considered robust to heterogenous variances in such situations (Underwood, 1997) and significant results were interpreted cautiously. There was no significant difference between the live and half controls so data was pooled (unpaired *t-test*, t=0.117, p=0.907).

13.3 Results

<u>13.3.1 Fouling</u>

The majority of coatings inhibited both barnacles and oyster spat (Figure 13.1 & 13.2) and coating B9 inhibited all types of fouling including amphipods and silt over the 24 week period of the trial. Despite the overall level of fouling being low because it was the dry season a clear antifouling effect was seen at 7 weeks (Appendix 4.7). At 12 weeks there were four times as many barnacles on control shell than on coated shell (Figure 13.1). Coatings B2-B6 and B9 had significantly less oyster spat than controls (Figure 13.2, one-way ANOVA, $F_{11,200}$=6.310, p<0.001, Tukey's test, α=0.01) (Pictures in Appendix 4.8).

The fouling surveys at 19 and 24 weeks were hampered by factors outside of the trial. Two longlines tangled together which resulted in several panels and many shell being lost and fouling and coating being abraded off the shells (Table 13.2). However the trends seen at 12 weeks continued at 19 and 24 weeks. At 19 weeks coatings B9, B2 and B4 had the best antifouling efficacy with no barnacle settlement (Figure 13.1 and Appendix 4.9). Oyster spat was the most common fouling organism with an average of 10-20 spat on control shell and ~ 5 on coated shell (Figure 13.2 and Appendix 4.9). Again, coating B9 performed well against oyster spat. The same trend was seen at 24 weeks as at 19 weeks (Figure 13.1, 13.2 and Appendix 4.10).

After cleaning, a qualitative re-assessment of fouling and coating integrity found that coatings B1-B7 and B9 were removed very easily from the shell and all types of fouling were removed easily with the coating. However, coatings B8 and B10 were only partially removed or not at all (images in Appendix 4.10b). Fouling on control shell was not removed at all by water pressure and required manual chipping.

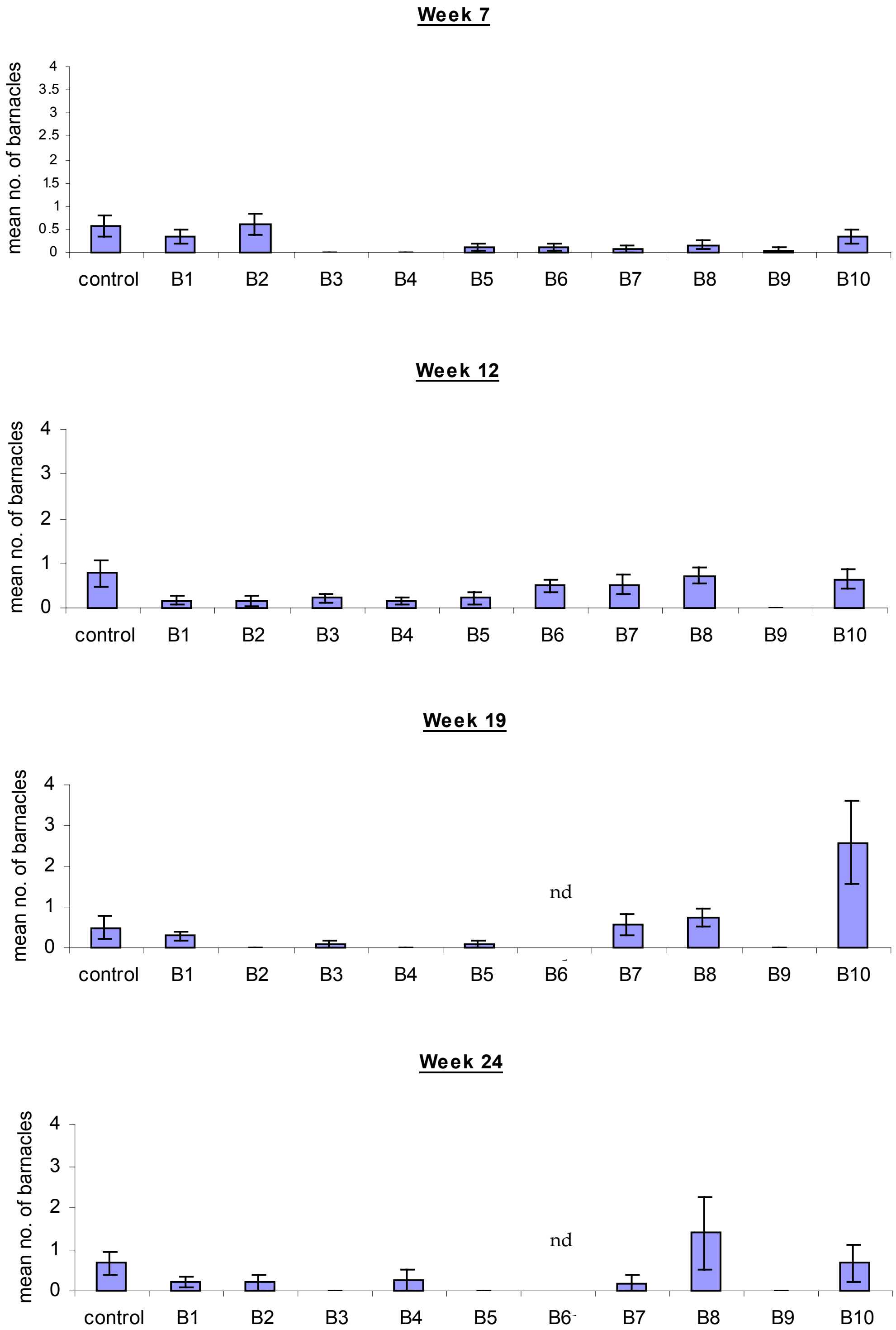


Figure 13.1. Average number of barnacles on control and coated shell over 24 weeks immersion. (Data are mean ± S.E., replicate numbers are shown in Table 13.2, nd = not determined due to a loss of replicates).

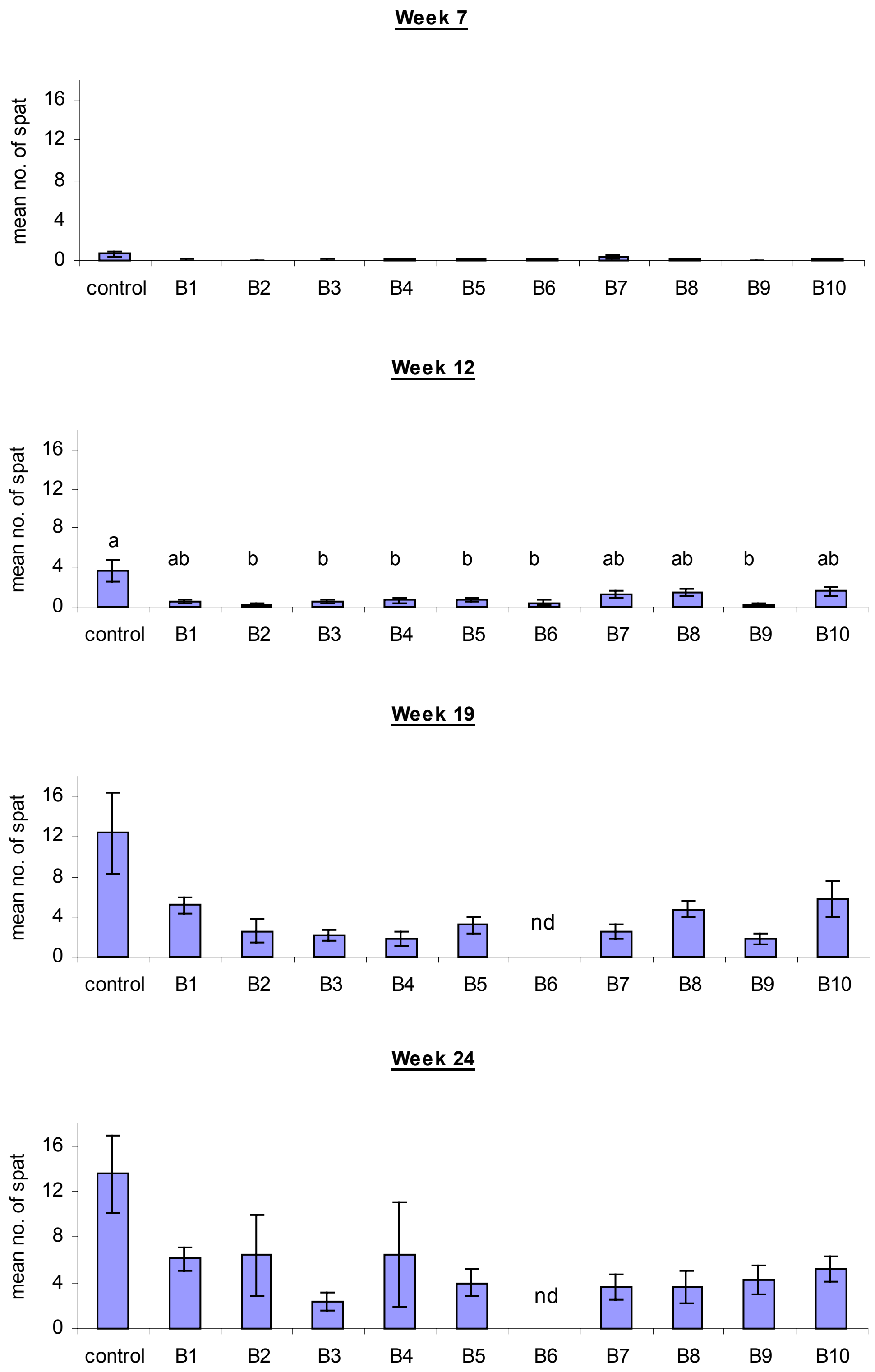


Figure 13.2. Average number of spat on control and coated shell over 24 weeks immersion (data are mean ± S.E., replicate numbers are shown in Table 13.2, one-way ANOVA followed Tukey's tests on 12 week data only, treatments with the same letter do not differ at α=0.01, nd = not determined due to a loss of replicates).

Table 13.2. Number of replicate shell from which data was obtained for each sampling period.

Treatment	Number of replicate shell[1]			
	t=0	t=12	t=19	t=24
Live control	18	18	12	pooled 15
Half shell control	18	16	3	
1	18	17	14	10
2	18	18	8	5
3	18	18	11	6
4	18	18	10	4
5	18	18	11	9
6	18	18	4	1
7	18	18	14	11
8	18	18	16	5
9	18	18	5	4
10	18	17	14	9

[1] Loss of replicates was due either to missing shell or shell that had lost all coating.

13.3.2 Coating integrity

Coating integrity was variable and was related to the different additives in each coating. Additive 1 did not influence coating integrity. However, coatings containing additive 2 such as B4, B9, B2 and B3, became brittle over the period of the trial, resulting in surface cracking and the coating eventually lifting off the shell. Coatings such as B7 and B10 are more malleable and were more resistant to cracking and therefore persisted on the shell (images in Appendix 4).

13.4 Summary

- Trial 4 was successful in determining several coatings which deterred settlement of both barnacles and oyster spat for 6 months over the dry season.
- Coating B9, containing additive 3, was the best performing coating inhibiting barnacles and spat as well as ascidians and silt.
- Coating B3 also performed well against oyster spat, the most common fouling organism, limiting fouling to less than 4 spat per shell compared with 12 on controls at 24 weeks.
- All coatings containing additives had better antifouling efficacy than their unmodified counterparts (B8 and B10).
- However some of the additives lead to poorer adhesion and abrasion resistance qualities.
- Cleaning with high pressure water at the end of the trial removed both coating and fouling from most of the treatments.

14. Field Trial 5. Testing over summer

14.1 Introduction

The same matrix that was tested in Trial 4 was then tested at Cone Bay, WA and Bynoe Harbour, NT, over the 2000/2001 summer fouling period. The trial was commenced in late October to early November 2000.

14.2 Materials and Methods

Ten coatings were trialed at Cone Bay, WA, and a smaller matrix of coatings tested at Bynoe Harbour, NT (Table 14.1). This allowed a comparison of efficacy across locations. The complete matrix was not tested at Bynoe Harbour as there was a limited number of shell available for coating.

Table 14.1. Coatings tested at Cone Bay and Bynoe Harbour over summer 2000/2001

Coating	Low surface energy (1)	Coating hardness (2)	AI (3)	Cone Bay	Bynoe Harbour
coating 1	B50 (10%)	0.5%	-	18	18
coating 2	A50 (10%)	0.5%	-	18	18
coating 3	A1000 (10%)	0.5%	-	18	-
coating 4	A50 (5%)	0.5%	-	18	-
coating 5	A1000 (10%)	-	-	18	-
coating 6	C (10%)	0.5%	-	18	-
coating 7	B50 (9%)	0.75%	3%	18	18
coating 9	-	-	-	18	-
coating 10	A50	-	-	18	18
Control half shell				18	18

The coatings trialled (Table 14.1) are essentially the same as those trialled in Section 13, with some small modifications. The base coating is Standard in all cases. To address poor integrity issues, coating hardness was reduced from 0.75% to 0.5% of the formulation and the coating referred to as B9 in Section 13 is now termed coating 7.

All coatings were applied onto dry half shell *via* dipping as in previous trials with 18 replicate shell divided between three, six pocket panels. Fouling and coating integrity were evaluated at both sites after 6 weeks and at the conclusion of the trials at 12 weeks. Fouling was measured at Cone Bay as number of barnacles per shell, however at Bynoe Harbour the numbers of barnacles were too great (> 100 barnacles per shell on controls, pers. obs.) so percent cover was recorded.

For both sites, at 12 weeks all treatments were cleaned using high pressure to determine how much fouling and coating could be removed. All panels were cleaned in accordance with standard cleaning practices at these farms. Fouling was recounted after cleaning.

Analyses

Six week data from Cone Bay was not formally analysed as there was a very clear effect of the coatings on barnacle settlement (Figure 14.1) and consequently the data did not fit the assumptions of ANOVA due to the large number of zeros in the data. Six week data (%) from Bynoe Harbour was sqrt-arcsine transformed prior to a one-way ANOVA followed by Tukey's *post hoc* tests.

A similar pattern to that at week 6 was seen at 12 weeks at Cone Bay with a very strong inhibition of fouling seen across all the coated treatments. In order to highlight individual coating performance in terms of fouling inhibition and also to assess whether cleaning improved the overall efficacy of the coatings, control data was excluded from analysis (the control data was interfering with the overall assumptions of ANOVA). The remaining treatment data was log (1+x) transformed prior to a two-way ANOVA with the factors coating type and cleaning, followed by Tukey's *post hoc* tests.

For 12 week data collected from Bynoe Harbour, controls were included in the analysis as the pattern was not as strong, the data sqrt-arcsine transformed, and a two-way ANOVA using the same factors was performed.

14.3 Results

14.3.1 Fouling

There was a dramatic effect of all coatings on barnacle fouling after 6 weeks at both sites. At Cone Bay, control shell had an average barnacle cover of 90 per shell whilst the coatings reduced this to 1-5 barnacles per shell (Figure 14.1 and Appendix 4.11). After 12 weeks at Cone Bay, all coatings again strongly inhibited fouling by barnacles, with an average of 5-10 barnacles per shell as compared with controls which had an average of 80 barnacles per shell (Figure 14.2 and Appendix 4.12). All the coatings performed very similarly to each other with respect to antifouling efficacy except for Coating 9 which was found to be significantly more fouled than the other coatings (two-way ANOVA, $p<0.001$, Table 14.2A).

After 6 weeks at Bynoe Harbour all coatings significantly inhibited barnacle settlement, reducing barnacle fouling to 10-20% cover of surface area of the shell as compared with controls which had an average barnacle cover of 80% (Figure 14.3 and Appendix 4.13, one-way ANOVA, $F_{4,82}=118.450$, $p<0.001$, Tukey's test $\alpha=0.05$) As many as 140 barnacles were counted from one valve of a control shell at Bynoe Harbour (pers obs.).

Again at 12 weeks all the coatings were significantly less fouled than the controls (Figure 14.4a and Appendix 4.14, Table 14.2B). Coating 7, containing the active ingredient, inhibited fouling to 50% cover as compared with controls which were 100% fouled. Coatings 1, 2 and 10 performed similarly to each other and restricted fouling to 70%.

The antifouling efficacy of coatings varied across the two sites. Coating 7 and 10 performed well at both Cone Bay and Bynoe Harbour. Coating 1 and 2 were not as effective at Bynoe Harbour as they were at Cone Bay. This may reflect the greater fouling pressure seen at Bynoe Harbour. There were ~80 barnacles on controls at Cone Bay whereas up to 140 barnacles were counted from controls at Bynoe Harbour. In addition, all coatings worked well at inhibiting barnacle settlement, however they did not inhibit soft fouling organisms such as ascidians.

14.3.2 Cleaning

At the end of the trial all treatments including controls, at both sites, were put through an industry cleaning machine to establish how much fouling and coating could be removed. At both sites, cleaning with water pressure removed a further 10-25% of the fouling cover for most of the coatings but did not seem to improve others, and cleaning did not significantly improve coating efficacy at Cone Bay (Table 14.2A, Figure 14.2, Appendix 4.12).

However at Bynoe Harbour cleaning with water pressure significantly reduced the percent cover of fouling for several of the coatings (Table 14.2B). Furthermore, formulation differences between the coatings had a significant influence on the effectiveness of cleaning to remove fouling. For example a further 50% of fouling was removed for coating 10 but <5% removed on coating 7 (Figure 14.4, Appendix 4.14).

Cleaning removed a small amount of fouling (10%) on control shell at Bynoe Harbour (Figure 14.4) but not at Cone Bay. A possible reason for the difference in the effectiveness of cleaning between the two sites may be that there was some spat fouling on all treatments at Bynoe Harbour and as this is limited to the top of the coating, it may have been more easily removed by cleaning and fouling was quantified as % cover rather than individual counts.

Fouling on the panels holding the shells was considerable after 12 weeks. The panels were heavily fouled by large barnacles (~2cm diam.) which were not removed using the cleaning machine. It may not be possible to leave shell completely unattended for 3 months until an antifouling solution has been developed for the panels.

At the end of the trial barnacle samples were sent to the WA Museum for identification. The dominant barnacle species at both sites was *Balanus cirratus*. The size and growth rates of this species differed between the two sites and thus fouling results varied.

14.3.3 Coating integrity

Across both sites coatings 7 and 10 had the best coating integrity maintaining coverage after 12 weeks. Of those tested at Cone Bay, coating 6 and 9 were the best performing coatings (Appendix 4.12). The modifications of the constituents within each coating was more evident in the coating integrity results than in fouling efficacy. For example, coating 10 was a more flexible coating and was more resistant to breakup by barnacles. Coating 7 was far more brittle as was coating 2 so that by the completion of the trial the coating was completely removed from the shell.

Cleaning removed the majority of coating across all treatments. Some barnacles were able to grow through the coating and these were not removed by high pressure cleaning. Furthermore, anecdotal evidence suggested that by manipulating water pressure during the cleaning process most of the fouling could be removed and the coating remained intact. This has implications for increasing coating life and management strategies.

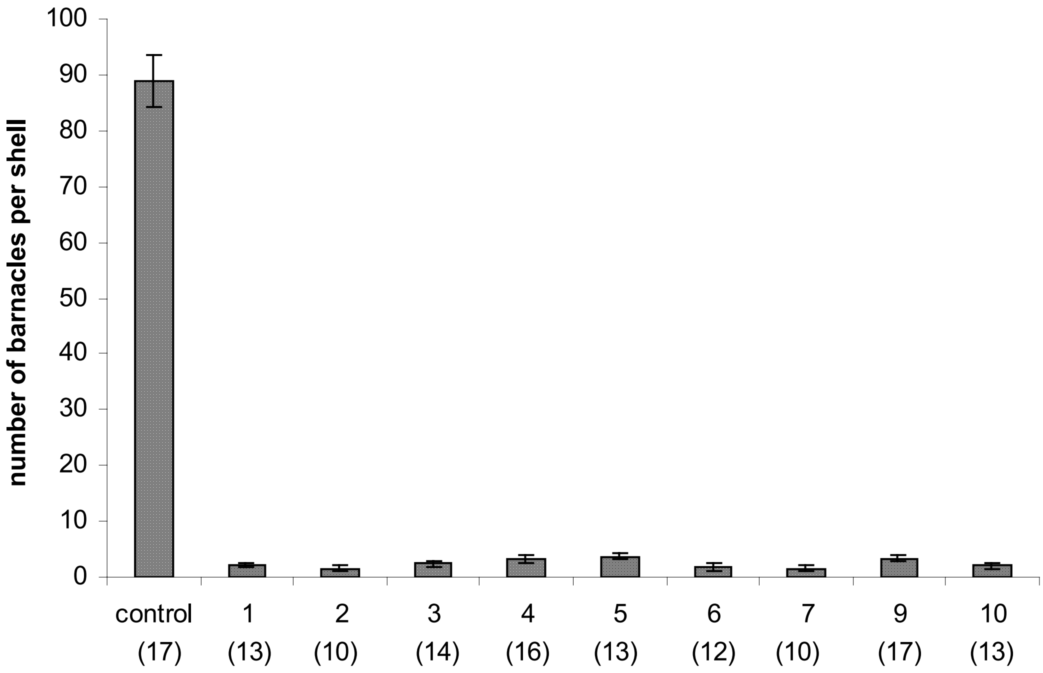


Figure 14.1. Number of barnacles per shell on control and coated shell after 6 weeks at Cone Bay. (Data are mean ± S.E., numbers in brackets refer to replicates).

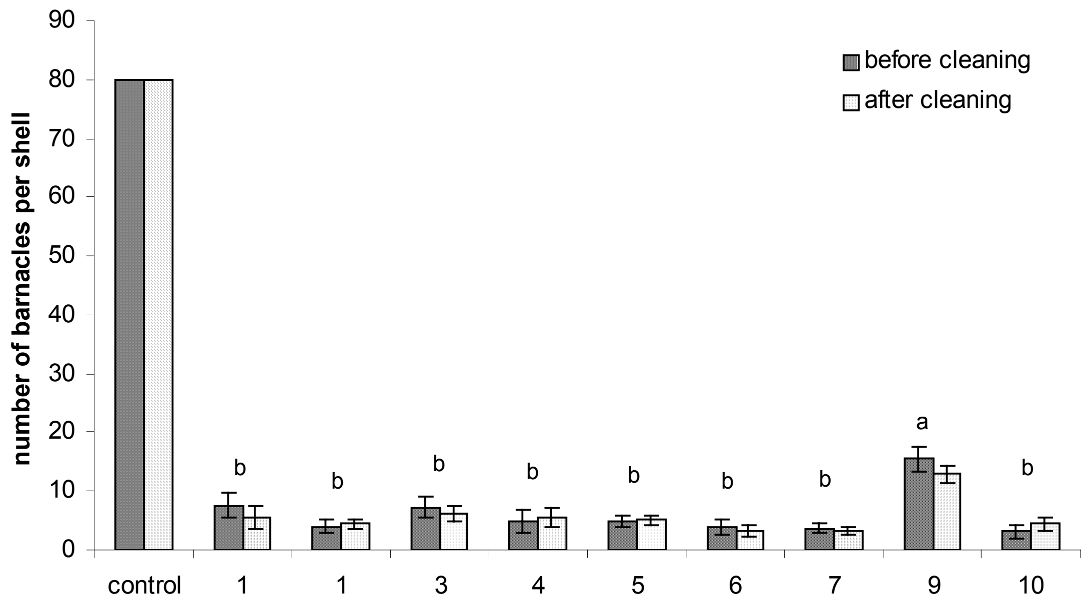


Figure 14.2. Number of barnacles per shell on control and coated shell, before and after cleaning, after 12 weeks at Cone Bay. (Data are mean ± S.E., n=18, bars sharing the same letter are not significantly different at α=0.05, controls were not included in analysis).

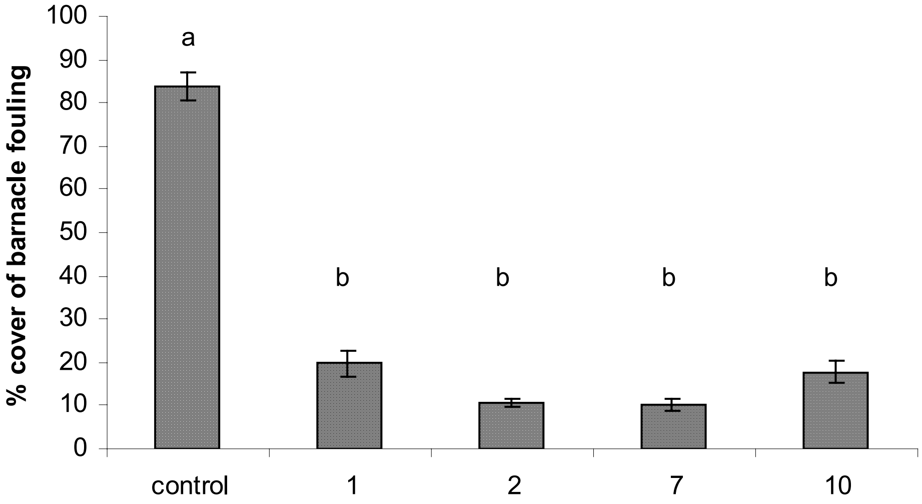
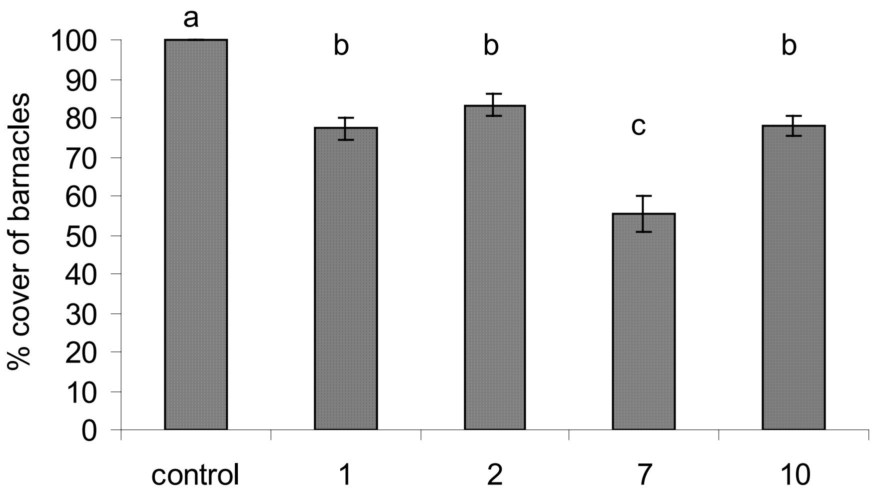


Figure 14.3. Percent cover of barnacles on control and coated shell after 6 weeks at Bynoe Harbour. (Data are mean ± S.E., n=18 except for Coating 2 where n=15, bars sharing the same letter are not significantly different at α=0.05).

Table 14.2 Results of two-way ANOVA (coating X cleaning) comparing barnacle settlement on different coatings and before and after cleaning at Cone Bay (A) and Bynoe Harbour (B) after 12 weeks. Data were sqrt-arcsine transformed prior to analysis.

A. Cone Bay	Sum of Squares	df	Mean Square	F	p
Coating	9.354	8	1.169	9.949	0.000
Cleaning	0.0135	1	0.0135	0.115	0.735
Coat*Clean	0.222	8	0.0278	0.237	0.984
Residual	34.789	296	0.118		
B. Bynoe Harbour					
Coating	7.263	4	1.816	77.475	0.000
Cleaning	1.811	1	1.811	77.275	0.000
Coat*Clean	0.549	4	0.137	5.855	0.000
Residual	3.984	170	0.0234		

A. Before cleaning



B. After cleaning

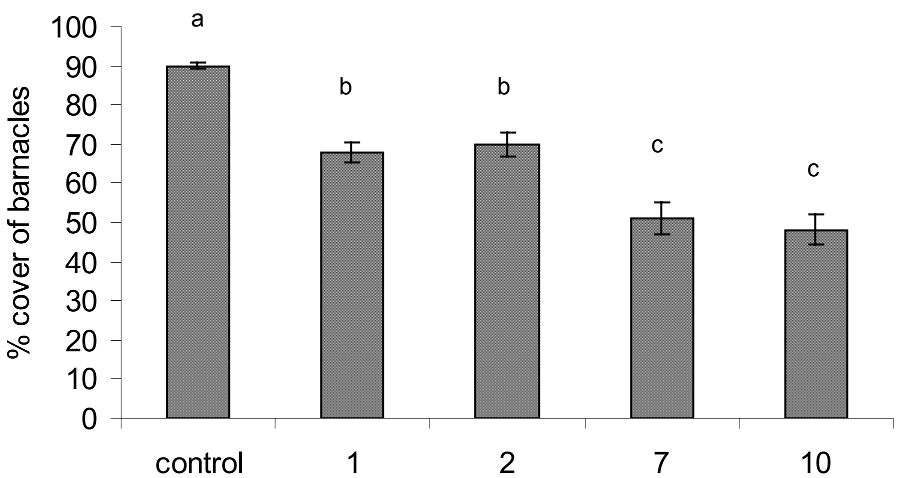


Figure 14.4. Percent cover of hard fouling organisms (barnacles and oyster spat) on control and coated shell, before (A) and after cleaning (B), after 12 weeks at Bynoe Harbour, NT. (Data are mean ± S.E., n=18)

14.4 Summary

- This trial demonstrated coatings that were effective at inhibiting barnacle settlement for 12 weeks at Cone Bay and for 6 weeks at Bynoe Harbour.
- Fouling pressure at the Northern Territory site was greater than at the Western Australian site as reflected by the different efficacies of the coatings.
- The addition of an active ingredient (coating 7) resulted in enhanced antifouling activity.
- The modification of low surface energy parameters did not have a significant impact on antifouling efficacy.
- Although coating 7 had enhanced antifouling efficacy coating 10 had better coating properties as it is a more malleable style of coating. This means that if a barnacle does settle and then grow through the coating, it does not cause secondary cracking throughout the remaining coating. This does occur on all the other coating types including coating 7. Once the coating has been cracked this then allows more barnacles to settle.
- Cleaning with water pressure was effective at removing fouling and coating and manipulation of the water pressure may mean that it is possible to easily wash fouling off while retaining the coating.
- The barnacle, *Balanus cirratus* was the dominant fouling species at both sites.

15. New Generation Coatings

15.1 Introduction

Coatings in previous trials have focused on hot-melt formulations. While these coatings have an immediate drying time they need to be applied to dry shell which can result in increased handling and is impractical for large scale application. Therefore Wattyl, in consultation with industry, undertook to develop coatings that can be applied to wet shell at ambient temperatures and the oysters immersed directly after coating. This trial aimed to test the first versions of these types of coatings on blacklip pearl oysters (*Pinctada margaritifera)*, specifically addressing survival. The trial was commenced in July 2002, at James Cook University's field station at Orpheus Is. (OIRS).

15.2 Materials and Methods

Table 15.1 Treatments tested at OIRS in July 2002.

Treatment	No. of shell
Epoxy SF	6 live shell
	3 half shell
Epoxy AF	6 live shell
	3 half shell
Control	6 live shell

Two coatings, Epoxy SF and AF (containing an active ingredient) were supplied by Wattyl. Coatings were applied to *Pinctada margaritifera*. Before coating all shell were washed with high pressure water to remove soft fouling and any remaining fouling was chipped off. Shell were patted dry using paper towels to remove excess water and then air dried for ~ 10 mins while the coatings were prepared. The shell remained damp. Six live and three half shell were coated for each treatment plus six live controls. Controls were cleaned, dried and handled as for coated oysters.

The coatings comprised of two parts so in each case, all of part A was poured into a bucket and then all of part B was added. The formulation was mixed slowly by hand for ~ 2 mins. The resulting coating was applied to shell using a paint brush within 10 mins of mixing and shell were returned to water immediately after coating. Shell were out of the water for a maximum of 20 mins for the entire process.

Shell were kept in a flowing seawater system overnight before being placed into 15 pocket mesh panels and returned to a longline ~ 30 hours after coating. The longline is in ~6m of water and the panels were suspended to a depth of 3m. Treatments were not randomised due to the small scale of the trial.

Fouling, coating performance and mortality was assessed visually at five weeks. At ten weeks the main fouling organisms, bivalves, were counted for each replicate. The effect of coating on bivalve fouling after ten weeks was compared by one-way ANOVA and Tukey's *post hoc* tests, after log(1+x) transformation of the data.

At 16 weeks all replicates were cleaned with high pressure water, coating integrity was visually assessed and panels returned to the longline.

At 33 weeks, panels containing control and SF and AF coated shell were washed with high pressure water to assess a) whether the predominant fouling organisms, bivalves, could be removed from coated and control shell and b) the coating integrity of SF and AF. Shell were washed using high pressure water for a standardised length of time.

15.3 Results

All oysters survived the coating process and there was 100% survivorship for the duration of the trial. The visual survey at 5 weeks found that all shell coated with AF maintained 100% coating cover and all but one of the replicates for SF maintained 100% cover. Fouling was minimal on both AF and SF with a low level cover of silt while control shell had a medium to high cover of silt and algae. Hard fouling organisms such as barnacles and bivalves were not present after 5 weeks.

By week 10 bivalves were the major fouling organism. The coatings encourage the settlement of bivalves as AF coated shell had an average of 13 bivalves per shell compared with 3 and 1.6 per shell for SF and controls respectively (Figure 15.1). AF coated shell were significantly more fouled by bivalves than coating SF and controls (one-way ANOVA, $F_{2,14}$=47.520, p=0.000, Tukey's test, α=0.05, Figure 15.1). In addition there was a large difference in fouling between live and half shell for AF but not for SF. The average cover of bivalves was 13 on live shell compared with only 6 on dead shell.

When cleaned using high pressure water at 16 weeks both coatings maintained 100% coating cover (Figure 15.2).

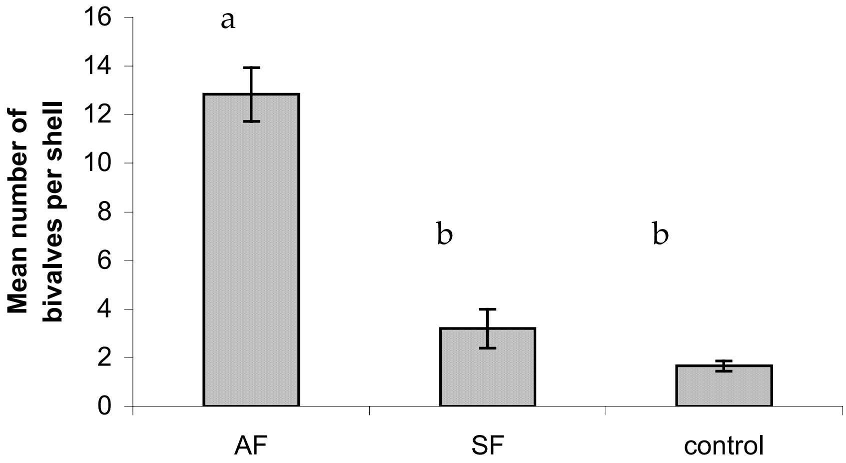


Figure 15.1. Mean number of bivalves on live shell after 10 weeks (Data are mean ± S.E., n=6, bars sharing the same letter are not significantly different at α=0.05).

Figure 15.2. Panel with shell, after washing with high pressure water at 16 weeks. SF coated shell are in the top three rows and controls in the bottom two rows. Bivalve fouling can be seen on coated shell.

After 33 weeks there was 100% survival of coated oysters however there was bivalve fouling across all treatments, including controls, ranged between 4 – 6 oysters per shell (Figure 15.3). Again SF was the best performing coating and AF performed similarly to controls. After high pressure washing, all soft fouling such as ascidians and algae was removed however washing was unable to remove the majority of oysters (Figure 15.3).

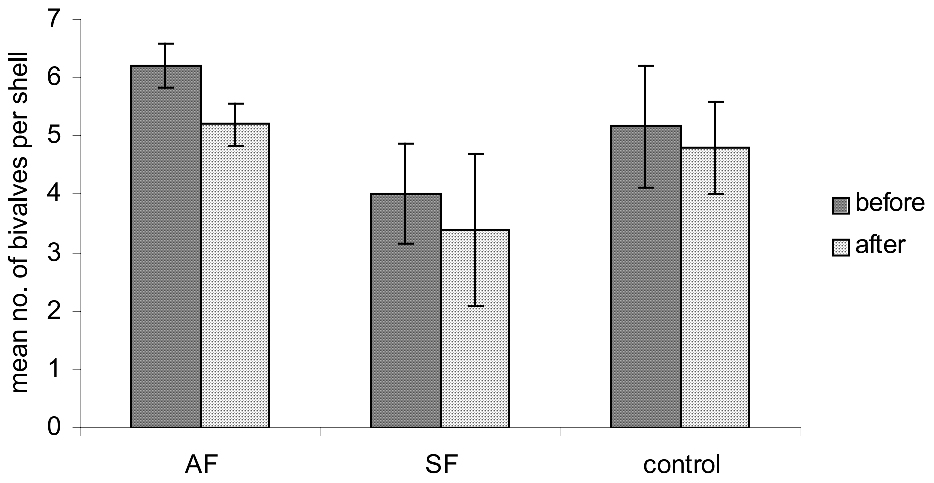


Figure 15.3. Mean number of bivalves before and after washing for control and coated shell after 33 weeks. (Data are mean ± S.E., n=6).

The coating integrity and surface characteristics of coatings AF and SF were excellent after 8 months immersion. There was some minor chipping of coating AF when prolonged high pressure water was applied but this did not occur with coating SF. In addition, when bivalves were removed from coated shell they had not disrupted the integrity of the coating as seen by the scar left by a bivalve in Figure 15.4.

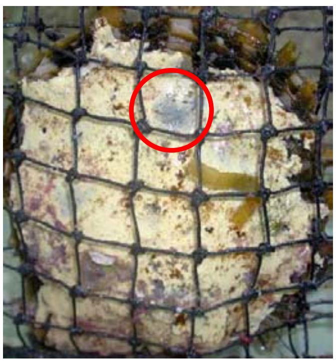


Figure 15.4. SF coated shell after 8 months (a bivalve scar is highlighted by the circle).

15.4 Summary

- The trial at OIRS was important for providing the foundation for the next generation of antifouling coatings that can be applied directly onto damp shell of pearl oysters.
- There was 100% survivorship in both coating treatments.
- The coatings are durable, long lasting and withstand regular cleaning with high pressure water.
- Bivalve fouling was removed easily without disrupting the surface integrity of the coating.
- There was no barnacle fouling during this trial so we are unable to predict accurately efficacy however the trial clearly demonstrates important coating characteristics that have been achieved.

16. Coated panels tested at Ross River, QLD

16.1 Introduction

The trial at OIRS established that there was no mortality associated with the new coating type and coating integrity was excellent. Therefore the aim of this trial was to improve on the antifouling activity of the coating. Further iterations of SF were tested on panels in the Ross River and live oysters were not used. The BX4429 series of coatings contain an active ingredient as well as other constituents which control release rate.

16.2 Materials and Methods

Panels coated in antifouling paint plus control panels were received from Wattyl (n=3) (Table 16.1). The panels were randomly allocated to racks (6 panels per rack) (Figure 16.1) and suspended 1 – 1.5 m below the water line on a floating pontoon in the Ross River in June, 2003.

Table 16.1 Antifouling panels tested in the Ross River in June, 2003.

Treatments	No. of panels
BX4429 – 1	3
BX4429 – 2	3
BX4429 – 3	3
BX4429 – 4	3
BX4429 – 5	3
BX4429 – 6	3
BX4429 – 7	3
BX4429 – 8	3
Control	3

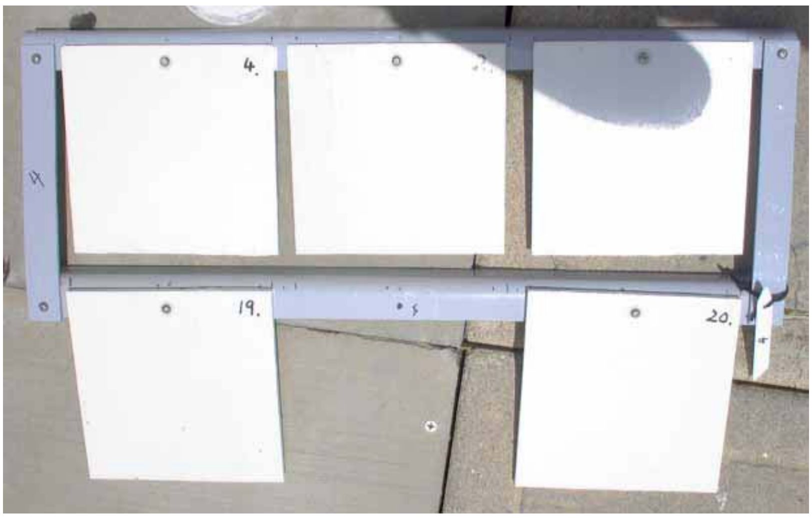


Figure 16.1. PVC rack with test panels, BX4429 series.

Fouling was assessed at 5, 8 and 13 weeks using the point intercept method. A clear sheet with 64 points, 1cm apart, was placed over each panel and bare space or fouling organisms were recorded under each point and then divided by the total number of points to obtain percent cover. The fouling categories were: the bryozoan *Bugula neretina*, tube building worms, encrusting bryozoans, diatoms, ascidians and bivalves. Barnacles were not present at this time.

At 13 weeks all panels were cleaned using high pressure water to determine how easily fouling could be removed and to test coating integrity under conditions similar to pearl farming cleaning practices. Percentage cover was recounted after cleaning.

Week 5 and 8 data were not formally analysed as there were too many zeroes in the data sets. For 13 week data, the percentage of bare space on control and coated panels after cleaning was compared using a one-way ANOVA, followed by tukey's *post hoc* tests. Data was sqrt-arcsin transformed prior to analysis.

16.3 Results

After 5 weeks the main fouling organisms were the arboreal bryozoan, *Bugula neretina* and a tube building polychaete worm. The best performing treatments were BX4429 - 2, 3, 5 and 7 which were completely unfouled (Figure 16.2). The remaining treatments had a 20 – 40% cover of fouling including controls which were 20% fouled, principally by tube worms.

After 8 weeks, controls had a much broader range of fouling organisms including ascidians, encrusting bryozoans and bivalves however the dominant groups were diatoms and tube worms (Figure 16.2, Appendix 4.15). Fouling increased on all other treatments and principally consisted of diatoms and *B. neretina*. Again treatments 2, 3, 5 and 7 were the best performing, maintaining 75 – 80% bare space (Figure 16.2).

By 13 weeks all treatments were fouled between 55 – 95% (Figure 16.2, Appendix 4.16). Treatments 2, 3, 5 and 7 became fouled with diatoms at this time however the proportion of hard fouling was still low (40% ± 4%) compared with controls at 73% ± 4.8% (Figure 16.2).

There was some preferential settlement with tube worms choosing to settle on controls over coated panels and the largest settlement of bivalves was also seen on controls (5% at 13 weeks) (Figure 16.2). The percentage cover of *B. neretina* is likely to be over-estimated using the point intercept method as when the panels are removed from the water for sampling the normally upright animal lays flat against the panel and therefore covers more area.

Cleaning using high pressure water was very effective at removing all types of fouling on the coated panels (16.3, Appendix 4.17). All coatings except for BX4429-8 had significantly more bare space than controls (Figure 16.4, one-way ANOVA, $F_{8,18}$ = 9.487, p=0.000). Coating BX4429-7 and 5 were 100% clean and coatings BX4429 – 2 and 3 had less than 5% (± 0.6%) cover after cleaning.

Cleaning did not remove all fouling on controls with a 38% (± 3.2%) fouling cover remaining (Figure 16.3, Appendix 4.17). The main fouling organisms remaining were tube worms (15.1 ± 9%), encrusting bryozoans (14.6 ± 3.4%) and bivalves (7.3 ± 5.8%).

16.4 Summary

- Four coating treatments (BX4429 – 2, 3, 5 and 7) inhibited fouling to less than 25% compared with controls which were 75% fouled after 8 weeks.
- At 13 weeks the majority of panels had become fouled. On the four best treatments the fouling was generally soft fouling such as diatoms and bryozoans.
- Cleaning with high pressure water significantly reduced the cover of fouling across all treatments (except BX4429-8) compared with controls.
- All coatings maintained excellent coating integrity for the duration of the trial and after cleaning.

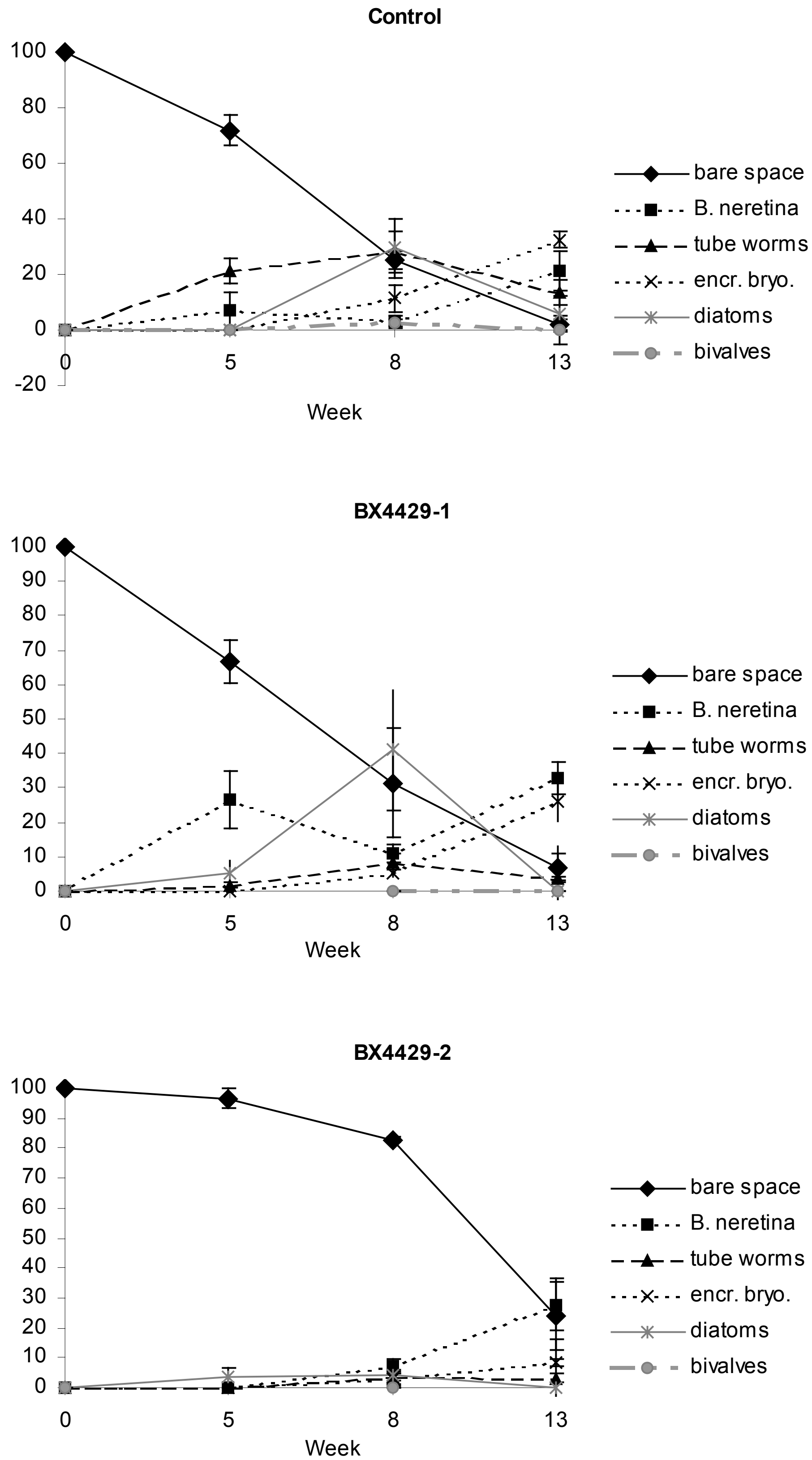


Figure 16.2 Cover of bare space and fouling organisms on control and BX4429 coated panels over 13 weeks. (Data are mean ± S.E., n=3).

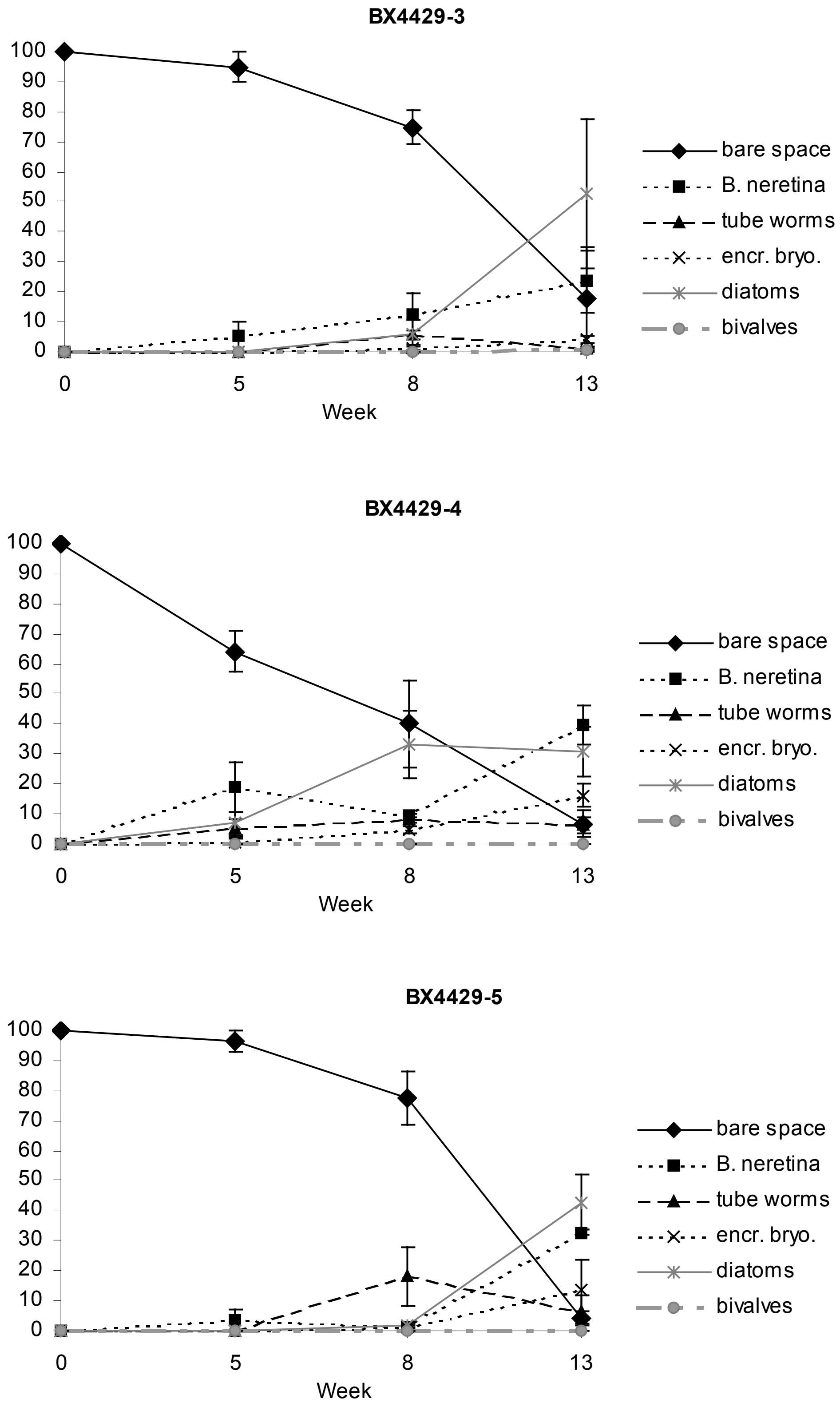


Figure 16.2 (con't) Cover of bare space and fouling organisms on control and BX4429 coated panels over 13 weeks. (Data are mean ± S.E., n=3).

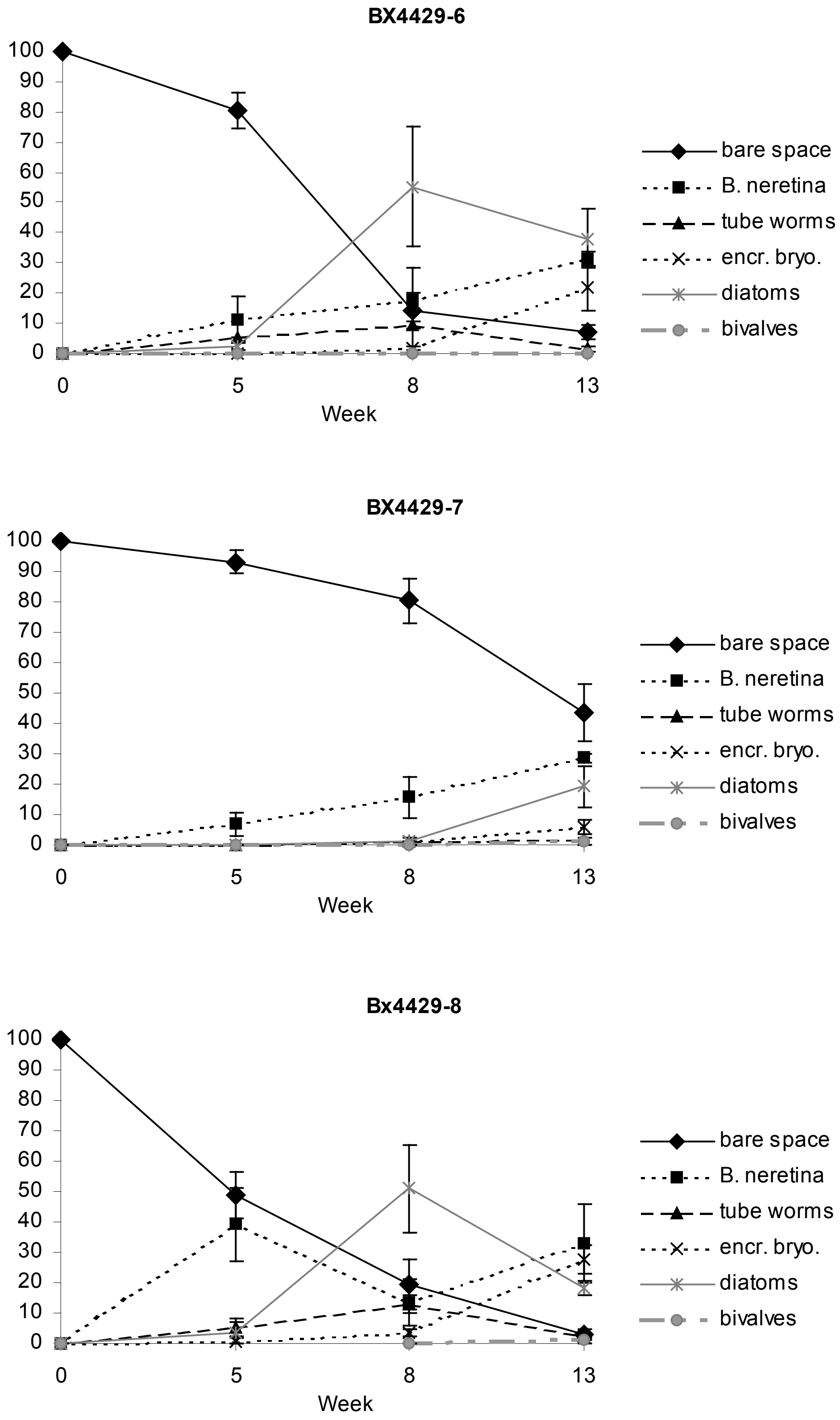


Figure 16.2 (con't) Cover of bare space and fouling organisms on control and BX4429 coated panels over 13 weeks. (Data are mean ± S.E., n=3)

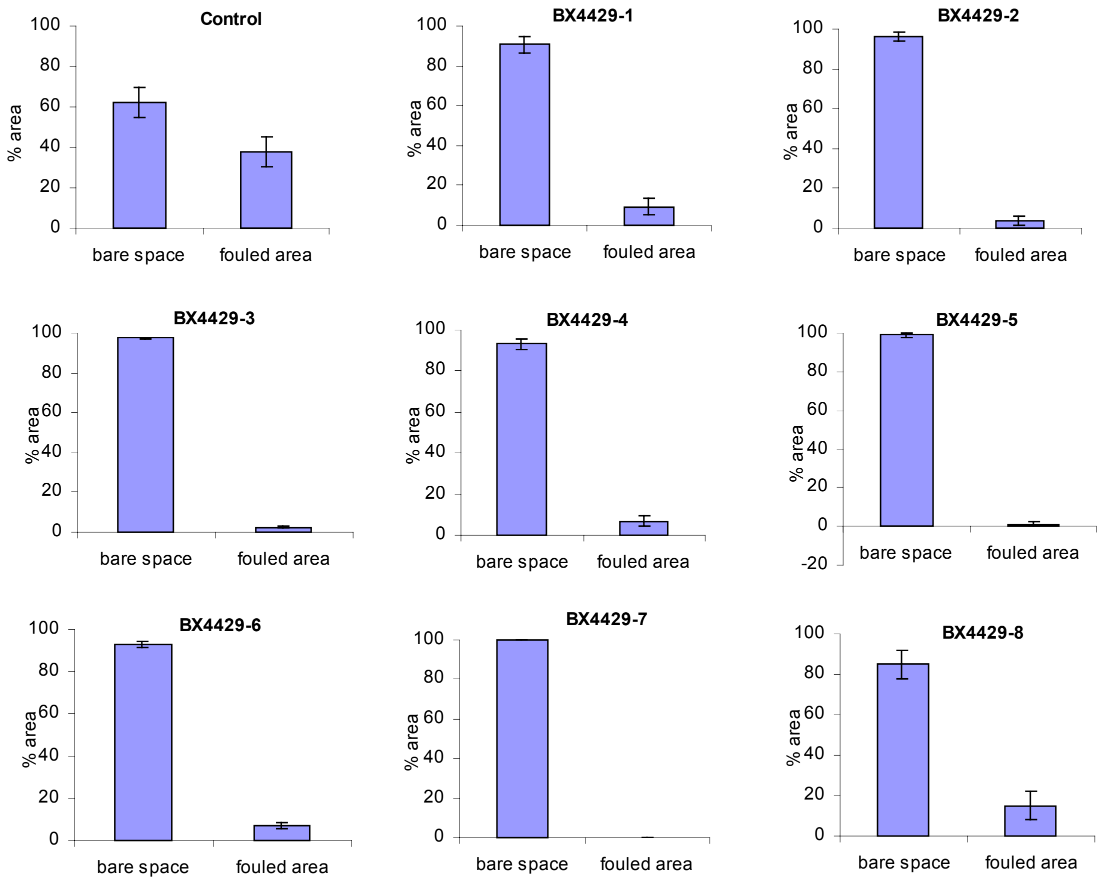


Figure 16.3 Comparison of bare vs fouled area of BX4429 coatings and controls after cleaning with high pressure water.(Data are mean ± S.E., n=3).

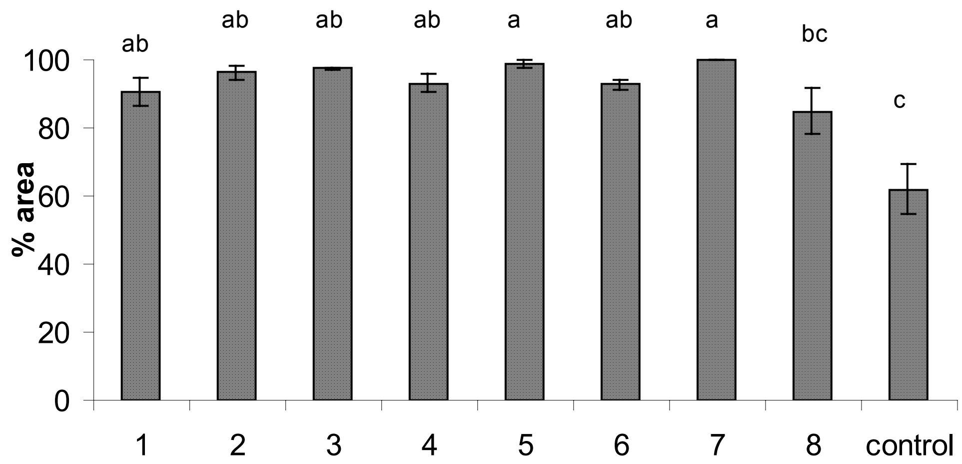


Figure 16.4 Percentage bare space after cleaning using high pressure water. (Data are mean ± S.E., n=3, bar sharing a letter are not significantly different at α =0.05).

17. Project Review

PEARLSAFE

The project saw the successful development of PearlSafe. PearlSafe is a significant and important innovation for the Australian pearl industry. It enables the industry to retain larger shell in which larger nucleii are implanted, rather than culling them. PearlSafe has been released as a commercial product and more than 30 000 shell have been treated. With a value of $350 per shell, approx. $10 million worth of shell have been protected to date (CRC, 2000).

PearlSafe is a coating designed specifically to combat infestations of clionid boring sponges including *Pione* and *Cliona* species which cost the industry millions of dollars each year in discoloured and deformed pearls (Moase *et al*., 1999). Clionid sponges target the hinge area of the oyster where the periostracum has been worn away, and erodes the shell layers. This causes hinge instability, brittleness, deformation of shell growth and blisters under the nacreous layer. The culling of infected shell is normal practise in commercial pearl culture operations and represents a significant financial loss, particularly as it is the older shell which yield the most valuable pearls that are most often heavily infected.

PearlSafe is extremely effective at treating clionid infections with a 90% success rate after the first application on shell with light to medium infections and an 83% success rate for treating heavily infected shell. Heavily infected shell would normally be culled. Furthermore, PearlSafe prevents the reinfestation of the hinge area by sponges and a second application on very heavy infestations is almost guaranteed to eradicate the sponge completely.

Several application methods were tested and optimised. The treatment method is simple and involves immersing the hinge or infected area of the pearl shell into PearlSafe for a few seconds. A short drying time is required and the shell can be placed back into the normal cycle. PearlSafe 'suffocates' the boring sponge and is also completely biodegradable.

PEARLCLEAR

Fouling efficacy

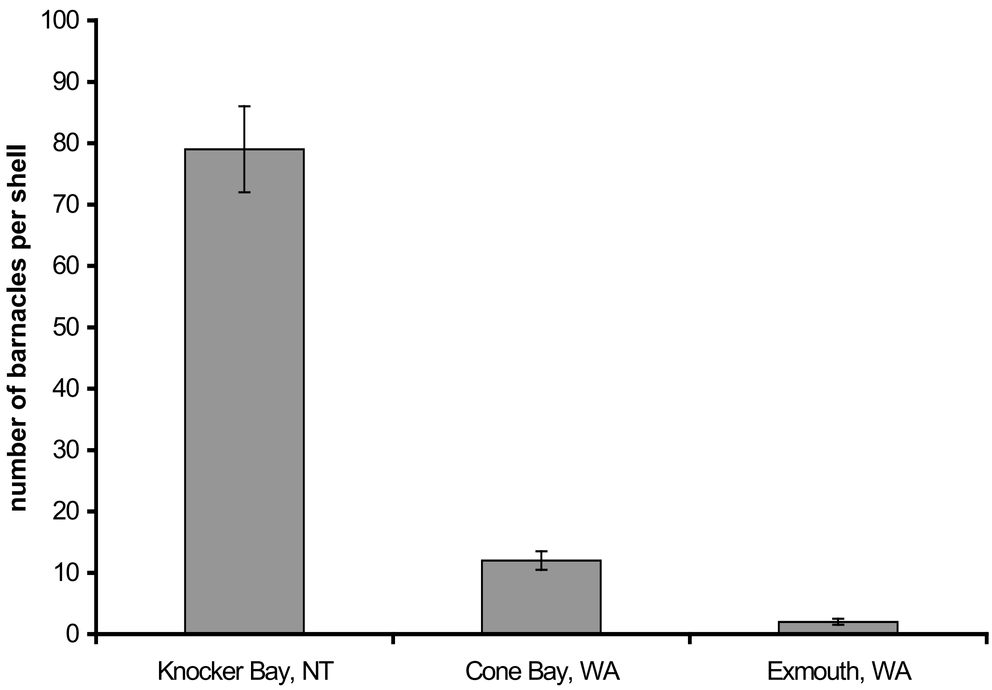
The project was successful in developing coatings (Coating 7 and 10) that significantly deterred barnacle settlement for 6 weeks over the wet season at both Cone Bay and Bynoe Harbour and for 12 weeks at Cone Bay (Section 14). The coatings were also effective at deterring oyster spat and barnacles for six months over the dry season (Section 13).

Over the four years of field and laboratory trials several antifouling technologies were investigated. A rapid application technique was used during the field trials with coatings hot-sprayed onto the shell surface. Laboratory analysis showed that the parameters for hot spraying (eg. film thickness and application temperature) significantly affected coating performance.

Based on surface-texture data, a rapid field trial was then conducted at Knocker Bay N.T., with coatings applied to perspex tiles (Section 10). Data from this trial confirmed that application technique, and the resulting surface texture, significantly affected biofouling development. Laboratory bioassays were then conducted to investigate the relationship between application technique, coating surface topography and barnacle settlement. Modified coating formulations were developed and tested in these bioassays and one type of coating, HB2, was particularly effective (Section 11). Based on this coating Trials 3 (Section 12), 4 (Section 13) and 5 (Section 14) were commenced with each trial testing improved formulations based on results from the previous trials. Testing occurred over both the wet and dry seasons.

Several coatings (B series) were effective at deterring barnacle and oyster spat fouling for six months over the dry season. Importantly, two coatings (Coating 7 and 10) were effective at deterring barnacle fouling for three months at Cone Bay and six weeks at Bynoe Harbour over the wet season.

Testing showed significant differences in fouling between each field site, with rapid and severe fouling at Knocker Bay, rapid but less severe fouling at Cone Bay, and limited fouling at Exmouth. Below is a comparison of barnacle fouling across the three sites after six weeks in Summer 1999.



During the wet season the dominant fouling organism at Bynoe Harbour and Cone Bay was the barnacle, *Balanus cirratus*. This species completely monopolised all available settlement surfaces. In the dry season the occurrence of oyster spat fouling was greater (the species were not identified) as were soft fouling organisms such as colonial ascidians. At the Exmouth site fouling was more varied, and included algae, ascidians, oysters and barnacles across all seasons.

This variation in fouling across the sites was strongly reflected in the coating's antifouling performance over the series of trials. At the Cone Bay site coatings generally performed better with a strong inhibition of fouling across all trials. At Bynoe Harbour and Knocker Bay, N.T., coatings were limited in their success, never completely inhibiting settlement of barnacles. However they did limit fouling to less than 10% cover compared with controls at 80% after six weeks over the wet season (Section 14).

The trials revealed that it is preferable to reduce the amount of time that the oysters needed to be out of water and also that coatings need to contain active ingredients to deter fouling, particularly in regions where fouling pressure is high. A new generation of coatings was developed that could be applied directly onto damp shell and as the coating cures in water, shell can be immersed immediately after coating. These coatings are also durable and abrasion resistant making them ideal for use in pearl aquaculture.

Initially the antifouling efficacy of the new generation coating was limited. At OIRS where oyster spat was the dominant fouling organism, coating AF was significantly more fouled than controls (Section 15).

However, the latest panel trial revealed several coatings, particularly BX4429-7, which had significant antifouling activity (Section 16). BX4429-7 maintained less than 20% fouling cover over 8 weeks compared with controls which had a cover of 75%. In addition, cleaning using high pressure water was very effective at removing all forms of fouling compared with controls. After cleaning coating BX4429-7 was 100% free of fouling.

The majority of the coatings (all those based on the HB2 formulation as well as SF and AF) inhibited or reduced the settlement of 'silt' and in general the coated shell remained very 'clean' compared with controls. Silt, defined by Swain and Schultz (1996) as "absorbed organic and inorganic chemicals, trapped silt and detritus and un-identified slimes" is an important step in the fouling community succession and may harbour or promote other larger fouling organisms or predators (Littlewood and Marsbe, 1990). As these types of coatings inhibit the settlement of silt they may be indirectly protecting the pearl oyster from further fouling and attack by boring organisms. In another study testing the relationship between parasites and fouling, Mouritsen and Bay (2000) found that snails that were infected by a parasitic worm also had significantly higher levels of biofouling, suggesting that the basic defence mechanisms of the snail were weakened making them more susceptible to fouling.

Throughout the trials no signs of disruption or deformation of shell growth ('double-back') was seen in any of the coating treatments and the oysters produced new growth processes similar to controls.

Coating technology

Early trials involved the testing of coating types whose antifouling efficacy was based on deterrence rather than toxicity. Among the significant benefits of these coatings is the move away from the reliance on heavy metals as fouling inhibiters resulting in obvious environmental benefits and moreover making the coatings ideal for application directly onto the shells of commercial bivalve species (de Nys *et al*., 2003).

Modes of fouling inhibition can be categorised into chemical, physical and mechanical. The deterrent activity of the first generation of coatings (PearlClear) is based on a combination of all these. Several of the application techniques tested in these trials, such as 'snowy', simulated surface roughness and were successful at deterring settlement. There is increasing evidence for the effect of physical micro-topography or surface 'roughness' and 'smoothness', on the settlement of organisms. Micro-textured surfaces ranging from 1μm (Scardino *et al*., 2003), to 30-100μm

(Callow *et al*., 2002; Berntsson *et al*., 2000a, b; Andersson *et al*., 1999) and up to mm's (Hills and Thomason, 1998) can significantly deter important biofouling species.

In addition, several of the coatings (for example, B10 in Section 12) were malleable coatings. Therefore if a barnacle did settle and then grow through the coating the malleable formulation prevented secondary cracking though-out the remaining coating.

PearlClear, HB2 and the B series of coatings have also been found to modify the behaviour of barnacle larvae using a combination of mechanical and chemical activities. Afsar *et al*. (2003) found that barnacle larvae exposed to PearlClear and HB2 coatings had modified behaviour compared with control surfaces. The barnacles were unable to explore the surface, which is vital for their settlement behaviour, and consequently turned upside down without settling. The nature of the chemical inhibition is not toxic but rather, makes the surface unattractive for settlement.

The issues of adhesion to the shell surface, abrasion resistance and minimum thickness required for a suitable length of activity need to be determined before successful commercialisation. However, the coatings were effective at deterring settlement for three months at Cone Bay and Exmouth over the wet season.

The new generation coatings tested at OIRS and Ross River have excellent coating characteristics. They are extremely abrasion resistant and have not shown signs of cracking or peeling after 12 months. Further, they can be treated with high pressure water to remove fouling and the coating remains intact. These will be developed further with industry support.

Application techniques
Several application techniques were trialled including dipping the oyster into the coating, spraying and painting onto the surface. For the PearlClear and HB2 series (*ie* A1-A10 and B1-B10) dipping was found to be the best method of application as this produced the best adhesion as well as fewer flaws within the coating. There are several possible application techniques for the new generation coatings, including spraying, but more research is required to optimise this process.

Survivorship
The effect of the coatings on survivorship of the pearl oyster varied across the trials. In the majority of trials none or very low levels of mortality were seen and the coating process was within the tolerance range for these animals. In the trial where high mortality was observed issues related to oyster condition prior to coating as well as handling contributed to the levels of mortality (Section 12).

There is very little information regarding the temperature tolerances of pearl oysters although their physiological activity is regulated by water temperature (Tomaru *et al*., 2001). Wada (1991) suggested that condition of the oyster, oxygen content and salinity were important factors influencing tolerance to high temperatures for *Pinctada fucata*. The upper critical water temperatures for ciliary movement and heartbeat for *P. fucata* have been reported as 35°C for prolonged exposure (Wada, 1991).

In another study using *Pinctada fucata*, Otu and Shimizu (1961) tested the effects of exposure time, up to 60 mins, on oyster survival and pearl quality. They found that mortality only occurred in high numbers (24.4%) when the shell had been exposed for 60 mins under the direct sun when the ambient air temperature was also high. They also found no effect of exposure time on pearl quality. In all the current coating trials exposure times were limited to less than 30 mins and all shell were dried in the shade.

Pearl oysters also appear to be susceptible to higher mortality when handled during the colder months (Pass *et al*., 1987; Yamashita, 1986). Furthermore, the species and size of the bivalve may also influence survivorship after handling (Arakawa, 1990). Pass *et al*. (1987) found that exposure to colder ambient temperatures weakened *Pinctada maxima* making them less resilient to handling practices, more susceptible to disease and subsequently high mortality was seen. Yamashita (1986) also recommended performing major handling operations in the warmer months.

The first generation of coatings were applied *via* dipping and spraying at a range of temperatures from 80-120°C. It is important to note that the maximum temperatures reported were the temperatures inside the reservoir of the spray apparatus and are much less when the coating adheres to the shell. Coatings applied *via* dipping immersed the shell in the coating for less than a few seconds which is within the tolerance range for other bivalve species (de Nys *et al*., 2003; Nel *et al*., 1996; Arakawa, 1990).

In a separate study, the antifouling efficacy PearlClear was tested on the Akoya pearl oyster, *Pinctada fucata*. Umetani (2001) tested the effect of coating temperature at 85°C, 95°C and 105°C on survival and found that the highest mortality was seen in the lowest temperature treatment. This is suggested to be due to the thickness of the coating where there is an inverse relationship between viscosity and temperature. At lower temperatures the oysters were unable to open their valves. Coating at 105°C did not lead to any mortalities and furthermore, encouraged faster byssus production than the other treatments, which is a recognised index of physiological activity (Van Winkle, 1970).

The new generation coatings do not need to be heated and have no effect on survivorship. Coated *Pinctada margaritifera* at OIRS have been monitored for 12 months with no mortality.

Equipment
The first generation of coatings were applied to panel nets to establish if fouling could be prevented on associated farming equipment. These coatings were not effective as they did not adhere to the panels and were abraded off after a few weeks.

Fouling on the panels was extreme after three months during Trial 5 at Cone Bay (Section 14). Large barnacles (2cm diam.) were common and these were not removed using high pressure water. Soft fouling, such as ascidians, was also abundant but this was removed with cleaning. Lodeiros and Himmelman (1996) studied combinations of panel and/ or shell cleaning on growth and survival of the scallop *Euvola ziczac* and found that growth was strongly affected when panels became fouled. They also found that fouling on shell contributed more to mortality than fouling on panels. Scallop culture is generally conducted using pearl nets (also called lantern nets) so the dynamics of water flow, fouling and food availability differ from pearl culture in panel nets. However, juvenile pearl oysters (*Pinctada margaritifera* and *P. fucata*) are often cultured in lantern nets and therefore the impact of panel fouling on growth and survival can be significant (Southgate and Beer, 2000; Wada, 1973).

There is data suggesting that fouling on panels does not have a significant effect on food availability (Ross *et al*., 2002; Lesser *et al*., 1992), however it is generally considered undesirable by pearl growers (M. Doroudi, pers comm.) and furthermore creates drag on the longline and associated farming equipment (Hardy, 1991; Dharmaraj and Chellam, 1983). The new generation coatings have yet to be trialled on equipment.

Given the significant fouling observed on panels, antifouling research solutions for pearl oysters and panels need to be conducted in unison. There has been some early research on antifouling panels for use in aquaculture (His *et al*., 1996) however some of these technologies have relied on the use of heavy metals (Lee, 1992; Huguenin and Huguenin, 1982) and these have an adverse effect on shellfish (Claisse and Alzieu, 1993; Paul and Davies, 1986) and the surrounding environment (Gibson and Wilson, 2003).

In recent years there has been a strong research emphasis on alternatives to heavy metals including inherently antifouled polymers (Anon, 2003) and new antifouling technology for inanimate submerged surfaces (Anon, 2001; Jelvestam *et al*., 2003). Environmentally benign active ingredients which degrade quickly, do not bioaccumulate and can be easily incorporated into a number of delivery systems have been developed (Anon, 2001; Callow, 1999). In addition, research has also focused on deterring the settlement of fouling organisms by manipulating the topography of surfaces (Berntsson *et al*., 2000a, b; Callow *et al*., 2002; Andersson *et al*., 1999).

Integrated farming practices
The application technique for any antifouling coating that is developed will involve some time out of the water for the oyster. The new generation coatings mean that the shell surface does not have to be dry for the coating to adhere, however the coating process will take place out of the water and should not exceed 20 mins. Pearl oysters are taken out of the water regularly during the production cycle for cleaning and operational procedures and are fairly robust (Otu and Shimizu, 1961).

For ideal coating adhesion the shell surface will have to be free of fouling organisms, mud and silt. This is achieved currently using high pressure water abrasion and will be part of the coating process.

Mortality data collected from the trials suggests that there may be optimum times to perform coating operations. Data collected in these trials and reports from the literature (Pass *et al*., 1987; Yamashita, 1986) show that pearl oysters are susceptible to stress when handled during the colder months of the year. While there needs to be more research into the impact of coating on oyster condition it is recommended that coating application be performed in the warmer months. This correlates well with the increase in fouling pressure.

The goal of the coating would be one that is effective for six months. The new growth processes on the lip area will become uncoated but are themselves a passive form of defence against predators (Gervis and Sims, 1992; Crossland, 1911) and have their own antifouling and antiboring cover of periostracum (Scardino *et al*., 2003).

FRDC Project No. 2003/206 is the continuation of this work. Coating development, antifouling efficacy, application techniques, oyster survival and effects on pearl quality will be further investigated.

18. Benefits and Adoption

Direct Benefits and Beneficiaries

The immediate benefits and beneficiaries will be the Western Australian and Northern Territory pearl operations. Other beneficiaries will be the Queensland industry. Use of the product PearlSafe means that growers will benefit by retaining the larger and consequently more valuable shell that otherwise would be culled due to clionid sponge infestation. PearlSafe has been adopted by the Australasian industry with over 5000 litres sold since the project commenced (2000-2004, data provided by Wattyl Pty Ltd).

Growers will benefit from antifouling technology through decreased labour costs associated with removal of fouling and will therefore continue to compete internationally in the production of high quality pearls.

Flow of Benefits

Fisheries managed by	Commercial Sector	Recreational Sector	Traditional Fishing
NSW			
QLD			
SA			
TAS			
VIC			
WA	70%		
NT	30%		
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%		

19. Further Development

This project clearly identified key performance indicators for an effective antifouling coating for the pearling industry. A coating must be:

1. Effective for six months for any period of the year, in particular the wet (summer) season when fouling is at its peak.
2. Be able to applied to damp shell
3. Be able to cure underwater directly following application
4. Be applied in an automated process at on-farm and at sea

The first step in this process was achieved in this project with the development of a water curing epoxy-resin coating that adheres to damp shell, cures underwater and has a coating lifetime of more than 18 months. To further develop this coating a new industry driven project has begun in 2003 (FRDC 2003-206, Antifouling solutions for the Australian pearling industry: coatings for shell and equipment). The aim of this project is to extend the efficacy of the epoxy-based coatings to six months and develop functional and cost effective application methods resulting in the commercial supply of an effective antifouling coating that meets industry criteria.

The project aims to complete this development within three years including the trialing of the product on-farm. These trials will provide a quantitative measure of the impacts of the coating on the environment and on the quality of pearl production. The project will develop a much stronger industry focus and participation based on the identification of this being an area of improvement. The project will be developed and monitoring by an industry focused steering committee that maintains four monthly reviews of the goals and directions of the project.

20. Planned Outcomes

The product PearlSafe addresses the project outcomes of reduced mortality and faster oyster growth by targeting and eradicating boring sponge infestations. These infestations occur on the hinge region of the shell and cause brittleness, disruption of the nacreous layer and potentially the death of the oyster. The use of PearlSafe means that oysters that would otherwise be culled can now be retained in the production cycle.

The outcomes of the project substantially contributed to other CRC and FRDC research with the cross fertilisation of ideas and technology resulting in the development of OysterClear, a product designed to inhibit fouling on Sydney rock and Pacific oysters.

Finally, key performance indicators have been identified for the further development of antifouling coatings.

21. Conclusions

This project has made a very significant contribution to the control of fouling in the pearling industry. It has had a direct commercial outcome in terms the development of PearlSafeTM, an effective control agent of boring sponges in pearl aquaculture. This product is now available and is in use within the industry. The project also developed coatings that deter the settlement and growth of fouling organisms with efficacy varying on a geographic scale. Fouling was prevented for up to six months in WA sites but for only three months in NT. However, during this process it became clear that application methods required significant development to apply any coating on any scale. The subsequent development of water-curing epoxy-based coatings in this project has facilitated the further development of antifouling coatings for pearl oysters and pearling equipment with the aim of developing a coating that is both effective and able to be applied in the field under automated procedures. Furthermore, we have for the first time developed a comprehensive database of the impacts of biofouling on the pearling industry and methods used to control and remove fouling. This is available in the public domain and will facilitate further investigation of the nature and process of fouling in the pearling industry and lead to sustainable technologies to control fouling.

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23. Acknowledgements

The outcomes achieved in this project would not have been possible without the support and contribution of many groups and individuals. Maxima Pearling Company strongly supported the original concept of antifouling coatings applied to pearl oysters and were involved prior to the commencement of FRDC funding, enabling much of the early coating testing to happen onsite at Cone Bay. Thanks to Dave Jackson, Nick Miller, Scott, Alan Wilmot, Patrick Moase, Medhi Doroudi and the staff at Cone Bay for the use of boats and crew and valuable feedback. With the commencement of FRDC funding we acknowledge the support of Paspaley and Kailis who contributed much in the way of test sites and infrastructure including boats and crew as well as valuable feedback about the coatings success and practical application. Thanks to David Mills at Paspaley and the staff at Bynoe Harbour and Knocker Bay. Thanks also to Richard McCulloch and Patrick Moase at Broome Pearls and the staff at Swift Bay and Exmouth Gulf.

We also formally acknowledge the support of:
Wattyl Australia Pty. Ltd.
Pearl Producers Association – Brett McCallum, Mick Buckley
The Aquaculture CRC
The FRDC for support with this project and Project 98/314 with technical support and intellectual cross-fertilisation.
The Centre for Marine Biofouling and Bio-Innovation, University of New South Wales.

24. 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 of existing antifoulants and consistent with this the agreement does not give the FRDC equity in the antifouling products themselves.

The intellectual property related to wax based coatings 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 licensed by Unisearch to Wattyl Australia Pty. Ltd.

The intellectual property relating to epoxy based coatings is covered under the project agreement FRDC 2003/206 Antifouling solutions for the Australian pearling industry: coatings for shell and equipment.

Appendix 2 - Staff

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Andrew Bissett

Appendix 3 – Project Outputs

Products

PearlSafe TM (pg 92-93 brochure)

Industry Presentations

Amwing Pearl Producers Assoc. Industry Workshop, Perth, 30-31 Oct 1999.

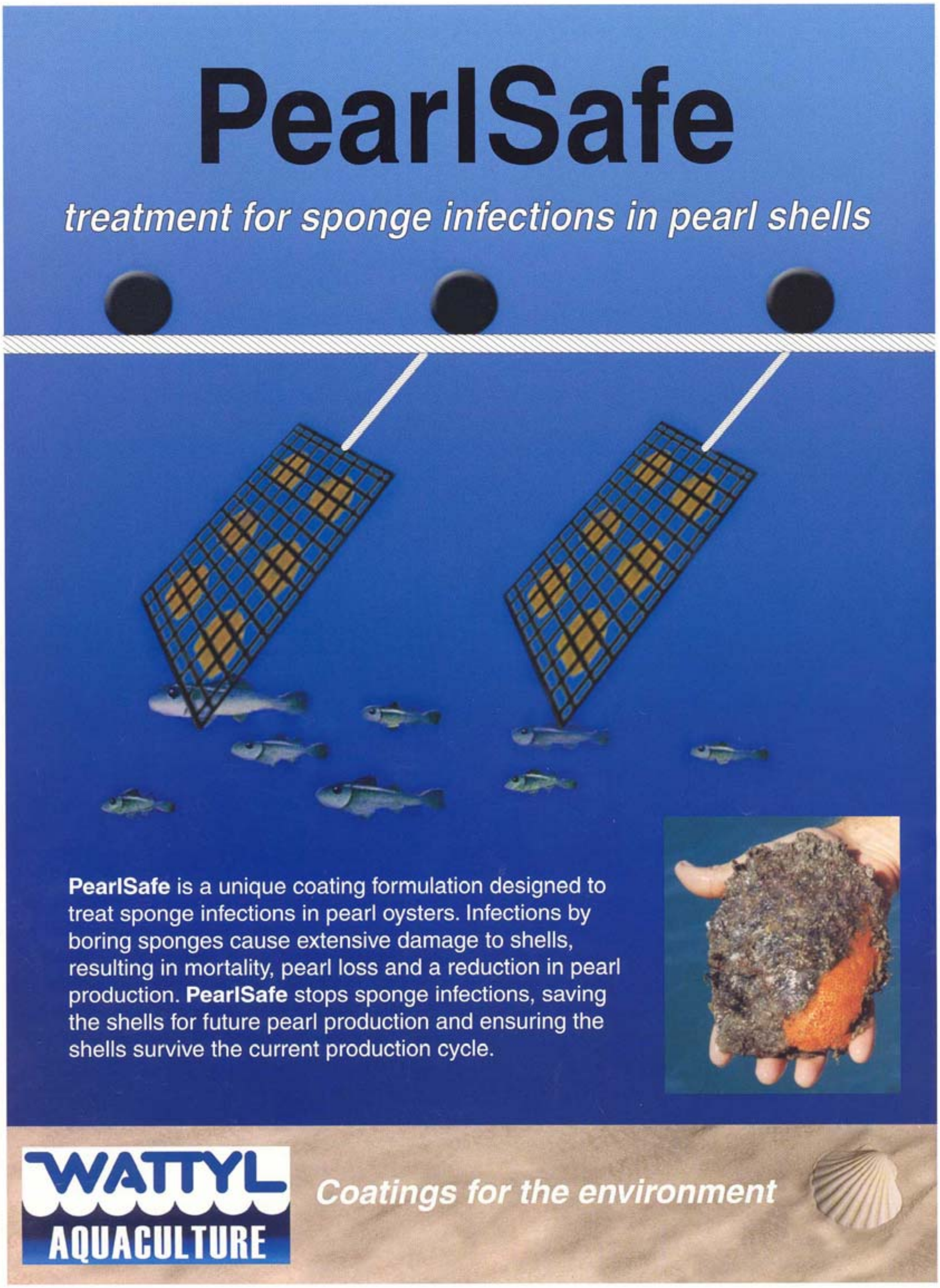
Conference Presentations

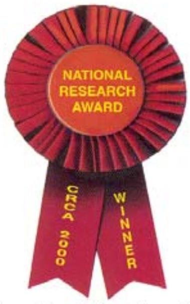
- de Nys, R., Ison, O., McCloy, S., Hodson, S. and Steinberg, P. (1999) Biofouling in Aquaculture – novel methods of control. World Aquaculture '99. 26 April – 2 May 1999.
- de Nys, R., Ison, O., McCloy, S., Hodson, S. and Steinberg, P. (2000) Preventing biofouling in shellfish aquaculture. AquaTas
- de Nys, R., Ison, O., McCloy, S., Hodson, S. and Steinberg, P. (2000) Preventing Biofouling in Aquaculture. Australian Marine Sciences Association Annual Conference. Sydney, July 2000
- 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.

Publications

Ison, O., Southgate, P. C. and de Nys, R. Fouling and fouling prevention in the pearling industry: A review. *Biofouling* (in prep).

de Nys, R and Ison, O. Biofouling and its impacts on the pearling industry. In Pearl Oyster Biology and Culture. Southgate, P.C. and Lucas J. (eds). Elsevier Press (in prep).


PearlSafe
treatment for sponge infections in pearl shells
PearlSafe is a unique coating formulation designed to treat sponge infections in pearl oysters. Infections by boring sponges cause extensive damage to shells, resulting in mortality, pearl loss and a reduction in pearl production. **PearlSafe** stops sponge infections, saving the shells for future pearl production and ensuring the shells survive the current production cycle.
WATTYL
AQUACULTURE
Coatings for the environment



Developed in collaboration with the University of NSW and the Cooperative Reseach Centre for Aquaculture.

Instructions for Use

Remove the oysters from the water and clean off any surface fouling from the areas affected by *Cliona*. (e.g. using hydraulic water jets) Allow the oysters to dry for approximately 15 minutes until no surface water is visible. Each oyster is then to be individually dipped in the solution of PearlSafe as follows.

1. Hold the oyster by the top and dip the shell into the solution of PearlSafe such that the whole of the affected area is covered with solution.

2. Dip for a period of 2 seconds.

3. Remove the oyster from solution and leave to dry (prefably standing the oyster in an upright position) before returning to the water. It is essential that both the oysters are as clean and dry as possible prior to applying PearlSafe and that the PearlSafe is given sufficient time to dry before re-submerging the oyster into the ocean. It is also essential that the duration for which the oyster is out of the water be kept to a minimum.

Note: Times are approximate and based on application to *Pinctada maxima* at 35˚C in greater than 90% humidity.

PACKAGING	COVERAGE	APPLICATION METHOD	CLEAN UP
20 Litre	25 mls per shell (150 mm diameter shells)	Dipping	Methylated Spirits

SHELF LIFE (cool dry place) : 12 months
PACKAGING GROUP: 11
UN NO: 1866
HAZ CHEM CODE: 3[Y]E
SAFETY PRECAUTIONS - provide adequate ventilation
- flammable. Do not use in the presence of naked flames
- refer to material safety data sheet

All Wattyl Aquaculture Coatings are manufactured from high-grade materials to rigid specifications, but since we have no control over the conditions under which these products are transported, stored, handled or used, customers are advised to thoroughly test them before adapting them to their own uses. Customers must read the manufactures standard terms and conditions of sale as all information provided is subject to those conditions as well as the advice given on this sheet and Material Safety Data Sheets (MSDS). Subject to the provisions of the Trade Practices Act, the Company's liability in relation to defective product shall be limited to replacement or repair of the product or the supply of equivalent product