The adhesion of the barnacle *Elminius modestus* (Darwin) to fouling-release coatings.

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Abstract

The main aim of this thesis was to investigate the potential of *Elminius modestus* (= *Austrominius modestus*) for evaluating the performance of fouling-release (FR) coatings. A secondary aim was to explore how the membranous-basis of this species influences the fracture mechanics and release from FR coatings in comparison to *Balanus amphitrite* (= *Amphibalanus amphitrite*), a barnacle with a calcareous-basis and widely adopted as a model for antifouling and FR studies. The critical removal stress (CRS) − the force required to remove fouling organisms, normalised by contact area − is a standard measure to evaluate FR coatings using either barnacles with calcareous-bases or metal studs ('pseudobarnacles'). Testing FR coatings against a diverse range of fouling organisms is necessary to evaluate the global effectiveness of a coating.

The percentage settlement of cyprids, growth rate, and CRS of laboratory-cultured barnacles were evaluated on polydimethylsiloxane (PDMS) standard coatings (Silastic T-2 and Sylgard 184). The percentage settlement on the PDMS coatings between the two species did not significantly differ, however, there were differences in the growth rate and CRS. When grown on Silastic T-2 and Sylgard 184 and fed *Tetraselmis suecica* algae, *E. modestus* grew at a faster rate than that of *B. amphitrite*. There was also a significant coating effect on the growth of *E. modestus* with barnacles on Sylgard 184 growing to larger size than those grown on Silastic T-2. The CRS of *E. modestus* was less than that for *B. amphitrite* but only for the coating Sylgard 184.

Using high-speed photography, the separation processes of *E. modestus* and *B amphitrite*, from Silastic T-2 and Sylgard 184 coatings was observed. Four distinct separation patterns were characterised; lift, peel, adjacent peel and twist. These were based on the location of the initial separation and direction of propagating instabilities in respect to the direction of detachment force. The observed differences in the separation patterns between species may have more to do with the variations in shape and structure of the barnacle shell than to the type of basis. However, the flexibility of the membranous-basis of *E. modestus* was important for the propagation of the fracture as it hindered the formation of fingering instabilities as they progressed through the adhesive interface.

The bulk properties of five polysiloxanes and three fluoropolymers were modified by changing the polymer chain length and cross-linker density, which provided coatings with a modulus ranging from 0.31 to 19.73 MPa. These were used to investigate whether laboratory assays were a good predictor of a coatings performance in the field, in terms of settlement/recruitment and CRS. Two field populations (Fairlie Quay and Burnham-on-Crouch) over two years (2010 and 2011) were compared to a laboratory culture of *E. modestus* barnacles. There were similarities between the laboratory settlement/field recruitment and CRS of *E. modestus* from the two field populations and the laboratory culture across the eight coatings. This made it possible to discriminate between the coatings. Although, the CRS measurements did significantly differ between locations and years, where the general pattern from highest to lowest in terms of CRS between the locations was Fairlie Quay > laboratory > Burnham-on-Crouch.

These eight coatings were also used to investigate the degree in which the elastic modulus of a coating can influence the CRS of *E. modestus*, compared to the CRS of *B. amphitrite*. The regression analysis confirmed that as the modulus increases the CRS for both species increases. There were marked differences in the removal of barnacles from the high modulus fluoropolymers. *B. amphitrite*, unlike *E. modestus*, failed to detach and left the basis on the coating's surface. As *E. modestus* can differentiate between the coatings in terms of FR efficacy and was amenable to laboratory culture with a comparable growth rate to *B. amphitrite*, this species is recommended as an additional model for FR studies.

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Abbreviations

FR	**Fouling-release**
CRS	**Critical removal stress**
PDMS	**Polydimethylsiloxane**
TBT	**Tributyl tin**
IMO	**International Maritime Organisation**
NIS	**Non-indigenous species**
GHG	**Greenhouse Gas**
SPC	**Self-polishing Co-polymer**
CDP	**Control depletion Paint**
γ_{sw}	**Interfacial energy of water and substrate**
γ_{s}	**Energy from the substrate**
γ_{w}	**Energy from water**
Φ	**Interaction constant**
Wa	**Work of adhesion**
G	**Griffith fracture energy**
P	**Critical pull-off force**
t	**Thickness**
K	**Bulk modulus**
E	**Elastic modulus**
a	**Distance (Griffiths fracture)**
a	**Contact radius (Kendall's model)**
$2A$	**Area**
dW	**Work done**
dWe	**Stored elastic energy**
PC	**Probe contact**
CS	**Complete separation**
RO	**Reverse osmosis water**
ASW	**Artificial seawater**
FSW	**Filtered seawater**
SD	**Standard deviation**
SE	**Standard Error**
AFM	**Atomic Force Microscopy**

Chapter 1. Development of a Test Species for Fouling-Release Research: An Introduction.

1.1. Introduction

Biofouling is a term used to describe the collective community of molecular, micro- and macrofouling organisms on surfaces immersed in water in the natural environment (Wahl 1989; Callow & Callow 2002). This description relates principally to the accumulation of organisms on surfaces in fresh and marine waters. However, biofouling, and the accumulation of a biofilm (an assemblage of molecular and micro organisms on a surface {Callow & Callow 2002}), is also prevalent on surfaces in living tissues, tooth surfaces, medical devices and implants (Chen et al. 2013).

The accumulation of a biofouling community particularly concerning that on artificial surfaces in the marine environment, for example ship's hulls and propellers, aquaculture systems, coastal power stations, marine sensors, or oil platforms, are regarded as being detrimental to the purpose and performance of such structures. Potent biocides such as tributyl-tin (TBT) were efficient in ameliorating the effects of fouling on ship's hulls; however, small concentrations (see page 15) of TBT were discovered to be lethal to non-target organisms in the marine environment. Environmental regulations banning the use of such toxic paints were introduced spurring investigations into environmentally benign alternatives, such as silicone fouling-release (FR) systems (Afsar et al. 2003; Sun et al. 2004; Wiegemann & Watermann 2004). The success of silicone coatings is assumed to depend on their physical surface properties, including but not limited to, surface energy, elastic modulus and thickness (Brady & Singer 2000; Anderson et al. 2003; Wendt et al. 2006). Measuring the adhesive strength of fouling organisms to silicone coatings is an effective method for evaluating the capability of the FR coating (Swain & Schultz 1996). Barnacles are said to be the most important species with regard to fouling on ship's hulls (Christie & Dalley 1987; Aldred & Clare 2008; Briand 2009) with their presence being reported on up to 84% (Christie & Dalley 1987) and 87% (Woods Hole

Oceanographic Institute 1952) of fouled vessels. Thus barnacles are the main focus of many fouling studies. At present, the majority of research in this area has centred on calcareous-based barnacles such as *Balanus amphitrite* (= *Amphibalanus amphitrite*) (Clare & Høeg 2008). The issue being that there is a variety of fouling organisms with approximately 4000 species identified (Yebra et al. 2004; Holm et al. 2006) each possessing a unique adhesive mechanism. Testing FR coatings with a diversity of sessile organisms was deemed necessary to comprehend the global effectiveness of the coating (Holm et al. 2006). The aim of this thesis was to explore the suitability of the membranous-based barnacle, *Elminius modestus* (= *Austrominius modestus*) (Buckeridge 1982), for screening of FR coatings. This included a comparison between laboratory assays and field immersion tests, particularly focusing on the difference in the critical removal stress of barnacles from both environments. In addition, it examined how the nature of the membranous-basis influenced the fracture and detachment processes of this barnacle from silicone coatings compared to the fracture process in the calcareous-based *B. amphitrite*.

1.2. Biofouling

The process in which a substrate in the marine environment is colonised is complex and dynamic (Wahl 1989). The colonisation of a new substrate (primary succession) starts the moment it is exposed or submerged in natural seawater. New substrates can occur naturally for example when rocks in the intertidal and subtidal environments fracture or after a volcanic event which creates lava flows; new surfaces can also be created through artificial means by the submersion of structures such as oil rigs, aquaculture nets or ship's hulls (Jenkins & Martins 2010). When any new substrate (natural or artificial) is immersed it begins to absorb organic compounds from the water, these consist mostly of macromolecules including glycoproteins and polysaccharides (Wahl 1989; Abarzua & Jakubowski 1995). The accumulation of absorbed compounds or 'conditioning film' is rapid and is said to occur within seconds or minutes after immersion (Clare et al. 1992; Callow & Callow 2002). Next, the surface is colonised by bacteria, cyanobacteria and unicellular algae such as diatoms, a process that is alleged to take a matter of hours following immersion (Wahl 1989; Abarzua & Jakubowski 1995; Callow & Callow 2002; Dobretsov et al. 2006). The

microbial organisms which become established on a surface form a biofilm and are often referred to as microfouling (Callow & Callow 2002; Anderson et al. 2003). This bacterial colonisation can be broken down into two phases; the first a reversible attachment phase normally termed 'absorption' and the second a non-reversible attachment phase called 'adhesion' (Wahl 1989; Kerr & Cowling 2003). The process which controls the development of the conditioning film and the initial steps of bacterial colonisation include physical forces such as Brownian motion, Van-der-Waals forces, gravity and electrostatic interactions (Wahl 1989; Clare et al. 1992; Abarzua & Jakubowski 1995). The adhesion phase involves the production of extracellular polymeric substances (EPS) by the bacteria and unicellular algae, which enables them to adhere to the substrate. Wahl (1989) provides a comprehensive explanation for both of these processes.

Eventually a macrofouling community will become established on the surface. This can consist of algae and such animals as anemones, bryozoans, hydroids, mussels, tubeworms and barnacles (Callow & Callow 2002; Anderson et al. 2003). Formerly, macrofoulers were considered to take several days to weeks to become established on a new surface (Wahl 1989). However, it is now understood that some groups of macrofoulers are capable of attaching permanently to a surface within hours, supporting the concept that fouling is not a strict sequential or successional process (Roberts et al. 1991; Clare et al. 1992).

The composition of a fouling community on any given surface depends on multiple factors including the type of substrate, its colour and topography, local hydrodynamics i.e. water flow and turbulence, the temperature and salinity; these are often dictated by the geographical location and season (Thomason et al. 1998; 2002a; Callow & Callow 2002; Yebra et al. 2004). There are biological factors which can influence the fouling composition such as propagule or larval supply and availability, the growth and longevity of the organisms, as well as competition and predation (Dayton 1971; Breitburg 1985; Callow & Callow 2002). Yet the composition of a fouling community is subject to change following physical (for example wave exposure) or biological disturbances (for example predation) removing settled organisms, creating new spaces and allowing for secondary succession of other organisms (Dayton 1971).

Fouling on artificial surfaces is a great concern (Armstrong et al. 2000). One of the more high profile sectors particularly troubled with fouling is the shipping industry. The collection of fouling organisms on ship's hulls and propellers increases the roughness of the surface, resulting in high frictional resistance and drag. The frictional drag created by fouling along the hull increases with the severity of the fouling, and reductions in speed up to 86% have been reported for ships with calcareous macrofouling assemblages (Schultz 2007). Along with the increase in drag, the added weight of the fouling can contribute to the reduction of the speed and manoeuvrability of the vessel. To compensate for the reduction in the power of the vessel more fuel is consumed. The fuel consumption for a US naval mid-sized ship with a heavy coverage of biofilm (slime) can increase by 10.3%, whereas a ship with coverage of calcareous fouling and weeds can increase fuel consumption up to 20.4% (Schultz et al. 2011). Considering a heavily fouled vessel the predicted cost of this increase in fuel consumption to the US naval fleet can be up to $400M - $540M annually (Schultz et al. 2011).

A consequence of this increase in fuel consumption is an increase in greenhouse gas (GHG) emissions (International Maritime Organisation 2010). Until recently, international shipping was not included in a Nations CO_2 emissions budget due to the complex global activities of the shipping industry. However the International Maritime Organisation's (IMO) Marine Environment Protection Committee (MEPC) have introduced new regulations to aid the reduction of GHG emission from international shipping. These regulations added to Annex VI of the International Convention for the Prevention of Pollution from Ships (MARPOL) introduces a mandatory Energy Efficiency Design Index (EEDI) for new ships and a Ship Energy Efficiency Management Plan (SEEMP) for all ships (International Maritime Organisation 2012). The EEDI involves technical development to improve the performance and therefore improve the fuel efficiency of new ships, whereas the SEEMP involves operational developments to improve the efficiency of all vessels (International Maritime Organisation 2012). These regulations were set to come into force from 1st January 2013 for all vessels over 400 gross tonnage (International Maritime Organisation 2012).

Vessels which accumulate fouling require frequent dry-docking, where the hull is mechanically cleaned, repaired and repainted (Brady & Singer 2000). These processes are costly and generate large volumes of waste (Yebra et al. 2004). An

additional area of concern is that of biosecurity and involves the management and control of species invasions (Davidson et al. 2016). International shipping has been credited as being the most common pathway for the introduction of marine invasive or non-indigenous species (NIS) specifically via the transportation on ship's hulls and in the ballast water, with the former being the more significant contributor (Reise et al. 1999; Gollasch 2002; Minchin & Gollasch 2003; Molnar et al. 2008). Marine invasive species have caused ecological and economical damages through competition with native and commercial species, introduction of parasites and diseases, and fundamentally altering the food webs and community structure (Ruiz et al. 1997; Gollasch 2002; Minichin & Gollasch 2003; Molnar et al. 2008; Piola et al. 2009). For example the introduction of the Asian bivalve *Potamocorbula amurensis* to San Francisco Bay (Carlton et al. 1990) or the Eurasian zebra mussel *Dreissena polymorpha* in the Great Lakes (Ricciardi et al. 1997) where both species have altered the community structure through competition with other suspension feeding and deposit feeding fauna. The rates of invasions are growing, not only through increased reporting and awareness but also through an increase in shipping traffic (Figure 1.1) (Ruiz et al. 1997; Minchin & Gollasch 2003; Piola et al. 2009). Ports and harbours where shipping frequency is at its highest have the greatest occurrence and abundance of invasive species (Piola et al. 2009). For example 116 species have been introduced in Chesapeake Bay, 137 in the Great Lakes and 253 in San Francisco Bay (as reviewed in Ruiz et al. 1997; Reise et al. 1999; Gollasch 2002). By comparison the North Sea has a much lower number of NIS with approximately 80 reported invasive species introduced to the area by means of shipping (Reise et al. 1999; Gollasch 2002). One example of a species that has been introduced into the North Sea is focus of this study, the barnacle *Elminius modestus.*

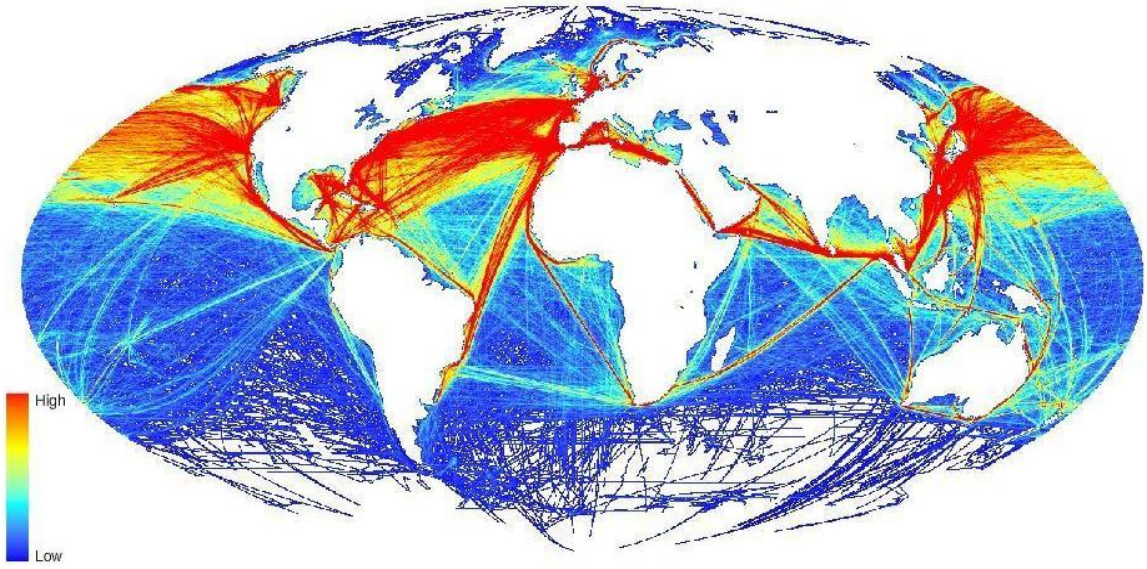

Figure 1.1. Image of global shipping routes. Data collected for 12 months from October 2004 of 3374 commercial and research vessels, representing 11% of the merchant ships over 1000 tonnes at sea in 2005. *Source:* Natural Centre of Ecological Analysis and Synthesis, http://www.nceas.ucsb.edu/globalmarine/impacts.

1.3. *Elminius modestus:* An introduction

Elminius modestus (Darwin) (= *Austrominius modestus*; Buckeridge 1982; see Table 1.1) is an acorn barnacle (Figure 1.2A). The distinguishing characteristics of the species include four whitish-grey calcified wall plates that form a low conical shell with an average size of 5mm diameter; they possess a wide diamond-shaped operculum and a membranous-basal plate (Figure 1.2B) (Moore 1944).

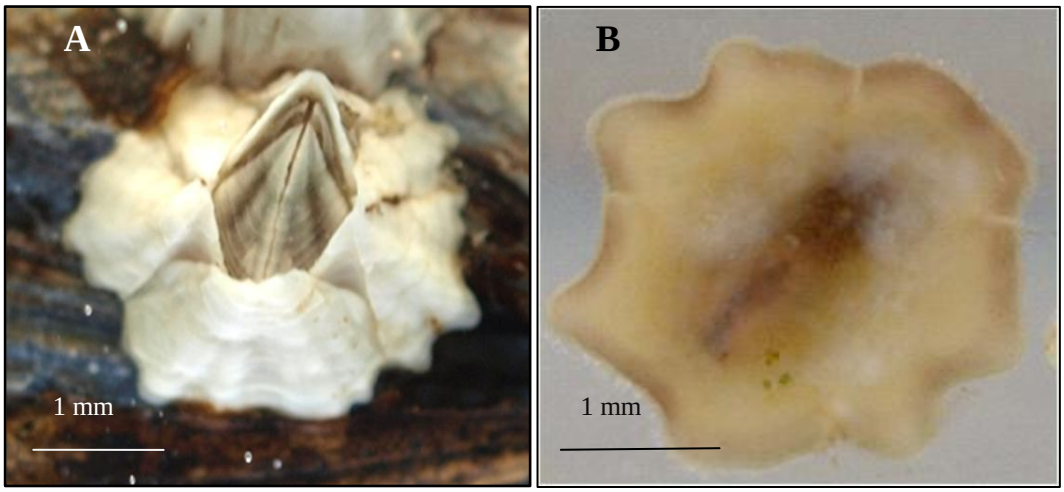

Figure 1.2. A picture of the barnacle *Elminius modestus* (A) on the shell of *Mytilus edulis* and an image of the membranous-basis settled on Silastic T-2 (B). Images taken by author.

Table 1.1. Systematic classification of *Elminius modestus* including the revised classification (highlighted section) of the species.

	Classification (Southward 2008)	Revision of classification (in Buckeridge & Newman 2010)
Phylum:	**Arthropoda**	**Arthropoda**
Class	**Maxillopoda**	**Maxillopoda**
Subclass:	**Cirripedia**	**Cirripedia**
Superorder:	**Thoracica**	**Thoracica**
Order:	**Sessilia**	**Sessilia**
Suborder:	**Balanomorpha**	**Balanomorpha**
Superfamily:	**Balanoidea**	**Tetraclitoidea**
Family:	**Archaeobalanidae**	**Austrobalanidae**
Subfamily:	**Elminiinae**	**Elminiinae**
Genus:	***Elminius***	***Austrominius***
Species:	***modestus***	***modestus***

The distribution of this barnacle species prior to the 1940s was confined to the southern temperate seas, specifically southern Australia and New Zealand (Darwin 1854; Moore 1944). Then in 1944 Bishop (1947) first recorded their presence along the British coastline in Chichester Harbour. Subsequent populations were later discovered in the rivers Crouch, Colne, Roach and Blackwater during 1946, then along the shoreline in Lowestoft, Dorset and Poole in 1947 (Crisp & Chipperfield 1948; Knight-Jones 1948). Crisp (1958) provided a detailed account on the spread of *E.*

modestus throughout the UK and mainland Europe. Further documented accounts on the distribution of *E. modestus* include north-west and western coast of France (Barnes & Barnes 1963; 1969), northern Spain, north and south coasts of Portugal (Barnes & Barnes 1963; O'Riordan & Ramsay 1999; 2013), Shetlands (Hiscock et al. 1978), Helgoland in Germany (Harms & Anger 1989), Lough Hyne located on the southern coast of Ireland (Lawson et al. 2004), the Gulf of Venice (Casellato et al. 2007) and the Isle of Sylt in Denmark (Witte et al. 2010). There are also brief accounts of *E. modestus* in other locations including the Azores and South Africa (Sandison 1950; Crisp 1958; Newman & Ross 1976; Buckeridge 1982). In addition, there is a personal observation of *E. modestus* on the rocky outcrops on Whitley Bay beach, North-East England in 2013, which previously has no documented accounts of *E. modestus* being present.

The distribution of *E. modestus* is predominantly restricted to north-west European and Australian and New Zealand waters (Figure 1.3 and Figure 1.4). However, *E. modestus* has been reported on the hulls of vessels docked in Japan, although, no population has yet to be established on Japanese shores, *E. modestus* is considered to be a high risk for future invasions (Otani et al. 2007). *E. modestus* have also been reported as being a high risk of invasion to the Atlantic coast of North America (Carlton et al. 2011).

The introduction of *E. modestus* to UK and European coastlines has been attributed to remote dispersal as fouling on the hulls of ships (Bishop 1947; 1951; Knight-Jones 1948; Stubbings 1950; Crisp 1958; Hiscock et al. 1978). Samples of *E. modestus* were collected from the hulls of ships docked in Portsmouth during 1944 (Stubbings 1950) and from the hulls of the S.S. *Queen Elizabeth* (August, 1946) and M.V. *Empire Wansbeck* (October, 1946) while stationed at Southampton and Harwich, respectively (Bishop 1947). Once established in these locations, *E. modestus* is thought to have spread along the shore by marginal dispersal at a rate of approximately 20 – 30km/yr (Crisp 1958).

Figure 1.3. Global distribution of *Elminius modestus*, using the accounts from Moore 1944; Sandison 1950; Crisp 1958; Barnes & Barnes 1963; 1969; Newman & Ross 1978; Hiscock et al. 1978; Foster 1982; Harms & Anger 1989; Lawson et al. 2004; Casellato et al. 2007; Witte et al. 2010.

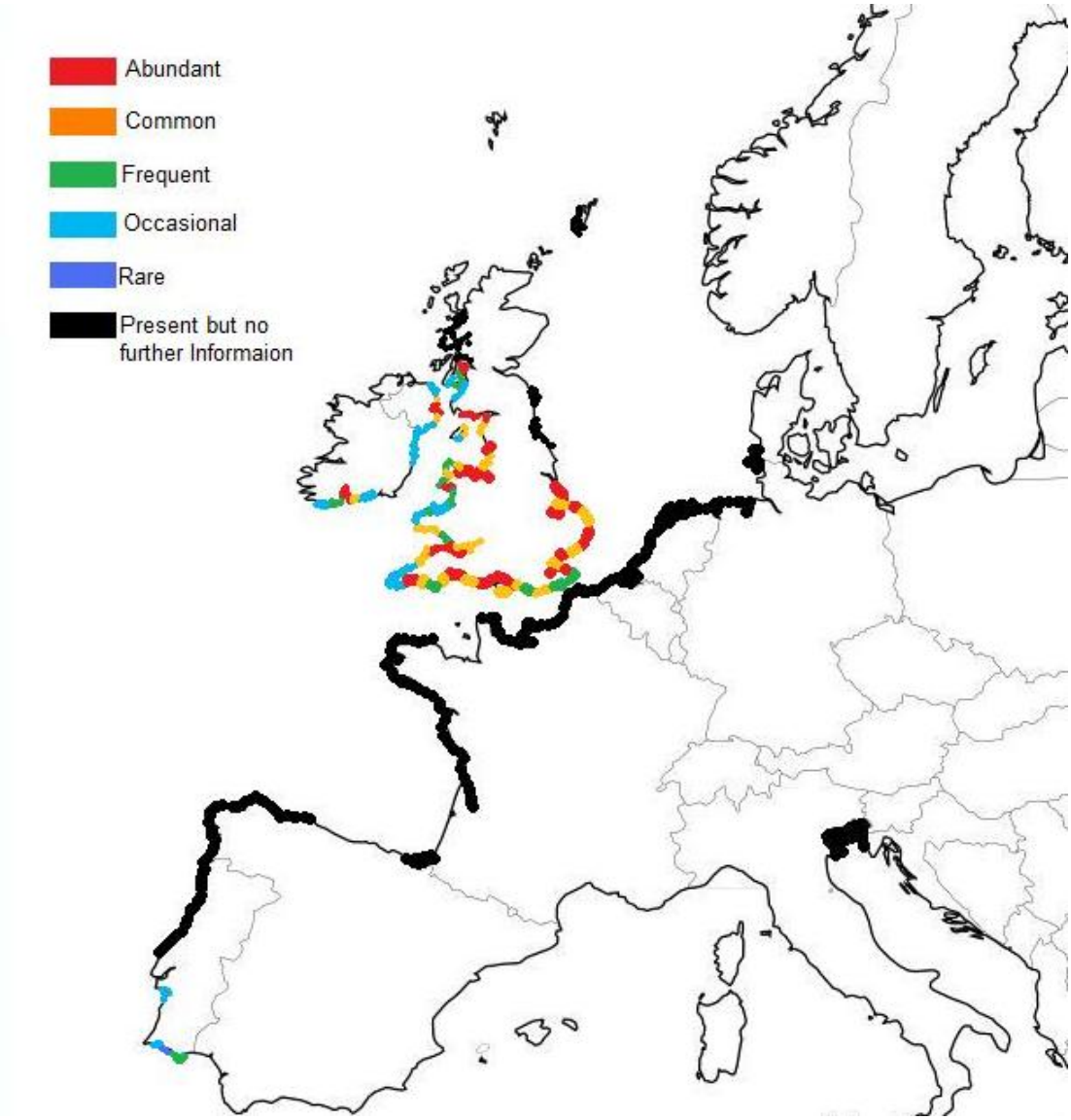

Figure 1.4. Spread of *Elminius modestus* around Europe using the accounts of Crisp 1958; Barnes & Barnes 1963; 1969; Hiscock et al. 1978; Harms & Anger 1989; Lawson et al. 2004; Casellato et al. 2007; Witte et al. 2010; O'Riordan & Ramsay 2013. Abundant: adult density at ≥ 100dm^{-1}, Common: density 10 – 100dm^{-1}, Frequent: density 1 – 10dm^{-1}, Occasional: density 0.01 – 1dm^{-1}, Rare: density below 0.01dm^{-1}, Present: *Elminius modestus* have been reported but no data on the abundance is available.

E. modestus is an intertidal barnacle more common on the mid to low shore (Rainbow 1984). Their position on the shore has been described by Moore (1944) as 'versatile' as they can occupy a wide range of tidal heights extending to the high water mark and the sub-littoral to a depth of '5 fathoms' (9.1m) (Crisp & Chipperfield 1948; Knight-Jones 1948). However, Flowerdew (1984) deemed that the occurrence of *E.*

modestus at these depths was erroneous, claiming that confusion between two *Elminius* species; *E. modestus* and *E. covertus.* These two barnacles occur alongside each other, and may explain the apparent large intertidal range, yet Flowerdew (1984) did not offer further clarification as to whether *E. modestus* occupies the lower or upper tidal range.

E. modestus are able to tolerate fluctuations in salinity and temperature by closure of the opercular valves at particular external stimulus and are dominant in euryhaline environments such as estuaries and harbours (Moore 1944; Flowerdew 1984; Rainbow 1984). *E. modestus* are able to reproduce continuously throughout the year; provided with optimal conditions they can produce broods of nauplii every ten days with the reproductive activity being only slightly reduced during the colder winter months (Crisp & Davies 1955). After settlement, *E. modestus* can reach maturity and reproduce within eight weeks, at an approximate size of 4 – 6mm in diameter (Crisp & Davies 1955; Hui & Moyse 1982). With fast growth rates and short generation times there is a continuous supply of larvae, and with gregarious settlement, this barnacle is capable of forming dense aggregations; for example Crisp & Davies (1955) recorded the settlement of 50 – 100 spat cm^{-2} during a week's exposure in June and July along the river Crouch. This dense coverage of *E. modestus* has been claimed to have a detrimental impact on native species, primarily other barnacle species, for example, competing for space and resources with *S. balanoides* higher along the shoreline and then with *B. improvisus* below the tidal line (Knight-Jones 1948), this can result in changes in the ecosystem structure and its functioning (Bracewell et al. 2012). In contrast, a recent study has demonstrated that *E. modestus* are able to co-exist with native species (Gallagher et al. 2016). Gallagher et al. (2016) demonstrated that despite the relatively high abundances and dominance of *E. modestus* along the test sites in south-west Ireland, this invasive species did not entirely displace or outcompete the native species (*S. balanoides* and *Chthamalus montagui*). The co-existence between the native and invasive barnacle species could be possible through niche partitioning or adaptation of either species to fill an alternative niche (Gallagher et al. 2016).

Despite the potential co-existence of *E. modestus* to native species, where *E. modestus* is present, it often dominants new exposed surfaces, which had either been created through disturbance and removal of previously settled organisms (Gallagher et al. 2015; 2016) or on new artificial structures (Bracewell et al. 2012; 2013). Artificial structures are able to provide a novel environment for opportunistic invasive species

and potentially contribute to further dispersal by providing a 'stepping stone' across unsuitable substrates (Bracewell et al. 2012; 2013). With the proliferation of artificial structures through increasing urbanisation, coastal defences and renewable energy initiatives, Bracewell et al. (2013) predicts an increase in the abundance and distribution of *E. modestus*.

1.4. Antifouling

The problems associated with fouling on shipping vessels is said to have been recognised for over 2000 years (Yebra et al. 2004). One of the earliest examples for preventing fouling (referred to as antifouling) comes from a piece of well preserved, lead-sheathed timber from a wrecked Phoenician galley dating back to 700 B.C. (Lunn 1974). Tar, pitch, tallow and arsenic are just some of the concoctions (Table 1.2) that were said to have been used by ancient civilisations (Woods Hole Oceanographic Institution 1952; Yebra et al. 2004). Yet it was not until the use of copper sheathing, introduced in the 16th century, that a successful antifouling method existed or was recorded. Yebra et al. (2004) acknowledged that the first authenticated use of copper sheathing was on the frigate HMS Alarm in 1761; however, Lunn (1974) quotes 1758 as the year this occurred. The success of copper at preventing fouling involves the release of copper ions (Cu^{2+}) from its surface when immersed in seawater. The Cu^{2+} ions are bioavailable and are capable of crossing biological membranes into the cells disrupting the cells functions and causing a toxic effect (Brooks & Waldock 2009). Attempts were made to sheath iron clad ships in copper following their introduction in the late 18th century; however, due to the corrosive effect of copper on iron this was not possible and the use of copper as an antifoulant was nearly discontinued (Yebra et al. 2004; Almeida et al. 2007). It was this, that is alleged to have renewed interest in antifouling compositions (Yebra et al. 2004) and lead to the development of the first antifouling paints in the mid 19th century (Almeida et al. 2007). During the 1950s the first organometallic paints (containing tin, arsenic, mercury etc) were developed and following numerous advancements gave rise to tributyl-tin (TBT) (Almeida et al. 2007).

Table 1.2. A brief history of antifouling techniques.

Time period	*Civilisation/ Nation*	*Antifouling product*	*Reference*
700 B.C.	**Phoenicians**	**Lead sheathing**	**Lunn 1974; Yebra et al. 2004.**
-	**Phoenicians,**	**Tar, wax and asphalt**	**Almeida et al. 2007.**
-	**Carthaginians**	**Pitch**	
500 B.C.	**-**	**Coatings of arsenic and sulphur mixed with oil used to combat shipworm**	**Yebra et al. 2004.**
300 B.C.	**Greeks**	**Tar, wax and lead sheathing**	**Yebra et al. 2004; Almeida et al. 2007.**
10 A.D	**Vikings**	**Seal Tar**	**Yebra et al. 2004.**
13th – 15th Century		**Pitch blended with oil, resin and tallow.**	**Yebra et al. 2004.**
1618	**Danish**	**One of the first references of copper being used underwater on the keel of a vessel.**	**Lunn 1974; Yebra et al. 2004.**
1625	**English**	**William Beale patented the use of copper as an antifoulant.**	**Yebra et al. 2004.**
16th Century	**Spanish**	**Vessels sheathed with lead to protect against shipworm and fouling.**	**Lunn 1974.**
1758 -1761	**English**	**Copper sheathing was used on the Frigate HMS Alarm, by the instruction of Admiral Anson.**	**Lunn 1974; Yebra et al. 2004.**
1780		**Copper was widely used in the British Navy.**	**Lunn 1974; Yebra et al. 2004**
1784	**French**	**Zinc alloy sheathing was used on *Le Meilleur Ami***	**Lunn 1974.**
18th Century		**Introduction of iron clad ships. Efficacy of copper sheathing lead to attempts to plate iron ships with copper, but due to the corrosive effect of the copper on the iron, copper sheathing was discontinued.**	**Lunn 1974; Yebra et al. 2004.**
1824	**English**	**Sir Humphry Davy demonstrated that it's the dissolution of copper in seawater which prevented fouling.**	**Yebra et al. 2004.**
Mid 19th Century		**Some of the first antifouling paints emerged.**	**Almeida et al. 2007; Anderson et al. 2003; Townsin 2003.**
1847	**English**	**William John Hay invented a paint which contained copper compounds; these were isolated from the iron hull by the use of a non conductive varnish as a binder.**	**Yebra et al. 2004.**
1950-1960		**First appearance of organometallic paints (containing tin, mercury,**	**Almeida et al. 2007; Yebra et al. 2004.**

		arsenic). Initially used as a co-toxicant with copper paints.	
1974	**English**	**Milne and Hails patented tributyl-tin self-polishing co-polymer (TBT-SPC) paint.**	**Milne & Hails 1977; Anderson et al. 2003; Yebra et al. 2004.**

1.5. Tributyl-tin (TBT)

Tributyl-tin self polishing copolymer (TBT-SPC) patented (Patent GB 1457590A, International Paint Ltd.) in 1974 by Milne and Hails (Milne & Hails 1976; Yebra et al. 2004), was held to be the most effective coating to have been developed (Clare et al. 1992; Löschau & Krätke 2005). These paints were based on an acrylic copolymer (usually methyl methacrylate); the TBT groups are bonded to the polymer by means of an ester link (Yebra et al. 2004; Almeida et al. 2007; Finnie & Williams 2010). Once immersed, the seawater starts to dissolve the surface layer of the paint. Once this layer has been worn away dissolution on the subsequent layer begins. In the presence of a flow of water this dissolution polishes the paints, making it smoother and allows for a slow and controlled release rate of the biocide particles (Christie & Dalley 1987; International Maritime Organisation 2002; Townsin 2003). The release rate or polishing rate was approximately 5 – 20μm a year (Almeida et al. 2007) and depending on the initial thickness of the coating, this could allow for dry docking intervals of up to five years (Anderson et al. 2003; Finnie & Williams 2010). These paints were largely a success and by the late 1970s TBT-SPC was universally adopted as the antifouling technology for most marine vessels (International Maritime Organisation 2002; Finnie & Williams 2010).

TBT is a biocide and was highly effective at killing a range of aquatic organisms, not just those restricted to ship's hulls. With their universal use, large quantities of TBT were released into the marine environment and many non-target organisms were significantly affected (Alzieu 1998). TBT was and still is persistent in the marine environment (Clare et al. 1992). The half-life of the biocide in seawater, which can be highly dependent on pH, temperature, turbidity and light, was estimated to range from a few days in warmer, clear waters, to many weeks in turbid cold waters (Alzieu 1998). Yet the half-life of TBT in sediment was estimated to be between 1 – 4 years, and perhaps as much as 19 years (Alzieu 1998). In areas where there is increased shipping

activity, such as ports and harbours, large concentrations of TBT had the potential to accumulate (Armstrong et al. 2000; International Maritime Organisation 2002). According to Alzieu (1998) water samples taken in the late 1980s from European harbours had high concentrations of TBT ranging from 10 – 1500ngl^{-1} and concentrations of the biocide in the harbour sediments ranged from 1 – 2mgkg^{-1} dry weight. However, it only took low concentrations of TBT to cause malformations in marine organisms. Those that were particularly affected included marine bivalves and gastropods (Alzieu 1998). For example, a concentration of 1ngl^{-1} caused the development of male characteristics (referred to as imposex) in female dogwhelks (*Nucella lapillus*) resulting in sterility and a decline in the population (Alzieu 1998; International Maritime Organisation 2002; Yebra et al. 2004). Concentrations of 2 – 20ngl^{-1} were responsible for shell calcification anomalies and reproduction disturbances in the oyster *Crassostrea gigas.* This had large implications for the Arcachon Bay oyster industry, which suffered a decline in production of 7000 tonnes from 1979 to 1981 and a reported loss of 880 million Francs (Alzieu 1998; Yebra et al. 2004).

In 1988, the IMO were requested to introduce measures for restricting the use of TBT on seagoing vessels (International Maritime Organisation 2002). In 1990, a resolution adopted by the IMO – MEPC recommended that the use of TBT-based paints be abolished on non-aluminium hulled vessels less than 25m in length and restrict the release rate of the biocide to be no more than 4μg per cm^2 per day (International Maritime Organisation 2002; Yebra et al. 2004). On the 5th October 2001 at an International Convention on Control of Harmful Anti-fouling Systems on Ships, it was resolved that following the 1st January 2003 application of TBT-containing antifouling paints was to be banned, and that the presence of these paints on ships was eliminated from 1st January 2008 (Yebra et al. 2004). This only came into legal force on the 17th September 2008 (Finnie & Williams 2010).

1.6. Alternative antifouling paints

The mounting environmental concern for TBT-SPC paints in the run-up to the ban generated significant investment into the research and development of new, 'environmentally friendly' alternatives (Yebra et al. 2004; Almeida et al. 2007). The void left by TBT was filled by controlled depletion paints (CDP) and tin-free SPC (TF-

SPC) coatings such as copper acrylate copolymers and silyl acrylate copolymers (Finnie & Williams 2010). However, these were not as efficient as TBT; for example the service life was shorter, they were only suitable in low fouling environments, and the release rate was not constant (CDP mostly) (Yebra et al. 2004; Chambers et al. 2006; Almeida et al. 2007). Co-biocides or booster biocides were often incorporated to help increase the length and functionality of the paints (Thomas 2001; Chambers et al. 2006). The use of such coatings is likely to continue into the foreseeable future, however, concern over the environmental impact of the biocidal component of the paints is increasing and with the movement towards stricter legislations, the use of some biocidal paints will be prohibited (Finnie & Williams 2010; Webster & Chisholm 2010). There was, and is, a need for the development of fully biocide-free, environmentally benign alternatives (Almeida et al. 2007).

1.6.1. Novel alternatives

There has been, and will continue to be, a considerable effort into the research and development of novel non-toxic or low toxicity antifoulants. The following is a concise summary of alternative antifouling technologies:

1.6.1.1. Micro-topography

Micro-scale surface topographies can be used to deter the colonisation of fouling organisms (Bers & Wahl 2004; Carman et al. 2006; Schumacher et al. 2007; Scardino et al. 2008). By altering the surface topography, the surface wettability can be transformed; this consequently affects the adhesion of fouling species to the surface. Examples of the topographies which have been tested include simple ridges, pillars and pits and Sharklet AF™. Sharklet AF™ are biologically inspired patterns (biomimetic) modelled on the skin of fast-moving sharks. They have been shown to reduce the colonisation of *Ulva* zoospores by up to 86% (Carman et al. 2006) and barnacle cyprid larvae by 97% (Schumacher et al. 2007). However, the success of the surface at preventing settlement is dependent on the dimensions of the geometry of the surface in relation to the size of the specific organism (Schumacher et al. 2007; Scardino et al.

2008). The implication of surface topographies for use as a marine antifoulant is unclear. The added roughness of the surface and therefore frictional resistance means its application on ship's hulls seems unlikely, however, there is potential for its application on stationary structures and aquaculture systems (Scardino & de Nys 2011).

1.6.1.2. Natural products

Certain marine macroalgae and organisms including octocorals and sponges are able to resist becoming fouled. Of the adaptations employed, the use of secondary metabolites either produced by the organisms themselves or via micro-organisms living on their surfaces, has received substantial attention for their antifouling potential (Clare et al. 1992; Armstrong et al. 2000; Rittschof 2000; Hellio et al. 2004; 2005; Dobretsov et al. 2006). The metabolites produced by the marine species have been demonstrated to inhibit bacterial growth (Hellio et al. 2001; 2005 Burgess et al. 2003) and the settlement of diatoms, tunicates, mussels and barnacle cypris larvae (Clare 1996; Armstrong et al. 2000; Hellio et al. 2001; 2004; 2005). However, to produce sufficient quantities for the commercial application of antifouling products, would require vast numbers of animals to be collected and destroyed in order to extract enough metabolites (Clare 1996; Armstrong et al. 2000). Instead the culture of micro-organisms that produces the metabolites (Clare 1996; Armstrong et al. 2000) or synthetic productions of the secondary metabolites (Todd et al. 1993; Qian et al. 2010) have the potential for providing a sustainable production, however, there may be difficulties in producing sufficient quantities of the compounds (Qian et al. 2010). Although these products are naturally occurring the new chemicals discovered from marine species are still classed as toxic and the registration process for these chemicals would be restrictive, expensive and time-consuming (Rittschof 2000; Webster & Chisholm 2010).

1.6.1.3. Enzymes

Enzymes, specifically protease enzymes, have shown antifouling potential. Enzymes function by hydrolysing the protein, glycoprotein and polysaccharide adhesives of fouling organisms (Pettitt et al. 2004). This has been shown to inhibit the

settlement and adhesion of fouling organisms such as the barnacle *B. amphitrite* and the green alga *Ulva linza* (Pettitt et al. 2004; Aldred et al. 2008; Tasso et al. 2012). The problem being how to incorporate this into a coating for commercial application and how long the enzymes stay active in such coatings. The registration process of the enzymes for application in antifouling coatings may also be restrictive, expensive and time-consuming (Olsen et al. 2007).

1.7. Fouling-release coatings

Fouling-release (FR) coatings were formulated and patented by Milne in 1977 (GB1470465A International Paint Ltd) (Milne 1977; Anderson et al. 2003). They became commercially available in the 1990s after the launch of Intersleek 425 by International Paint Ltd following the successful trial of this coating on the vessels 'Tropic Lure' and the Navy submarine *HMAS* Collins in 1993 (Finnie & Williams 2010). Since the ban on TBT-SPC, the use of FR coatings has become highly favoured (Brady 2001; Berglin & Gatenholm 2003; Yebra et al. 2004; Finnie & Williams 2010).

The initial commercial FR coatings were based on silicones. Silicones, siloxanes or poly(organosiloxanes) are all names for polymers which have a backbone with alternating silicon and oxygen atoms with organic side groups such as methyl groups (Polydimethylsiloxane, PDMS) (Finnie and Williams 2010). Fluoropolymers are a second group of polymers which are now available as commercial FR coatings; fluoropolymers are polymers containing fluorinated groups such as fluorinated polyurethanes or perfluoropolyethers (PFPE) (Finnie and Williams 2010). The principle of FR coatings is that they interfere with the organism's capacity to adhere to the coatings surface (Clare 1998; Holm et al. 2006; Wendt et al. 2006). The adhesion of the organisms to the coating is suitably weakened so that they can be easily removed by (1) the shear and tensile forces generated as the vessel moves through the water; (2) by the weight of the organisms being sufficient allowing them to 'slough off' and (3) by predatory or grazing fish and crabs (Schultz et al. 1999; Berglin et al. 2003).

Of the physical properties regarded as the reason for the coatings FR ability, there are three which have been the primary focus and these include the surface energy, the elastic modulus and the coatings thickness (Kohl & Singer 1999; Brady & Singer

2000; Singer et al. 2000; Anderson et al. 2003; Berglin et al. 2003; Sun et al. 2004; Yebra et al. 2004; Chaudhury et al. 2005; Wendt et al. 2006; Kim et al. 2007; 2008).

1.7.1. Surface energy

The surface free energy or critical surface tension (γ) of a coating is fundamental to bioadhesion. Defined by Anderson et al. (2003) it is "the excess energy of the molecules on the surface compared with the molecules in the thermodynamically homogeneous interior". Molecules in the interior or bulk of the coating are stable within a matrix, in which neighbouring molecules are able to interact with one another. Molecules at the surface, on the other hand, are unbalanced only being able to react with molecules in the bulk, directly adjacent. The excess energy that results is available to interact with molecules approaching the surface, so when immersed in water, it is available to interact with water molecules (Callow & Fletcher 1994). The magnitude of the surface energy is determined by the surface's ability to interact with approaching molecules per unit area (Anderson et al. 2003). Hence surfaces with high energies can interact more than surfaces with low energies.

To calculate the surface energy of a coating, the contact angles (θ) of a series of liquids (with known surface tensions) on the coatings' surface are measured (Baier 1970; Callow & Fletcher 1994; Packham 2003). This can be done using an instrument called a goniometer. On surfaces with high energies, the test liquids will have a tendency to spread across the surface, wetting it, resulting in a low contact angle. Surfaces of this type are referred to as hydrophilic. However, on surfaces with low energies, the test liquids have a tendency to bead, providing a higher contact angle (Figure 1.5). Surfaces of this type are referred to as hydrophobic (Callow & Fletcher 1994).

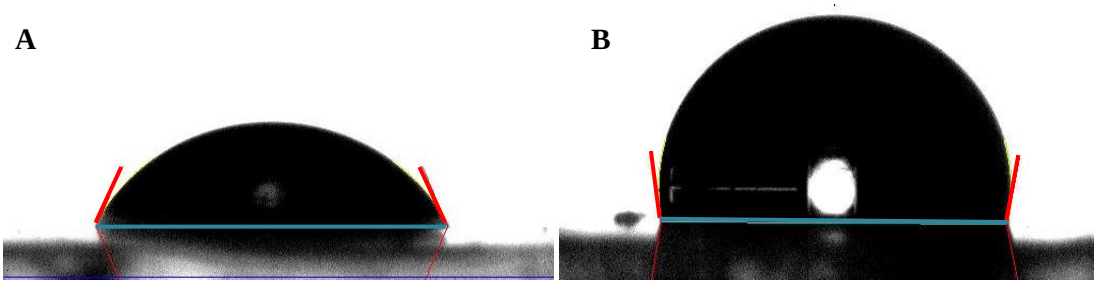

Figure 1.5. Illustration of the contact angle of water on the surface of two silicone coatings with (A) a high surface energy and low contact angle (65 θ) and (B) lower surface energy and a high contact angle (100 θ).

Relative adhesion to a surface is a function of the surface free energy. However, to predict adhesion the surface energy from the substrate (γ_s), the water (γ_w) and their interfacial energy (γ_{sw}) are required (Brady 1999). The interfacial energy being equivalent to the sum of surface tension of the substrate and the water minus the geometric mean of these values multiplied by an interaction constant Φ (Brady 1999) as demonstrated by Eq. (1); the Goods-Girifalco equation.

$$\gamma_{sw} = \gamma_s + \gamma_w - 2\Phi_{sw}\left(\gamma_s \gamma_w\right)^{1/2} \quad (1)$$

When an adhesive is deposited on a surface, the substrate-water interface is interrupted and two new interfaces are created, the substrate-adhesive and adhesive-water interface (Brady 1999). The work required to separate the substrate and water, otherwise known as the work of adhesion (W_a) is equal to the surface energy of the substrate and the water minus the interfacial energy (Callow & Fletcher 1994);

$$W_a = \gamma_s + \gamma_w - \gamma_{sw} \quad (2)$$

A low surface energy results in low work of adhesion. However, a crucial feature of this relationship, as demonstrated by the Baier curve (Figure 1.6), is that the lowest relative adhesion does not occur on surfaces with the lowest surface free energy (Baier et al. 1968; Brady 1999; Anderson et al. 2003; Baier 2006). Rather the lowest relative adhesion occurs on surfaces with a surface tension/surface free energy of 22 –

24mN/m, which is approximately equivalent to the dispersive force of water (Baier 2006; Magin et al. 2010).

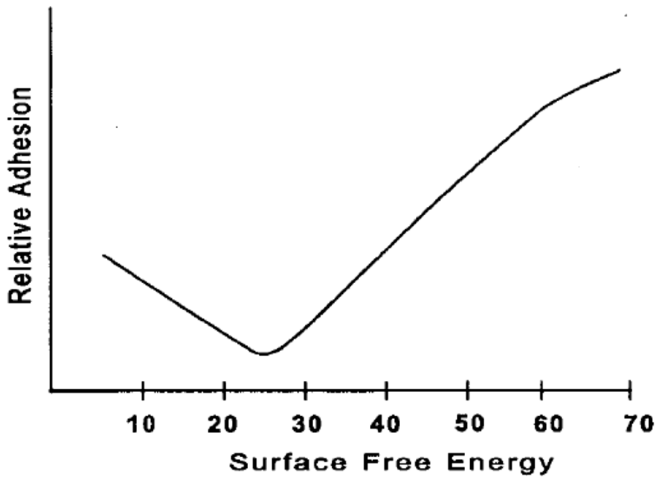

Figure 1.6. Baier Curve. The association between surface free energy and relative adhesion. *Source:* Brady 1999.

1.7.2. Elastic modulus and thickness

The elastic modulus of the coating refers to its ability to deform elastically when subjected to an external force. The surface energy is important as it inhibits adhesion. However, the elastic modulus and the thickness of the coating are important with regard to the removal of fouling organisms. The process of this removal has often been described in terms of fracture mechanics (Brady & Singer 2000). Fracture mechanics is the study of crack propagation and it is used to describe the fracture or crack forming between the adhesive of a fouling body and the coating's surface. The energy required for the formation and completion of the fracture can be calculated using Griffith's (1921) fracture criterion and Kendall's (1971) model. Griffith's (1921) fracture criterion explains that the increase in energy needed to propagate a crack the distance a, forming two new interfaces with an area of $2A$, comes from the difference between the work done dW by an external force and the change in stored elastic energy dU_e in the stressed object. A necessary condition for the crack to propagate can be described with:

$$-\frac{\delta(W-U_e)}{\delta A} \geq G \tag{3}$$

Or

$$-\frac{\delta(U_T)}{\delta A} \geq 0 \tag{4}$$

where *G* is the Griffith fracture energy, *W* is the work, *A* is the area, *Ue* is the elastic energy and U_T is the total energy. Using Griffiths fracture principles Eq. (3 & 4), Kendall (1971), modelling the adhesion of elastomers, developed a formulae for removal of a rigid cylindrical stud on an elastomeric coating.

$$Pc = \pi a^2 \left(\frac{2GK}{t}\right)^{1/2} \tag{5}$$

where *K* is the bulk modulus, *Pc* is the critical pull-off force and *t* is the thickness (Kendall 1971). This model demonstrates that as a force or stress is applied to the stud, on an elastomeric surface, the surface will deform by a certain degree until the stress reaches a critical point in which a fracture will occur. Surfaces with a high elastic modulus are hard with a reduced ability to deform; on these surfaces the type of fracture that separates the adhesive and the coating is a shear fracture. In contrast surfaces with a low modulus have a greater ability to deform; the type of fracture that occurs on these surfaces is a peel fracture. The energy required for a peel fracture is less than the energy that is needed to complete a shearing fracture (Brady 2001).

With regard to the influence of thickness on removal force, put simply, the pull-off force decreases as the thickness of the coating increases (Kohl & Singer 1999; Brady & Singer 2000). However, Kendall's model (1971) shows that the importance of thickness on the critical pull-off force depends on its relationship to the contact area of the stud. When the thickness of the coating is much greater than the contact radius ($t>>a$) the critical pull-off force becomes independent of the thickness (Kim et al. 2007).

For thick coatings ($t>>a$) Eq. (5) becomes:

$$Pc = \sqrt{2\pi EGa^3/(1-v)^2} \quad (6)$$

where v is the Poisson's ratio. Using Eq. (6) Brady & Singer (2000) explained that surface energy (expressed in Eq. 3 by G), and the elastic modulus are both important during a fracture; such that the removal force correlates better to the product $(E\gamma)^{1/2}$ than to either the modulus (E) or the surface energy (γ) (Figure 1.7) (Brady & Singer 2000).

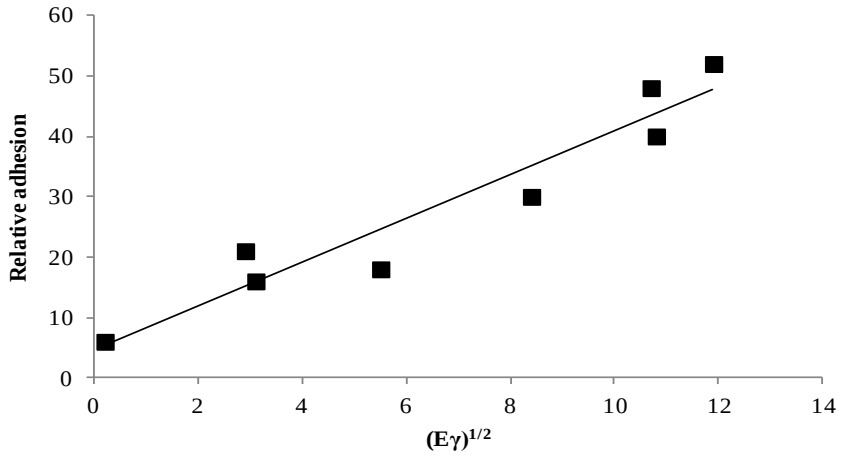

Figure 1.7. Relative adhesion as a function of the square root of the product of critical surface energy (γ) and elastic modulus (E). *Source:* Brady and Singer 2000.

Kendall's model has frequently been used to illustrate the removal of biofouling organisms specifically barnacles, from FR coatings (Kohl & Singer 1999; Sun et al. 2004; Wendt et al. 2006; Kim et al. 2007).

1.7.3. Chemical composition of fouling-release coatings

Silicone and fluoropolymers are two polymers that have been shown to possess the necessary properties essential for fouling-release. However, their different chemical structure results in them achieving effective fouling-release through different mechanisms (Brady 1999; 2001). The differences discussed relate to the difference in surface energy and modulus of the polymers. Fluoropolymers limit the bonding of adhesives to their surface by their arrangement of the functional groups such as perfluoroalkyl. These groups are cross-linked together which minimises re-arrangement within the polymer and this prevents the infiltration of marine adhesives on the surface of the polymer. This causes a weak bond to form with the adhesive, resulting in the coating having a lower surface energy than in silicones (Brady 2001; Yebra et al. 2006). Fluoropolymers also have limited rotation of the C – C backbone of the polymer because of the presence of the fluorine atoms. This means the fluoropolymers have limited flexibility and a much higher elastic modulus than silicones (Brady 2001; Yebra et al. 2006). Silicones, in addition, have a longer bond length between the Si – O bond and a larger bond angle between the Si – O – Si link than along the C – C fluoropolymer chain in fluoropolymers. The silicones backbone also has a low rotation energy which results in high chain flexibility (Figure 1.8). All this contributes to the silicone having a very low modulus (Brady 1999; Finnie & Williams 2010; Webster & Chisholm 2010).

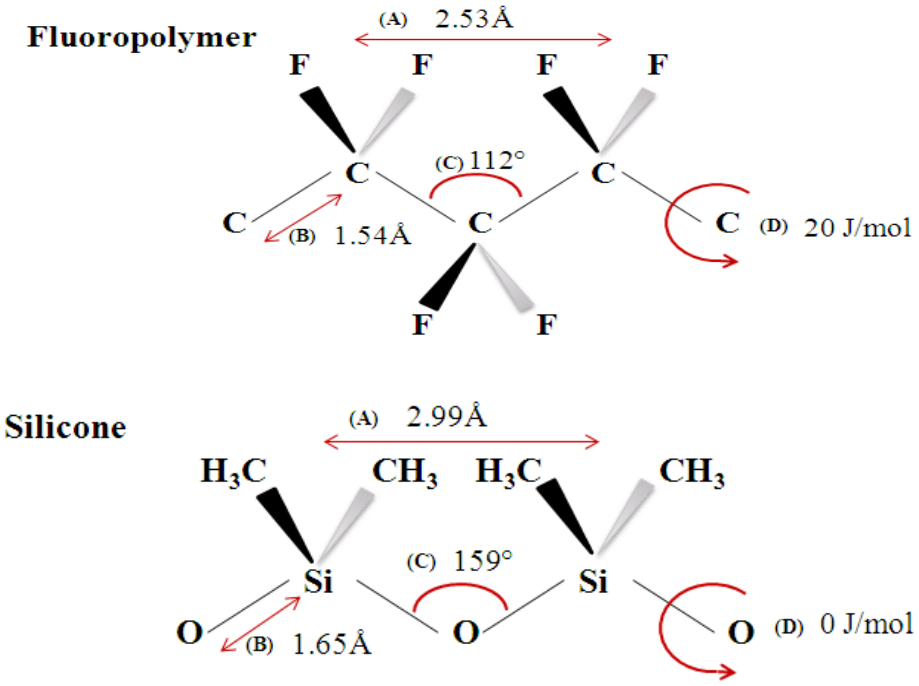

Figure 1.8. An example of the typical molecular structure of a fluorocarbon (poly(tetrafluoroethylene)) and a silicone (polydimethylsiloxane), illustrating the difference in A) link length, B) bond length, C) bond angle and D) rotation energy (adapted from Brady 1999).

1.8. Recent developments in fouling-release research

Many of the past and present commercial FR coatings are based on silicones. Nevertheless, silicones do have disadvantages; they are mechanically weak and are therefore easily damaged; they are only beneficial to high-speed vessels (> 15 knots) in order for fouling to be released (Kavanagh et al. 2001; Marabotti et al. 2009; Callow & Callow 2011). They have poor adhesion to substrates and require a tie-coat primer, increasing the expense and application time (Webster & Chisholm 2010). Recent focus has been to try and improve the mechanical properties of silicone FR coatings without detracting from their FR properties (Beigbeder et al. 2008; Marabotti et al. 2009; Kaffashi et al. 2012). A brief summary of some of the avenues being investigated includes reinforcing the silicone polymer matrix with fillers such as carbon nanotubes, natural sepiolite (Beigbeder et al. 2008) and silica (Kaffashi et al. 2012). These have all shown some promise in improving the mechanical properties of the silicone coatings.

However, in the case of silica, the introduction of the filler changed the bulk properties by increasing the elastic modulus and thus reduced the FR properties of the coating. Another avenue included the combination of silicones with polyurethane (Ekin et al. 2007; Pieper et al. 2007; Fang et al. 2010; Sommer et al. 2010). Adding polyurethane to the silicone improves its adhesion to substrates and increases the silicones mechanical resistance (Ekin et al. 2007). However, when they were immersed in water the urethane groups rearrange near the surface altering the wettability and converted the coating from a hydrophobic one to a hydrophilic one (Pike et al. 1996). The addition of cross-linkers to stabilise siloxane-polyurethane have been shown to prevent the rearrangement of the urethane groups and have demonstrated excellent FR properties and perform equally well in laboratory assays and in field trials (Ekin et al. 2007; Pieper et al. 2007; Fang et al. 2010; Sommer et al. 2010; Stafslien et al. 2016). Also under investigation are combined silicone and fluorinated polymers. The idea was to combine the advantages of both silicones and fluoropolymers (Marabotti et al. 2009). Fluorosilicones have shown significant improvements on release characteristics against fouling algae and barnacles when compared to a silicone coating (Marabotti et al. 2009).

The examples discussed above are all based on hydrophobic systems; an additional area of research is on amphiphilic coating systems (Krishnan et al. 2006; Martinelli et al. 2011; 2012; Wang et al. 2011). Amphiphilic coatings possess both hydrophobic and hydrophilic functionalities across its surface, the level of complexity this creates on a surface has been demonstrated to inhibit the adhesion of marine fouling, specifically the green macroalgae *Ulva linza*, the diatom *Navicula incerta* and cyprids of the barnacle *Balanus amphitrite* (Krishnan et al. 2006; Martinelli et al. 2011; 2012; Wang et al. 2011; Zhou et al. 2014). The early amphiphilic coatings were composed of hyperbranched fluoropolymers and poly(ethylene glycol) (PEG) (Gudipati et al. 2004); and today a FR coating based on a similar system is available on the commercial market (Intersleek ® 900) (Finnie & Williams 2010). More recent formulations demonstrating significant potential includes diblock or triblock copolymers which combine a polysiloxane block and an amphiphilic PEGylated-fluoroalkyl modified polystyrene block often dispersed in a PDMS matrix (Martinelli et al. 2011; 2012; Zhou et al. 2014; Stafslien et al. 2015), in an attempt to combine the benefits of both amphiphilic and silicone coating systems.

1.9. Methods for assessing fouling-release coatings

The physical properties (e.g. hardness, tensile strength, texture, modulus), chemical properties (e.g. surface energy, toxicity) and finally, biofouling property (Swain 1997), of every new coating formulation needs to be rigorously tested to evaluate its performance. The biofouling property regards the ability of the coating to prevent settlement and reduce the adhesion of settled organisms. This is potentially one of the more important qualities, as it assesses whether the coating can do what it is designed for.

1.9.1. Pseudobarnacles

One tool that has been used to measure biofouling properties, specifically the adhesion to a coating, is the pseudobarnacle test. A pseudobarnacle is a cylindrical stud that can be epoxy, wood or metal. The stud is fixed to a coating using synthetic adhesives, for example, epoxy adhesive Araldite®. After this cures, the removal stress of the pseudobarnacle from the coating can be measured (Berglin & Gatenholm 1999; Kohl & Singer 1999; Singer et al. 2000; Stein et al. 2003; Chisholm et al. 2007; Kim et al. 2007). The removal stress of pseudobarnacles was thought to replicate the removal stress of real barnacles on silicone coatings (Berglin & Gatenholm 1999). Pseudobarnacles were beneficial as they provided rapid assessment of coatings and could be used to demonstrate the fracture process modelled by Kendall (1971). However, the synthetic adhesives used for pseudobarnacle tests do not possess comparable viscoelastic and multi-layered properties of barnacle adhesives (Sun et al. 2004). Barnacles when grown on silicones have been shown to develop an atypical, thick 'gummy' adhesive and calcareous-based barnacles specifically have been shown to develop an abnormal 'cupped' basis (Wiegemann & Waterman 2003; 2004; Sun et al. 2004; Wendt et al. 2006; Ramsay et al. 2008). The development of cupped basal plates is thought to be the result of the growth of the parietal plates of the barnacles exerting a downward pressure on the substrate. This causes the barnacle to lift off the silicone coatings which makes the basal plate grow into a cup shape (Wiegemann & Watermann 2003). The thick adhesive fills the gap between the cupped basis and the substrate to help the barnacle maintain attachment to the substrate (Wiegemann & Waterman 2003; Ramsay et al. 2008). Adhesives with a thicker 'gummy' state have been shown to

possess a lower modulus than the typical adhesive produced, and this can influence the adhesion of barnacles (Sun et al. 2004). Furthermore, Kendall's (1971) model assumes that the stud is rigid, however the calcareous-basis of the barnacle *Balanus amphitrite* exhibits a flexural rigidity of 0.002NM, this was orders of magnitude less than that of a pseudobarnacle (Ramsay et al. 2008). For example, Ramsay et al. (2008) found that a steel plate pseudobarnacle with a thickness of 100μm had a flexural rigidity of ~0.02Nm, which increased to ~20Nm as the thickness of the plate increased to 0.0001m. This means during the fracture process and detachment from a coating, there is greater flex in a barnacle than in a pseudobarnacle. This flexure that occurs during the fracture reduces the force required to detach a real barnacle rather than a pseudobarnacle (Chung & Chaudhury 2005; Ramsay et al. 2008). Previous comparisons have shown that there is a large discrepancy between the removal stress of pseudobarnacles and barnacles (Sun et al. 2004). The use of pseudobarnacles is going out of favour; as they are unable to replicate the adhesives properties and growth malformations of barnacles from FR coatings and they do not show the same flexural rigidity of barnacles.

1.9.2. Choice of marine organisms

Although pseudobarnacles provide rapid assessment, there is a requirement for realistic assessment by testing FR coatings with marine fouling organisms. The methods used for measuring the settlement and the removal stress depend on the choice of organism being tested. There are a number of species which have been used to evaluate a coating's performance (Table 1.3); however, there are two primary candidates that have been more extensively investigated. The first is the green macroalga *Ulva linza* (formerly, *Enteromopha linza*) (Callow et al. 1997; Finlay et al. 2002; Chaudhury et al. 2005; Cassé et al. 2007; Pieper et al. 2007; Beigbeder et al. 2008; Marabotti et al. 2009; Fang et al. 2010; Sommer et al. 2010; Martinelli et al. 2011; 2012). This macroalga is a dominant species of the upper intertidal zone along shorelines throughout the world and has been reported to be the most common macroalga that fouls artificial structures, specifically ship's hulls (Callow et al. 1997; Chaudhury et al. 2005; Marabotti et al. 2009). The second group frequently used to assess FR coatings are barnacles. Species including *Balanus amphitrite* (= *Amphibalanus amphitrite*) (Pettitt et al. 2004; Wendt et al. 2006; Beigbeder et al. 2008;

Conlan et al. 2008; Kim et al. 2008; Marabotti et al. 2009; Sommer et al. 2010; Martinelli et al. 2012; Stafslien et al. 2012), *B. eburneus* (Wynne et al. 2000; Kavanagh et al. 2001; Sun et al. 2004; Holm et al. 2006), *B. improvisus* (Berglin & Gatenholm 1999; Singer et al. 2000; Berglin et al. 2001; Wiegemann & Watermann 2004) and *Elminius modestus* (Wiegemann & Watermann 2004; Robson et al. 2009) are just some examples of barnacle species used in coating research. However, *B. amphitrite* is more universally used for testing FR coatings than any other (Aldred & Clare 2008). The distribution of *B. amphitrite* is widespread throughout the sub-tropics, where it is considered a problematic fouling species as it is able to rapidly colonise submerged artificial structures (Aldred & Clare 2008; Marabotti et al. 2009). This species has been reported as being an excellent model for laboratory testing as they are able to produce larva all year round, and their cyprids readily settle in static conditions (Branscomb & Rittshcof 1984; Rittschof et al. 1992; Aldred & Clare 2008). Barnacles in general are considered to be one of the more, if not the most, important groups of organisms fouling ship's hulls (Briand 2009). Their relatively large size, gregarious settling behaviour and their propensity to settle on any hard surface has contributed to this reputation (Christie & Dalley 1987; Briand 2009). As the nature of this thesis is to investigate barnacle adhesion the following will concern barnacles only.

Table 1.3. A brief account of the marine test species used for removal stress measurements for antifouling and fouling-release research.

Fouling Group	*Species*	*Assay type*	*Adhesion method*	*Culture method*	*References*
Bacterium	***Cellulophaga lytica (Cytophaga lytica)***	**Percent removal**	**Water Jet**	**Laboratory culture**	**Ekin et al. 2007; Sommer et al. 2010; Stafslien et al. 2015; 2016.**
	Halomonas pacifica	**Percent removal**	**Water Jet**	**Laboratory culture**	**Ekin et al. 2007; Stafslien et al. 2015.**
Diatoms	***Amphora coffeaeformis***	**Percent removal**	**Flow channel**	**Laboratory culture**	**Schultz et al. 2000; Holland et al. 2004.**
	Craspedostauros australis	**Percent removal**	**Flow channel**		**Holland et al. 2004.**
	Navicula incerta	**Percent removal**	**Water Jet**	**Laboratory culture**	**Pieper et al. 2007; Sommer et al. 2010; Stafslien et al. 2015; 2016.**
			Flow channel		**Zhou et al. 2014.**
	Naviucla perminuta	**Percent removal**	**Flow channel**	**Laboratory culture**	**Holland et al. 2004; Pettitt et al. 2004; Statz et al. 2006; Wang et al. 2011.**
Macroalgae	***Ulva linza (*formerly *Enteromorpha linza)***	**Sporeling (~6 day old) percent removal**	**Water Jet**	**Laboratory culture**	**Finlay et al. 2002; Cassé et al. 2007; Ekin et al. 2007; Pieper et al. 2007; Fang et al. 2010; Sommer et al. 2010; Wang et al. 2011.**
			Flow channel		**Pettitt et al. 2004; Chaudhury et al. 2005; Statz et al. 2006; Beigbeder et al. 2008;**

					Marabotti et al 2009; Martinelli et al 2011; 2012; Zhou et al. 2014.
	Ectocarpus crouaniorum	**Percent removal**	**Flow channel**	**Laboratory culture**	**Evariste et al. 2012.**
Macrofouling fauna: **Barnacles**	***Balanus amphitrite***	**Cyprid adhesion**	**Water jet**	**Laboratory culture**	**Aldred et al. 2010.**
		Adult adhesion	**ASTM D-5618**	**Laboratory culture**	**Wendt et al. 2006.**
			Automated adhesion system		**Beigbeder et al. 2008; Marabotti et al. 2009; Martinelli et al. 2012.**
			Tensile removal stress		**Waterman et al. 1997.**
			ASTM D-5618	**Reattached:**	**Ekin et al. 2007; Kim et al. 2008; Rittschof et al. 2008; Sommer et al. 2010; Stafslien et al. 2012.**
	Balanus crenatus	**Adult adhesion**	**ASTM D-5618**	**Static immersion site**	**Wiegemann & Watermann 2004.**
	Balanus eburneus	**Adult adhesion**	**ASTM D-5618**	**Static immersion site**	**Swain & Schultz 1996; Wynne et al. 2000; Kavanagh et al. 2001; 2003; 2005.**
	Balanus improvisus	**Cyprid adhesion**	**Tensile strength fixing a fibre to the cyprid measured with a microbalance**	**Wild nauplii collected and cultured in a laboratory**	**Berglin et al. 2001.**
		Newly metamorphosed and	**Tensile strength**		**Berglin et al. 2001.**

		juvenile barnacles	**fixing a fibre to the barnacle measured with a tensiometer**		
		Adult adhesion	**ASTM D-5618**	**Laboratory culture**	**Berglin & Gatenholm 1999; Larsson et al. 2010.**
				Static immersion site	**Wiegemann & Watermann 2004.**
	Balanus variegatus	**Adult adhesion**	**ASTM D-5618**	**Static immersion site**	**Kavanagh et al. 2005.**
	Elminius modestus	**Adult adhesion**	**ASTM D-5618**	**Static immersion site**	**Wiegemann & Watermann 2004; Robson et al. 2009.**
	Pseudobarnacles		**Automated pull-off system**		**Kohl & Singer 1999; Chisholm et al. 2007; Kim et al. 2007; 2008; Sommer et al. 2010; Kaffashi et al. 2012.**
Tubeworms	***Hydroides dianthus***	**Adult adhesion**	**ASTM D-5618**	**Static immersion site**	**Kavanagh et al. 2001; Holm et al. 2006.**
	Hydroides elegans	**Adult adhesion**	**ASTM D-5618**	**Static immersion site**	**Holm et al. 2006.**
Bivalve mollusc	***Crassostera virginica***	**Adult adhesion**	**ASTM D-5618**	**Static immersion site**	**Kavanagh et al. 2001; Holm et al. 2006.**
	Mytilus galloprovincialis	**Pediveliger adhesion**	**Flow channel**	**Laboratory culture**	**Carl et al. 2012.**

1.9.3. Barnacles

Barnacles are sessile crustaceans in the subclass Cirripedia. Within this subclass there are four orders; the Ascothoracica, parasitic barnacles of anthozoans and echinoderms; Rhizocephala, parasitic barnacles primarily of decapods; Acrothoracica, burrowing barnacles and Thoracica (Newman & Ross 1976; Newman 1987). The latter order, which Newman (1987) described as the 'principle order of the Cirripedia' contains three suborders: the Lepadomorpha, the stalked barnacle; the Verrucomorpha, the asymmetrical barnacle and the Balanomorpha, the symmetrical acorn barnacle (Newman & Ross 1976; Newman 1987). The Balanomorpha has the greatest diversity of species within it, and include the species more common on the rocky shore and in the fouling community on ship's hulls (Newman & Ross 1976).

In brief, an acorn barnacle's life history includes six planktonic nauplius stages in which the last five are planktotrophic (feeding), followed by a non-feeding cypris stage which settles and metamorphoses into a sessile adult (Figure 1.9) (Clare & Matsumura 2000; Phang et al. 2006; Aldred & Clare 2008). The cyprid is a particularly important stage in the barnacle's lifecycle, as it is the cyprid that explores and selects a substratum for its suitability for settlement. Acorn barnacles are simultaneous hermaphrodites possessing both male and female reproductive systems**,** thus proximity between individuals is important in order for the barnacles to cross fertilise, potential partners need to be within range of the "acting males" extensible penis (Barnes et al. 1977; Clare & Matsumura 2000). Selection of a site for settlement is dependent on many factors, for example, the presence of adult conspecifics (Knight-Jones & Stevenson 1950; Larman & Gabbot 1975; Barnett & Crisp 1979), presence and age of a molecular film and biofilm (Wieczorek et al. 1995; Thompson et al. 1998; Olivier et al. 2000), local hydrodynamics (Rittschof et al. 1984; Eckman et al. 1990), surface contour and texture (Wethey 1986; Kerr & Cowling 2003; Schumacher et al. 2007), surface chemistry (Roberts et al. 1991; Callow & Fletcher 1994) and surface colour (Yule & Walker 1984a; Robson et al. 2009).

In the process of exploring a surface, a cyprid uses a temporary adhesive secreted by the unicellular antennulary cement glands, this serves to hold the cyprid onto the substratum to prevent it being dislodged (Clare & Matsumura 2000; Khandeparker & Anil 2007). As a cyprid explores a surface with their antennules, they

leave behind "footprints" of temporary adhesive, these acts as a settlement cue to subsequent exploring larvae (Walker & Yule 1984). Once a suitable site has been selected the cyprid settles and using a proteinaceous cement it attaches permanently to a surface. The cyprid then metamorphoses into the juvenile barnacle. It can take several days for the adult adhesive to be released as the secondary cement glands develop; in the barnacle *Semibalanus balanoides*, for example, this can take up to 40 days after settlement (Yule & Walker 1987). Once developed the adult adhesive is released through a system of ducts opening at the periphery of the basis of the barnacle forming rings of cement as the barnacle grows/moults (Yule & Walker 1987; Wiegemann 2005).

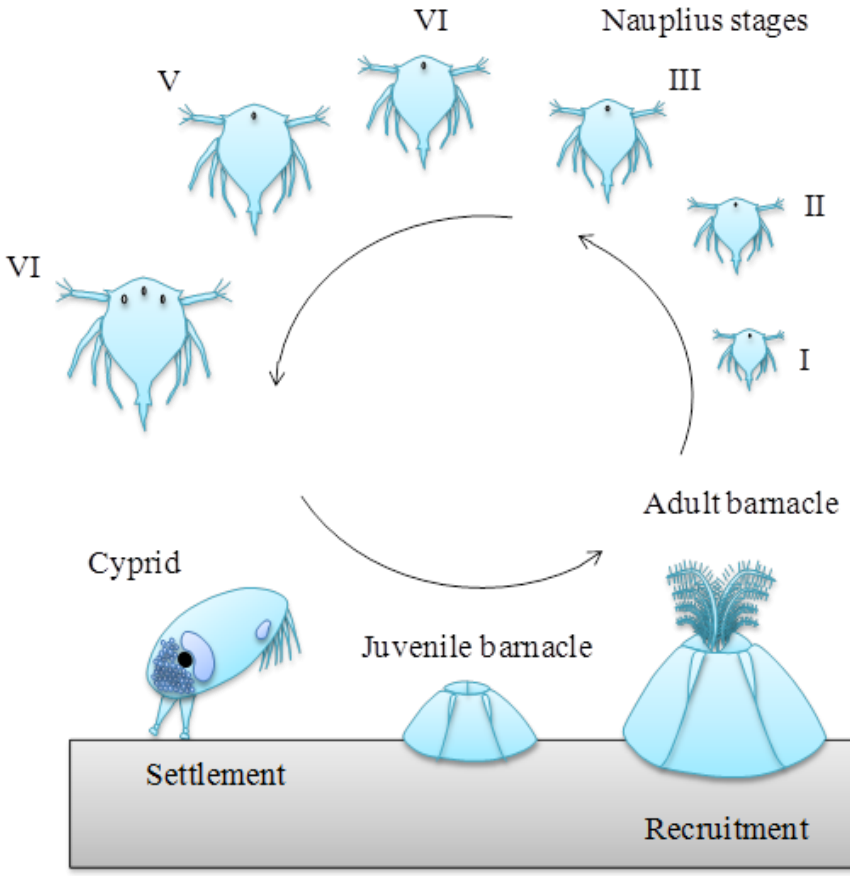

Figure 1.9. Life cycle of a barnacle, displaying six nauplii stages, a non-feeding cyprid stage and metamorphosis to a juvenile and adult barnacle.

1.9.3.1. Laboratory culture of barnacles

A laboratory culture of barnacles involves the lifecycle of the barnacle, from nauplii stage I (being released from adult barnacles) to nauplii stage VI through to the cyprid stage and settlement and eventually to adult, being completed under controlled laboratory conditions. Although not every barnacle species can be cultured in a laboratory, for example, *S. balanoides*. *S. balanoides,* a boreo-arctic species, has an annual breeding cycle and a very long larval development time (Barnes 1962), the attempts at culturing this species in a laboratory have so far been unsuccessful (Kirby 2006). Whereas *B. amphitrite*, a sub-tropical barnacle, has been shown to be an excellent laboratory species especially for evaluating antifouling and FR technologies. With a well maintained adult broodstock *B. amphitrite* are capable of reproducing all year round producing multiple batches of nauplii. This barnacle has a short larval development time with the nauplii metamorphosing into cyprids after 5 days of being fed daily on a diet of the diatom *Skeletonema* spp., incubated at 28ºC (Rittschof et al. 1992; Hellio et al. 2004).

B. amphitrite cyprids from laboratory cultures are often used to evaluate the capacity of antifouling technologies to inhibit settlement; for example, in toxicity assays (Rittschof et al. 1992); microtopography (Schumacher et al. 2007; Aldred et al. 2010); enzymes (Pettitt et al. 2004; Tasso et al. 2012) and now FR coatings (Beigbeder et al. 2008; Marabotti et al. 2009; Wang et al. 2011; Martinelli et al. 2012). However to evaluate FR coatings the critical removal stress (CRS) of the organism from the coating is the desired measurement. The CRS of laboratory cultured cyprids (Berlin et al. 2001; Aldred et al. 2010), newly metamorphosed barnacles (Berglin et al. 2001) and adult barnacles grown in the laboratory (Waterman et al. 1997; Wendt et al. 2006; Beigbeder et al. 2008; Marabotti et al. 2009; Martinelli et al. 2012) are current methods used to assess antifouling technologies and FR coatings.

However, Briand (2009) stated that "*no laboratory bioassay could hope to replicate such a complex process*" that is biofouling, suggesting that laboratory adhesion assays are not valid. The settlement process of barnacles is influenced by multiple factors (see section 1.9.3.) each of these being potentially inhibitory or facilitatory. For example, the presence of a 1 – 3 day old biofilm, as Wieczorek et al. (1995) demonstrated, can inhibit the settlement of *B. amphitrite*, however as the film

aged it was shown to facilitate cyprid settlement. The presence of a biofilm has also been shown to increase the adhesion and CRS of cyprids from a surface (Neal & Yule 1994a; Zardus et al. 2008). Once the barnacles have settled and have been recruited into the fouling community they are subjected to external physical and biotic factors, for example water turbulence/flow and predation, which have been shown to influence the barnacles adhesion to the substratum (Swain et al. 1998). If the CRS of barnacles can be influenced by multiple physical, chemical and biological factors such as the settlement and post-recruitment processes are influenced by, laboratory assays would not replicate the true performance of coatings on ship's hulls and therefore may not be valid (Briand 2009).

1.9.3.2. Field immersion trials

The use of static immersion sites in the field for natural colonisation is common practice for testing recruitment and CRS to antifouling and FR coatings (Becka & Loeb 1984; Swain et al. 1992; 2000; Swain & Schultz 1996; Wood et al. 2000; Wynne et al. 2000; Kavanagh et al. 2001; 2003; Sun et al. 2004; Wiegemann & Watermann 2004; Holm et al. 2006; Robson et al. 2009). Field immersion trials may best reflect the recruitment and CRS of marine organisms found on the hulls of ships, and therefore provide a more realistic idea of the coating performance. However, field trials are criticised for being costly, requiring a large volume of coating samples. It has also been claimed that field trials require several months submersion time to allow for colonisation and growth for adhesion testing (Webster et al. 2007; Rittschof et al. 2008; Stafslien et al. 2012). There are problems with seasonality of specific fouling organisms for example the barnacle *S. balanoides*, whose strict reproduction cycle sees this species only releasing nauplii in time for the spring diatom bloom (Barnes 1962). There are also environmental factors such as fluctuating sea temperatures which can result in low larval availability and poor recruitment (Harms & Anger 1989; Gallagher et al. 2015), and problems with predation removing organisms which had settled (Swain et al. 1998; Rittschof et al. 2008).

Laboratory assays would not be affected by weather, predation or low larval availability. Laboratory evaluations are considered to be beneficial as they are able to down-select coating formulations for field tests, thus reducing the number of different

samples needed to be immersed in the field (Swain 1997; Rittschof et al. 2008; Martinelli et al. 2012; Stafslien et al. 2012). There are a few studies that have compared the discriminatory abilities of barnacle cyprids from laboratory cultures and in the field, often comparing the percentage settlement in the laboratory to the percentage coverage or the total number recruited from the field (Rittschof & Costlow 1989; O'Connor & Richardson 1996; Thompson et al. 1998; Matsumura et al. 2000; Martinelli et al. 2012). Where settlement is defined as the permanent transition of planktonic larvae to the benthic community, and recruitment being defined as when the presence of the 'recruits' (the species being studied) have been observed on the substratum (Keough & Downes 1982; Pawlik 1992). However, there has yet to be a study that compares the CRS of adult barnacles settled and grown in the laboratory to those settled and grown in the field. One study did investigate the removal stress of re-attached calcareous-based barnacles to those recruited from the field on polyurethane coatings (Stafslien et al. 2016). Re-attached barnacles are ones that are reared from cyprid stage, settled on a polysiloxane elastomer for example Silastic ® T-2 until they reach > 5mm in diameter, they are then removed and placed on a new test coating where they begin to re-attach themselves (Rittschof et al. 2008; Stafslien et al. 2012). Re-attached barnacles have been shown to have a positive correlation to results from the field (Rittschof et al. 2008: Stafslein et al 2016). As Stafslien et al. (2016) discusses, the adhesion of laboratory re-attached *B. amphitrite* correlated well for five out of eight coatings to the adhesion of *B. amphitrite* from one field site tested. However, they were a much lower degree of correlation between the adhesion of laboratory re-attached *B. amphitrite* to the adhesion two different calcareous-based barnacles species (*B. crenatus* and *B. eburneus*) from two alternative field sites.

One objective of this thesis (Chapter 4) was to provide a view into laboratory and field assays, comparing the CRS of membranous-based *E. modestus* barnacles from two field sites in two years to a laboratory culture of *E. modestus* barnacles, settled and grown on the test coatings. With the intention to validate whether laboratory assays can discriminate between coatings in a way that is representative of that which would be seen in the field.

1.10. Critical removal stress of barnacles

The CRS of barnacles and other fouling organisms to a coating is calculated by measuring the force required to completely remove the organism, normalised by its contact area. This is referred to as the adhesion strength or the critical removal stress (CRS) and is measured in megapascals (MPa) (Callow & Fletcher 1994; Swain et al. 2000; Wiegemann & Watermann 2004).

1.10.1. Critical removal stress of adult barnacles

The CRS of barnacles and pseudobarnacles can be a measure of the stress needed to pull-off the organism from the coating, this is the tensile stress (Grenon et al. 1979; Becka & Loeb 1984; Swain et al. 1992; Watermann et al. 1997; Chisholm et al. 2007; Kim et al. 2007). However, the use of shear or push-off stress as measured following the ASTM D-5618 (1994), is more common at present (Watermann et al. 1997; Swain et al. 2000; Kavanagh et al. 2001; Sun et al. 2004; Wiegemann & Watermann 2004; Wendt et al. 2006; Conlan et al. 2008; Martinelli et al. 2012; Stafslien et al. 2012). The ASTM D-5618 (1994) is the "Standard Test Method for the Measurement of Barnacle Adhesion in Shear". It is the standard method which uses a probe to apply a force parallel to the surface, to the base of a single barnacle, at rate of approximately $4.5Ns^{-1}$ (1 lb s^{-1}) (ASTM D-5618 1994; Kavanagh et al. 2001). The probe is attached to a spring force gauge which measures the force (N) required to detach the animal. The CRS is then calculated by dividing this force by the size of the barnacles' base plate. The recommended size of barnacles suggested in the ASTM D-5618 ranges between 5 to 20mm in diameter, as size begins to become a factor affecting the accuracy of the CRS measurements for barnacles outside this size range (Kavanagh et al. 2001). Motorised adaptations of the force gauge have been developed (Stein et al. 2003; Wendt et al. 2006; Kim et al. 2008; Stafslien et al. 2012) including a fully-automated computer controlled equivalent (Conlan et al. 2008). The fully-automated method is beneficial as it is capable of a high-through-put of samples, provides an accurate measure of the basal area of the barnacle and is capable of measuring the CRS of barnacles with a smaller diameter of 3.6mm thus requiring a shorter period for growth (Conlan et al. 2008).

The ASTM D-5618 is specific for measuring barnacles, but it has been adapted and used to measure the removal force of other calcified fouling organisms, such as the tubeworms *Hydroides dianthus* and *H. elegans*, and the oyster *Crassostera virginica* (Kavanagh et al. 2001; Holm et al. 2006) (see Table 1.3).

It should be noted that the removal force calculated by Kendall's (1971) model is a tensile force. However, using the relationship:

$$T = 3S \tag{7}$$

where T refers to a tensile force and S is a shear force (Wynne et al. 2000); Kendall's (1971) model can be adapted and used to estimate the removal force in shear.

1.10.2. Removal stress of cyprids

The cyprid is the critical stage for selecting a settlement site, and being able to remove settled cyprids and newly metamorphosed barnacles would be very beneficial. However, due to the soft body of the cyprid and newly metamorphosed barnacles the method used for adult adhesion measurement is not suitable. The adhesion of cyprids has previously been measured by fixing a fibre to the side of a cyprid using synthetic adhesive and measuring the tensile force required to detach the cyprid from a surface using a microbalance (Yule & Walker 1984a; Berglin et al. 2001). This method has also been used on newly metamorphosed barnacles (Berglin et al. 2001). However, the use of water-jetting (impact pressure) and flow channels (wall shear) which are often used for testing the adhesion of biofilm and macroalgal fouling (see Table 1.3) (Schultz et al. 2000; Finlay et al. 2002; Pettitt et al. 2004; Chaudhury et al. 2005; Beigbeder et al. 2008) have also been used successfully for measuring cyprid adhesion to test coatings (Eckman et al. 1990; Zardus et al. 2008; Aldred et al. 2010).

1.11. Research gap

There is a large diversity of marine fouling organisms with approximately 4000 separate species identified (Yebra et al. 2004; Holm et al. 2006); so the variety of

different mechanisms for adhesion is extensive. The challenge for paint manufacturers is to develop a coating that will significantly inhibit the adhesion of all these organisms (Brady 1999; Brady & Singer 2000; Holm et al. 2006). Therefore it is necessary to screen FR coatings against as wide a diversity of fouling organisms as possible (Holm et al. 2006).

With regard to barnacle adhesion studies a significant proportion have centred on barnacles with a calcareous-basis, for example the model species *B. amphitrite* (Pettitt et al. 2004; Wendt et al. 2006; Beigbeder et al. 2008; Conlan et al. 2008; Kim et al. 2008; Marabotti et al. 2009; Sommer et al. 2010; Martinelli et al. 2012; Stafslien et al. 2012); but also including *B. eburneus* (Wynne et al. 2000; Kavanagh et al. 2001; Sun et al. 2004; Holm et al. 2006), *B. improvisus* (Berglin & Gatenholm 1999; Singer et al. 2000; Berglin et al. 2001; Wiegemann & Watermann 2004) and *B. crenatus* (Wiegemann & Watermann 2004). While other species of barnacles such as *E. modestus* and *S. balanoides* which have a membranous-basis, have been mostly neglected in FR studies. The literature that is available on the species *E. modestus* has speculated that the membranous-basis would influence the removal of the barnacle from FR coatings (Wiegemann & Watermann 2004; Robson et al. 2009). It has been mentioned above that the flexibility of the calcareous-basal plate influences the removal stress of barnacles from silicone coatings when compared to the rigid pseudobarnacle studs, where the greater the flexibility the less force is required for removal (Chung & Chaudhury 2005). The membranous-basis of barnacles such as *E. modestus* would have a greater flexibility than calcareous-based barnacles and therefore would require even less force for removal as established by Wiegemann & Watermann (2004).

Griffith's (1921) fracture criterion and Kendall's (1971) model of fracture mechanics no longer seem suitable for predicting the detachment of real barnacles from silicone coatings (Sun et al. 2004; Ramsay et al. 2008). Recent efforts have been directed towards developing an understanding of the release behaviour of real barnacles from silicones in order to devise a new model more suitable for the detachment of real barnacles (Kavanagh et al. 2005; Hui et al. 2011). Kavanagh et al. (2005) investigated the release mechanisms in two calcareous-based barnacles (*B. eburneus* and *B. variegatus*) from PDMS coatings using a high-speed camera. Whereby it was possible to visualise the viscous properties of the adhesive, detailing its characteristics and behaviour during detachment as well as visualising the fracture process and crack

propagation. As the flexibility of the structure is important for the fracture process, the release mechanics of membranous-based barnacles can be assumed to behave differently than calcified barnacles. One objective of this thesis (Chapter 3) was to investigate how the basal structures, calcified or membranous, influenced the release mechanics and fracture behaviour of barnacles.

1.11.1. Elminius modestus: *As a test species*

E. modestus have successfully been cultured under laboratory conditions for studies in settlement behaviour specifically in relation to con- and allo-specific settlement cues (Moyse 1960; Tighe-Ford et al. 1970; Billinghurst et al. 2001; Kirby 2006). However, there has not been research into the adhesion of *E. modestus* in relation to FR coatings using laboratory-reared barnacles. Current adhesion studies on *E. modestus* have used static field immersion sites to settle and grow barnacles to adulthood (Wiegemann & Watermann 2004; Robson et al. 2009). At present no commercial FR coating has been developed through the testing phase using a barnacle with a membranous-basis. However barnacles with a membranous-basis (*Semibalanus* spp and *Elminius* spp) are important members of fouling communities (Moyse 1960; Buckeridge 1982; Southward 2008). *Elminius modestus*, for example, is important due to its abundance and dominance in the fouling community on natural and artificial structures and due to its successful invasion of European waters it is an ecologically and economically important species (Crisp & Chipperfield 1948; Knight-Jones 1948; Crisp 1958). Thus in this capacity, the development of *E. modestus* as a test species is warranted and, moreover, it would provide a valuable comparative species for studies of adult adhesion.

1.12. Thesis objectives

The objectives of this thesis were:

1) To ascertain the potential of a laboratory culture of *E. modestus* to evaluate FR coatings. This step involved an investigation in the practicality of *E. modestus* as a test species for laboratory cultures. This focused on the settlement of laboratory-

cultured cyprids, the length of time required to grow the barnacles to a testable size for CRS, the minimum size of *E. modestus* barnacles needed for CRS (Chapter 2). The calcareous-based barnacle *B. amphitrite* was used as a comparator to measure the performance of *E. modestus* against.

2) To examine how the membranous-basis influenced the adhesion and removal of barnacles from coatings compared to a calcareous-basis (Chapter 3). A high-speed camera was used to investigate the fracture process of *E. modestus* from two PDMS coatings and compared this to the fracture of *B. amphitrite*. This provided a detailed account of the separation processes of the two barnacle species.

3) To compare the use of laboratory assays and field immersion trials for evaluating FR coatings (Chapter 4). This included comparisons of the settlement and recruitment, and critical removal stress (CRS) of *E. modestus* reared in the laboratory and field environments. Furthermore, experiments to examine why there were potential differences in the CRS of the barnacles between the two culture environments were undertaken. This included examining the CRS of *E. modestus* grown on PDMS coatings at a range of different temperatures (12, 15, 19, 20°C) and on a biofilmed and an un-biofilmed surface. An additional objective was to investigate the CRS of a second membranous-based species, *Semibalanus balanoides*, from FR coatings in comparison to *E. modestus*. S. *balanoides* have a strict annual breeding season and cannot be cultured in a laboratory (Kirby 2006), thus it was necessary to use field immersion for settlement on the test coatings

4) The final objective was to examine the CRS of *E. modestus* from silicone and fluoropolymers with different surface and bulk properties (Chapter 5). This was to measure how the coating's properties influenced the adhesion of this membranous-based barnacle compared to *B. amphitrite.* This concluded whether *E. modestus* was capable of discerning between coatings for FR evaluations and whether it was suitable as a test species for future FR research.

Chapter 2: An Assessment of *Elminius modestus* (Darwin) - a Barnacle with a Membranous-Basis - as a Model Species for Evaluating Fouling-Release Coatings.

2.1. Abstract

Adult barnacles with a calcareous basis such as *Balanus amphitrite* are highly utilised in assessing the efficacy of fouling-release (FR) coatings through settlement and adhesion analysis. In contrast, species with a membranous-basis, for example *Elminius modestus,* are mostly neglected for such studies. The aim of this chapter was to examine the practicality of *E. modestus* for a laboratory culture and as a model test species, compared to the barnacle *B. amphitrite*. The percentage settlement of cyprids, rate of growth and critical removal stress (CRS) of adult barnacles from Silastic T-2 and Sylgard 184 silicone test coatings for *E. modestus* and *B. amphitrite* were evaluated. The CRS was measured using both the ASTM D 5618-94 method and an automated version of the test in shear. The percentage settlement on the silicone test coatings of the two test species did not differ. However, settlement across the repeated cultures for both species showed distinct variations and was un-predictable. When grown on Silastic T-2 and Sylgard 184, and fed *Tetraselmis suecica* algae, *E. modestus* grew at a faster rate than *B. amphitrite*. There was also a significant coating effect on the growth of *E. modestus* with barnacles on Sylgard 184 growing to larger size than those grown on Silastic T-2. The CRS of *E. modestus* was less than that for *B. amphitrite* but only for Sylgard 184. These differences likely reflect the differences in the respective basis-substratum interfaces (hard-hard vs. hard-soft) for the two species. It was concluded that *E. modestus* does provide a valuable comparative species for studies on adult barnacle adhesion in the context of FR studies.

2.2. Introduction

Elminius modestus (= *Austrominius modestus*) (Buckeridge 1982) has been described as an important fouling organism (Knight-Jones & Crisp 1953; Southward 2008). *E. modestus* is able to reproduce almost continuously throughout the year and has a short larval development period. It is therefore amenable to culture in the laboratory (Moyse 1960; Tighe-Ford et al. 1970; Billinghurst et al. 2001; Kirby 2006). With the call for testing new coatings with a broader range of fouling species (Holm et al. 2006), the previously expressed opinion that *E. modestus* is a candidate for such tests (Moyse 1960) should be revisited.

A method currently employed to evaluate the efficacy of fouling-release (FR) coatings is to measure the critical removal stress (CRS) of the barnacles attached to them, i.e. the force per unit area of basis required to completely remove the barnacle from the coating (Swain & Schultz 1996; Swain et al. 2000; Wiegemann & Watermann 2004). The ASTM D-5618 (1994) is a standard for measuring barnacle adhesion in shear. *Balanus amphitrite* (= *Amphibalanus amphitrite*) (Clare & Høeg 2008) in particular has become a model species for fouling studies, principally that of laboratory-based assessments in settlement and adhesion (Rittschof et al. 1984; 1992; Hellio et al. 2004; Aldred & Clare 2008; Conlan et al. 2008). Other species that have been used in fouling studies, but which have focussed on barnacles that have settled in the field, include *Balanus crenatus* (Wiegemann & Watermann 2004), *B. eburneus* (Swain et al. 2000; Kavanagh et al. 2005), *B. improvisus* (Singer et al. 2000; Wiegemann & Watermann 2004) and *E. modestus* (Wiegemann & Watermann 2004; Robson et al. 2009). *E. modestus* has a membranous-basis whereas the aforementioned barnacles all have a calcareous-basis. A solid (basis)-coating interface may be expected to behave differently than a soft (membrane)-coating interface in terms of fracture mechanics yet only a limited number of studies have employed *E. modestus* (Wiegemann & Watermann 2004; Robson et al. 2009). These studies were done at static field immersion sites where settlement and growth of barnacles to adulthood occurred on test coatings in the natural environment. However, field tests of coatings generally require a larger volume of test material and are criticised for being relatively time consuming compared to laboratory tests (Webster et al. 2007). Laboratory evaluations are considered useful for down-selecting coatings for field tests and for empirical studies of

model coatings (Swain 1997; Rittschof et al. 2008; Martinelli et al. 2012; Stafslien et al. 2012).

Presently, no commercial fouling-release coating has been developed through the testing phase using a barnacle with a membranous-basis. Yet barnacle genera with a membranous-basis (*Semibalanus* spp and *Elminius* spp) are important members of fouling communities (Moyse 1960; Buckeridge 1982; Southward 2008). The development of *E. modestus* as a test species is justified and it would provide a valuable comparative species for studies of adult adhesion.

The aim of this chapter was to examine the practicality of *E. modestus* as a test species including laboratory cultures. To achieve this, this chapter focused on the settlement of laboratory-cultured cyprids, the length of time required growing the barnacles to a testable size for CRS measurements and the minimum size of *E. modestus* barnacles needed to determine CRS. To rate the performance of *E. modestus*, the settlement, growth and CRS was compared to that of the calcareous-based barnacle, *B. amphitrite*. Finally, CRS values of *E. modestus* from two standard silicone coatings was measured and compared to those of *B. amphitrite*. The hypotheses to be tested are 1) that *E. modestus* is suitable as a test species and comparable in terms of settlement and growth to *B. amphitrite*, but due to its membranous-basal plate, 2) *E. modestus* would more easily be removed (lower CRS) from silicone elastomer coatings.

2.3. Materials and methods

2.3.1. Coating preparation

Glass microscope slides (76mm x 26mm x 1mm, Fisherbrand) were coated with Silastic® T-2, and Sylgard® 184 (Dow Corning). These are both commercially available polydimethylsiloxanes (PDMS) often used as standards in fouling-release studies, but should not be confused with commercial FR coatings (Sun et al. 2004; Holm et al. 2005; Statz et al. 2006; Wendt et al. 2006; Conlan et al. 2008; Ramsay et al. 2008; Rittschof et al. 2008). The coatings were sourced by International Paint Ltd., Felling, UK and prepared at Newcastle University, UK. These coatings were used for the settlement, growth and CRS measurements.

Microscope slides were acid-washed in 70% nitric acid solution for 12 hrs then rinsed with reverse osmosis (RO) water and air dried. These slides were fixed in rows to adhesive vinyl sheets on a level surface. A base coat of Dow Corning 1200OS Primer (polydimethylsiloxane tetrapropyl orthosilicate) was applied using laboratory blue roll and then dried. This primes the glass surface to allow the silicones to adhere to the microscope slides. Silastic T-2 and Sylgard 184 both consist of a two-part system of a base and a curing agent. For Silastic T-2, a ratio of 100 parts of the base silicone to 10 parts of the curing agent was mixed thoroughly in aluminium containers and applied to the microscope slides using an extra smooth, gloss paint roller. Sylgard 184 was supplied as a two-part liquid component kit, in which the volume of base and curing agent was pre-measured with set ratio of 10:1, Part A (base silicone) to Part B (curing agent). The mixing and application to the microscope slides were as before for Silastic T-2. Once the slides had been coated with the respective silicones they were left to cure for up to 48 hrs at room temperature (~19°C). The thicknesses of the coatings were measured using digital callipers at six points across each slide (Conlan et al. 2008). Before use, all coated slides were leached in RO water for 14 days, with a change of water after 7 days. After leaching, the slides were rinsed in fresh RO water and immersed in artificial seawater (ASW, 32 – 34 salinity Tropic Marin) for 1 hr. The slides were then air dried and used immediately for settlement assays.

2.3.2. Maintenance of adult barnacles

Six white polypropylene pipes (3 x 300mm long, 3 x 350mm long, all 40mm in diameter, from RS Components) were suspended from a raft at Burnham-on-Crouch, Essex (51º 37.5' N, 0º 49.3' E) on 8th April 2009 for colonisation by *E. modestus*. On 29th March 2010 the pipes together with their fouling were collected and transported back to the laboratory aquarium at Newcastle University, UK. These provided the adult brood stock that all subsequent laboratory cultures were raised from. The adults were maintained in 2 plastic aquarium tanks (one 21 x 28 x 37cm containing 18L, the second 22 x 25 x 47cm containing 20L of ASW) in an aquarium system with re-circulating ASW, at 19 ± 1 ºC on a 16:8 hour light and dark (L:D) cycle. The individual tanks were aerated and the barnacles were fed newly hatched *Artemia* sp. nauplii (Great Salt Lake Brine Shrimp Co.) daily. Every two weeks the tanks and adults were cleaned with fresh water, removing any build-up of detritus with a stiff bristled brush.

Adult *B. amphitrite* were obtained from Duke University Marine Laboratory, Beaufort, North Carolina, USA. New populations of adults were received approximately every 5 – 7 months. These were maintained in semi-static culture at Newcastle University in ~15L of aerated 1µm filtered, UV-treated seawater, at 28ºC on a 16:8 L:D cycle; the seawater was collected from the North-East coast of England. The adults were fed *Artemia* sp., and were cleaned and the water changed daily.

2.3.3. Larval culture

2.3.3.1. Elminius modestus

The method used for the culture of *E. modestus* was modified from the protocol established by Kirby (2006). Initially, the adult barnacles were cleaned and gently scrubbed using a stiff bristled brush under freshwater. They were removed from water overnight, but were covered by moistened laboratory blue roll and the tanks were covered with aluminium foil to prevent desiccation. The following morning, the adults were returned to the tanks that had been filled with 50µm-filtered ASW. External to the tanks, a point fibre-optic cold light source was positioned close to the water's surface. After being released from the adults the phototactic nauplii swam towards this light. The larvae were then collected by pipette and transferred to a beaker containing ASW and *Skeletonema marinoi* (Seasalter Shellfish (Whitstable) Ltd., UK). Once collected, the nauplii were counted. Larval releases of a 4 hr duration produced between 12,000 and 35,000 nauplii. The nauplii were transferred to 7L of 1µm filtered (glass fibre filter) ASW treated with antibiotics (21.9mgl^{-1} penicillin G and 36.5mgl^{-1} streptomycin sulphate; Sigma Aldrich (Rittschof et al. 1992)) and aerated. A maximum of 20,000 nauplii was added to each 7L of ASW. The culture was maintained in an incubator set at 22 ± 1°C with a 12:12 L:D cycle. The nauplii were fed with 600ml *S. marinoi* daily at a concentration of ca. 1 x 10^5 cell ml^{-1} (Kirby 2006).

On day four of the culture the larvae were removed using a tier of mesh filters (300µm, 250µm and 160µm). Nauplii that were retained in the 250µm and 300µm filters were placed in 7L of fresh 1µm filtered ASW treated with antibiotics and returned to the 22°C incubator. Following the protocol established by Kirby (2006), any nauplii retained in the 160µm filter were discarded to minimise asynchronous cultures. *E. modestus* larvae reached the cypris stage on day seven or eight of the

culture. The cyprids were filtered as described previously and retained on the 250µm filter. These cyprids (aged day zero) were used immediately for settlement assays as cyprids of this age provided the optimal level of settlement (Kirby 2006) (cf. *B. amphitrite* below).

2.3.3.2. Balanus amphitrite

The procedures for release and collection of nauplii followed those described for *E. modestus*. On collection the nauplii were transferred to 7L of 1µm filtered seawater, maintained at 28°C on a 16:8 L:D cycle, treated with antibiotics (see above) and fed *S. marionoi*. Cyprids were present after five days of incubation. The culture was filtered through the mesh series (300µm, 250µm and 160µm) and cyprids retained in the 300µm and 250µm filters were stored for three days in the dark at 5 – 6°C prior to settlement assays (Rittschof et al. 1992; Billinghurst et al. 1998).

2.3.4. Influence of the culture medium on the settlement of Elminius modestus

Preliminary attempts to culture *E. modestus* were carried out to assess the influence of the culture medium on the settlement of cyprids. After collection and collation of the nauplii (> 12,000), the culture was divided in two. Half was placed in 7L of 1µm filtered ASW and half in 7L of 1µm filtered seawater (FSW); both were treated with antibiotics as before. The cultures were maintained at 22 ± 1°C with a 12:12 L:D cycle and fed *S. marinoi* daily. On day four of the culture, the separate cultures were filtered through the filter series and returned to fresh ASW and FSW, respectively. After the appearance of cyprids, settlement assays (see below) were conducted using sterile, polystyrene 24-well plates (Iwaki®) with ten cyprids per well containing 2ml of the appropriate culture medium filtered to 0.2µm. The experiment was repeated three times.

2.3.5. Settlement assays

2.3.5.1. 24-well plate assays

The competence of laboratory-reared *E. modestus* cyprids to settle was initially assessed using untreated, sterile, polystyrene 24-well plates (Iwaki®). To test for competency to settle, ten cyprids were placed in each well (n = 12) with 2ml of 0.2µm filtered ASW. After 24 and 48 hrs of incubation at 22 ± 1°C, the number of settled cyprids was counted and the average percentage of individuals settled per well was calculated. For each culture of cyprids, the competence to settle was assessed in this manner as a baseline for settlement on coated surfaces.

For comparison, settlement of laboratory-reared *B. amphitrite* cyprids was assessed against settlement of *E. modestus* cyprids. The culture of *E. modestus* was run in parallel to the culture of *B. amphitrite* for each culture number, so the settlement assay was performed at the same time. As with *E. modestus*, ten *B. amphitrite* cyprids were placed in each well (n = 12) with 2ml of 0.2µm filtered ASW. After 24 and 48 hrs of incubation at 28 ± 1°C, the percentages of settled individuals were calculated and the average recorded. The settlement of three repeat cultures of *B. amphitrite* and *E. modestus* were compared.

2.3.5.2. Settlement on coated surfaces

The propensity for *E. modestus* cyprids to settle on standard silicone coatings was assessed. For this, eight slides coated with Silastic T-2 and eight slides coated with Sylgard 184 housed in quadriPERM® culture vessels, were each seeded with 20 cyprids. These were pipetted into a 2ml droplet of 0.2µm filtered ASW centred on the coated slides. The settlement of *B. amphitrite* cyprids was assessed for comparison on a different set of Silastic T-2 and Sylgard 184 coated slides. The settlement of three repeat cultures of *E. modestus* and *B. amphitrite* cyprids, the same three repeat cultures of cyprids used for the settlement on the 24-well plates, were used for the settlement on the silicone surfaces. Slides with cyprids of *E. modestus* and *B. amphitrite* were incubated at 22 ± 1°C and 28 ± 1°C, respectively for 48 hrs when the numbers of settled individuals were recorded. Having an increased number of cyprids within the 2ml

droplet should increase the prospect for settlement on the silicone coatings (Clare et al. 1994; Elbourne et al. 2008).

After recording the number of settled individuals, 15ml of *Tetraselmis suecica* was added to each chamber of the culture vessels. The newly settled juvenile barnacles were then grown on for use in CRS tests, whereby they were maintained at their respective temperatures on a 12:12 L:D cycle and fed 15ml of *T. suecica* (~3 x 10^5 cells ml^{-1}) three times a week (Monday, Wednesday and Friday); their water being changed at each feeding.

2.3.6. Growth measurements

The growth of 1) a single culture of *E. modestus*, and 2) a culture of *E. modestus* grown in parallel to a culture of *B. amphitrite*, all on Silastic T-2 and Sylgard 184 coated microscope slides, was measured over time. The basal areas of the *E. modestus* and *B. amphitrite* barnacles were recorded starting 7 days after settlement and then on a weekly basis for a minimum 18 weeks. Over the growth period barnacles needed to be removed to prevent overcrowding. From the first and second cultures of *E. modestus* a total of 35 and 19, and 25 and 17 barnacles were removed from the Silastic T-2 and Sylgard 184 coatings, respectively. From the *B. amphitrite* culture a total of 36 and 29 barnacles were removed over the growth period from Silastic T-2 and Sylgard 184, respectively. The barnacles which were removed, were selected based on their proximity to another individual barnacle or to the edge of the slide. The silicone-coated microscope slides with the settled barnacles were scanned (HP Scanner 5400C) from beneath the slides at a resolution of 1200dpi. The area (mm^2) of the basis of each barnacle was calculated from digital images using ImageJ software (Rasband 1997; Abramoff et al. 2004). With the first culture of *E. modestus* at 14 weeks, when the average basal diameter was ~3mm (basal area ~7mm^2), *Artemia* sp. were fed to the barnacles maintained in quadriPERM® culture vessels. However, subsequent to this change of diet there was 40% mortality in the dishes. Consequently, the feed reverted to *T. suecica*. The second culture of *E. modestus* and single culture of *B. amphitrite* were settled and grown at the same time and maintained on the same diet of *T. suecica.*

2.3.7. Critical removal stress measurements

2.3.7.1. Influence of size of Elminius modestus *on the critical removal stress*

To assess the minimum size for *E. modestus*, the CRS from multiple cultures of cyprids settled on Silastic T-2 were collated. Four batches of Silastic T-2 (produced at different times) were used for the settlement of the cyprids. These were grown for varying lengths of time between 13 to 28 weeks, to produce a range of individuals with sizes from ~2.57mm to 5.78mm in diameter. The CRS was measured using the ASTM D-5618-94 (a manual method) and a bespoke, automated instrument (Advanced Analysis and Integration Ltd, Manchester, UK) according to Conlan et al. (2008). Briefly, this entailed a sample slide with settled barnacles being placed within a motorised platform above an inbuilt camera which captured a silhouette image of the barnacles and the software determined the barnacles basal area (mm^2). The platform moves towards a fixed push-off bar, where the rate of advance of the platform was set at a constant speed of 90mm min^{-1}. When the platform came into contact with the barnacle the force (N) needed to remove the barnacle from the slides surface/coating was recorded. The in-built software standardises the removal force (N) by dividing it by the basal area (mm^2) to provide the CRS measurements of megapascals (MPa). For the manual method, a hand-held spring force gauge (PSM-2K, IMADA Co. Ltd, 0.2kg KgF) was used to measure the force (N) to remove the barnacles. The instrument was positioned at the base of the barnacle and the force applied parallel to the surface at a rate of ~ 4.5Ns^{-1} as per the ASTM D-5618 (1994) standard for measuring barnacle adhesion in shear. The barnacles were scanned (HP Scanner 5400C) prior to detachment and ImageJ software (Rasband 1997; Abramoff et al. 2004) was used to calculate the basal area (mm^2) from the digital images. The manual and automated methods were used to measure the CRS of 196 and 135 barnacles, respectively. The CRS data was pooled and ranked in order of magnitude and the averages were taken of every 10 (automated method) and 15 individuals (hand-held force gauge). The data for *E. modestus* that were incompletely removed, where the basal membrane remained attached to the surface, were discarded.

2.3.7.2. The critical removal stress of Elminius modestus *and* Balanus amphitrite

For the critical removal stress (CRS) measurements, one culture of *E. modestus* cyprids and one culture of *B. amphitrite* cyprids were settled and grown on separate Silastic T-2 and Sylgard 184 coated microscope slides. The barnacles were grown for a period of 20 weeks, with an approximate average size of 4.5mm and 3.9mm in diameter for *E. modestus* and *B. amphitrite*, respectively. The CRS was measured using the automated method (Conlan et al. 2008).

2.3.8. Statistical analysis

2.3.8.1. Laboratory settlement assays

Data sets were arcsine transformed and tested for a normal distribution (Kolmogorov-Smirnov test) (Ennos 2012) and a homogeneous variance (Levene's test) (Quinn & Keough 2002). Two, two-factor repeated measures ANOVAs with a 0.05 significance level and a *post hoc* Tukey's comparison including repeat cultures (3 repeats) and time (24 hr and 48 hr) as co-factors in both tests (Quinn & Keough 2002), was used to test the null hypotheses: 1) there was no difference in the percentage settlement of *E. modestus* cyprids cultured in ASW or FSW and 2) there was no difference in the percentage settlement of *E. modestus* and *B. amphitrite* cyprids. A three-factor ANOVA with 0.05 significance level and a *post hoc* Tukey's comparison including repeated cultures (3 cultures), time (24 hr and 48 hr) and species (*E. modestus* and *B. amphitrite*) as co-factors, was used to test the null hypothesis that there was no difference in the percentage settlement of *E. modestus* cyprids and *B. amphitrite* cyprids.

2.3.8.2. Growth

The data sets were checked for a normal distribution and a homogeneous variance using a Kolmogorov-Smirnov test (Ennos 2012) and Levene's test (Quinn & Keough 2002), respectively. For the single culture of *E. mod*estus, a repeated measures ANOVA with a 0.05 significance level and a Bonferroni pairwise comparison was used to compare the size (basal area) of the barnacles at 6, 12, 18 and 28 weeks, on Silastic T-2 and Sylgard 184 coated surfaces. This was to test the null hypothesis that there was no difference in the growth of *E. modestus* on Silastic T-2 compared to Sylgard 184. A

two-factor repeated measures ANOVA with a 0.05 significance level and a Bonferroni pairwise comparison was used to measure the growth of *E. modestus* and *B. amphitrite* at 6, 12 and 18 weeks, on the two silicone coatings (Silastic T-2 and Sylgard 184). This was to test the null hypothesis that there was no difference in the growth of *E. modestus* compared to the growth of *B. amphitrite* across the two coatings.

2.3.8.3. Critical removal stress

The null hypothesis examined was that there was no difference in the CRS of the two barnacle species when grown on the test coatings. Data were transformed using log10 after an initial Kolmogorov-Smirnov and a Levene's test showed that the distribution and variance were neither normal nor homogeneous. A three-factor nested ANOVA with a 0.05 significance level was then used on the transformed data, including the interaction effect of species x coating (Quinn & Keough 2002).

2.4. Results

2.4.1. Influence of the culture medium on the settlement of Elminius modestus

The settlement data were normally distributed (24 hrs: $df = 15$, $D = 0.143$, $P = 0.094$ and 48 hrs: $df = 15$, $D = 0.119$, $P = 0.150$) with homogeneous variance (24 hrs: $df1 = 2$, $df2 = 13$, $F = 0.27$, $P = 0.845$ and 48 hrs: $df1 = 2$, $df2 = 13$, $F = 1.10$, $P = 0.418$). The null hypothesis that there was no difference in the percentage settlement of *E. modestus* cyprids cultured in ASW to the percentage settlement of cyprids cultured in FSW, was confirmed. Therefore, the culture medium had no influence on the settlement of *E. modestus* cyprids ($df = 1$, $F = 0.027$, $P = 0.871$) (Figure 2.1; Table 2.1). However, there was a difference in the percentage settlement between each of the three repeat cultures ($df = 2$, $F = 21.938$, $P \leq 0.001$). The percentage settlement of cyprids from culture 2 was higher than the settlement of cyprids from cultures 1 and 3. The settlement of cyprids from culture 1 was higher than the settlement of cyprids from culture 3 (culture 1 vs 2 Tukey's $P = 0.003$; culture 1 vs 3 Tukey's $P = 0.006$; culture 2 vs 3 Tukey's $P \leq 0.001$). There was also an interaction effect of water x culture number ($df = 2$, $F = 0.7362$, $P = 0.007$). This suggests that there was a difference in the

settlement of cyprids between the two types of water, Figure 2.1, reveals that the clearest difference in the percentage settlement of cyprids in ASW compared to FSW was for culture 1 at 48 hrs in which ASW was higher than FSW. The replicate assays were, however highly variable and this was especially the case for the other cultures and times.

In addition the percentage of settled cyprids increased over time, with a significantly greater percentage settling after 48 hrs than after 24 hrs (df = 1, F = 28.295, $P < 0.001$). However the interaction of time x culture number (df = 2, F = 3.178, P = 0.075), water x time (df = 1, F = 2.270, P = 0.144) and water x time x culture number (df = 2, F = 1.718, P = 0.199) demonstrates that this was not the case across the three repeat cultures for both types of water; settlement did not increase significantly from 24 to 48 hrs in all circumstances.

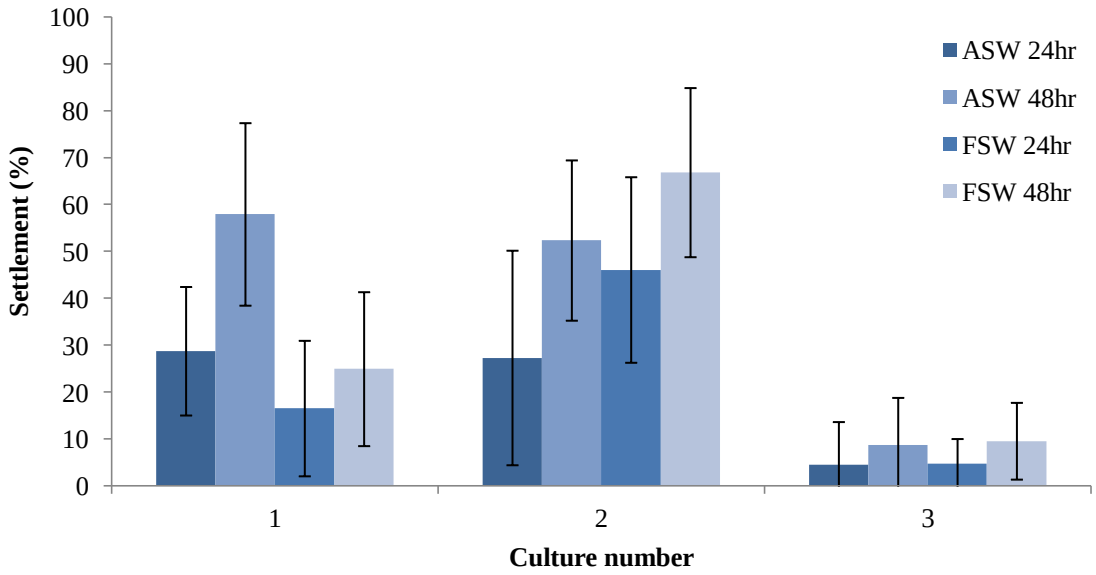

Figure 2.1. Mean percentage settlement (±1 SD) of *Elminius modestus* cyprids cultured in 1µm filtered artificial seawater (ASW) and 1µm filtered seawater (FSW) at 24 and 48 hrs in polystyrene well plates.

Table 2.1. ANOVA table of results for the settlement of *Elminius modestus* cultured in 1µm filtered artificial seawater (ASW) and 1µm filtered seawater (FSW).

	Sum of Squares	***Mean Square***	***df***	***F-value***	***P-value***
Water	**0.001**	**0.001**	**1**	**0.027**	**0.871**
Culture number	**2.121**	**1.060**	**2**	**21.938**	**≤ 0.001**
Time	**0.498**	**0.498**	**1**	**28.295**	**≤ 0.001**
Time x culture number	**0.112**	**0.059**	**2**	**3.178**	**0.075**
Water x culture number	**0.662**	**0.331**	**2**	**7.632**	**0.007**
Water x time	**0.041**	**0.041**	**1**	**2.270**	**0.144**
Water x time x culture number	**0.062**	**0.031**	**2**	**1.718**	**0.199**

2.4.2. Settlement of Elminius modestus *and* Balanus amphitrite

The data were normally distributed (24 hrs: $df = 70$, $D = 0.178$, $P = 0.051$ and 48 hrs: $df = 70$, $D = 0.117$, $P = 0.095$) with homogeneous variance (24 hrs: $df1 = 4$, $df2 = 66$, $F = 1.702$, $P = 0.147$ and 48 hrs: $df1 = 4$, $df2 = 66$, $F = 1.185$, $P = 0.326$). The null hypothesis that the percentage settlement of *E. modestus* cyprids would be equal to the percentage settlement of *B. amphitrite* was not supported. There was a significant difference in the settlement between the two barnacle species, with the settlement of *B. amphitrite* being higher than that of *E. modestus* ($df = 1$, $F = 9.971$, $P = 0.003$) (Figure 2.2; Table 2.2). However, the interaction of species x culture number demonstrates that this was not the case across all three repeat cultures, with the percentage settlement of *E. modestus* and *B. amphitrite* being equal in some but not all circumstances ($df = 2$, $F = 2.595$, $P = 0.090$). From Figure 2.2, the percentages of settled cyprids between the two species for culture 1 and 2 were similar.

There were significant differences in the settlement across the three repeat cultures for both species, with the settlement of culture 1 being less than that for culture 2 and culture 3 (culture 1 vs 2 Tukey's $P = 0.002$; culture 1 vs 3 Tukey's $P < 0.001$ and culture 2 vs 3 Tukey's $P = 0.075$). For *E. modestus* and *B. amphitrite* the percentage of settled cyprids increased over time with a significantly greater percentage settling after 48 hrs than after 24 hrs ($df = 1$, $F = 137.00$, $P < 0.001$). The interaction effect of time x culture number shows this to be true across all three of the repeat cultures ($df = 2$, $F =$

10.038, $P < 0.001$). However the interaction effects of species x time ($df = 1$, $F = 0.042$, $P = 0.838$) and species x time x culture number ($df = 2$, $F = 1.834$, $P = 0.176$) suggests that the differences between the settlement after 24 and 48 hrs are not equally significant for both *E. modestus* and *B. amphitrite* across the three cultures. From Figure 2.2, there appears to be a greater difference in the settlement of *E. modestus* between 24 and 48 hrs specifically for cultures 2 and 3.

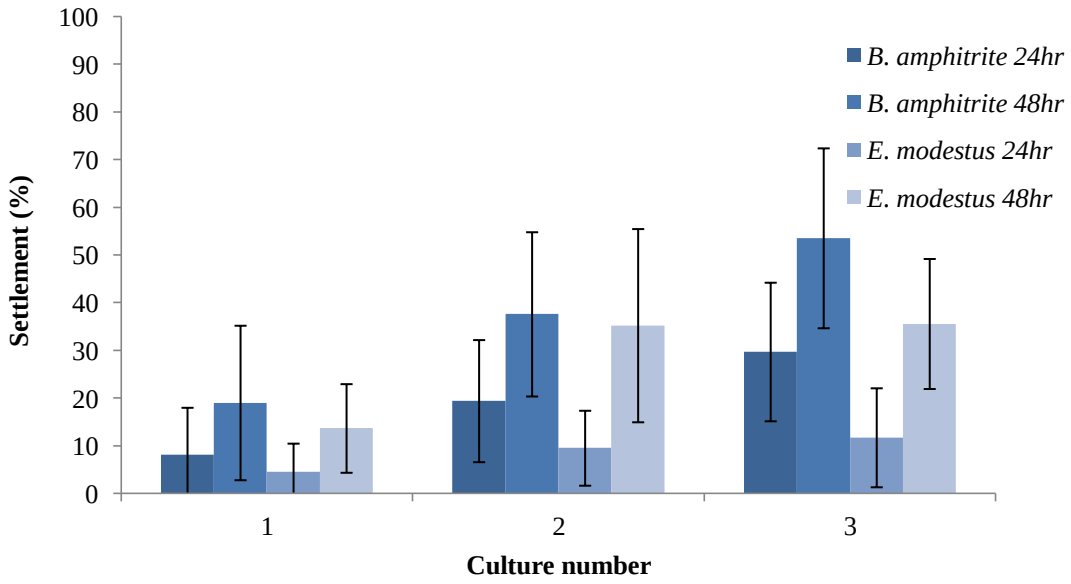

Figure 2.2. Mean percentage settlement (±1 SD) of *Elminius modestus* and *Balanus amphitrite* cyprids in Iwaki 24-well plates after 24 and 48 hrs.

Table 2.2. ANOVA table of results for the settlement of *Elminius modestus* and *Balanus amphitrite*.

	Sum of Squares	*Mean Square*	*df*	*F-value*	*P-value*
Species	**0.477**	**0.477**	**1**	**9.971**	**0.003**
Culture number	**1.533**	**0.767**	**2**	**19.120**	**≤ 0.001**
Time	**1.718**	**1.718**	**1**	**137.691**	**≤ 0.001**
Species x culture number	**0.248**	**0.124**	**2**	**2.595**	**0.090**
Time x culture number	**0.251**	**0.125**	**2**	**10.038**	**≤ 0.001**
Species x time	**0.001**	**0.001**	**1**	**0.042**	**0.838**
Species x time x culture number	**0.037**	**0.018**	**2**	**1.838**	**0.176**

The settlement data for *E. modestus* and *B. amphitrite* on silicone coated slides were normally distributed (*df* = 95, *D* = 0.099, *P* = 0.200) with homogeneous variance (*df1* = 11, *df2* = 84 *F* = 2.013, *P* = 0.056). The null hypothesis that the percentage settlement of *E. modestus* cyprids on silicone coatings would be equal to the settlement of *B. amphitrite* cyprids was confirmed. The settlement of *E. modestus* on the silicone-coated slides (Figure 2.3) was consistent with the settlement of *B. amphitrite* (*df* = 1, *F* = 4.713, *P* = 0.062) (Table 2.3). The settlement between the two silicone coatings was also consistent (*df* = 1, *F* = 2.513, *P* = 0.117). However, the settlement between the repeat cultures of *E. modestus* and *B. amphitrite* cyprids differed (*df* = 2, *F* = 5.809, *P* = 0.004), with settlement of cyprids from culture 1 being lower than for cyprids from culture 2 (culture 1 vs 2 Tukey's *P* = 0.003; culture 1 vs 3 Tukey's *P* = 0.099; culture 2 vs 3 Tukey's *P* = 0.402). The interaction effects between species x coating (*df* = 1, *F* = 2.850, *P* = 0.095), species x culture number (*df* = 2, *F* = 0.655, *P* = 0.528), culture number x coating (*df* = 2, *F* = 0.316, *P* = 0.730) and culture number x coating x species (*df* = 2, *F* = 0.530, *P* = 0.590) had no significant influence on the percentage settlement of the barnacles.

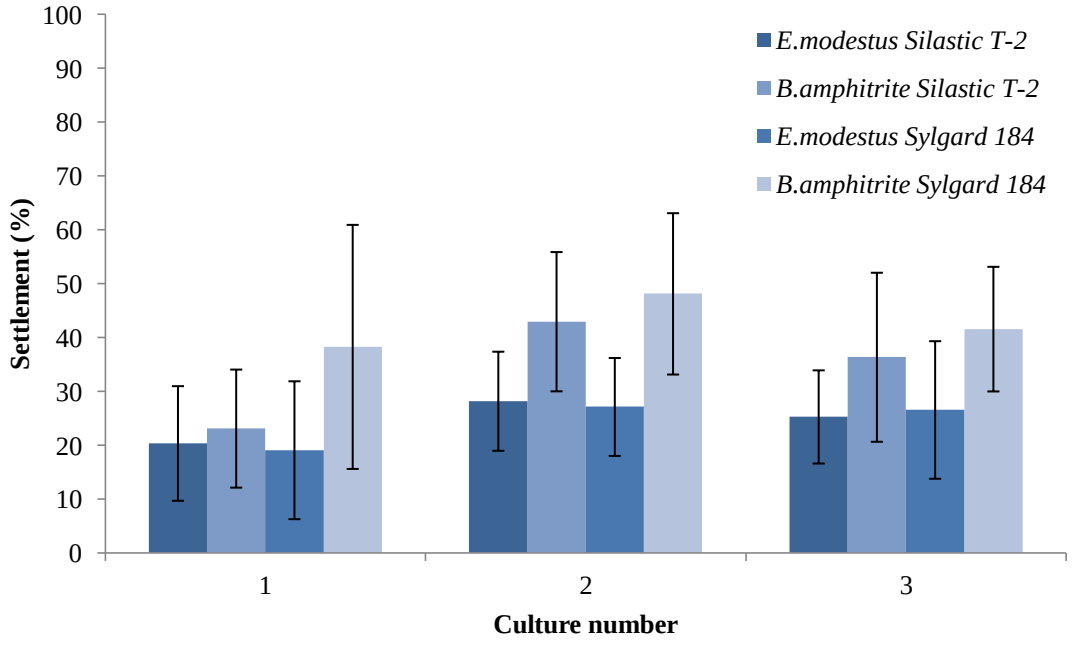

Figure 2.3. Mean percentage settlement (±1 SD) of *Elminius modestus* and *Balanus amphitrite* cyprids on Silastic T-2 and Sylgard 184 coated microscope slides after 48 hrs.

Table 2.3. ANOVA table of results for the settlement of *Elminius modestus* and *Balanus amphitrite* cyprids settled on Silastic T-2 and Sylgard 184 coated microscopes slides.

	Sum of Squares	*Mean Square*	*df*	*F-value*	*P-value*
Species	**0.059**	**0.059**	**1**	**4.713**	**0.062**
Culture number	**0.238**	**0.119**	**2**	**5.809**	**0.004**
Coating	**0.051**	**0.051**	**1**	**2.513**	**0.117**
Species x culture number	**0.026**	**0.013**	**2**	**0.644**	**0.528**
Species x coating	**0.058**	**0.058**	**1**	**2.850**	**0.095**
Culture number x coating	**0.013**	**0.006**	**2**	**0.316**	**0.730**
Species x culture number x coating	**0.022**	**0.011**	**2**	**0.530**	**0.590**

2.4.3. Growth

Figure 2.4 illustrates the average growth of *E. modestus* from the first culture on Silastic T-2 and Sylgard 184 over a period of 28 weeks. The growth data for *E. modestus* on both coatings presented a normal distribution (df = 58, $D \geq 0.963$, $P \geq 0.093$) with homogeneous variance ($df1$ = 2, $df2$ = 56, $F \geq 1.523$, $P \geq 0.222$). The focus of this statistical test was to compare the growth of barnacles between two silicone coatings, the null hypothesis being that the basal growth of *E. modestus* barnacles on Silastic T-2 was equal to that on Sylgard 184. This hypothesis was not confirmed, there was a difference in the basal area of *E. modestus* barnacles grown on the two silicone coatings, with those grown on Sylgard 184 growing to a larger size (df = 1, F =23.646, $P \leq 0.001$) (Table 2.4). However, the interaction effect of coating x time was not significant, suggesting the difference in the size of the barnacles between the two coatings was not significant at every time point tested (df = 3, F = 0.749, P = 0.524). The basal area of *E. modestus* changed over the 28 weeks; at each measurement (6, 12, 18 and 28 weeks) the size of *E. modestus* was significantly larger than the time before i.e. the average size of barnacles at 12 weeks were larger than barnacles at 6 weeks, barnacles at 18 weeks were larger than those at 12 (and 6) weeks and barnacles at 28 week were larger than those at 18 (and 6 and 12) weeks (df = 3, F = 324.792, $P \leq 0.001$; all pairwise comparisons $P \leq 0.001$).

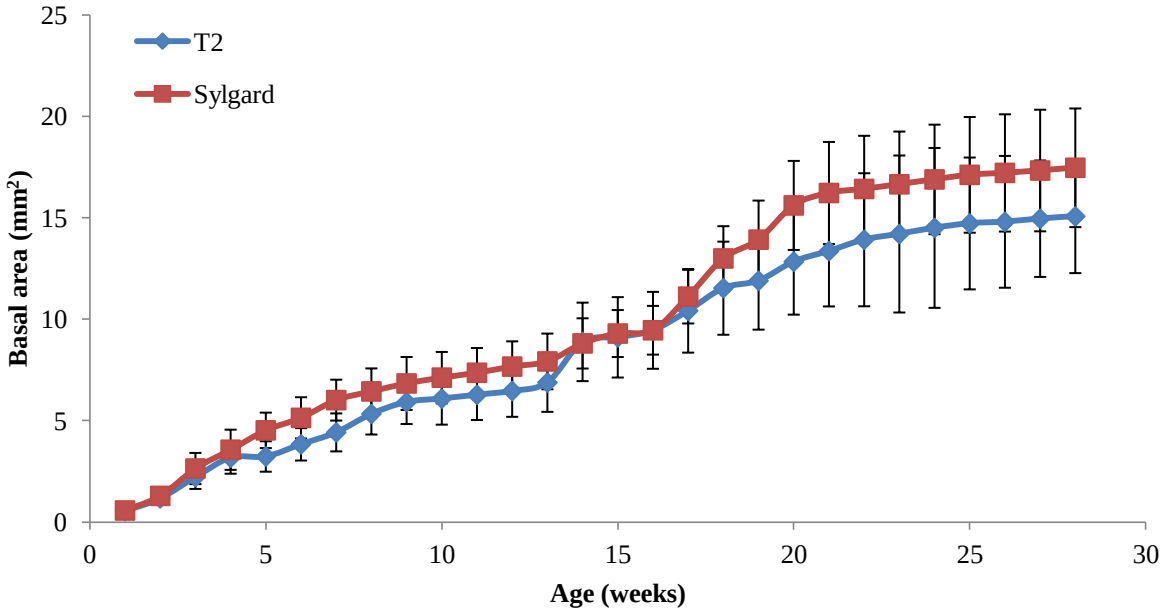

Figure 2.4. The mean weekly growth rate (± 1 SD) of *Elminius modestus* on Silastic T-2 and Sylgard 184 (n ≥ 31 and 28, T2 & Sylgard, respectively).

Table 2.4. ANOVA table of results for the growth of *Elminius modestus* on Silastic T-2 and Sylgard 184 coated microscope slides.

	Sum of Squares	***Mean Square***	***df***	***F-value***	***P-value***
Time	**3414.258**	**1138.086**	**3**	**324.792**	**≤ 0.001**
Coating	**109.140**	**109.140**	**1**	**23.646**	**≤ 0.001**
Coating x time	**7.876**	**2.625**	**3**	**0.749**	**0.524**

Figure 2.5 shows the average growth of *E. modestus* and *B. amphitrite* cultures on Silastic T-2 and Sylgard 184. The growth data for *E. modestus* and *B. amphitrite* on both coatings presented a normal distribution (*E. modestus; df* = 48, $D \geq 0.150$, $P \geq 0.200$ and *B. amphitrite; df* = 48, $D \geq 0.176$, $P \geq 0.124$) with homogeneous variance (*E. modestus*; *df1* = 4, *df2* = 44, $F \geq 3.147$, $P \geq 0.067$ and *B. amphitrite*; *df1* = 4, *df2* = 44, $F \geq 3.571$, $P \geq 0.056$). The null hypothesis that the growth of *E. modestus* was equal to the growth of *B. amphitrite* across the two coatings, was not supported (*df* = 1, *F* = 38.658, $P \leq 0.001$) (Table 2.5). The basal area of *E. modestus* was found to be significantly greater than the basal area of *B. amphitrite*, indicating a quicker rate of

growth. Overall, the basal areas of the barnacles significantly increased over the growth period (df = 2, F = 107.410, $P \leq 0.001$), however the pairwise comparison shows that this was only significant between barnacles at 6 and 12 weeks, in which the latter was larger than the former ($P \leq 0.001$), whereas, the size of barnacles at 18 weeks were not significantly larger than those at 12 weeks (P = 0.268). The interaction effect of time x species was significant, indicating that there was a distinct difference between the basal area of the two barnacle species across the three time periods (df = 2, F = 16.841, $P \leq$ 0.001). There was also a significant difference due to the coating, in which the basal areas of the barnacles were larger for those grown on Sylgard 184 than on Silastic T-2 (df = 1, F = 12.176, P = 0.001). Although the interaction effects of coating x species (df = 1, F = 0.279, P = 0.598), time x coating (df = 2, F = 0.098, P = 0.906), and time x coating x species (df = 2, F = 0.120, P = 0.887) demonstrates that this was not the case in all circumstances. From these interactions, it suggests that the differences between the two silicone coatings were not significant for both barnacle species or at each time point. In Figure 2.5 there appears to be a greater difference in the size of *E. modestus* between the two coatings than for *B. amphitrite,* and that the differences in size between the coatings is less distinct at 6 weeks than it is at 12 and 18 weeks, and this is more apparent for *E. modestus*.

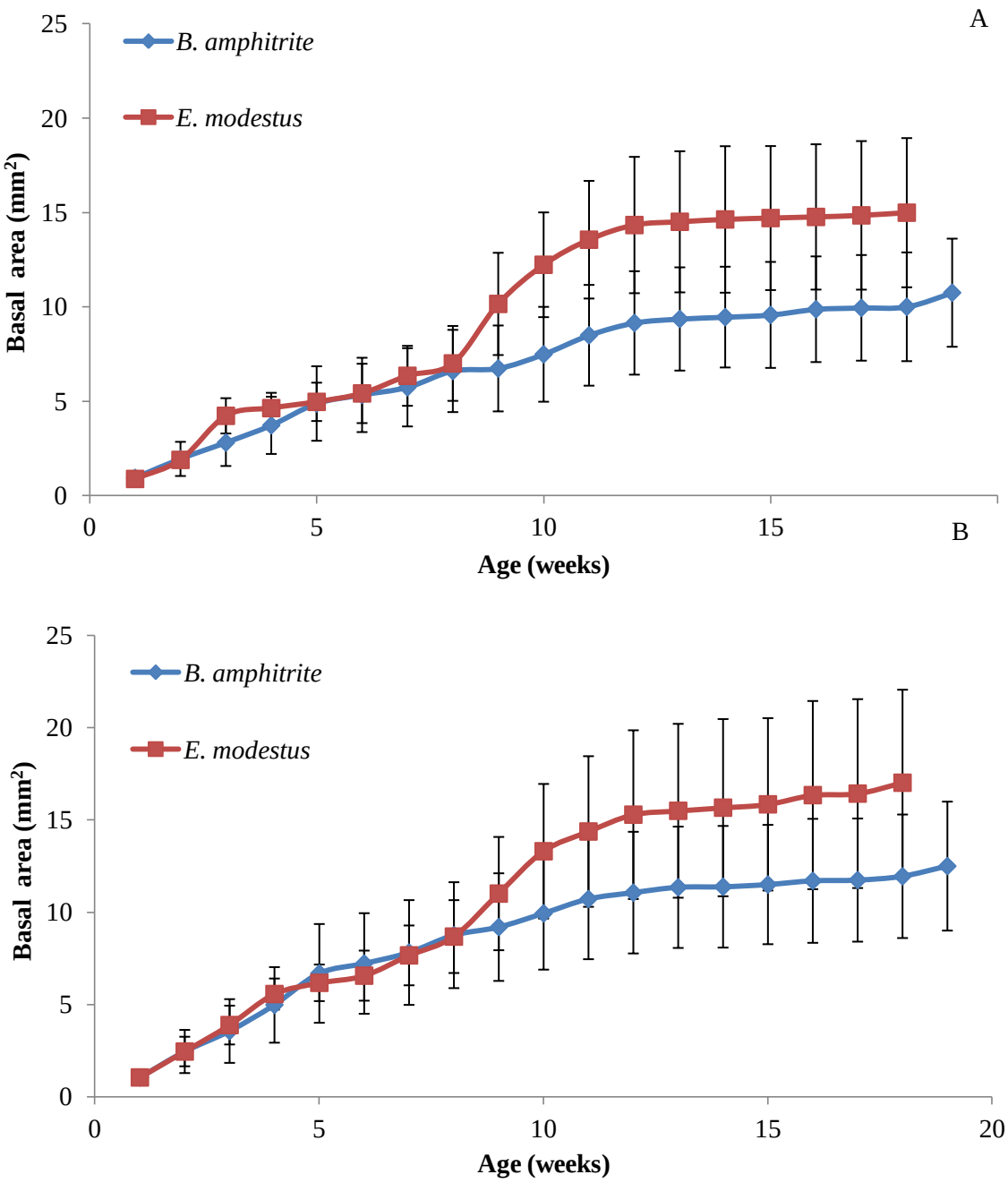

Figure 2.5. The mean weekly growth rate (± 1 SD) of *Balanus amphitrite* and *Elminius modestus* cultured in 2009 on Silastic T-2 (A) and Sylgard 184 (B). The total number of barnacles used to measure growth is presented in Table 2.6.

Table 2.5. ANOVA table of results for the growth of *Elminius modestus* and *Balanus amphitrite* on Silastic T-2 and Sylgard 184 coated microscope slides.

	Sum of Squares	*Mean Square*	*df*	*F-value*	*P-value*
Species	**404.095**	**404.095**	**1**	**38.658**	**≤ 0.001**
Time	**18000.996**	**900.498**	**2**	**107.410**	**≤ 0.001**
Coating	**127.281**	**127.281**	**1**	**12.176**	**0.001**
Time x species	**282.389**	**141.195**	**2**	**16.841**	**≤ 0.001**
Time x coating	**1.650**	**0.825**	**2**	**0.098**	**0.906**
Coating x species	**2.921**	**2.921**	**1**	**0.279**	**0.598**
Time x species x coating	**2.017**	**1.009**	**2**	**0.120**	**0.887**

Table 2.6. Numbers (n) of *Balanus amphitrite* and *Elminius modestus* barnacles at 6, 12 and 18 weeks, on Sylgard 184 and Silastic T-2 coated microscope slides. Total number of barnacles collated from multiple slides.

Weeks	*Balanus amphitrite*				*Elminius modestus*			
	Sylgard 184		*Silastic T-2*		*Sylgard 184*		*Silastic T-2*	
	number of barnacles	*number of slides*	*number of barnacles*	*number of slides*	*number of barnacles*	*number of slides*	*number of barnacles*	*number of slides*
6	**66**	**12**	**100**	**11**	**42**	**12**	**30**	**12**
12	**59**	**12**	**63**	**10**	**40**	**12**	**26**	**10**
18	**53**	**12**	**51**	**10**	**33**	**10**	**21**	**9**

2.4.4. Critical removal stress measurements

2.4.4.1. Influence of size of Elminius modestus *on the critical removal stress*

The diameter of the bases of the 135 barnacles tested using the automated machine, ranged from 2.5mm to 5.8mm with CRS values of 0.041MPa to 0.335MPa. Both size and CRS ranges were greater than the barnacles removed using the manual method. Of the 196 barnacles removed using the manual method, the diameter of the barnacles varied from 2.9mm to 5.1mm with a range in the CRS from 0.048 MPa to 0.228MPa (Figure 2.6).

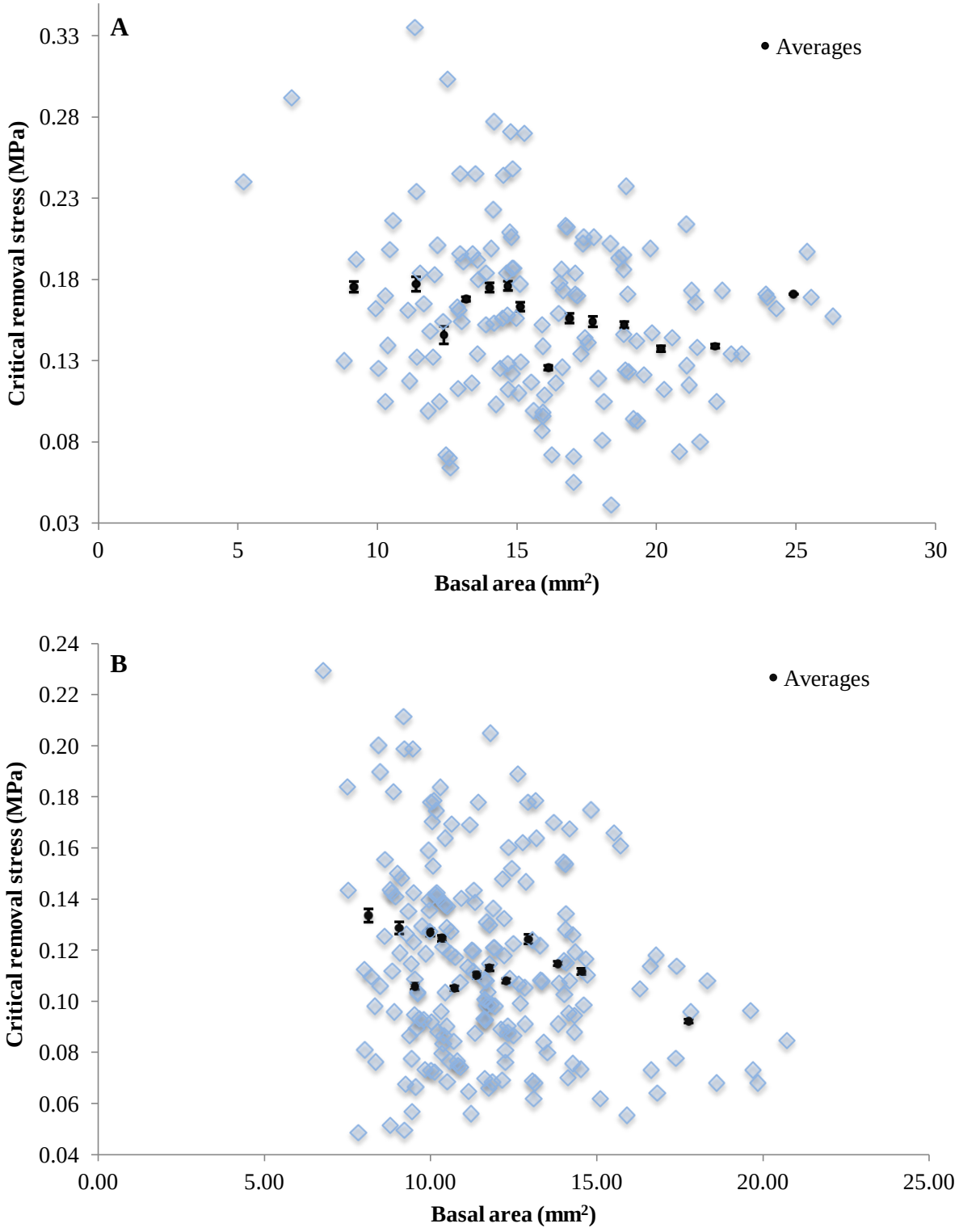

Figure 2.6. The critical removal stress of *Elminius modestus* from Silastic T-2 when using the (A) automated method (n = 135) and (B) manual method (n = 196) as a function of basal area. The averages (± variance) were calculated from every 10 (A) and 15 (B) individuals which were ranked according to size.

The variability in the CRS of barnacles removed using the automated method reduced initially for barnacles approximately 4.1mm in diameter (~13mm^2), as seen by a decrease in the variance. For the manual method, the variability in the CRS reduced for barnacles approximately 3.6mm in diameter (~10mm^2). (For comparison data on *B. amphitrite* see Conlan et al. 2008).

2.4.4.2. A comparison of critical removal stress of Elminius modestus *and* Balanus amphitrite

The CRS data were transformed using log10, after which they were normally distributed (df = 129, D = 0.043, P = 0.200) with homogeneous variance ($df1$ = 27, $df2$ = 101, F = 1.446, P = 0.097). The null hypothesis that the CRS of the two barnacle species would be equal, was not supported. The CRS of *E. modestus* was significantly less than that of *B. amphitrite* (df = 1, F = 4.046, P = 0.046) (Figure 2.7; Table 2.7). The interaction effect of species x coating (df = 1, F = 1.104, P = 0.295) shows that this is not the case for both silicone coatings; there was only a significant difference between the two barnacle species for one of the silicone coatings – Sylgard 184 (Figure 2.7). Nevertheless, the two coatings did not differ significantly with respect to the CRS values overall (df = 1, F = 0.249, P = 0.619).

The CRS data was generated using barnacles across eight slides per coating, yet there were no nesting effects due to a difference between slides for barnacles removed from Silastic T-2 and Sylgard 184 (df = 7, F = 1.034, P = 0.432).

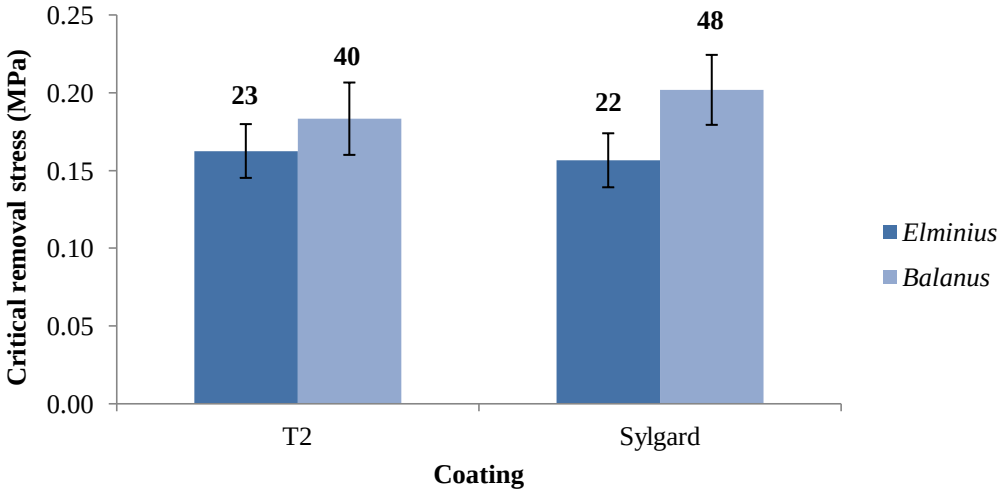

Figure 2.7. The mean critical removal stress (± 95% confidence interval) of *Elminius modestus* and *Balanus amphitrite* from Silastic T-2 and Sylgard 184 using the automated method. Numbers (n) of barnacles presented above the columns. Data presented in the graph are the original, un-transformed data.

Table 2.7. ANOVA table of results for the critical removal stress of *Elminius modestus* and *Balanus amphitrite* on Silastic T-2 and Sylgard 184 coated microscope slides.

	Sum of Squares	*Mean Square*	*df*	*F-value*	*P-value*
Species	**0.108**	**0.108**	**1**	**4.046**	**0.046**
Coating	**0.007**	**0.007**	**1**	**0.249**	**0.619**
Coating x species	**0.029**	**0.029**	**1**	**1.104**	**0.295**
Slide number	**0.318**	**0.045**	**7**	**1.034**	**0.432**

2.5. Discussion

The aim of this chapter was to examine the potential of laboratory cultures of *E. modestus* for evaluating the performance of FR coatings. This was accomplished by focusing on the settlement of laboratory-cultured cyprids, the length of time required to grow the barnacles to a testable size for critical removal stress (CRS) measurements and comparing these to the performance of the calcareous-based barnacle *B. amphitrite*. Within this study the settlement of *E. modestus* on polystyrene surfaces and silicone coatings was consistent with that of *B. amphitrite* under most circumstances. The

growth rate of *E. modestus* was higher than that of *B. amphitrite*, with *E. modestus* reaching a larger size after 18 weeks when fed on a diet of *T. suecica*.

The minimum size recommended for CRS measurements of *E. modestus* using the automated machine is 4.1mm in diameter. This is 0.5mm more than the minimum size of *B. amphitrite* recommended by Conlan et al. (2008) (3.6mm in diameter). Finally, the CRS values of adult barnacles from two silicone coatings (Silastic T-2 and Sylgard 184) were investigated. The CRS of *E. modestus* was less than that of *B. amphitrite* for only one of the two coatings, Sylgard 184.

2.5.1. Influence of the culture medium on the settlement of Elminius modestus

Kirby (2006) working on *E. modestus* and Rittschof et al. (1984; 1992) working on *B. amphitrite* used natural filtered seawater (FSW) to culture barnacle larvae to the cyprid stage. This was initially the preferred method in this study, with FSW having elements understood to be essential for the development of cyprids, which could not be replicated in artificial seawater (ASW). Preliminary trial cultures were undertaken using FSW. However, due to unforeseen circumstances (a failure with the seawater pump system, occurring on more than one occasion) FSW was unavailable. Consequently, cultures of *E. modestus* using ASW were attempted and did successfully yield cyprids.

It became necessary to understand whether or not, and if so, to what degree, the culture medium influenced the development and ultimately the settlement of the cyprids. This study found that the settlement of cyprids reared in FSW did not differ from those that were reared in ASW. However, it was evident that the settlement between the three repeat cultures differed significantly, with those from the third culture producing the lowest percentage of settled individuals.

Previous studies have demonstrated that the concentration of algae is important for the development of barnacle nauplii and the settlement of the cyprids (Qiu & Qian 1997; Thiyagarajan et al. 2002). If the food supply is low, the nauplii may not receive sufficient energy to moult through the naupliar stages and metamorphose to the cyprid stage, increasing the development time. The larvae may also be unable to build up the lipid and protein reserves required to transform from cyprid to the juvenile barnacle thus

reducing settlement (Moyse 1960; Tighe-Ford et al. 1970; Thiyagarajan et al. 2002; Maréchal et al. 2012). However the quality of the algae is also important for the reproductive development and larval fitness of marine invertebrates (Ban et al. 1997; Caldwell et al. 2002; 2005). Investigations have shown that diatoms such as *Skeletonema* spp. and *Nitzschia* spp. can reduce the egg viability and hatching success in other marine invertebrates including several copepod species (from the genera *Acartia, Calanus, Centropages and Temora*) (Ban et al. 1997; Miralto et al. 1999), the brine shrimp *Artemia salina* (Caldwell et al. 2003), the echinoderms *Paracentrotus lividus* (Miralto et al. 1999), *Psammechinus miliaris* and *Asterias rubens* (Caldwell et al. 2002; 2005), and the polychaetes *Nereis virens* and *Arenicola marina* (Caldwell et al. 2002; 2005). These diatoms can produce toxic substances including aldehydes (for example 2, 4-decadienal) which are released when the cells are damaged through activities such as grazing (Miralto et al. 1999; Caldwell et al. 2002; 2005).

Skeletonema spp. was used in this investigation as previous studies have shown that cyprids reared on this diatom appear after a shorter period of time (the shortest being 5 days) and are able to readily settle (Moyse 1963) in comparison to other diatoms species investigated, including *Phaeodactylum* spp. (Wisely 1960; Moyse 1963) or the flagellates *Isochrysis* spp. and *Rhodomonas* spp. (Stone 1988). The repeat cultures in the present study were given the same volumetric quantity of algae with an approximate concentration of 1×10^5 cell ml^{-1}. However, the effect of grazing pressure in the cultures and thus the potential release of any toxic aldehydes, which is not likely due to the strain of *Skeletonema* spp. selected, may none-the-less be a factor contributing to the variation in the settlement success between the different cultures. Additional comparisons on the settlement of these two barnacle species whilst monitoring the algae quantity and quality could provide evidence to explain the variability between the cultures.

Despite the difference between the repeat cultures, it may be concluded that the culture medium (ASW or FSW) does not influence the settlement of *E. modestus* cyprids. This is beneficial as laboratories would not necessarily be restricted to having a supply of natural seawater in order to culture cyprids and that ASW and FSW could be interchanged depending on availability.

2.5.2. Settlement of Elminius modestus *and* Balanus amphitrite

Rittschof et al. (1984) stated that *B. amphitrite* was an 'excellent model organism' for use in antifouling studies, in part due to their 'predictable settlement'. The settlement of *E. modestus* on the two silicone coatings (Silastic T-2 and Sylgard 184) was consistent with that of *B. amphitrite*. However, there were differences in the settlement between the two barnacle species on the polystyrene surfaces, with the settlement of *B. amphitrite* being higher than that of *E. modestus,* although not for every culture repeat. The consistent settlement on the silicone coatings between the two species does show an indication of the potential of *E. modestus* as a model species, especially in examining the influence of coatings, but, in assays using polystyrene surfaces, for example toxicity assays (Rittschof et al. 1992), *E. modestus* may be less suitable as a model species. Settlement in this study did not conform to the 'predictable' pattern that Rittschof et al. (1984) described for *B. amphitrite,* with both species showing an equal degree of variability between the three repeat cultures. As mentioned above, the quantity and quality of the algae used as feed could be influencing the development of the nauplii and subsequent settlement of the cyprids. There may be other aspects of the experimental set-up of the cyprid culture which may influence settlement, for example light and turbulence of the cultures (Barnes & Barnes 1982; Pawlik 1992; Franco et al. 2016). These factors have previously been shown to influence the growth of nauplii and their development to the cyprid stage, and therefore the settlement rates. On the other hand, the type of assay used may have contributed to the variable settlement rates. Settlement assays utilising polystyrene 24-well plates and microscope slides (for drop assays) have been highlighted as having poor settlement rates and high variability. This has been attributed to the cyprids becoming 'trapped' within the air/seawater interface or, with regard to drop-assays, having restricted movements in a confined droplet of water (Qiu et al. 2008; Petrone et al 2011; Di Fino et al. 2014). Settlement assays using Falcon™ (1006) Petri-dishes or glass vials are alternatives to 24-well plates and are said to provide a greater volume of water and a larger area for cyprid exploration and settlement, and in the case of the Falcon™ (1006) Petri-dishes can remove the air/seawater interface thus preventing the cyprids becoming trapped (Rittschof et al. 1984; Qiu et al. 2008; Petrone et al 2011; Di Fino et al. 2014). Petri-dishes and glass vials have been shown to have superior and more reliable settlement rates when compared to 24-well plates with *B. amphitrite* cyprids (Qiu et al. 2008; Petrone et al 2011) and *B. improvisus* cyprids (the Falcon (1006) Petri-dish assay,

only) (Di Fino et al. 2014). Whether these alternative methods would improve the settlement rate and reliability when assessing the competency of cyprids of *E. modestus*, warrants investigation.

2.5.3. Growth

The time required to grow *E. modestus* to a suitable size to measure the CRS was monitored. The recommended size for measuring the CRS in shear for *B. amphitrite* is between 5 – 20mm in diameter (ASTM D-5618 1994; Swain 1997), however, *E. modestus* is a smaller barnacle. In nature the average diameter has been reported at 5mm (Moore 1944) with the largest specimens being discovered with diameter of up to 10 (Darwin 1854) and 13mm (Moore 1944). By comparison the average basal diameter of adult *B. amphitrite* can range between 7.3 and 15.5mm (Barnes et al. 1970). The natural average size of *E. modestus* is within the parameters set by the ASTM D-5618 (1994) test method, and therefore in theory this test method could be used with *E. modestus*. However, previous work on the CRS of *E. modestus* included smaller individuals than recommended (Wiegemann & Watermann 2004; Robson et al. 2009). For example, Wiegermann & Watermann (2004) tested *E. modestus* with an average diameter of 4.5mm that were grown in the field for six weeks on Sigma Glide. By comparison the *Balanus* spp. tested in their study attained an average basis diameter of 8mm after the six weeks of field immersion.

Under laboratory feeding regimes (*T. suecica* and *Artemia* sp.) *B. amphitrite* can grow to the recommended size for testing of 5mm in 12 weeks (Wendt et al. 2006, Conlan et al. 2008). In this study, *E. modestus* attained an average basis diameter of 4.4 ± 1mm and 4.7 ± 1mm diameter on Silastic T-2 and Sylgard 184, respectively, in 28 weeks growing approximately 0.54 and 0.63mm^2 per week, for Silastic T-2 and Sylgard 184, respectively. The 12-week time frame for *B. amphitrite* growth is with the inclusion of *Artemia* sp. nauplii in the feed mix. *Artemia* sp. nauplii are a common source of food for laboratory reared *B. amphitrite* (Wendt et al. 2006, Conlan et al. 2008, Rittschof et al. 2008), being introduced to the barnacles after 2 – 3 weeks when they have reached an approximate basal diameter of 2mm. *Artemia* sp. was added to the feed of *E. modestus* when they had reached approximately 3mm diameter; larger than that recommended for *B. amphitrite* and therefore assumed to be capable of feeding on the

nauplii. Indeed nauplii did become depleted and orange faecal pellets were present in *E. modestus* culture vessels suggesting the barnacles were feeding on them. However, after introducing *Artemia* sp. nauplii, there was a 40% mortality of the barnacles. Consequently the food was switched back to *T. suecica*, only.

The growth rate of a second culture of *E. modestus* was monitored and this time in parallel with a culture of *B. amphitrite*, in which they were both fed *T. suecica* at the same time and same concentrations. When *E. modestus* was fed just *T. suecica* its weekly average growth on Silastic T-2 and Sylgard 184 was 0.83 and 0.94mm^2 reaching 4.36 and 4.65mm diameter in 18 weeks, respectively. When *B. amphitrite* was fed just *T. suecica* its average weekly growth on Silastic T-2 and Sylgard 184 was 0.55 and 0.64mm^2, reaching an average diameter of 3.86 and 4.01mm in 19 weeks, respectively. *E. modestus* attained a much larger size than *B. amphitrite* after the growth period, suggesting a much faster rate of growth. However, it is important to comment on the population density of the cultures tested. Although the populations were not uniform throughout for each slide and coating tested, there was a greater number of *B. amphitrite* per slide than for *E. modestus* on equivalent slides. With an increased population size there can be a slower rate of growth as the competition for food and space increases (Crisp 1960). Therefore, the slower rate of growth of *B. amphitrite* in this study may be due to an increase in competition for food and less space for basal growth. The interaction of population density and basal area was not investigated in this study, therefore the extent of the influence of population on the overall growth of the barnacles is uncertain. Crisp (1960) noted that differences in growth (measured by weight) of *Semibalanus balanoides* caused by changes in population density were "rather small", as individuals which were crowded grew in height not basal area and thus weight was not greatly affected. The basal area was measured in this investigation as it was thought to be least disruptive to the barnacle specimens, i.e. involve the least amount of handling time of the slides and a short time out of the water. Although for a more robust investigation into the growth of the different barnacle species, measures of height in addition to basal area would be beneficial.

The same concept of population size can be used to explain the difference in growth of *E. modestus* from culture 1 on the Silastic T-2 and Sylgard 184 coatings. There were fewer individuals per slide coated with Sylgard 184, than on Silastic T-2, therefore there was less competition between individuals with a potentially quicker rate

of growth. But this is not the case for the second culture of *E. modestus*, in which there were more individuals on the Sylgard 184, but they grew at a faster rate than those on less populated Silastic T-2. The growth of *B. amphitrite* was not affected by the type of coating. This suggests that *E. modestus* was more sensitive to the type of coating than *B. amphitrite*.

Negating population densities, and merely considering the practicality of growing barnacles for screening FR coatings, the growth rate of *E. modestus* in the second culture was still slow in comparison to published accounts of *B. amphitrite* in the laboratory (when fed *Artemia* sp.) (Wendt et al. 2006). This in turn would result in a slower throughput of coating test samples. In this study the incorporation of the preferred feed (*Artemia* sp.) led to 40% mortality. A different culture set-up, in which the barnacles are maintained in larger volumes of seawater either aerated or with a flow system may reduce the mortality and improve the growth rate. For example, as demonstrated with *S. balanoides*, an increase in hydrodynamic flow and turbulence increases the cirral beats and thus feeding, which can consequently increase the rate of growth (Barnes & Barnes 1982: Sanford et al. 1994). This is a topic that would benefit from further investigation, in order to establish a laboratory protocol that would provide a faster rate of growth for laboratory-cultured of *E. modestus*.

2.5.4. Critical removal stress

The basal area of such barnacles as S. *balanoides, B. improvisus, B. eburneus* and *E. modestus* has previously been reported to influence the force (N) required for detachment (Yule & Walker 1984b; Berglin et al. 2001; Kavanagh et al. 2001; Robson et al. 2009). The ASTM D-5618 (1994) test method recommends 5 – 20mm in diameter, as barnacles with basal diameters outside this range result in larger variances in the CRS measurements. Conlan et al. (2008) recommended a minimum size for *B. amphitrite* of 3.6mm diameter (basis) using the automated method. This was beneficial as it could reduce the growth time of *B. amphitrite* from 12 weeks to between 7 and 9 weeks (Conlan et al. 2008). The automated method is perhaps better suited to CRS measurements for the smaller and slower growing *E. modestus*. The minimum diameters of the basis recommended for *E. modestus* in this study were 4.1mm for the automated method and 3.6mm for the hand-held force gauge; results from the manual

method are consistent with those established for *B. amphitrite* (Conlan et al. 2008). The time required to grow *E. modestus* individuals to a testable size in the laboratory could be reduced to approximately 10 – 18 weeks, based on the two cultures of *E. modestus*.

The critical removal stress (CRS) of *E. modestus* on Sylgard 184 (0.156 MPa ± 0.017 95% CI) and Silastic T-2 (0.162 MPa ± 0.017 95% CI) was lower than the CRS value of *B. amphitrite* on the same coatings (Sylgard 184; 0.201 MPa ± 0.022 95% CI and Silastic T-2; 0.181 MPa ± 0.021 95% CI). However, this was only significant in the case of Sylgard 184. Wiegemann & Watermann (2004) found the CRS of field-grown *E. modestus* was lower than two field-grown calcareous barnacles: *B. improvisus* and *B. crenatus* on Sigma Glide (Sigma) silicone. They speculated that the differences in removal stress might relate to size; the *Balanus* spp. were 8mm diameter whereas *E. modestus* were much smaller at 4.5mm diameter (Wiegemann & Watermann 2004). Alternatively, the difference in CRS might have been the result of species-specific differences in the adhesive, or a mechanical effect of the calcareous-basis offering a greater resistance to detachment. The CRS values of *B. amphitrite* from the two silicone coatings (Silastic T-2 and Sylgard 184) attained in the study were similar to that reported in previous studies (Sun et al. 2004; Holm et al. 2005; Wendt et al. 2006; Conlan et al. 2008; Rittschof et al. 2008). Size is unlikely to be a reason for the difference in CRS on the coatings in this study, as both species were on average 4.5 ± 2mm in diameter. Therefore, the difference could be a mechanical effect of the type of basis or species-specific difference in the adhesives as Wiegemann & Watermann (2004) speculated. However, the reason why only a difference between the CRS of the two barnacle species was discernible for Sylgard 184 and not Silastic T-2 is unclear. The two coatings do have minor differences in their bulk properties according to the product data information sheets (Dow Corning). The difference in bases and adhesives may be reacting in a different manner to the properties of the coating not only in terms of CRS but also growth. Expanding the number of coatings with variations in their surface and bulk properties would provide a better understanding of the relationship between the type of basis and the CRS.

When barnacles with a calcareous-basis are grown on low modulus, low surface energy coatings a proportion produce a thick rubbery or ‘gummy’ adhesive as well as a concave basis (Berglin & Gatenholm 2003; Sun et al. 2004; Ramsay et al. 2008). This rubbery adhesive has different mechanical properties and chemical content to the

adhesive produced by barnacles grown on coatings with a higher modulus (Berglin & Gatenholm 2003). Wiegemann & Watermann (2004) commented that *E. modestus,* when grown on PDMS, produces adhesive that is less thick and hydrated than that produced by *Balanus* spp. on the same coatings. However, there have been no further studies on the adhesive of *E. modestus* or any other membranous-based barnacle specifically when grown on FR coatings (or any other surfaces for that matter). A recent study has been published on the adhesive of a second membranous-based barnacle *Tetraclia japonica formosana*, but not in relation to growth on a coating. This study by Lin et al. (2014) found *T.j. formosana* to be lacking a common cement protein (CP-20K), which is present in calcareous-based barnacles such as *B. amphitrite, B. albiocostatus* and *Megabalanus rosa*. It was suggested that the absence of CP-20K in *T.j. formosana* contributes to a difference in the mechanism for substratum attachment compared to calcareous-based barnacles (Lin et al. 2014). Whether this is cement protein is present or absent in *E. modestus* requires further investigation.

Investigations on the structure and mechanical properties of the adhesive of *E. modestus* using such techniques as atomic force microscopy (AFM) and scanning electron microscopy (SEM) to image the structure of the adhesive, and X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy to understand the composition of the adhesive (Wiegemann & Watermann 2004; Dickinson et al. 2009; Sullan et al. 2009; Barlow et al. 2010), would be beneficial to elucidate the mechanisms of the adhesion of this species in comparison to *B. amphitrite*. Furthermore, the nature of the adhesive has a pivotal role in the removal process of the barnacle from FR coatings. Examining the adhesive of *E. modestus* would clarify whether there are species-specific differences in the adhesives, which influence the detachment processes.

2.6. Conclusion

The practicality of using *E. modestus* for laboratory culture and as a model test species for evaluating FR coatings was explored. The percentage settlement of *E. modestus* on the two standard silicone coatings was comparable to that of *B. amphitrite.* This was not the case when polystyrene was tested; settlement of *E. modestus* cyprids was lower for some of the repeat cultures. *E. modestus* does have potential as a test

species in settlement assays especially with regard to silicone coating evaluations, but it is clear from the un-predictable settlement results that the settlement assay for this species needs to be optimised.

The growth rate of *E. modestus* in this study was shown to be faster than that of *B. amphitrite*, when fed on a diet of *T. suecica*. But more interestingly was the coating effect on the growth of *E. modestus*, in which those grown on Sylgard 184 grew larger and faster, than those on Silastic T-2. In addition, the difference in the CRS between the two barnacles was only present for Sylgard 184. *E. modestus* was more sensitive to the coating type than *B. amphitrite*, and therefore *E. modestus* is possibly superior to *B. amphitrite* as a test species for discriminating between the performance of coatings. Whether this difference reflects the contrasting types of bases (membranous versus calcified) requires further investigation. Nevertheless, it is reasonable to conclude that *E. modestus* is a good model species for laboratory evaluations of FR surfaces.

Chapter 3. High-Speed Video Analysis of the Detachment of Barnacles with Membranous and Calcareous Bases.

3.1. Abstract

The flexibility of a barnacle's basis is important with regard to the fracture mechanics and release properties from an elastomeric coating, as a more flexible basis requires less energy for removal. Using high-speed photography, the separation processes of *Elminius modestus* and *Balanus amphitrite,* from two polydimethysiloxane (PDMS) coatings, (Silastic T-2 and Sylgard 184), were observed under wetted and de-wetted conditions. Four distinct separation patterns were characterised: lift, peel, adjacent peel and twist. These were based on the location of the initial separation and direction of propagating instabilities in respect to the direction of detachment force. The model separation pattern for *E. modestus* was a lift separation, whereas *B. amphitrite* displayed a peel separation. The observed differences in the separation patterns between species may have more to do with the variations in shape and structure of the barnacle's shell than to the type of basis. However, the flexibility of the membranous-basis of *E. modestus* was important for the propagation of the fracture as it hindered the formation of fingering instabilities as they progressed through the adhesive interface. The time for initial separation occurred sooner and the CRS was lower for *E. modestus* compared to *B. amphitrite*. There were also significant interaction effects of degrees of wetness and coatings for the removal times and CRS for *E. modestus* but not for *B. amphitrite*, suggesting that the detachment process of *E. modestus* may be more easily influenced by environmental variations.

3.2. Introduction

Kendall's (1971) model for examining the force required to remove a cylindrical stud or a pseudobarnacle from an elastomeric coating is often used in studies detailing the mechanics of release from fouling-release (FR) coatings (Berglin & Gatenholm 1999; Kohl & Singer 1999; Singer et al. 2000; Kim et al. 2007). Many of these studies focus on the effect of different permutations of surface and bulk properties (i.e. thickness and elastic modulus) of the coatings in terms of release characteristics (Kohl & Singer 1999; Berglin et al. 2003; Wendt et al. 2006; Kim et al. 2007). However, Kendall's model is not appropriate to assess the release mechanics of real barnacles. The synthetic adhesives do not possess comparable viscoelastic and multi-layered properties of barnacle adhesive (Sun et al. 2004), furthermore, when grown on silicones the barnacle adhesive can develop an atypical 'gummy' nature (Wiegemann & Waterman 2003; Sun et al. 2004; Wendt et al. 2006; Ramsay et al. 2008). In addition, Kendall's model assumes that the stud is rigid whereas the calcareous-basis of the barnacle *Balanus amphitrite* has flexural rigidity many orders of magnitude less than that of pseudobarnacles meaning that there is greater flex in the basis on release (Ramsay et al. 2008). In a model developed by Chung and Chaudhury (2005) it was shown that studs with greater flexibility required less force to be removed from an elastomer, thereby providing an explanation for the discrepancy between the actual removal stress of real barnacles and that predicted by Kendall's model (Sun et al. 2004).

Attention has been directed on developing an understanding of the release behaviour of real barnacles from silicone coatings in the hope of devising a new model more suitable for the detachment process of real barnacles (Kavanagh et al. 2005; Hui et al. 2011). Kavanagh et al. (2005) investigated the release mechanisms in two calcareous-based barnacles (*Balanus eburneus* and *B. variegatus*) from polydimethylsiloxane (PDMS) coatings using a high-speed camera. It proved possible to visualise the viscous properties of the adhesive, detailing its characteristics and behaviour during detachment as well as visualising the fracture process and crack propagation.

The work by Ramsay et al. (2008) on the flexibility of barnacle bases and that of Kavanagh et al. (2005) on the release mechanisms focussed on barnacles with calcareous-bases. The uncalcified membranous-bases of barnacles such as *Elminius modestus* and *Semibalanus balanoides* would obviously have greater flexibility than

their calcified counterparts. It seems reasonable to suggest that the release mechanics of membranous-based barnacles will not conform to Kendall's model.

The aim of this chapter was to examine and compare the detachment processes of the membranous-based barnacle *E. modestus* and the calcareous-based barnacle *B. amphitrite* from silicone coatings. A high-speed camera was used to investigate the fracture processes of *E. modestus* and *B. amphitrite*, from two PDMS coatings. This provided a detailed account and comparison of the separation processes of the two barnacle species. The hypotheses to be tested are: 1) the membranous-basis does influence the fracture process; and 2) there are clear differences in the separation of a membranous-based barnacle compared to a calcareous-based barnacle from the silicone coatings in terms of the timings of the separation processes and critical removal stress.

3.3. Materials and methods

3.3.1. Preparation of coated slides and barnacle settlement

Silastic® T-2 and Sylgard® 184 (Dow Corning) were coated on microscope slides to an average thickness of 130μm and 140μm, respectively (see Chapter 2 for coating preparation). The coatings were leached in a tank of static reverse osmosis (RO) water with a carbon filter (Fluval filter) for 14 days. The water was changed after 7 days. After leaching, the slides were rinsed in fresh RO water and immersed in artificial seawater (ASW, 32-34 salinity Tropic Marin) for 1 hr. Once removed from ASW, the slides were air dried. Laboratory-cultured *E. modestus* and *B. amphitrite* cyprids were settled on the coated microscope slides (see Chapter 2 for methods of cyprid culture and settlement procedure). Two cultures of *E. modestus* and two cultures *B. amphitrite* cyprids were used. Once settled on the coatings, the barnacles, maintained in quadriPERM® culture vessels, were fed 15ml of *Tetraselmis suecica* three times per week. The water was changed at each feeding. The first batches of *E. modestus* and *B. amphitrite* cyprids settled in February 2009 within 2 days of each other and were grown for 3 months to a mean size of 4.47mm in diameter (± 0.161 SE) and 3.9mm in diameter (± 0.06 SE) (*E. modestus* and *B. amphitrite*, respectively). The second cultures were started in October 2009 and were grown for 4 months until their mean size was 5.6mm

in diameter (± 0.108 SE) and 5.4mm in diameter (± 0.252 SE) (*E. modestus* and *B. amphitrite*, respectively).

Once the barnacles had reached a suitable size for removal experiments (see Chapter 2 section 2.5.4.), they were cleaned in fresh water, air dried for 5 minutes and then scanned (HP Scanner 5400C) at 1200 dpi resolution.

3.3.2. High-speed video set-up

The high-speed video set-up was adapted from a method used by Kavanagh et al. (2005), where a built-to-purpose structure was designed around the equipment provided by Newcastle University, UK (Figure 3.1). The set-up involved a single slide being positioned on a glass platform above an Olympus *i*-SPEED digital camera with a 50mm lens (SIGMA F2.8 EX DG). This recorded the detachment process of a barnacle at up to 800 frames per second (fps). A hand-held force gauge (PSM-2K, IMADA, Inc.) was used to apply the force in shear to the base of the barnacle's shell plates in parallel to the slides surface, as described in the standardised method, ASTM D-5618 (1994). A fibre optic annular ring light was positioned over the lens to provide sufficient light to capture the image due to the cameras very fast shutter speed. The CRS and time for removal were recorded for each barnacle tested. ImageJ software (Rasband 1997; Abramoff et al. 2004) was used to calculate the basal area of each barnacle in order to calculate the CRS. The time for removal was the time taken from the moment the probe of the hand-held force gauge contacted the shell of the barnacle, referred to as 'probe contact' (PC) to the time that the barnacle had been moved by exactly the diameter of the shell, referred to as 'complete separation' (CS) (Figure 3.2). The video recorded the time for the entire detachment event, the removal time was then calculated by CS – PC.

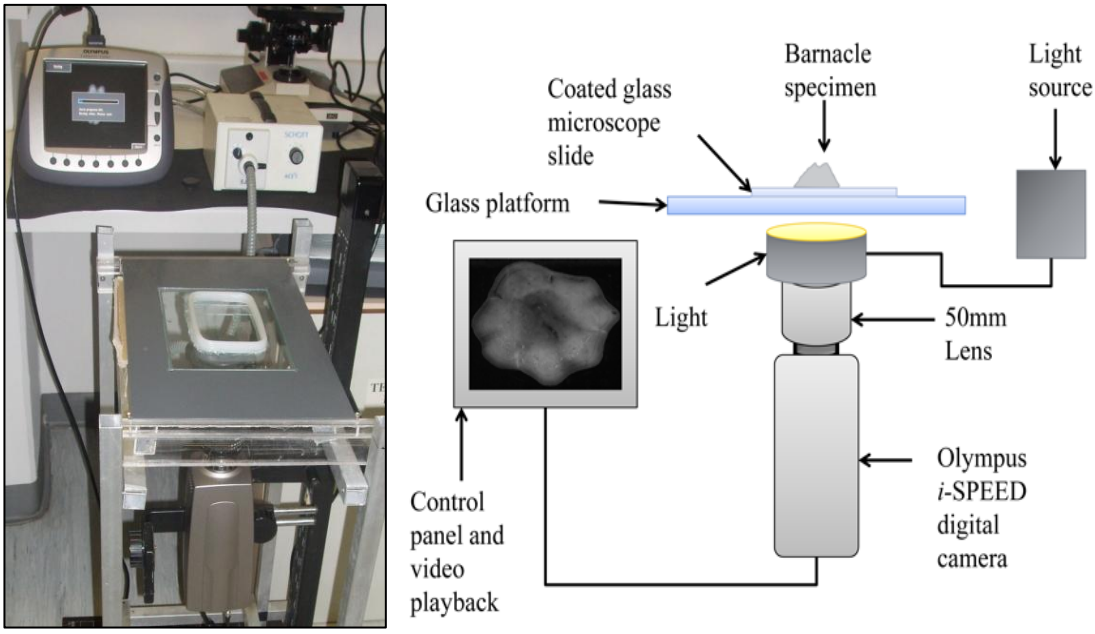

Figure 3.1. Photograph and schematic diagram (not to scale) of the high-speed image capture equipment set-up.

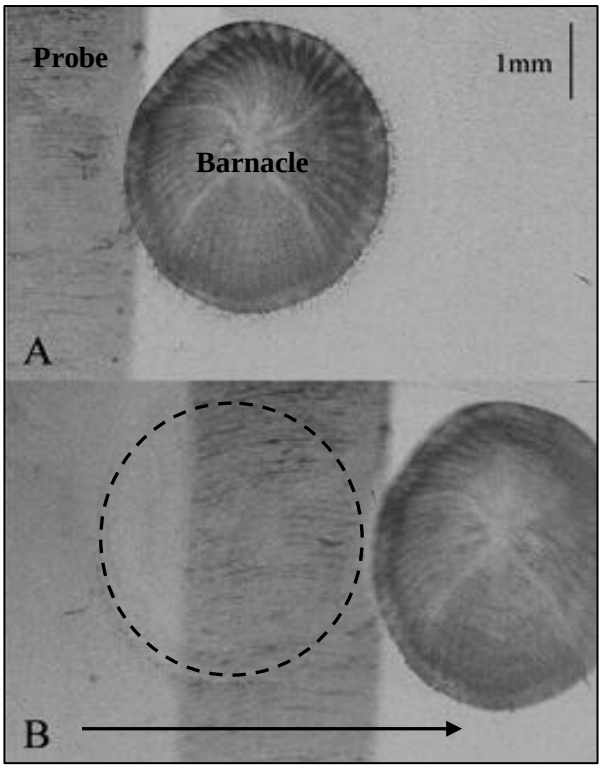

Figure 3.2. Example of 'time for removal' recorded from probe contact (A) to complete separation (B) as viewed from underneath the basal plate of a barnacle. Movement of probe from left to right indicated by the arrow. Dashed circle in B indicates the original location of the barnacle.

Observations of the detachment process were made of de-wetted and wetted barnacles. De-wetted (also referred to as dry) entailed the removal of moisture that surrounded the barnacles using laboratory blue roll and air drying for 5 minutes. Wetted barnacles were submerged in 2ml of artificial seawater (ASW) mixed with green food dye; this formed a droplet of water, not capable of fully submerging an individual barnacle but it was sufficient to observe the influence of water on the detachment process. The additional contrast from the food dye made it possible to visualise the influx of water underneath the basis (Kavanagh et al. 2005). The glass platform which held the microscope slides had a raised silicone and plastic perimeter which prevented the water escaping from the platform and on to the camera below.

3.3.3. Statistical analysis

The times for initial separation and for complete removal, and the CRS of *E. modestus* and *B. amphitrite* from the two PDMS coatings under de-wetted and wetted conditions were recorded and analysed. The time for initial separation and the CRS data were $\log_{10}$ transformed after a Kolmogorov-Smirnov test (Ennos 2012) and Levene's test (Quinn & Keough 2002) revealed that the data sets did not present a normal distribution nor an equal variance. The time for complete removal data did have a normal distribution with an equal variance. Three separate, three-factor nested ANOVAs with 0.05 significance levels were used to test the null hypotheses: 1) that the initial separation times of *E. modestus and B. amphitrite* were equal; 2) the times for complete removal of *E. modestus and B. amphitrite* were equal; and 3) the CRS of the two barnacle species was equal. These tests included the interaction effects of species x coating, species x wetness and species x wetness x coating.

3.4. Results

High-speed video recordings were made of the detachment of 59 *E. modestus* and 93 *B. amphitrite* from the coatings Silastic T-2 and Sylgard 184. See Table 3.1 for the number of barnacles removed per coating and for the numbers removed while de-wetted and wetted.

Table 3.1. Total number of *Elminius modestus* and *Balanus amphitrite* detached from the coatings Silastic T-2 and Sylgard 184, under the de-wetted and wetted condition.

Barnacle species	***Silastic T-2***		***Sylgard 184***	
	De-wetted	***Wetted***	***De-wetted***	***Wetted***
Elminius modestus	21	11	15	12
Balanus amphitrite	27	16	29	21

3.4.1. Removal process of barnacles from silicones

Barnacle removal from the silicone coatings followed a 5-step process, consistent with that described by Kavanagh et al. (2005):

1. Initial separation and cavity development;
2. propagating instabilities;
3. complex branching separation;
4. adhesive separation and adhesive failure;
5. complete removal.

The initial separation and cavity development refers to the first appearance of a separation in the barnacle's adhesive from the silicone coating (Figure 3.3A). In some instances this takes on the appearance of a cavity or an air pocket, occurring between the silicone coating and the barnacle's basis. The initial separation does not happen immediately upon applying the removal force; it occurs approximately mid-way through the length of time needed to detach the barnacle completely. As soon as the cavity develops it rapidly propagates from its source location, commonly around the periphery of the shell in an arc shape (Figure 3.3B). The cavity sometimes propagates

in more than one direction taking on a complex branching appearance (Figure 3.3C). When the instabilities have extended and the adhesive has separated from more than half of the barnacle's basal area, adhesive failure occurs, whereby the barnacle detaches from the coating (Figure 3.3D). Some of the barnacles rotated as they detached, however this appeared to be dependent on the pattern of adhesive detachment and the shape of the barnacle. Figure 3.3 illustrates the basic steps in the separation process of a barnacle from a silicone coating. The four subsequent figures (Figure 3.4, 3.5, 3.6 and 3.7) are still frames from the high-speed video of the detachment of two *B. amphitrite* and two *E. modestus*, providing examples of the separation process.

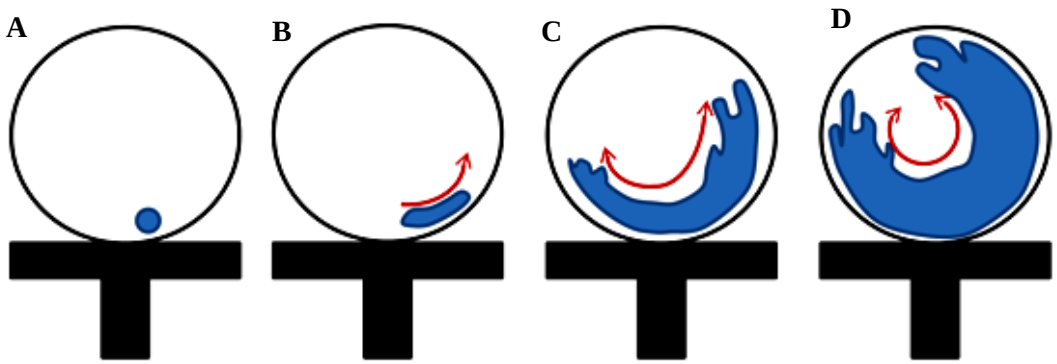

Figure 3.3. Diagrammatic representation of the typical process of a barnacle detaching from a silicone coating, exhibiting: A) initial separation and cavity development indicated by the blue circle; B) propagating instabilities (the red arrow indicating the direction of the spread); C) complex branching separation; and D) adhesive separation.

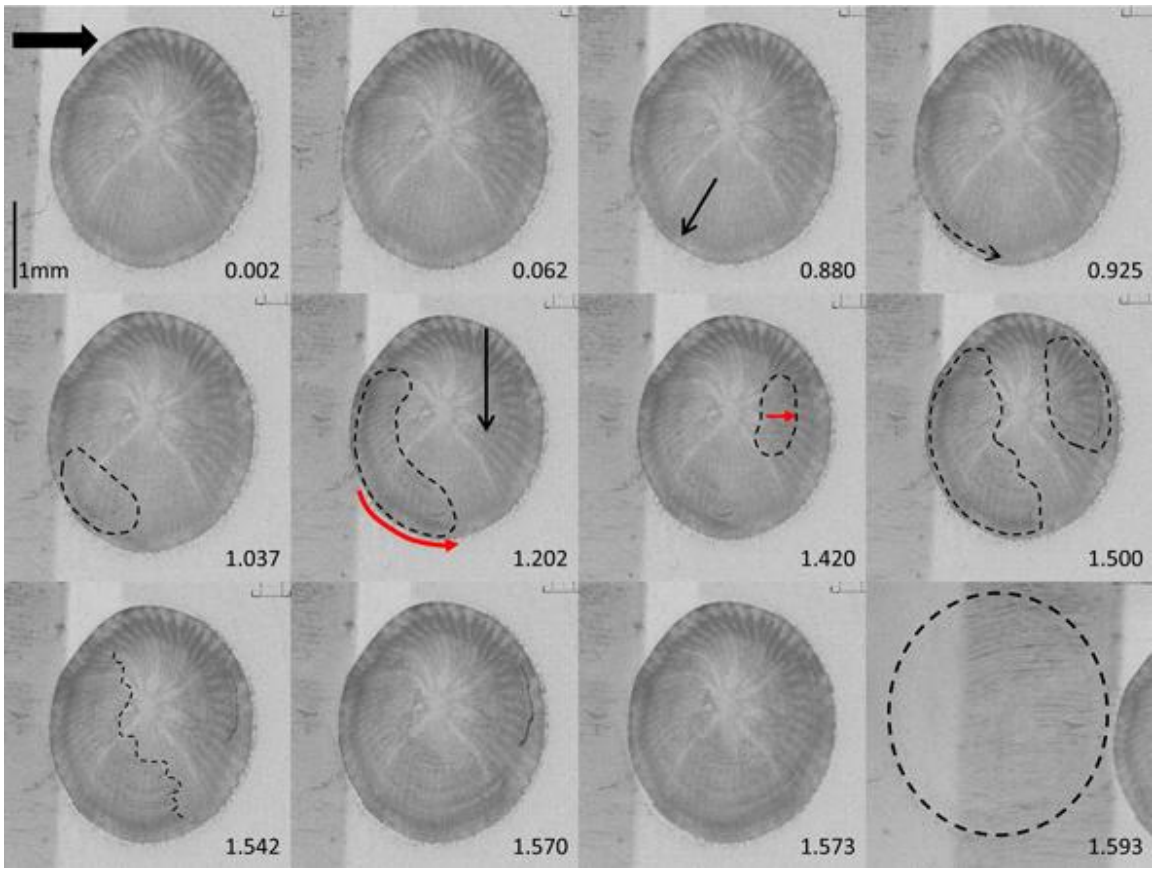

Figure 3.4. Detachment of *Balanus amphitrite* under shear force from Sylgard 184 while de-wetted. The numbers in the lower right corners represent the time in seconds. The black arrow at 0.002 seconds indicates the direction of the applied force. The initial separation began at 0.880 seconds, the location is indicated by the arrow. This cavity propagated in the direction of the dashed black arrow along the periphery of the shell at 0.925 seconds. The dashed areas from 1.037 and 1.202 seconds shows the growing instability complex moving in the direction indicated by the red arrow at 1.202 seconds. At 1.202 seconds an additional cavity front became clear. By 1.500 seconds the complex instabilities covered over 50% of the barnacle's basis. At 1.542 seconds, viscous fingering separations were clearly visible. Complete separation occurred at 1.593 seconds, the total time for removal being 1.591 seconds. After separation from the coating, a ring of adhesive remained on the silicone surface circled by the dashed ring (times specific to this detachment example).

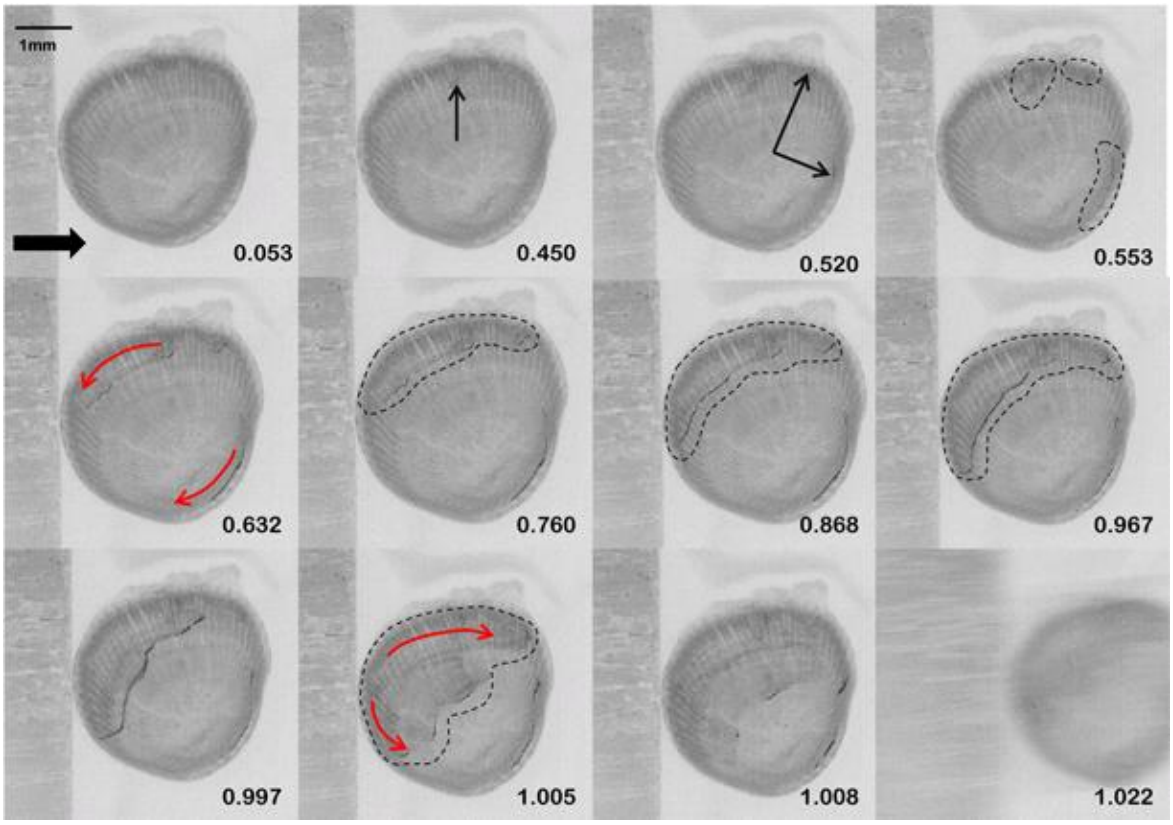

Figure 3.5. Detachment of *Balanus amphitrite* under shear force from Sylgard 184 while wetted. The numbers in the lower right corners represent the time in seconds. The black arrow at 0.053 seconds indicates the direction of the applied force. The primary cavity appeared at 0.45 seconds. Secondary instabilities developed at 0.520 seconds; these instabilities began to branch-out at 0.553 highlighted by the dashed areas. The red arrows at 0.632 seconds highlight the direction the instabilities moved as they developed. The dashed areas at 0.760, 0.868 and 0.967 seconds highlight the growing instability. The cavity front of the growing complex propagated from the left to the right of the barnacle in the same direction as the applied force in the direction indicated by the red arrows at 1.005 seconds. At 1.005 seconds, the cavity covered over 50% of the basal area. Complete separation occurred at 1.022 seconds, the total removal time being 0.969 seconds (times specific to this detachment example).

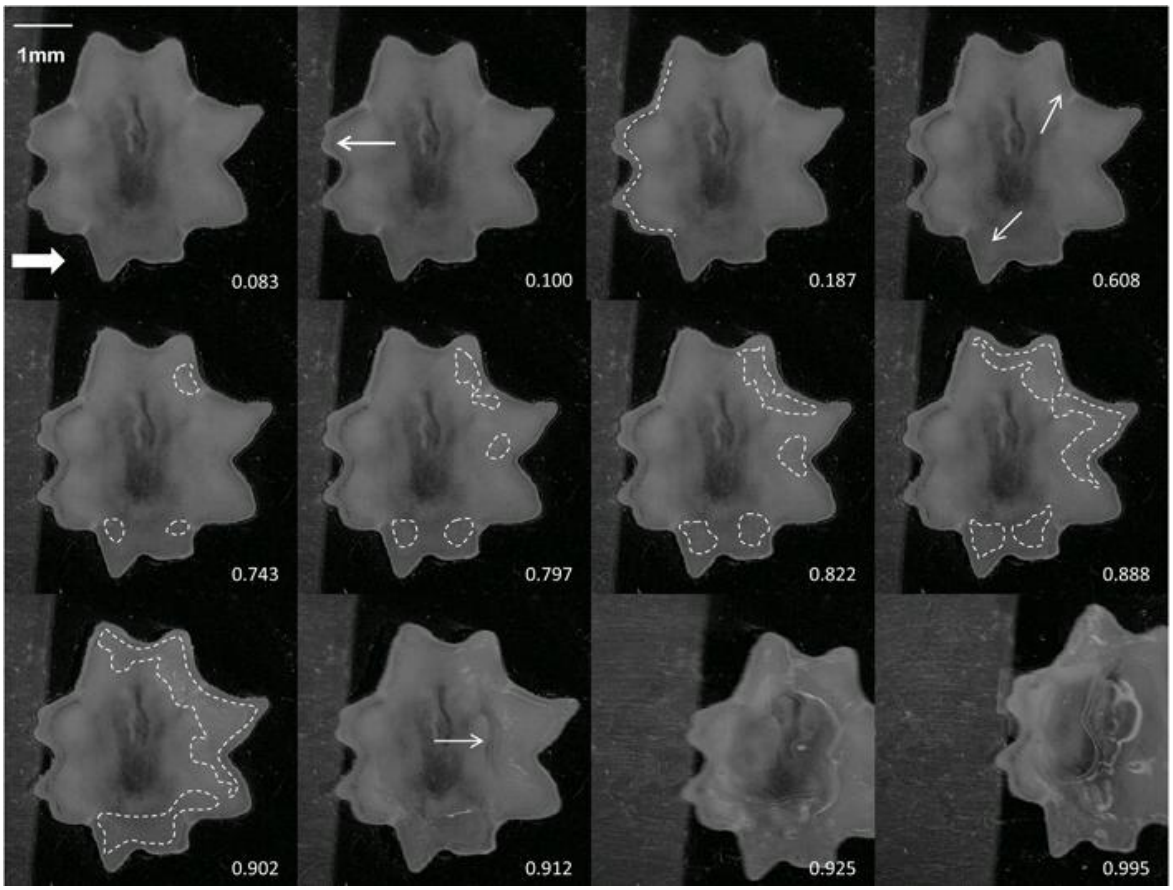

Figure 3.6. Detachment of *Elminius modestus* under shear force from Sylgard 184 while de-wetted. The numbers in the lower right corners represent the time in seconds. The arrow at 0.083 seconds illustrates the direction of applied force. As the pressure was applied, a black line appeared from the edge of the shell indicated by the arrow at 0.100 seconds. With increasing pressure this line spread along the periphery of the shell illustrated by the dashed line at 0.187 seconds. At 0.608 seconds a cavity appeared in the locations pointed to by the arrows. The dashed areas at 0.743, 0.797, 0.822, 0.88 and 0.902 show the development of the growing instability which covered two thirds of the basal area. At 0.912 seconds, a tear in the basal membrane appeared (arrowed), perpendicular to the direction of the force. The last two images shows the movement of the barnacle across the coating where it was completely separated at 0.995 seconds. The removal time being 0.912 seconds (times specific to this detachment example).

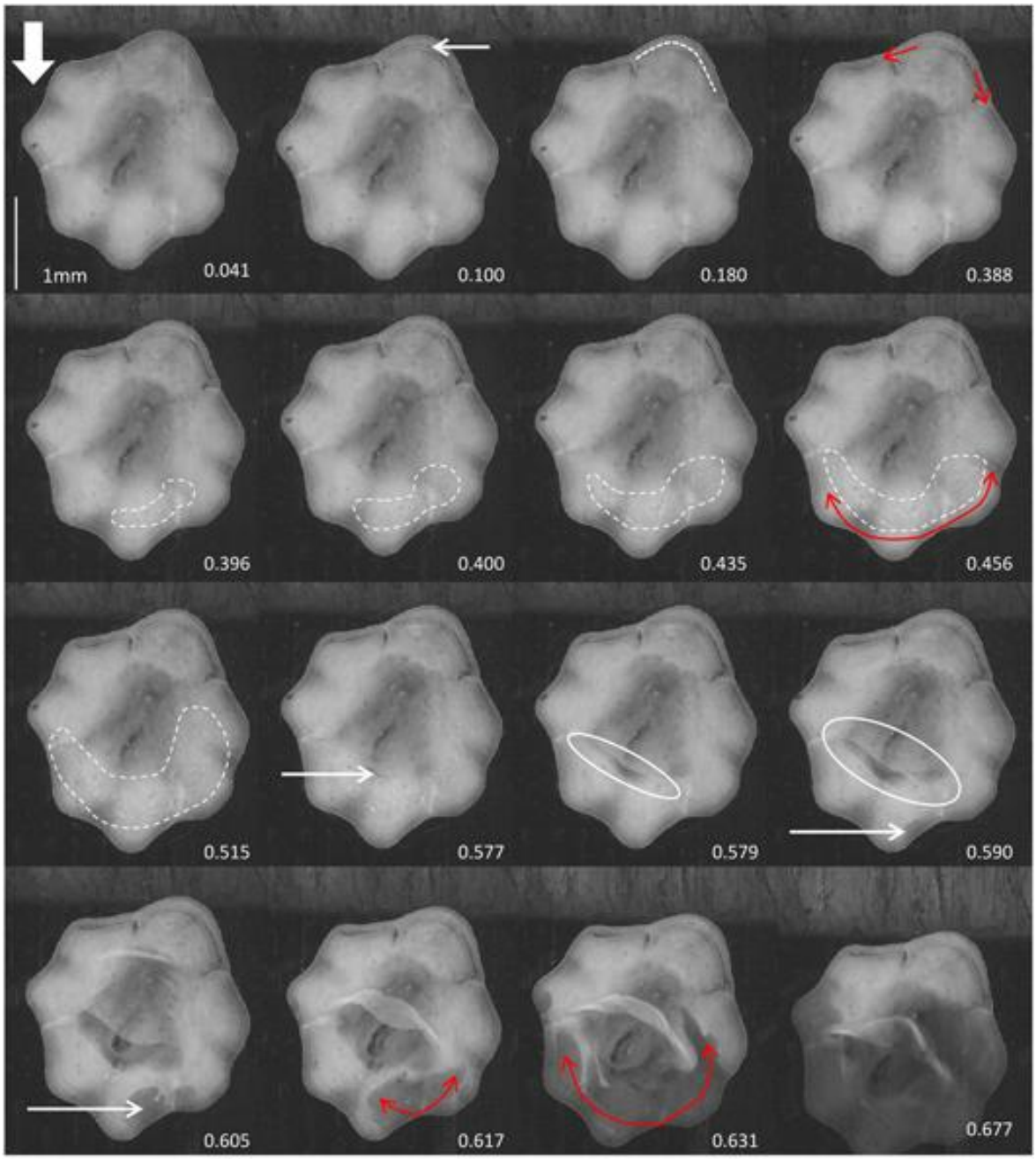

Figure 3.7. Detachment of *Elminius modestus* under shear force from Silastic T-2 while wetted. The numbers in the lower right corners represent the time in seconds. The arrow at 0.041 seconds illustrates the direction of the applied force. As the force increased, a black line seen at 0.100 seconds appeared from the edge of the shell. With increasing pressure this line spread along the periphery of the shell indicated by the red arrows. At 0.396 seconds a cavity appeared, highlighted by the dashed area. The growing instability complex developed moving in the direction illustrated by the red arrows at 0.456 seconds. At 0.577 seconds, a tear in the membranous-basis appeared (arrowed and ringed by a solid white line), developing further at 0.579 and 0.590 seconds. At 0.590 seconds the surrounding water began to percolate underneath the basal plate indicated by the white arrow. In subsequent images, the water seeped further under the basis, spreading in the

direction indicated by the red arrows and occupying the cavity space. After 0.677 seconds, the water had infiltrated under the basal plate covering over two thirds of the area. By 0.763 seconds (not shown in the picture) complete separation had occurred, the removal time being 0.722 seconds (times specific to this detachment example).

3.4.2. Patterns of separation

The location of the initial separation and the direction of the propagating instabilities (step A and B from section 3.4.1) in relation to the direction of the applied force and the point of contact of the force gauge probe on the barnacle, differed for each individual barnacle. However, it was possible to categorise them into four patterns of separation (Figure 3.8):

A. **Lift separation**. The initial separation occurred in the area of the basis furthest from the contact point of the force gauge probe. The instabilities spread towards the probe against the direction of the force, either along a single edge of the basis or along both edges.

B. **Peel separation**. The initial separation occurred in the area of the basis closest to the probe's contact point, with the instabilities spreading away from the probe in the same direction of the applied force. Barnacles exhibiting this pattern appeared to peel off the silicone surface. Again the instability spread down either one side or both the sides of the basis.

C. **Adjacent peel separation**. This is similar to pattern B as the instabilities propagated in the same direction as the applied force, but the initial separation occurred on the sides of the basis perpendicular to the direction of the applied force. Barnacles exhibiting this pattern frequently had two cavities developing at the same time on either side of the barnacle.

D. **Twist separation**. The initial separation appeared in two locations, often proximal and distal to the point of contact of the probe. The propagating instabilities moved either clockwise or anticlockwise around the periphery of the basis. Barnacles exhibiting this pattern appeared to be twisting off the surface and showed the greatest amount of rotation during the detachment process.

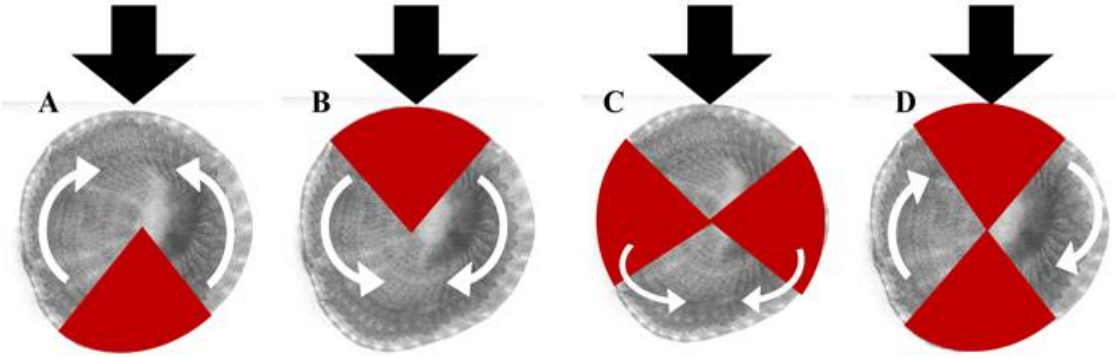

Figure 3.8. Four separation patterns of barnacles detaching from silicone coatings. The black arrow indicating the direction of the force and location of the probe of the force gauge, the red area indicates the region that the initial cavity develops in and the white arrows illustrate the direction of the propagating instabilities. A) Lift separation; B) peel separation; C) adjacent peel separation; and D) twist separation.

Of the 59 *E. modestus* tested, 57.5% exhibited separation pattern A. Pattern B was seen for 27.5% of the barnacles and only 7.5% presented pattern C. No individuals exhibited separation pattern D (Figure 3.9). However, 7.5% of the *E. modestus* showed no distinct separation. This meant it was not possible to see any cavity development or cavity propagation in the video. Of the 93 *B. amphitrite* tested, a larger percentage of barnacles (39.8%) showed no distinct separation and therefore was not classified under A, B, C or D. Pattern A was exhibited by 20.4% of the barnacles, while 25.8% showed pattern B, and 7.5% and 6.5% displayed patterns C and D, respectively. The wetted and de-wetted condition of the barnacles did not appear to influence the pattern of separation. In addition, there did not seem to be any correlation to the type of separation pattern and the type of PDMS coating used in this study.

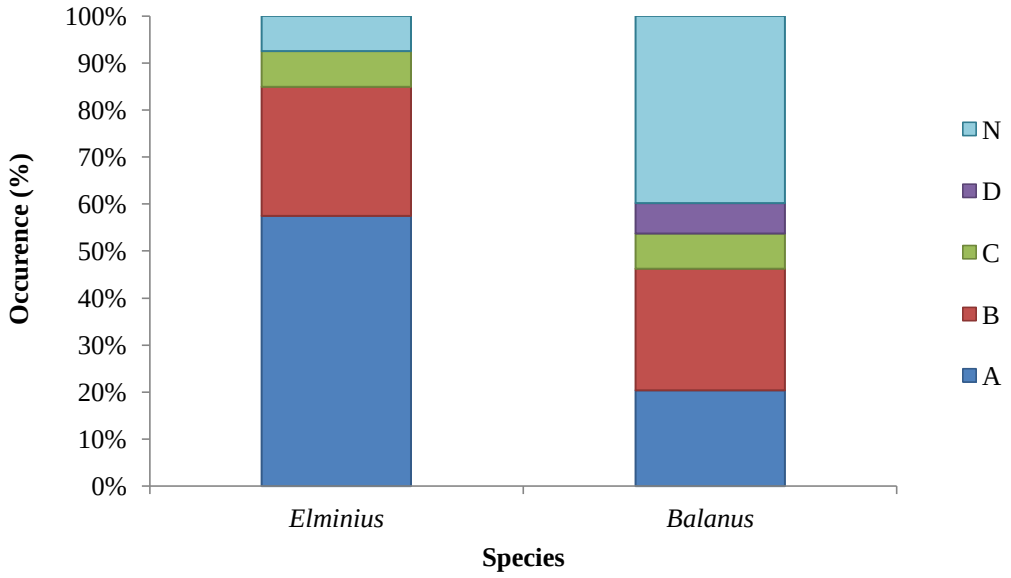

Figure 3.9. The percentage occurrence of separation patterns exhibited by *Elminius modestus* and *Balanus amphitrite* barnacles during removal from silicone coatings. Detachment pattern N is no distinct separation. Numbers (n) of barnacles = 59 and 93 *E. modestus* and *B. amphitrite,* respectively.

With *E. modestus,* an additional feature occurred immediately on application of the detachment force and prior to the initial separation that was not present in *B. amphitrite*. A black line appeared or extended from the edge of the basal margin (Figure 3.10). As the pressure of the force gauge was applied, this feature appeared from the basal margin, with increasing pressure the line became more defined, moving further away from the basal margin and along the periphery of the basis, eventually becoming rougher, more irregular and scalloped in appearance. This feature was present for every *E. modestus* that was removed whether in the wetted or de-wetted condition.

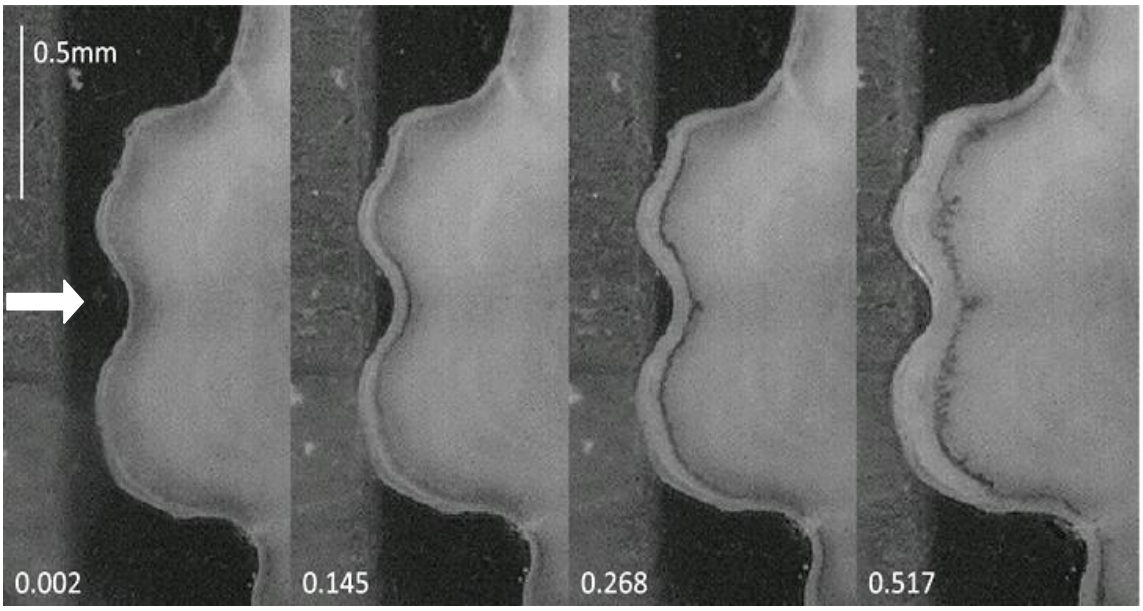

Figure 3.10. Still frames of an *Elminius modestus* on Sylgard 184 showing the appearance of a black line from the edge of the basal margin as the detachment force was applied. Direction of force indicated by the white arrow. With increasing pressure over time, the line becomes more defined and eventually more irregular. The time in seconds is presented in the lower left-hand corner of the still frames.

3.4.3. Initial separation

Figure 3.11 shows the average time in seconds for the onset of separation in both barnacle species. The data were transformed using $\log_{10}$ to attain a normal distribution (df = 96, D = 0.134, P = 0.070) with homogeneous variance ($df1$ = 6, $df2$ = 91, F = 1.887, P = 0.092). The null hypothesis that the initial separation time for both barnacle species would be equal was not supported; the initial separation occurred sooner for *E. modestus* than it did for *B. amphitrite* (df = 1, F = 14.319, P = 0.007) (Table 3.2). There was also a significant difference in the time of initial separation between barnacles removed while wetted compared to de-wetted, with the initial separation occurring sooner for barnacles subjected to wetted conditions in contrast to those that were de-wetted (df = 1, F = 40.563, P = 0.001). Yet, with regard to the effect of the coating, there was no difference in the initial separation time between the two silicone coatings (df = 1, F = 2.913, P = 0.161).

However, the interaction effects of species x wetness (df = 2, F = 14.969, P = 0.104), coating x species (df = 2, F = 2.514, P = 0.211), coating x wetness (df = 2, F =

2.843, P = 0.183), and coating x species x wetness (df = 3, F = 0.035, P = 0.882), demonstrate that the differences which were present in the timings between species and between degrees of wetness mentioned above, were not present in every circumstance. From Figure 3.11, the differences in the time of initial separation between *E. modestus* and *B. amphitrite* were significant for barnacles removed under wetted conditions, but not from de-wetted conditions, and this difference is more apparent for Silastic T-2. The differences between wetted and de-wetted conditions were significant for *E. modestus,* but not *B. amphitrite*, again this difference is more apparent for Silastic T-2.

The data were gathered from barnacles grown across multiple slides, to account for any pseudo-replication, the nested effect of slides was included. Yet, there was no nested effect of the different microscope slides for the initial separation time (df = 7, F = 5.695, P = 0.804).

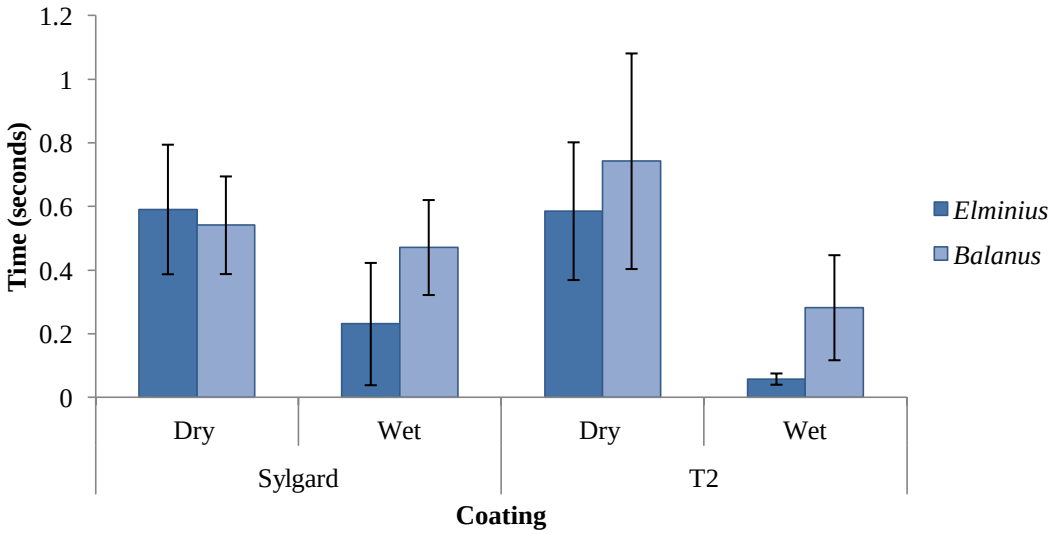

Figure 3.11. The mean time in seconds (± 95% confidence intervals) for the initial separation to appear during the detachment of *Elminius modestus* and *Balanus amphitrite* while de-wetted (Dry) and wetted (Wet) from the silicone coatings Sylgard 184 and Silastic T-2. (Data presented in the graph are the original, un-transformed data).

Table 3.2. ANOVA table of results of the time for initial separation of *Elminius modestus* and *Balanus amphitrite* on Sylgard 184 and Silastic T-2 coated microscopes slides, whilst de-wetted and wetted.

	Sum of Squares	*Mean Square*	*df*	*F-value*	*P-value*
Species	**0.555**	**0.555**	**1**	**14.319**	**0.007**
Wetness	**4.156**	**4.156**	**1**	**40.563**	**0.001**
Coating	**0.337**	**0.337**	**1**	**2.913**	**0.161**
Species x wetness	**0.523**	**0.523**	**2**	**14.969**	**1.104**
Coating x species	**0.110**	**0.110**	**2**	**2.514**	**0.211**
Coating x wetness	**0.703**	**0.703**	**2**	**2.843**	**0.183**
Coating x species x wetness	**0.005**	**0.005**	**3**	**0.035**	**0.882**
Slide number	**2.101**	**0.350**	**7**	**5.695**	**0.804**

3.4.4. Propagating instabilities

The manner in which the instabilities propagated across the basis appeared to differ between *B. amphitrite* and *E. modestus*. Figure 3.12 demonstrates a typical propagating instability in *B. amphitrite*, which has the classic form of viscous fingering. Viscous fingering is the instability between two fluids of differing viscosities, where the fluid with a lower viscosity penetrates with finger-like projections into fluid with a higher viscosity (Lemaire et al. 1991; Kavanagh et al. 2005). In *B. amphitrite* the cavities tended to flow in a more consistent wedge with an almost predictable trajectory; the finger-like projections advancing in a smooth motion, and the adhesive separating behind the cavity front. In *E. modestus* the cavity front had an irregular appearance but not with the typical finger-like projections seen in *B. amphitrite*. The movement of the cavity was more fluid and dynamic; and once the cavity had become established it looked and behaved as a bubble underneath the basal membrane. In some instances the bubbles changed the direction of the propagation or produced smaller bubbles that pinched off from the larger one. In both cases, the area behind the bubble, which had been separated from the surface, appeared to re-contact with the surface. Often, as the bubble grew, the appearance of the adhesive within the bubble seemed reticulated, whereas behind the cavity front in *B. amphitrite* it appeared more striated. Although the direction of the propagating cavity in *E. modestus* did appear more random, the bubble never crossed the area directly in the centre of the basis (marked by the black dashed line in Figure 3.13) where the body of the barnacle was positioned.

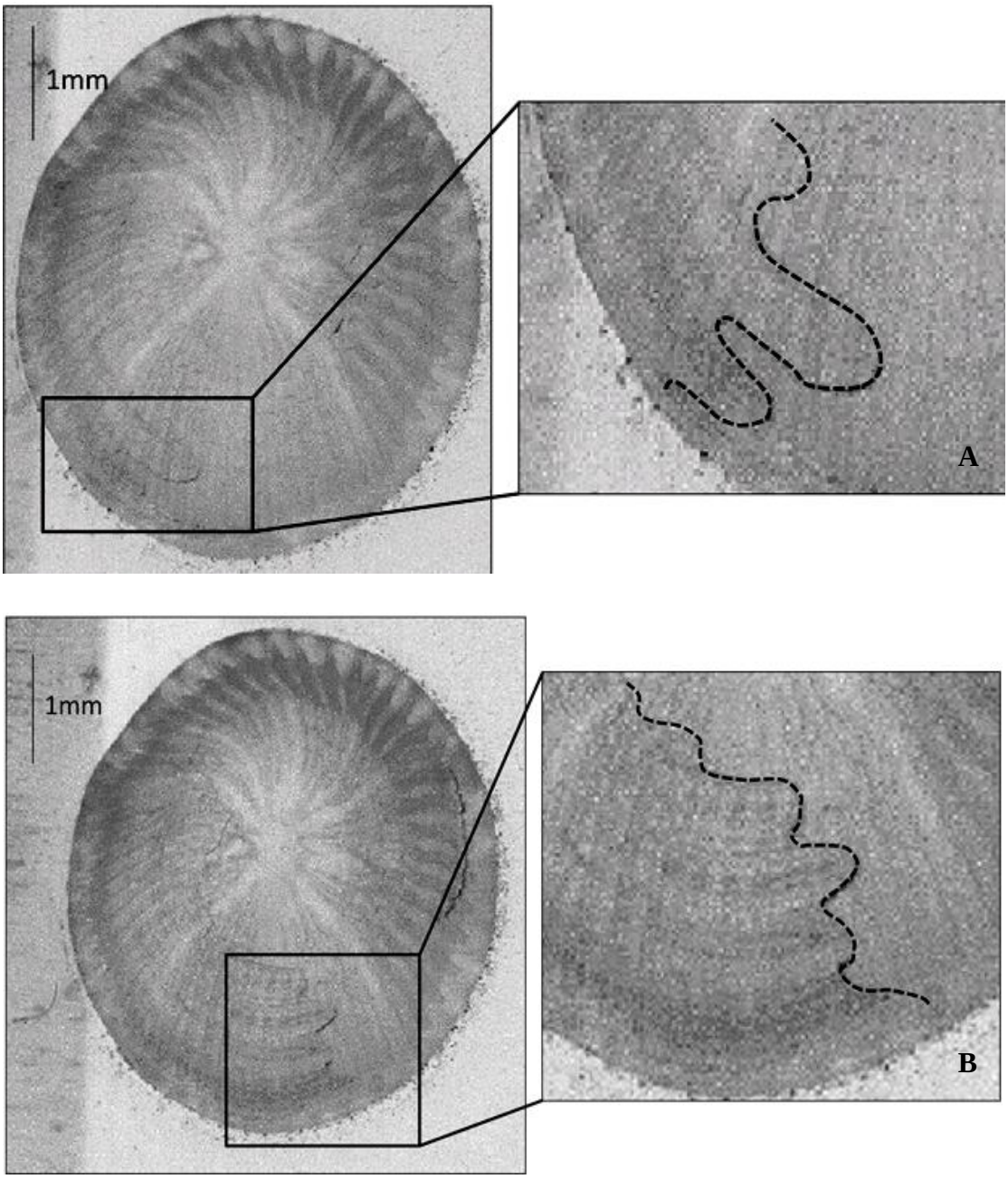

Figure 3.12. Propagating instabilities in *Balanus amphitrite* during removal from Sylgard 184 while de-wetted. The dashed black line highlights the cavity front, with the finger-like projections developing from picture A to B.

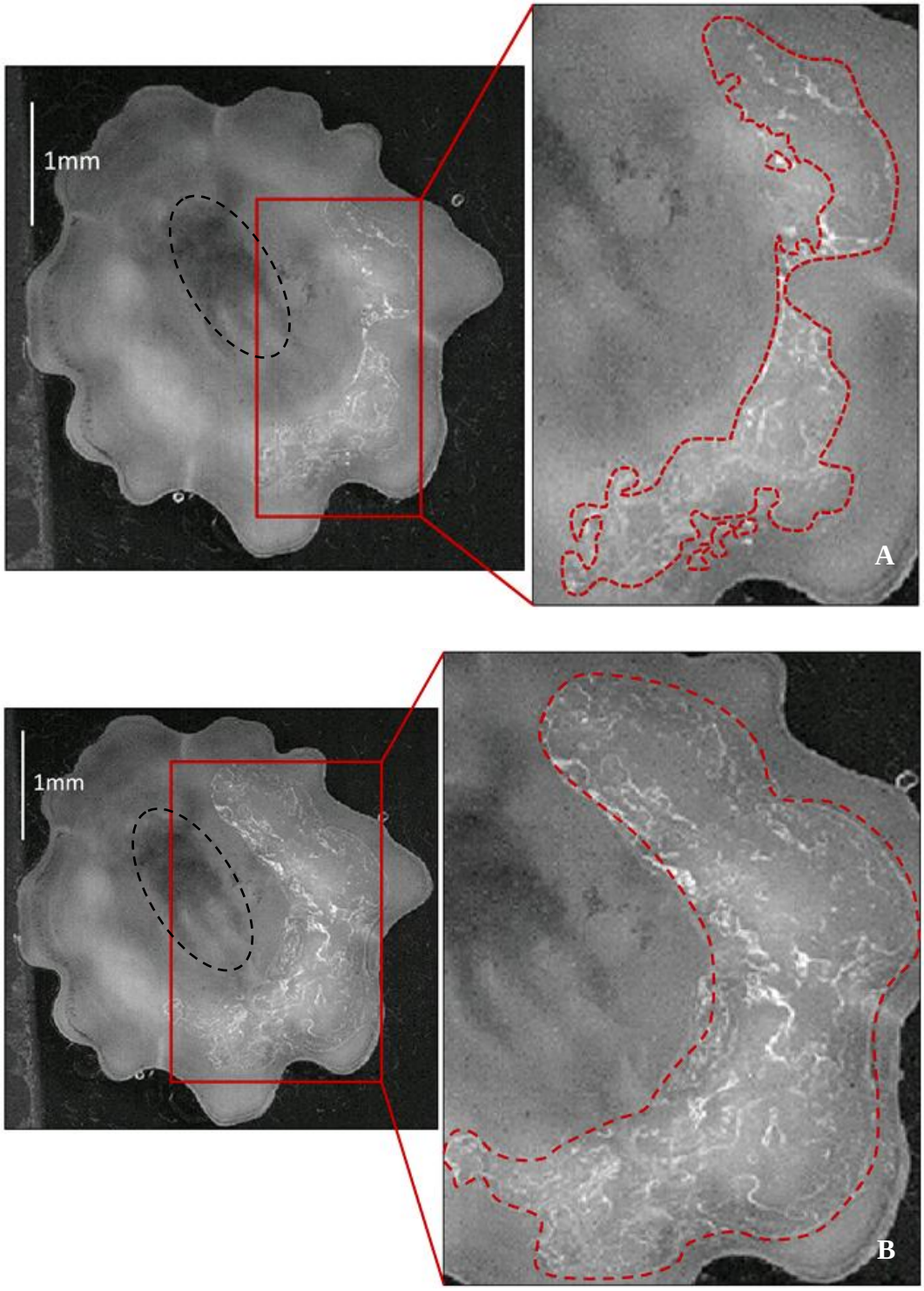

Figure 3.13. Propagating instabilities in *Elminius modestus* during removal from Sylgard 184 while de-wetted. The irregular cavity front highlighted by the dashed red line, developed from picture A to B.

3.4.5. Complete separation

During the final stages of separation, the membranous-basis of *E. modestus* tore perpendicular to the direction of force for 54.5% and 86.4% of all individuals removed from Silastic T-2 and Sylgard 184, respectively. Moreover, shell failure occurred in 4.5% and 13.6% of individuals on Silastic T-2 and Sylgard 184, respectively. Shell failure comprised of a fracture in the parietal plates that were in contact with the probe of the force gauge and preceded tearing of the membranous-basis. With *B. amphitrite* only 3.9% and 4.16% of barnacles removed from Silastic T-2 and Sylgard 184, respectively, showed shell failure where a fracture occurred in the basis, in parallel to the direction of force. The basal failure in *B. amphitrite* coincided with a fracture in the parietal plates. In addition, when the calcified-basis fractured, a proportion of the plate remained fixed to the surface. In no instance did a proportion of the membranous-basis of *E. modestus* remain on the surface.

Figure 3.14 shows the times for complete removal of *E. modestus* and *B. amphitrite* from Silastic T-2 and Sylgard 184 coatings while de-wetted and wetted. The data were normally distributed (df = 91, D = 0.072, P = 0.200), with homogeneous variance ($df1$ = 28, $df2$ = 62, F = 1.091, P = 0.377). The null hypothesis that the time for complete removal for both barnacle species would be equal was supported; there was no significant difference in the removal times between the two barnacle species (df = 1, F = 3.737, P = 0.057) (Table 3.3). Nor were there differences in the times for complete removal due to the type of coating (df = 1, F = 0.189, P = 0.665). However, there was an interaction effect of coating x species (df = 2, F = 4.578, P = 0.035). Figure 3.14 demonstrates that the complete removal is slower for *E. modestus* than *B. amphitrite* when removed from Sylgard 184, but not from Silastic T-2.

There was also an effect of wetness on the time for complete removal. Barnacles were removed under wetted conditions sooner than from de-wetted surfaces (df = 1, F = 9.382, P = 0.003). However, the interaction effects of wetness x coating (df = 2, F = 0.037, P = 0.859), wetness x species (df = 2, F = 0.024, P = 0.878) and coating x species x wetness (df = 3, F = 0.1.294 P = 0.259) show that the difference in timings due to wetness was not present in all circumstances. From Figure 3.14, it would seem that the difference due to degrees of wetness was present for *E. modestus* on Sylgard 184 and *B. amphitrite* on Silastic T-2.

The data was gathered from barnacles grown across multiple slides, however, there was no nested effect of the different microscope slides on the complete removal time (*df* = 7, *F* = 1.164, *P* = 0.521).

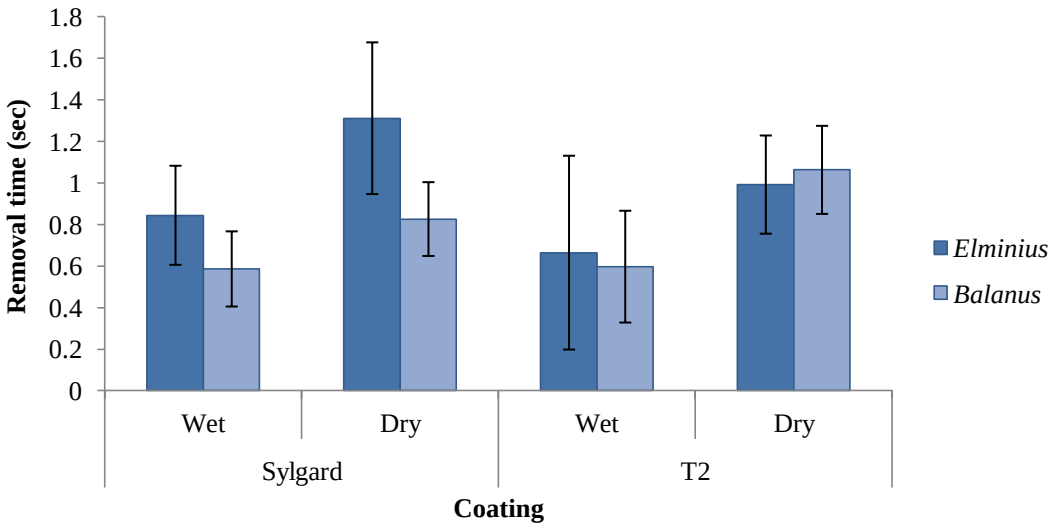

Figure 3.14. The mean time to complete removal (± 95% confidence intervals) of *Elminius modestus* and *Balanus amphitrite* while wetted (Wet) and de-wetted (Dry) from the silicone coatings Sylgard 184 and Silastic T-2.

Table 3.3. ANOVA table of results of the complete removal times of *Elminius modestus* and *Balanus amphitrite* on Sylgard 184 and Silastic T-2 coated microscopes slides, whilst de-wetted and wetted.

	Sum of Squares	*Mean Square*	*df*	*F-value*	*P-value*
Species	**0.670**	**0.670**	**1**	**3.737**	**0.057**
Wetness	**1.681**	**1.681**	**1**	**9.382**	**0.003**
Coating	**0.034**	**0.304**	**1**	**0.189**	**0.665**
Species x wetness	**0.004**	**0.004**	**2**	**0.024**	**0.878**
Coating x species	**0.820**	**0.820**	**2**	**4.578**	**0.035**
Coating x wetness	**0.007**	**0.007**	**2**	**0.037**	**0.848**
Coating x species x wetness	**0.232**	**0.232**	**3**	**1.294**	**0.259**
Slide number	**0.877**	**0.219**	**7**	**1.164**	**0.521**

3.4.6. Critical removal stress

Figure 3.15 shows the CRS of *E. modestus* and *B. amphitrite* from Silastic T-2 and Sylgard 184 coatings while de-wetted and wetted. The data was transformed using $\log_{10}$ to attain a normal distribution (df = 151, D = 0.057, P = 0.200) with homogeneous variance ($df1$ = 36, $df2$ = 114, F = 1.516, P = 0.166). The null hypothesis that the CRS of *E. modestus* and *B. amphitrite* were equal was not confirmed; the removal force to detach *E. modestus* was significantly less than the force to remove *B. amphitrite* (df = 1, F = 14.287, $P \leq 0.001$) (Table 3.4). There were, however, no significant differences in the CRS values between barnacles from wetted and de-wetted conditions (df = 1, F = 0.130, P = 0.719) and between the barnacles from the two silicone coatings (df = 1, F = 1.176, P = 0.280). There was an interaction effect of species x wetness (df = 2, F = 4.124, P = 0.044) and coating x wetness (df = 2, F = 4.353, P = 0.039) on the CRS of barnacles. There was no interaction effect, however, of coating x species (df = 2, F = 0.781, P = 0.378) and coating x wetness x species (df = 3, F = 1.590, P = 0.209). From Figure 3.15, the CRS of *E. modestus* was less than that of *B. amphitrite* when de-wetted but not when wetted. Also there appears to be a difference in the CRS of wetted and de-wetted *E. modestus* on Sylgard 184, in which the former is greater than the latter, but not on Silastic T-2. Yet, coating and degrees of wetness did not influence the CRS of *B. amphitrite* (Figure 3.15). Furthermore, there was no nested impact of the different slides (df = 7, F = 0.001, P = 0.982).

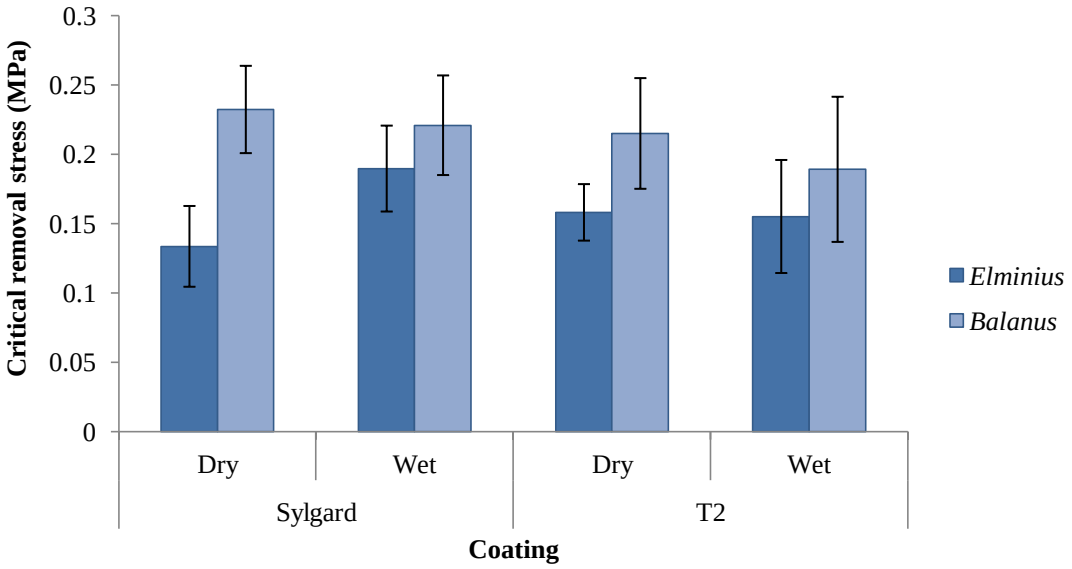

Figure 3.15. The mean critical removal stress (± 95% confidence intervals) of *Elminius modestus* and *Balanus amphitrite* on Sylgard 184 and Silastic T-2 whilst de-wetted (Dry) and wetted (Wet). (Data presented in the graph are the original, un-transformed data).

Table 3.4. ANOVA table of results of the critical removal stress of *Elminius modestus* and *Balanus amphitrite* on Sylgard 184 and Silastic T-2 coated microscopes slides, whilst de-wetted and wetted.

	Sum of Squares	*Mean Square*	*df*	*F-value*	*P-value*
Species	**0.508**	**0.508**	**1**	**14.287**	**≤ 0.001**
Wetness	**0.005**	**0.005**	**1**	**0.130**	**0.719**
Coating	**0.042**	**0.042**	**1**	**1.176**	**0.280**
Species x wetness	**0.147**	**0.147**	**2**	**4.142**	**0.044**
Coating x species	**0.028**	**0.028**	**2**	**0.781**	**0.378**
Coating x wetness	**0.155**	**0.155**	**2**	**4.353**	**0.039**
Coating x species x wetness	**0.057**	**0.057**	**3**	**1.590**	**0.209**
Slide number	**0.00184**	**0.00184**	**7**	**0.001**	**0.982**

3.5. Discussion

The aim of this chapter was to examine how the membranous-basis of *Elminius modestus* influenced the process of its detachment from silicone coatings compared to that of a calcareous-based barnacle, *Balanus amphitrite*. Using a high-speed camera it was possible to visualise and describe the detachment process of the two barnacle species.

3.5.1. Removal process of barnacles from silicones

The removal process involves a series of steps from initial separation, through spreading instabilities, to complete removal and these findings were consistent with those described by Kavanagh et al. (2005) for the removal of *Balanus eburneus* and *B. variegatus.* A new feature noted for *E. modestus*, however, was the appearance of a black line at the basal margin. This appeared to be an internal structure compressing with the application of pressure. The following diagram (Figure 3.16) shows a section through the shell of *E. modestus* illustrating some internal features close to the periphery of the shell. As this line appears within the growth zone of the basal margin, it may be that it was the compression of the basal-secreting cells and/or the epicuticle-secreting cells. As the force was applied, these cells were pushed or peeled from the shell wall (mural plate), the black colour being a density effect; i.e. with increasing pressure the cells compress more, becoming thicker and denser and thus appearing black in the image. As the pressure continues to build, the cells are forced further away and together causing them to crumple, in turn making the line become more irregular and scalloped in shape over time.

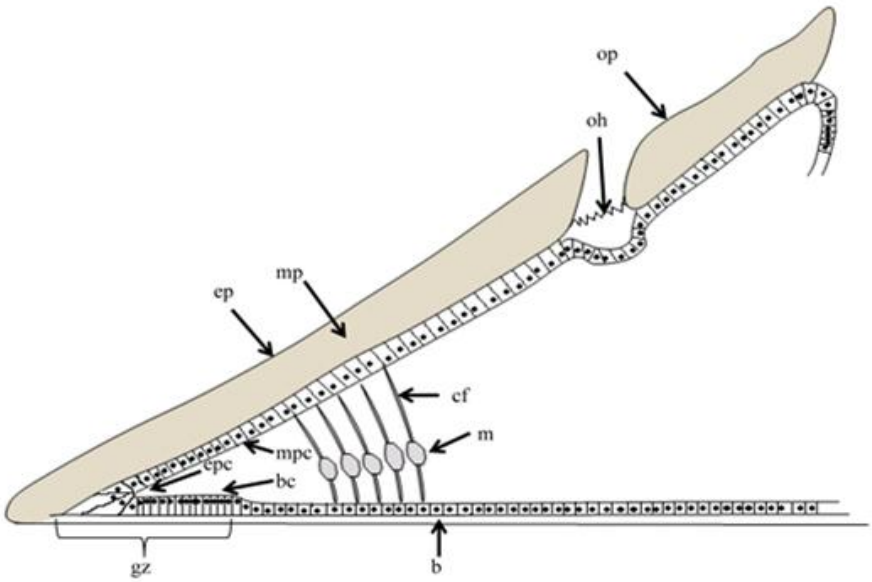

Figure 3.16. Cross sectional diagram of *Elminius modestus* through the peripheral part of the shell: b, basis; bc, basis-secreting cells; gz, growth zone; ep, epicuticle; epc, epicuticle-secreting cell; mp, mural plate; mpc, mural plate-secreting cells; m, muscle; cf, collagen fibres, oh, opercular hinge; op, opercular plate. Adapted from Bubel (1975).

3.5.2. Patterns of separation

One of the differences between *B. amphitrite* and *E. modestus* observed in this study was the pattern of separation and the proportion of each pattern per species. The incidence of barnacles displaying 'no distinct fracture' may in part be due to deficiencies in clarity and contrast in the videos, so any separation that occurred could have been missed. Before each detachment event, time was spent focusing the image of the basis on the high-speed video control panel. In spite of this, in some cases as soon as the detachment force was applied to the side of the barnacle, the pressure was sufficient to move the platform and send the image out-of-focus. It was not until reviewing the detachment events of the first culture that the incidences of poor focus became apparent. For the second cultures of *E. modestus* and *B. amphitrite*, additional supports were added underneath the platform and extra crossbeams added to the frame holding the platform to make the set-up sturdier; this reduced the incidences of poor focus. However, for *B. amphitrite* there was an additional issue with the concentric pattern of the growth bands on the basis adding to problems with the contrast. The presence of these growth bands on the basis made it difficult in some videos to clearly

see the separation and contributed to a higher proportion of *B. amphitrite* with 'no distinct fracture'. The relatively high number of barnacles tested (93 *B. amphitrite* and 59 *E. modestus*) [Kavanagh et al. (2005) used a combined total of 40 barnacles] compensated for the loss of information in some individuals through poor video quality.

Differences in shell shape and bases of *B. amphitrite* and *E. modestus* offer a possible explanation for the different separation patterns observed. The shell of *E. modestus* has a lower profile than that of *B. amphitrite*, which is taller with mural plates approaching closer to the vertical, as illustrated in Figure 3.17. When applying force to a steep (near vertical) side of *B. amphitrite* it could result in a lifting force causing the barnacle to pivot on the side furthest from the probe's contact point, resulting in peeling and separation patterns B and C. Chaudhury & Kim (2007) illustrated a similar peeling process with a rigid glass prism fixed to a silicone elastomer. The glass prism had a vertical surface, which the force was applied to. The lower angle formed between the parietes of *E. modestus* and the surface may translate to downward pressure on the shell when the probe makes contact. This force could cause the side furthest from the probe to lift contributing to a greater proportion of *E. modestus* with separation pattern A, the lift separation. The model detachment mode for *B. amphitrite* would therefore be a peel separation and the model detachment for *E. modestus* would be a lift separation. However, as stated above, 20% of *B. amphitrite* also exhibited pattern A. This was likely due to the barnacles ranging in size from small to average for this species (3.2 to 7.5mm in diameter). From personal observations, smaller-sized *B. amphitrite* have a relatively shallower profile than the larger specimens. Therefore, their shape is closer to the shell shape of *E. modestus* and this may explain why 20% of the *B. amphitrite* exhibited pattern A release. The height of the shell of each barnacle was not recorded in this study as this explanation for the variations in separation patterns was developed after the experiments. Further study is therefore required to examine possible relations between barnacle dimensions (height, slant height, width of basis) and the type of separation.

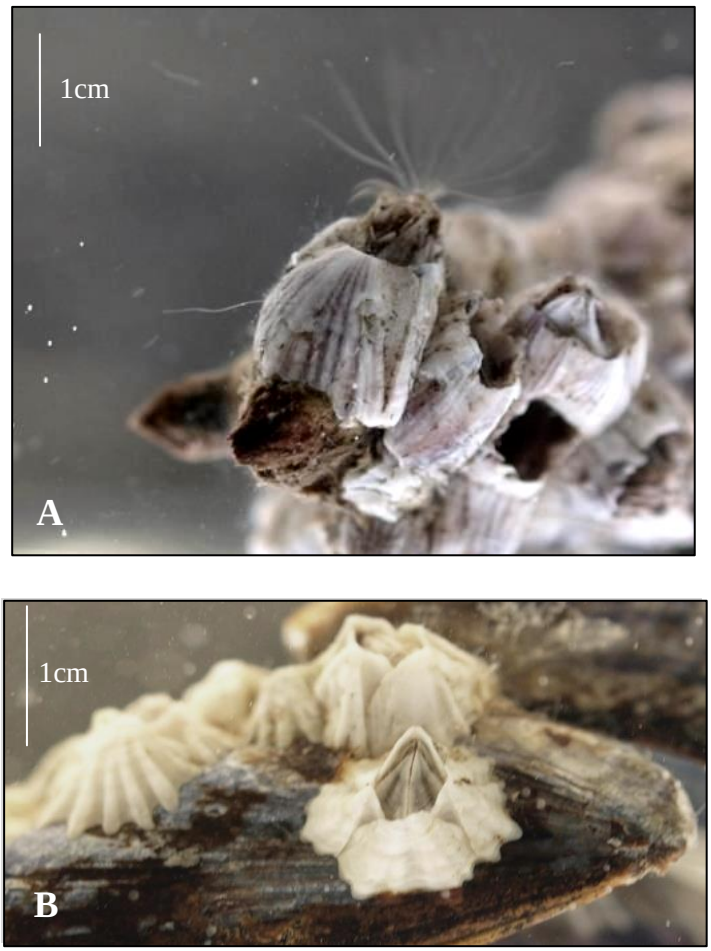

Figure 3.17. Pictures of *Balanus amphitrite* (A) and *Elminius modestus* (B), both collected from wild populations illustrating their different shell shapes. Images taken by author.

The bases of *B. amphitrite* and *E. modestus* also differ in shape. The basal margin of *E. modestus* is undulating, whereas that of *B. amphitrite* is comparatively smooth and circular. During the detachment of *E. modestus*, the force-gauge probe tended to bridge across two of the major ridges in the shell. Under these circumstances there were two points of contact of the probe on the barnacle and this contributed to a more stable contact area and therefore limited the incidence and angle of rotations. For example, when rotations did occur, there was one point of contact rather than two. In *B. amphitrite*, there was only ever one point of contact, contributing to the increased occurrence and degree of rotation. Examples of *B. amphitrite* exhibiting pattern D, where the barnacles were described as twisting off the coatings, demonstrated the greatest degree of rotation. The greater degree of stability offered by the two contact points in *E. modestus* could explain why no twisting separations occurred.

Each barnacle had a different shape and size, which potentially influenced the manner in which they separated from the coatings. There are, however, other factors to consider such as the layer of adhesive between the barnacle and coating and the thickness of the coatings. Kohl and Singer (1999) demonstrated that the separation of pseudobarnacles, via tensile forces, from coatings with a thickness gradient from 0.1mm to 0.9mm, begins in the area where the coating is at its thickest. In the present study, the coating thickness was intended to be constant; however, despite being prepared on a level surface, there were subtle variations in the thickness of the coatings. Measurements were taken of the coating thickness at six points across each slide. The thickness of the coating along the length and breadth of every slide varied by an average of 40µm for both Silastic T-2 and Sylgard 184 (average from 25 slides per coating). This is a much smaller difference than that discussed by Kohl and Singer (1999). As coating thickness was not a primary focus of this study, no tests for correlations between the thickness of the coatings and the patterns of separation were attempted, but it may warrant consideration in future investigations.

3.5.3. Propagating instabilities

Kavanagh et al. (2005) described the appearance of finger-like projections in the adhesive, which permeated across the basis during the detachment of *B. eburneus* and *B. variegatus* from silicone coatings. These fingering instabilities, or viscous fingering, portray the instability between two fluids of differing viscosities, where the one with a lower viscosity penetrates into the higher viscosity fluid (Lemaire et al. 1991; Kavanagh et al. 2005). Kavanagh et al. (2005) suggested the adhesive existed as a gradient of differing viscosities between the basis and the silicone coating. This supports the description Sun et al. (2004) provided of a multi-layered adhesive produced by barnacles when grown on silicones. Fingering instabilities were identified during the detachment of *B. amphitrite* in this study. They behaved in a similar manner to the description provided by Kavanagh et al. (2005) for *B. eburneus* and *B. variegatus*. However, the propagating instabilities seen for *E. modestus* did not take on the appearance of typical viscous fingering as seen in *B. amphitrite*. For the fingering instabilities to occur, the fluids with different viscosities need to be confined between two parallel planes (Saffman & Taylor 1958). In *E. modestus*, the membranous-basis may not offer sufficient resistance to confine the viscous adhesive during detachment

and therefore the characteristic finger-like projections are not produced. This theory assumes that the adhesive of *E. modestus* behaves in the same manner as that of *Balanus* spp. in terms of developing a graded or layered structure.

There has yet to be a study on the adhesive of *E. modestus*, hence it is merely speculation that the structure occurs in a graded form. What is known is that when grown on low modulus coatings, *E. modestus* produce a thicker more hydrated adhesive than when grown on a coating with a higher modulus (Wiegemann & Watermann 2004). The adhesive of *E. modestus* was, however, less hydrated than that of *Balanus* spp. Also, barnacles grown on low modulus coatings can develop a concave-shaped calcareous-basis (Wiegemann & Watermann 2003; Sun et al. 2004; Wendt et al. 2006). The thickness of the adhesive across the basal area is not uniform; the adhesive is thicker in the centre of the 'cupped' basis than at the edges (Berglin & Gatenholm 2003). However, the flexibility of the membranous-basis hinders the production of a concave shape. The space between the basis and substratum is diminished and therefore only allows for a thinner layer of adhesive than in calcareous-based examples (Wiegemann & Watermann 2004). The less hydrated adhesive and diminished gap (between basis and substratum) in *E. modestus* may be an additional factor explaining why *E. modestus* separation does not display fingering instabilities.

3.5.4. Complete separation

Shell damage occurred to a greater degree during detachment of *E. modestus* compared to *B. amphitrite*, reflecting the weaker shell strength of the former species. The shell of *B. amphitrite* is porous, with longitudinal channels interspersed with septa, strengthening the shell; whereas the shell of *E. modestus* is non-porous, more brittle and more liable to fracture (Barnes et al. 1970; Gubbay 1983). When force was applied to the shell of *E. modestus*, the entire structure seemed to flex and compress during detachment from the surface. This could be a factor of the membranous-basis not offering structural support. There is also a difference in the number of parietal plates, which could factor into the strength of the shell; *E. modestus* has four, whereas *B. amphitrite* has six plates. The reduction in the number of parietal plates is suggested to be an adaptation to prevent predation (Palmer 1982). The sutures between the shell's parietal plates are the weakest part of the shell and can separate when compressed (from

downward pressure on the opercular opening); therefore barnacles with fewer sutures are less vulnerable to being crushed (Barnes et al. 1970; Palmer 1982). However, the overall shell strength has been correlated to the strength of the sutures rather than the number. *E. modestus* have relatively weak butt sutures compared to the stronger mitred sutures in *B. amphitrite* (Barnes et al. 1970) and therefore *E. modestus* can be more prone to damage.

An additional observation during the detachment of *E. modestus* was that the position barnacle's body remains fixed in place. The shell itself was driven forward; the basal membrane on the side closest to the probe's contact point was compressed, whereas the membrane on the side furthest away was stretched. The basal membrane was stretched to the point where it failed and tore, often before the barnacles were detached. As soon as the membrane tore the barnacles were rapidly removed.

When *E. modestus* and *B. amphitrite* separated from the silicone coatings, remnants of adhesive remained on the surface. Kavanagh et al. (2005), who observed the same for *B. eburneus* and *B. variegatus*, suggested that adhesive was left behind due to cohesive failure of the adhesive. Sun et al. (2004) speculated that it was cohesive failure between the layers of the multi-layered adhesive in the same two species. This cohesive failure between the layers of the adhesive may be described as delamination, which is a mode of failure between layers of composite materials. As a fracture propagates, it progresses within a single plane, between the adhesive and the silicone coatings, i.e. interfacial failure. However, it is hypothesised that separation occurs between the adhesive layers and propagates moving from layer to layer following the mode of failure that offers the weakest mechanical resistance, as the cohesion between the adhesive layers fails, and it delaminates (Figure 3.18). In polymer science this type of delamination is referred to as a multiple-interface delamination (Li et al. 2010).

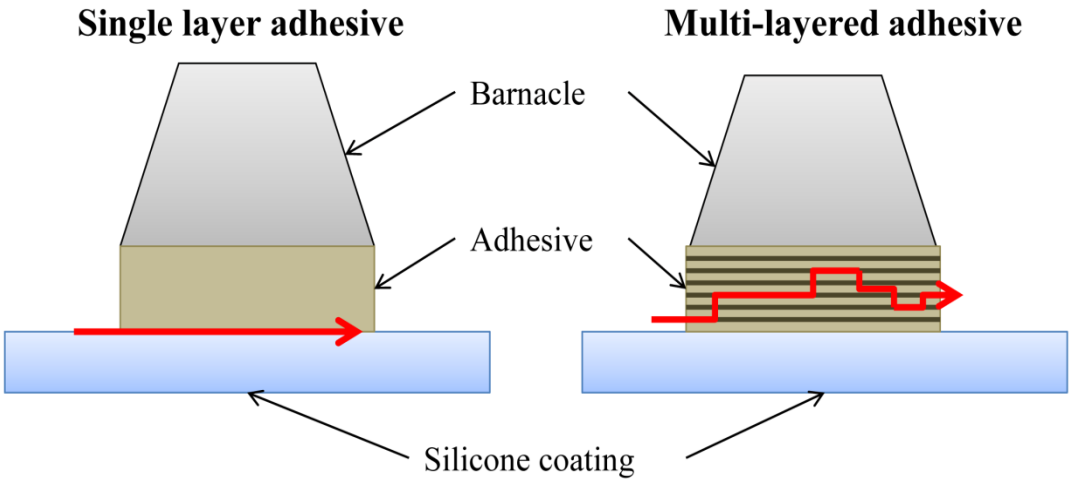

Figure 3.18. Diagram depicting the hypothesised delamination fracture between a single and multi-layered adhesive. The fracture (red arrow) in a single layered adhesive propagates on a single plane, whereas in a multi-layered adhesive the fracture propagates from layer to layer, following the path of least resistance.

3.5.5. The time for initial separation and complete removal

The time taken for initial separation to begin was less for *E. modestus* than for *B. amphitrite* and this difference was more prevalent under wetted conditions. Considering the flexible basis and the structure of the *E. modestus* shell, it may be that there is greater elastic deformation and less resistance to the application of force and, therefore, the initial separation occurs sooner. However, the time for complete removal was longer for *E. modestus* than *B. amphitrite*, but this difference was only present for Sylgard 184, for both wetted and de-wetted conditions. It could be that the greater degree of elastic deformation in *E. modestus* slows the propagation of the fracture once it has been initiated, whereas once the fracture begins in the more rigid structure of the *B. amphitrite* shell, it is more instantaneous. It is just speculation that the flexible nature of the *E. modestus* membranous-basis and shell structure contributes to the differences in timings. Factors other than physical differences in the shell's structure could be involved. For example, there may be differences in the adhesives of the two species. Unfortunately, the adhesive of *E. modestus* has yet to be investigated.

The time to initial separation and for complete removal was less for barnacles that were wetted as opposed to de-wetted. This difference was more apparent for *E.*

modestus. Barnacles create a seal with the coatings around the perimeter of the shell (Kavanagh et al. 2005), which prevents the influx of water underneath the basis. From the high-speed videos it is clear that water intrusion underneath the basis only began mid-way through the process of separation once the perimeter seal had been compromised. The water which percolates underneath the basis, filling the cavities, may have acted as a lubricant reducing the drag of the basis on the surface of the silicone coating. This provides a possible explanation for the reduction in complete removal time due to wetness but not necessarily for the reduction in the time to initial separation when wetted. During the time for initial separation to begin, the barnacles perimeter seal did not appear compromised; water did not appear to percolate underneath the barnacle, however, the moisture surrounding the barnacles still may have contributed to a lubricating effect aiding initial separation. Dehydration of the adhesive may also be a potential factor. Wiegemann & Watermann (2004) noted the CRS of calcareous-based barnacles (*B. improvisus* and *B. crenatus*) increases with dehydration of the adhesive, which consequently may require more time for the separation process to commence. For *E. modestus*, however, there no was significant increase in the CRS with dehydration (Wiegemann & Watermann 2004). The timings for dehydration that Wiegemann & Watermann (2004) investigated were 3 – 4 hours and 24 hours, whereas in this study, the barnacles were air dried for 5 minutes after initially removing the moisture surrounding the barnacles with laboratory blue roll. Therefore, it is unlikely that dehydration of the adhesive was a significant factor explaining the reduction in the initial separation time when wetted. It is not clear why the initial separation times for barnacles removed when wetted were less than barnacles which were de-wetted. This difference warrants further investigation.

3.5.6. The critical removal stress

The CRS results in this chapter support those found in Chapter 2, where the CRS of *E. modestus* was less than the CRS of *B. amphitrite*. In this chapter, this was the case for both silicone coatings, not just Sylgard 184, although this was only the case for barnacles that were de-wetted. Chung & Chaudhury (2005) stated that an object with a greater degree of flexibility and deformation requires less stress to be detached from elastomeric coatings. It would seem that the flexible attribute of *E. modestus*

membranous-basis and shell structure can contribute to a reduced removal force compared to a calcareous-based barnacle.

There was also an interaction of coating and wetness, in which the CRS values for *E. modestus,* while wetted, were greater than those that were de-wetted for Sylgard 184. This is in contrast to the work by Wiegemann & Watermann (2004), who demonstrated that, the removal stress of *E. modestus* while in water and after 1 hour of desiccation did not differ. It was surprising that the force to detach *E. modestus* when wetted was greater than de-wetted when the complete removal time for wetted individuals was less for de-wetted individuals. Considering that water percolating underneath the basis could potentially act as a lubricant aiding quick removal, it would be reasonable to assume that the removal force would be lower for wetted than de-wetted barnacles, but the opposite was found. Further investigation is necessary to answer this question. It may be that use of a motorised platform for the force gauge, similar to that used by Stein et al. (2003), which would provide a constant speed during detachment for each barnacle, would help remove human error in the application of the force. Whether (or not) the force gauge slipped for wetted barnacles and caused an increase in the detachment force is unknown. Regardless, a motorised platform may better highlight the difference in CRS and timings in regard to the degrees of wetness.

The type of coating did influence the removal times and the CRS values in this study. As mentioned in Chapter 2, the two coatings do have minor differences in their bulk properties. The difference in bases and adhesives may be reacting in a different manner to the properties of the coatings. However, only two silicone coatings were tested. Further experiments on a range of coatings with greater variability in surface and bulk properties should provide a better insight into the influence of substratum on the detachment mechanisms of membranous and calcareous-based barnacles.

3.6. Conclusion

This was the first study to use high-speed video analysis to examine, in detail, the detachment properties of a membranous-based barnacle. The flexible attributes of the membranous-basis of *E. modestus* appear to have contributed to the differences seen in the propagating instabilities and the time for removal when compared to the calcareous-based *B. amphitrite*. However, differences in the shape and structure of the

shell were likely to have made the greater contribution to the dissimilar patterns of separation i.e. lift separations in *E. modestus* and peel separations in *B. amphitrite*.

In this study, the influences of wetness and coating type on the removal times and the CRS were evident for *E. modestus*, in most cases, but less so for *B. amphitrite*. This suggests that the detachment processes of *E. modestus* is more easily influenced by variations in environmental stresses such as wetness and substrate type.

Questions remain regarding the nature of the adhesive of *E. modestus* and how this influences the release of barnacles from silicones. Kendall's (1971) fracture model for predicting the release of barnacles from elastomeric substrates has been deemed unsuitable for calcareous-based barnacles as they have a degree of flexibility greater than the rigid stud assumption of the model (Sun et al. 2004; Ramsay et al. 2008). Therefore, Kendall's model is likely even more unsuitable for predicting the release of membranous-based barnacles.

Chapter 4. A Comparison of Laboratory-based Assays and Field Performance Trials of Coatings: Bridging the Gap Between Laboratory and Field.

4.1. Abstract

Laboratory assays and field immersion trials are two approaches used to evaluate the efficacy of antifouling and fouling-release coatings in terms of settlement and adhesion of marine fouling. To determine whether laboratory assays are a good predictor of coating performance in the field, a series of eight coatings (five silicone and three fluoropolymer coatings) were used to compare the settlement/recruitment and critical removal stress (CRS) of the membranous-based barnacle *Elminius modestus* from a laboratory culture and two field populations (Fairlie Quay and Burnham-on-Crouch) over two years (2010 and 2011). A second membranous-based barnacle *Semibalanus balanoides* was abundant at the field location Fairlie Quay, thus recruitment and CRS was measured and compared to *E. modestus*. In addition, the influence of a 10-day-old biofilm and the influence of temperature (12 ºC, 15 ºC, 19 ºC and 22 ºC) on the CRS of laboratory cultured *E. modestus* were investigated.

Laboratory settlement/field recruitment and the CRS of *E. modestus* from the two field populations and the laboratory culture across the eight coatings had similarities. This made it possible to discriminate between the coatings and conclude that the silicone performs better than the fluoropolymers, with the silicone coatings having lower percentage coverage and lower adhesion measurements. Although the CRS measurements did differ significantly between locations and years, where the general pattern from highest to lowest between the locations was Fairlie Quay > laboratory > Burnham-on-Crouch. The presence of a biofilm and different temperatures did not influence the adhesion of *E. modestus* in this study, and thus provided no additional clarity as to why there were differences between laboratory and field results. Nevertheless, being able to differentiate between the coatings and determine which has the better FR properties is fundamentally the desired outcome for these tests.

4.2. Introduction

The process of biofouling, in which a submerged surface becomes colonised, is complex. The process is often described as sequential or "successional", suggesting a predictable series of events. In this model, the initial process, which commences seconds after immersion of a surface, is adsorption of a macromolecular conditioning film. Colonisation by bacteria occurs next, followed by attachment of microalgae and spores of macroalgae and ultimately the larval stages of invertebrates. However, the successional hypothesis may not be suitable. There are many conflicting reports on the influences of microfouling films or biofilms on the settlement of marine invertebrates and more specifically, on the settlement of barnacles (Todd & Keough 1994; Keough & Raimondi 1995; Wieczorek & Todd 1998). Dependent on the composition, density and age of the biofilm, it can have a facilitatory influence on cyprid settlement (Maki et al. 1988; 1990; Wieczorek et al. 1995) or it can have an inhibitory effect (Maki et al. 1988; 1990; Rittschof & Costlow 1989; Wieczorek et al. 1995; Olivier et al 2000), and therefore barnacles do not always require the presence of a biofilm for settlement. Colonisation ought to be regarded as a dynamic process (Wahl 1989; Clare et al. 1992), reflecting the availability of colonisation stages of fouling organisms and the interactions between colonisers and incumbents. The process of colonisation is heavily dictated by multiple physical, chemical and biological processes including light, water flow and turbulence, surface texture and colour, surface charge, surface area, wettability, biofilm composition, predation and competition (Yule & Walker 1984; Wethey 1986; Bourget 1988; Rittschof & Costlow 1989; Wahl 1989; Roberts et al. 1991; Hills & Thomason 1998; Thomason et al. 1998; Thompson et al. 1998; Wieczorek & Todd 1998; Bers & Wahl 2004; Prendergast et al. 2009; Robson et al. 2009). Not only does each factor alone exert an influence, interactions between factors have been shown to have a combined effect on the settlement and adhesion of colonisers such as barnacles (Thomason et al. 2002b; Prendergast et al. 2009; Robson et al. 2009).

Testing of antifouling and fouling-release (FR) coatings, in terms of laboratory settlement and adhesion, and field-testing through immersion trials, allowing for natural colonisation, are common practices (Becka & Loeb 1984; Swain et al. 1992; 2000; 2002; Swain & Schultz 1996; Wood et al. 2000; Wiegermann & Watermann 2004; Holm et al. 2006; Robson et al. 2009). Field immersion trials might be assumed

to be a better option over laboratory assays as the complex colonisation processes and interactions between physical, chemical and biological components are naturally occurring. Field trials, therefore, would best reflect the settlement and adhesion found on the ship's hulls and thus provide a more realistic idea of a coating's performance. However, field trials have been criticised as they require a large volume of coating sample and often require several months immersion time to allow the organisms to settle and grow to a sufficient size for adhesion testing (Rittschof et al. 2008; Stafslien et al. 2012). In addition, field trials can be restricted by the seasonality of certain fouling species and low larval availability resulting in poor settlement, and there may also be problems resulting from adverse weather conditions or predation which removes a portion of the organisms that had settled (Swain et al. 1998; Rittschof et al. 2008).

Laboratory adhesion assays still require a period of time to grow animals to testable size, but a large volume of coating is not required for testing and the assays are performed under controlled conditions; therefore these can allow for a systematic study of biofouling. Several studies have compared the settlement of barnacles reared under laboratory conditions to settlement in the field on antifouling and FR coatings (Rittschof & Costlow 1989; O'Connor & Richardson 1996; Thompson et al. 1998; Matsumura et al. 2000; Martinelli et al. 2012). The critical removal stress (CRS) of re-attached *Balanus amphitrite* from laboratory trials has also been compared to the removal stress of barnacles (*Balanus spp.*) from the field (Stafslien et al. 2016), which established a high level correlation in the adhesion of the barnacles between the two environments. There has, however, yet to be a study that compares the CRS of adult barnacles, including the membranous-based barnacle *E. modestus* that have been settled and grown in the laboratory on test coatings, to those recruited in the field.

The aim of the work presented in this chapter was to compare the use of laboratory assays and field immersion trials for evaluating FR coatings. The main focus was to compare the CRS of *E. modestus* barnacles reared in the laboratory and field environments, as well as comparisons in the laboratory settlement and field recruitment. The hypotheses to be tested are: 1) the settlement observed in laboratory assays would correlate well with the recruitment in the field; 2) the CRS measurements from laboratory-cultured barnacles would correlate well with those from the field and therefore 3) laboratory assays are a good predictor of a coating's performance in the

field. The influence of biofilm and temperature on the CRS of laboratory-raised *E. modestus* was investigated, in order to provide a possible explanation for any potential differences in the CRS of the barnacles between the two environments. In addition, the recruitment and CRS of a second membranous-based species, *Semibalanus balanoides* was compared to that of *E. modestus*. Field immersion trials are necessary for the settlement and growth of *S. balanoides* on the test coatings. This barnacle is an annual brooder. Naupliar release is synchronised with the spring diatom bloom (Barnes 1962) and laboratory cultures of this species, by virtue of the relatively slow rate of development of the larvae, have so far met with limited success (Kirby 2006).

4.3. Materials and methods

4.3.1. Coating selection

Preliminary trials for field assays were conducted during 2009 using Intersleek® 900, Intersleek® 700 and an Intersleek control coating referred to as Intersleek Clear, provided by International Paint Ltd, Felling, UK. All the coatings were applied to glass microscope slides (76mm x 26mm x 1mm, Fisherbrand). The coatings selected for field immersion trials and laboratory bioassays during 2010 and 2011 included: five silicones, four of which were polydimethylsiloxane (PDMS) coatings, with different molecular weights and crosslinking densities; one was a Polyether-silicone-polymer and three were fluoropolymers of different molecular weights and functional groups. These coatings were provided and prepared at International Paint Ltd, Felling, UK. These silicone and fluoropolymer coatings will be discussed in more detail in Chapter 5, and for the current purpose have been labelled S1, S2, S3, S4, S5, FP1, FP2 and FP3. Coatings used to investigate the influence of a 10-day-old biofilm and temperature on the adhesion strength of adult *E. modestus* were Silastic T-2, Rhodorsil 48V-750 PDMS, and Sylgard 184 (the latter was not used in the experiments that studied the influence of temperature).

4.3.2. Laboratory settlement assays

The coatings were leached for two weeks in a static tank of reverse osmosis (RO) water; the water was changed once a week and a carbon filter (Fluval filter) was immersed in the tank to absorb leachate. The coated slides were then immersed for 1 hr in artificial seawater (ASW) and left to air-dry. Once dried, the slides were used immediately for settlement assays with *E. modestus* cyprids. The method for the laboratory culture of *E. modestus* was discussed in Chapter 2. Cyprids were used immediately upon collection (day zero cyprids) whereby 20 cyprids were pipetted into a 2ml droplet of 0.2µm filtered ASW centred on each slide. The slides were placed in quadriPERM® culture vessels with the lids on to reduce evaporation. After 48 hrs, the number of settled cyprids was recorded. Next, 15ml of *Tetraselmis suecica* was added to the chambers of the culture vessels. The barnacles were maintained at 22°C on a 12:12 L:D cycle and fed 15ml of *T. suecica* at ~3 x 10^5 cells ml^{-1} three times a week. The barnacles were grown for five months and attained an average size of 4.4mm in diameter of the basis which was approximately consistent with the growth of the barnacles as discussed in Chapter 2.

4.3.3. Field assays

Two locations were selected to deploy samples in the field; 1) Burnham-on-Crouch, Essex (51° 37’ 14” N) and 2) Fairlie Quay, Ayrshire (55° 46’ 58” N) (Figure 4.1). Both are secure locations. Burnham-on-Crouch has a well established population of *E. modestus* (Crisp & Davies 1955; Crisp & Meadows 1962; Robson et al. 2009) and is the location of a commercial testing site for marine coatings (International Paint Ltd.). The fouling community at Fairlie Quay is dominated by *Semibalanus balanoides* and *E. modestus*.

Figure 4.1. Location of test sites for rack immersion. 1) Burnham-on-Crouch; 2) Fairlie Quay. * indicates the position of the rack within test site. Image composited from Google Maps website.

4.3.4. Design of test racks

The test racks were designed and purpose-built specifically for these field immersion experiments, and have subsequently been adopted by Thomason (2014). Coatings were applied to microscope slides and the design of the test racks only permitted settlement on the coated side of the slides. The racks were constructed of PVC sheeting (1000 x 500 x 4.5mm; RS Components Ltd) cut into strips of 105mm with two 20mm strips to hold the slides in place. Neoprene rubber (3mm thick; RS Components Ltd) was used as a cushion between the PVC and microscope slides, which helped to hold the slides firmly in place without damaging the glass slides or the coating. Zinc-plated steel roofing bolts (M8 x 30mm thread; RS Components Ltd) were spaced out along the racks to hold the three strips of PVC and slides in position

(Figure 4.2). A 50mm diameter hole was drilled at each end of the rack to secure the rack with rope to a fixture above the water's surface.

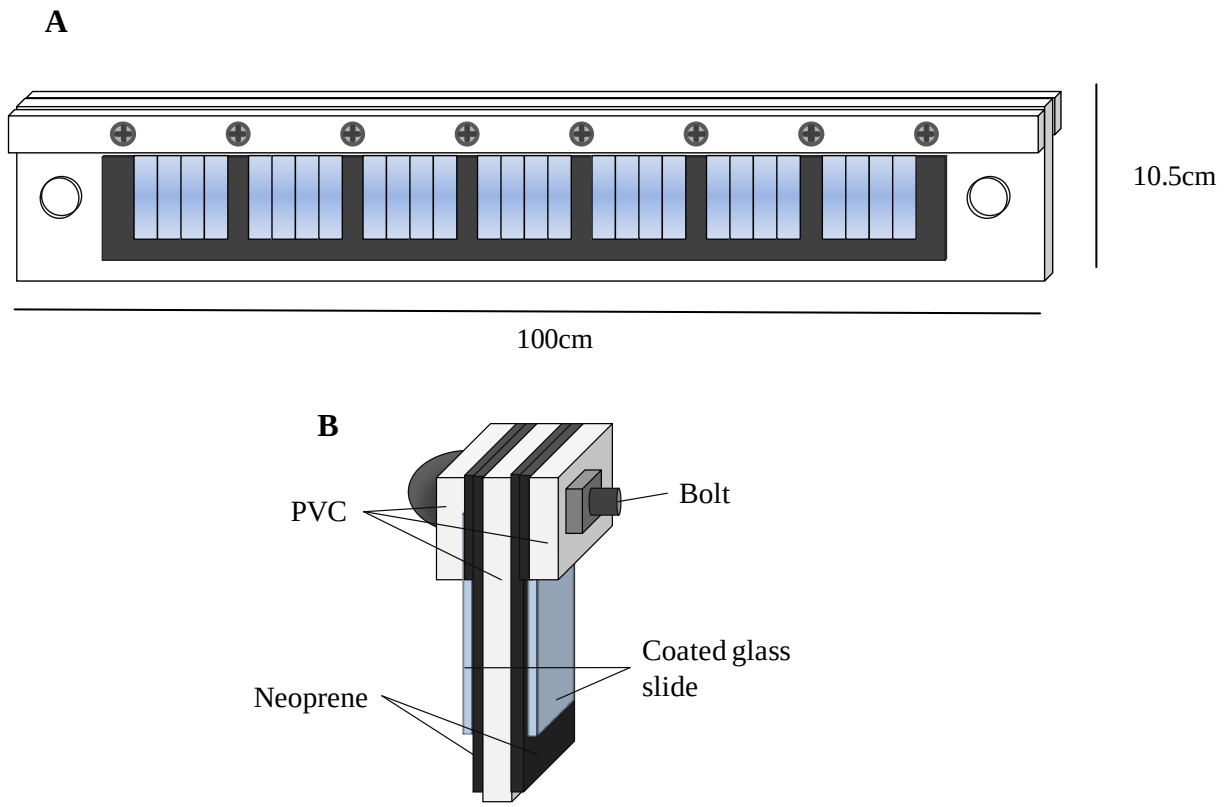

Figure 4.2. Design of the rack (A) and the cross-section (B) of the rack showing slides on both sides, immersed during 2009 at Burnham-on-Crouch and Fairlie Quay. Custom designed and purpose-built by R.C.Martin.

At each location the racks were suspended at a depth of 1m from the surface, fixed to the side of a raft at Burnham-on-Crouch and a pontoon in Fairlie Quay; this was thought to best represent the conditions experienced by a moored vessel. The racks were positioned horizontally so that the slides were maintained at an equal depth. A single rack held 56 coated microscope slides (28 microscope slides per side of the rack). Each type of coating was allocated a number and using a random number generator (Web references 1) the slides were randomly arranged along the racks with approximately an equal number of each coating per side of the rack. Each coated slide was labelled using a tungsten carbide pen (Fisher Scientific) to etch the name of the coating into the back of the slide.

The coatings deployed in 2009 at Fairlie Quay and Burnham-on-Crouch included the three Intersleek-based coatings; Intersleek 900, Intersleek 700 and Intersleek Clear. There was one immersion period in 2009, in which 50 slides per coating (150 slides in total) were immersed in March at Fairlie Quay and in April at Burnham-on-Crouch. In 2010 and 2011, eight coatings including five silicone and three fluoropolymer coatings (see section 4.3.1) were deployed. For both 2010 and 2011, there was one immersion period at Fairlie Quay during March, but two immersion periods in Burnham-on-Crouch, one during April and the second during June/July (Table 4.1). The number of slides immersed per coating per immersion period, was 16 (128 slides in total) in 2010 and 12 in 2011 (96 slides in total) (Table 4.2).

Table 4.1. Dates of rack immersions and collections.

Location	*Year*	*Date immersed*	*Date Collected*
Fairlie Quay	**2010**	**26th March**	**17th August**
	2011	**29th March**	**21st June**
Burnham-on-Crouch	**2009**	**20th April**	**7th July**
	2010	**19th /20th April**	**22nd June**
		22nd June	**19th October**
	2011	**14th April**	**12th July**
		12th July	**27th October**

Of the Intersleek-coated slides immersed at Burnham-on-Crouch on 20th April 2009 and collected on 7th July, 66% of the total were lost. Whereas 20% of the slides immersed in Fairlie Quay on 8th April 2009 and collected on 29th September were lost. Consequently, the racks were re-designed in-house with an extra strip of PVC to prevent loss of slides for the 2010 and 2011 immersion periods (Figure 4.3).

Table 4.2. Total number of slides that were deployed in the field and total number that were collected from Fairlie Quay and Burnham-on-Crouch from the years 2009, 2010 and 2011.

Location	*Year*	*Number of slides deployed*	*Number of slides collected*
Fairlie Quay	**2009**	**150**	**120**
	2010	**128**	**123**
	2011	**96**	**23**
Burnham-on-	**2009**	**150**	**51**
Crouch	**2010 - April**	**128**	**125**
	- June/July	**128**	**120**
	2011 - April	**96**	**74**
	- June/July	**96**	**80**

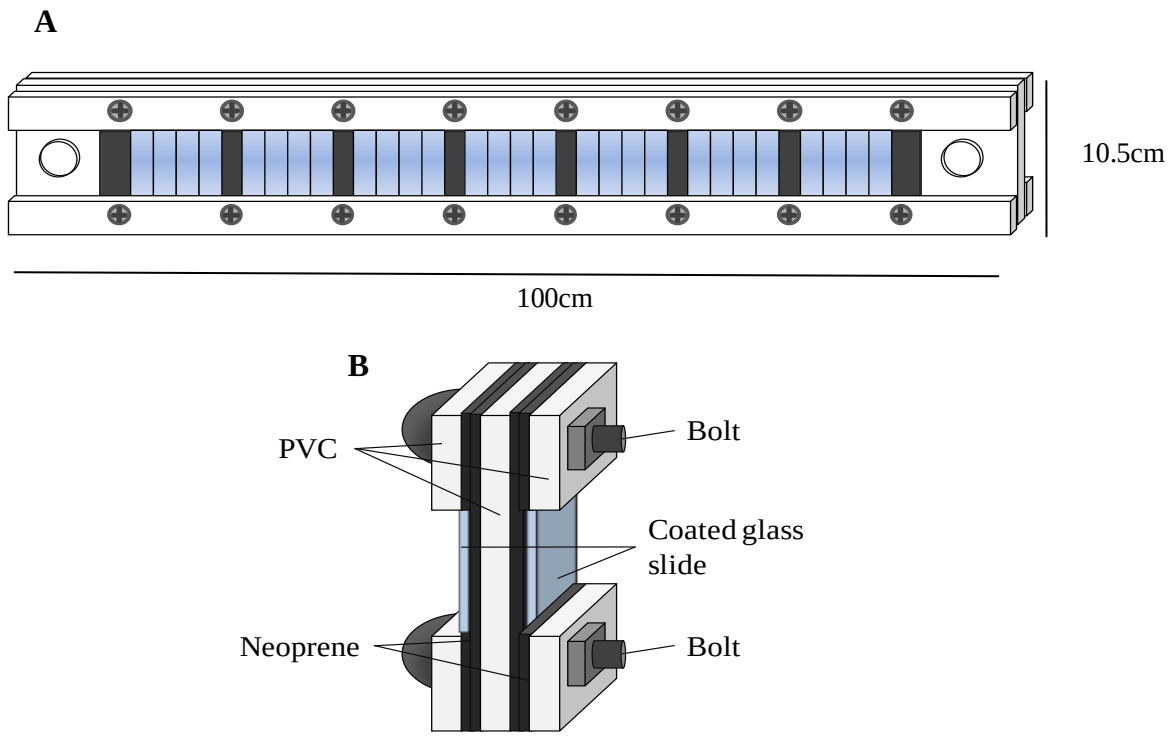

Figure 4.3. Modified design of the rack (A) and cross-section (B) deployed in 2010 and 2011 at Burnham-on-Crouch and Fairlie Quay. Redesigned by R.C.Martin.

4.3.5. Deployment of racks

The target species at the selected locations were *E. modestus* at Burnham-on-Crouch and *S. balanoides* and *E. modestus* at Fairlie Quay. The racks were immersed at Fairlie Quay in March/April (refer to Table 4.1 for dates) just prior to the predicted settlement season of *S. balanoides.* As *E. modestus* is able to reproduce continuously throughout the year with its peak between May and August (Crisp & Davies 1955) racks in Burnham-on-Crouch were immersed during April in 2010 and 2011, these were then collected in June/July and replaced with a second set of racks, which were collected in September/October.

There was no settlement of *S. balanoides* or *E. modestus* on the test coatings at Fairlie Quay in 2009, hence additional information regarding Fairlie 2009 will be omitted. The surrounding pier piling and rocky shore area was examined prior to deployment of the racks; as there were no newly settled juveniles it appeared that the settlement event had not been missed. When the racks were collected, the surrounding pier pilings were examined again and there were newly settled juveniles on the pilings but not on the sample microscope slides or on the PVC rack. Therefore, the chosen location i.e. suspension off the pontoon was not suitable. For 2010 the racks were fixed vertically to a pier piling, which was submerged during high tide and exposed during low tide. The design of the racks remained the same as those used at Burnham-on-Crouch in 2010 except that 70mm long zinc-coated steel roofing bolts (RS Components Ltd) were used to hold the PVC strips together. This was to allow the racks to stand approximately 30mm from the pier leg, permitting settlement on the underside of the racks. Settlement of *S. balanoides* and *E. modestus* at Fairlie Quay was achieved in 2010 with the re-designed racks.

4.3.6. Collection of racks

Upon collection, the slides were carefully removed from the racks and placed in quadriPERM® culture vessels; these were stacked in a cool box and transported back to the laboratory. Transportation took no more than 6 hours, after which the coated slides were placed in slide racks and immersed in holding tanks of artificial seawater (ASW). The slides were sorted into their coating group and digital images (photographs and scanned images) were taken to record the total number and

percentage cover of barnacles. The slides were then cleaned and any additional fouling that was present on the slides was removed. Where there was a heavy coverage of barnacles on the slides, these were thinned out to reduce overcrowding. The grouped slides were returned to the slide racks in 20L tanks of aerated ASW at 20 ± 2°C and maintained for up to five weeks being fed with a 1L mixture of *T. suecica* and *S. marinoi* three times a week. The tanks were cleaned and the water was replaced with fresh ASW every fortnight until the CRS testing. The barnacles returning from Burnham-on-Crouch in June 2010 (immersed in April 2010), were small with an average diameter of 1.8mm. These were maintained in the tanks for ten weeks to allow further growth before testing the CRS. Subsequent immersion periods in Burnham-on-Crouch were extended to allow for growth in the field as opposed to culture tanks in the laboratory.

4.3.7. Recruitment on coatings immersed in the field

Due to the large number of samples and the limited time available at the test locations, field recruitment was measured upon returning to the laboratory. This is not a true measure of the recruitment, as inevitably some barnacles were accidentally removed from the slides upon extraction from the racks and transportation from the field sites. The upmost care was taken to ensure that this was minimised. Recruitment is considered to be when presence of the individual organisms are observed and counted at a certain time (Keough & Downes 1982; Pawlik 1992) and in this study it refers to the total number of individuals that remained on the slides when returned to the laboratory. Using ImageJ software (Rasband 1997; Abramoff et al. 2004) on the digital images of the slides, the total number and the percentage area of barnacle cover per slide was calculated. The percentage cover of the slides was normalised by the total area of the slide that was available for settlement. However, due to the adverse weather conditions during 2011 at Fairlie Quay, a large number of slides were lost (see Table 4.3); therefore, a statistical comparison of the percentage coverage between 2010 and 2011 for the two barnacle species from Fairlie Quay was not possible. A comparison between *S. balanoides* and *E. modestus* could, however, be done for the 2010 data and comparisons of the percentage coverage between the 2010 and 2011 immersion periods could be done for Burnham-on-Crouch. The total number of barnacles recorded from the field is presented in Appendix 1, Table A1.1.

4.3.8. Critical removal stress

The critical removal stress (CRS) of the *E. modestus* barnacles reared in the laboratory and from Burnham-on-Crouch was measured using the automated method as described in Chapter 2. For *S. balanoides* and *E. modestus* from Fairlie Quay the hand-held force gauge (PSM-2K IMADE Co. Ltd) was used to measure the CRS. The sizes of the *S. balanoides* were on average too large for the automated instrument.

4.3.9. The influence of biofilm on the critical removal stress of **Elminius modestus**

Silastic T-2, Sylgard 184 and Rhodorsil 48V-750 coated microscope slides were used to investigate the influence of a laboratory cultured biofilm on the CRS of adult barnacles. The coated slides were divided into two sets (with an equal number of Silastic T-2, Sylgard 184 and Rhodorsil, per set), one set was immersed for ten days (Zardus et al. 2008) in a covered tank with a constant flow of unfiltered seawater at the Dove Marine Laboratory of Newcastle University, Cullercoats, to develop a natural biofilm. The second set was immersed in a covered tank of RO for 10 days. Prior to seeding with cyprids, the slides with and without a biofilm were air dried for up to 20 minutes. This was to allow the biofilm to dry in order to be able to pipette a 2ml droplet of 0.2µm filtered ASW in the centre on the slide. Approximately 20, day zero *E. modestus* cyprids were pipetted into this droplet for each slide. The slides, maintained in quadriPERM® culture vessels, were incubated at 22°C for 48 hrs, after which the chambers of the culture vessels were flooded with 15ml of *T. suecica*. The barnacles were cultured for 20 weeks, after which the CRS was measured by the automated method.

4.3.10. The influence of temperature on the critical removal stress of **Elminius modestus**

The influence of temperature on the CRS was investigated. Following the procedures described in Chapter 2 for the culture and settlement of cyprids, *E. modestus* day zero cyprids were settled on Silastic T-2 and Rhodorsil 48V-750 coated microscope slides. The cyprids were seeded to the coated slides and incubated at 22°C for 48 hrs, after which the wells of the quadriPERM® culture vessels were flooded

with 15ml *T. suecica*. The juvenile barnacles were cultured at 22°C in the culture vessels for two weeks on a diet of *T. suecica*, which was added three times per week. The water was changed at each feeding. After the two weeks, the slides were divided into four groups, with an equal number of Silastic T-2 and Rhodorsil slides and barnacles per group. The slides were placed in glass slide racks in one of four 1.5L containers of ASW and incubated at 22°C, 19°C, 15°C and 12°C. Each tank was fed on a diet of *T. suecica,* added once a week and the water was changed at each feeding. The slides were scanned (HP scanner 5400C) at 1200dpi resolution at two-week intervals. ImageJ software (Rasband 1997; Abramoff et al. 2004) was used to measure the basal area (mm^2) and the growth of the barnacles at the different temperatures over a 12-week period. The removal stress of the 14-week old barnacles was measured by the automated method.

4.3.11. Statistical analysis

4.3.11.1. Field recruitment and laboratory settlement

The field recruitment data, involving percentage cover of barnacles on the coated microscopes slides, were arcsine transformed and tested for normal distribution (Kolmogorov-Smirnov test) (Ennos 2012) and homogeneous variance (Levene's test) (Quinn & Keough 2002). However, the data were not normally distributed and did not have homogeneous variance; other transformations (log_{10} and square root) were tried but did not result in a normal distribution. The percentage coverage of the slides immersed in the field, at both Fairlie Quay and Burnham-on-Crouch, over the two years (2010 and 2011) was compared using a Kruskal-Wallis non-parametric test on the un-transformed data, with a 0.05 significance level and with a Mann-Whitney U *post-hoc* analysis test (Ennos 2012; Gao et al. 2016). For the racks immersed in Fairlie Quay, the null hypotheses investigated were: 1) there was no difference in the percentage cover between the two barnacle species *E. modestus* and *S. balanoides* in 2010, on each coating; 2) there was no difference in the percentage cover between the eight coatings for *E. modestus* and *S. balanoides*; and 3) there was no effect of the percentage coverage of *E. modestus* and *S. balanoides* due to the side and depth of the racks. For the racks immersed in Burnham-on-Crouch, the null hypotheses tested were: 1) there was no difference in the percentage cover of barnacle fouling between the four

immersion time points (April 2010, June 2010, April 2011 and July 2011) for a single coating and 2) there was no difference in the percentage cover on the eight coatings during a single time immersion period.

Comparisons of the percentage cover of *E. modestus* between Fairlie 2010 and the four immersion periods in Burnham-on-Crouch were carried out. The null hypothesis was that there was no difference in the percentage cover of *E. modestus* between Fairlie Quay in 2010 and Burnham-on-Crouch in April 2010, June/July 2010, April 2011 and June/July2011, on each coating.

The laboratory settlement data were tested for normal distribution (Kolmogorov-Smirnov test) (Ennos 2012) and homogeneous variance (Levene's test) (Quinn & Keough 2002). An ANOVA with 0.05 significance level and a *post hoc* Tukey's comparison was used to test the null hypothesis that there was no difference in the settlement of laboratory-cultured *E. modestus* cyprids across the eight coatings.

4.3.11.2. Critical removal stress

4.3.11.2.1. 2009 Preliminary trials

Data sets were transformed using a square root function after an initial Kolmogorov-Smirnov (Ennos 2012) and a Levene's test (Quinn & Keough 2002), showed that the distribution and variance were neither normal nor homogeneous. An ANOVA test with a 0.05 significance level was used to investigate the null hypothesis that there was no difference in critical removal stress (CRS) of *E. modestus* grown on Intersleek 900, Intersleek 700 and Intersleek Clear reared in the laboratory compared to those that grew in the field at Burnham-on-Crouch on the corresponding coatings during the 2009 preliminary field trials.

4.3.11.2.2. Comparison in the critical removal stress between laboratory and field cultured Elminius modestus.

Data sets were tested for normal distribution (Kolmogorov-Smirnov test) (Ennos 2012) and homogeneous variance (Levene's test) (Quinn & Keough 2002). A nested two-factor ANOVA with a 0.05 significance level and a *post hoc* Tukey's

comparison was used to test the null hypothesis that there was no difference in the CRS between *E. modestus* and *S. balanoides* barnacles from Fairlie Quay from 2010. The tests included the interaction effect of species x coating. The ANOVAs were nested to examine whether there was an impact of the microscope slides, the sides of the racks or the depth of the racks on the CRS of the barnacles. Comparisons between the years 2010 and 2011 for barnacles from Fairlie Quay were not possible (see section 4.4.1.1 for explanation). Additional ANOVAs with 0.05 significance levels were included to examine in more detail the potential differences between the two species for each coating separately.

A nested two-factor ANOVA test with a 0.05 significance level was used to test the null hypothesis that there were no differences in the CRS of *E. modestus* barnacles between the time points April 2010, June 2010, April 2011 and July 2011 from Burnham-on-Crouch. The test included the interaction effect of immersion period x coating. The test was nested to determine whether there was an impact of the microscope slides and the sides of the racks. Additional ANOVAs with 0.05 significance levels were included to examine in more detail the potential differences between the four immersion periods for each coating separately.

Finally, a two-factor nested ANOVA with a 0.05 significance level, including the interaction effect of location x coating, was used to test the null hypothesis that there was no difference in the CRS of *E. modestus* barnacles from the three locations: Fairlie Quay, Burnham-on-Crouch and the laboratory.

4.3.11.3. Influence of biofilm on the critical removal stress of Elminius modestus

The CRS of *E. modestus* from three PDMS coatings (Silastic T-2, Sylgard 184 and Rhodorsil 48V-750) was measured to investigate the influence of biofilm on the removal stress. The data sets were checked for normal distribution and homogeneous variance using a Kolmogorov-Smirnov test (Ennos 2012) and Levene's test (Quinn & Keough 2002), respectively. The data was transformed using $\log_{10}$. A two-factor nested ANOVA with a 0.05 significance level was used to test the null hypothesis that there was no difference in the CRS of barnacles removed from the coatings with a 10-day biofilm to the CRS of barnacles removed from coatings without a biofilm, including the interaction effect of biofilm x coating.

4.3.11.4. Influence of temperature on the size and critical removal stress of Elminius modestus

The data presented normal distribution (Kolmogorov-Smirnov test) (Ennos 2012) with homogeneous variance (Levene's test) (Quinn & Keough 2002). Two, two-factor nested ANOVAs with 0.05 significance levels and *post hoc* Tukey's comparisons were used to test the null hypotheses that: 1) there was no difference in the size of the barnacles at the end of the growth period, at the four temperatures (12°C, 15°C, 19°C and 22°C), on the coatings Silastic T-2 and Rhodorsil 48V-750; and 2) there was no difference in the CRS of the barnacles grown at the four temperatures (12°C, 15°C, 19°C and 22°C) for the two coatings, Silastic T-2 and Rhodorsil 48V-750. Both ANOVAs included the interaction effects of temperature x coating.

4.4. Results

4.4.1. Field recruitment

4.4.1.1. Fairlie Quay, Ayrshire

The racks immersed in 2010 were in the field for five months. After this time a sufficient number of *S. balanoides* and *E. modestus* barnacles had settled on the silicone and fluoropolymer coated slides (Figure 4.4). The racks were immersed in the same location in 2011 as in 2010; however, the immersion period had to be reduced due to bad weather. A total of 60% of the slides immersed at Fairlie Quay in 2011 were lost (Table 4.3). Those that remained on the outer facing side of the racks did not have barnacles on them. There were, however, signs of barnacle fouling in the form of barnacle-sized indentations in the soft silicone coatings and the presence of basal membranes remaining on the coatings surface (Figure 4.5). As a result of the damage to the sample a comparison in the percentage coverage for 2011 was not achievable.

Figure 4.4. Photograph of the re-designed racks fouled by *Semibalanus balanoides* and *Elminius modestus* after five months of immersion. The racks were attached to a pier piling at Fairlie Quay in 2010.

Table 4.3. The total number of slides that were collected from Fairlie Quay in 2010 and 2011. Total number of slides immersed in 2010 per coating was 16; total number immersed in 2011 per coating was 12.

Coating	*Total number of slides collected*	
	2010	*2011*
S1	**16**	**5**
S2	**15**	**2**
S3	**16**	**2**
S4	**15**	**4**
S5	**16**	**2**
FP1	**14**	**1**
FP2	**15**	**2**
FP3	**16**	**5**

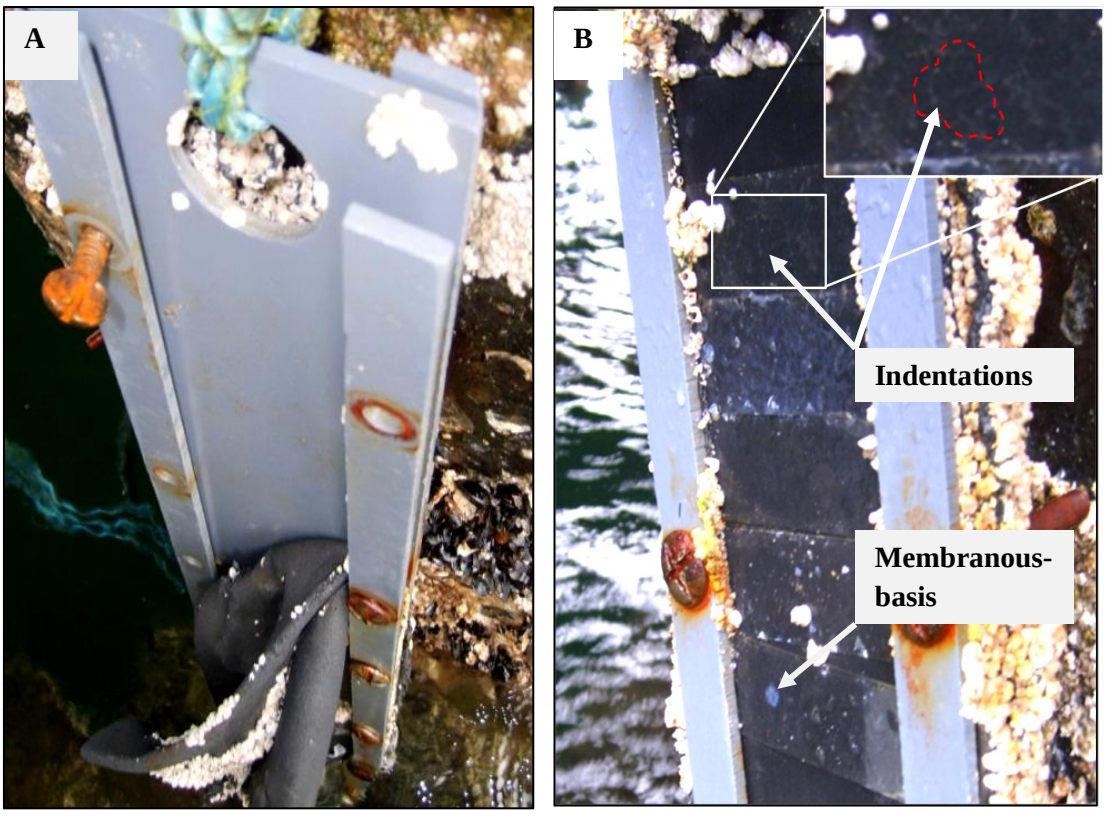

Figure 4.5. Examples of the damage to the racks and coated slides caused by extreme weather at Fairlie Quay during May 2011: A) illustrating the damage to the racks and loss of slides and B) microscope slides with indentations and membranous-bases remaining on the surface after removal of barnacles.

The data did not have a normal distribution (df = 246, D = 0.283, $P \leq 0.001$) nor homogeneous variance ($df1$ = 7, $df2$ = 238, F = 12.237, $P \leq 0.001$). The null hypothesis that there was no difference in the total percentage cover of *S. balanoides* and *E. modestus* in 2010 was supported for the coatings S1 (H = 3.778, P = 0.052), S3 (H = 0.112, P = 0.738) and S4 (H = 0.662, P = 0.414), but not for the coatings S2, S5, FP1, FP2 and FP3. The percentage cover of *S. balanoides* was significantly higher than that for *E. modestus* for the coatings S2 (H = 4.139, P = 0.042), S5 (H = 16.214, $P \leq$ 0.001), FP1 (H = 9.529, P = 0.002), FP2 (H =9.343, P = 0.002) and FP3 (H = 8.192, P = 0.004) (see Figure 4.6).

There was an effect of depth on the total percentage cover of *E. modestus* for two out of the eight coatings S5 (H = 8.060, P = 0.018) and FP1 (H = 10.088, P = 0.006) and of *S. balanoides* for three coatings S1 (H = 10.00, P = 0.007), S2 (H = 10.159, P = 0.006) and S4 (H = 7.797, P = 0.020), with a greater percentage cover of both species on slides which were on the lower section of the racks. The influence of the side of the rack on the percentage cover was only significant for one coating for *E. modestus* (FP1 H = 4.363, P = 0.037) and one coating for *S. balanoides* (S1 H = 7.333, P = 0.007), in which there were a greater number that had settled on the sheltered side of the rack, the side against the pier leg.

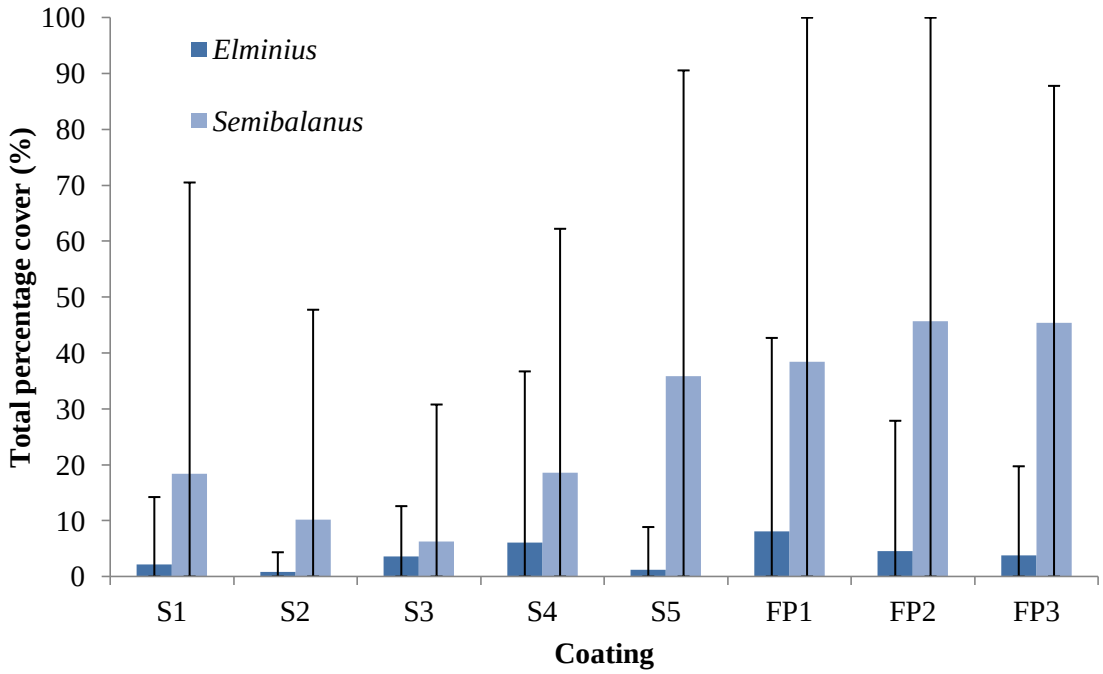

Figure 4.6. The total percentage cover (± range) of *Elminius modestus* and *Semibalanus balanoides* on five silicone and three fluoropolymer coatings immersed in Fairlie Quay during 2010.

The null hypothesis that there was no difference in the percentage settlement of *E. modestus* and S. *balanoides* across the eight coatings, was not confirmed. There were significant differences in the overall percentage cover between the coatings for both *E. modestus* (H = 16.095, P = 0.024) and *S. balanoides* (H = 23.942, P = 0.001). For *E. modestus* there was a greater percentage of barnacles settled on FP1 compared to

S2 and S5 ($U_{14, 15}$ = 48.00, P = 0.01; $U_{14, 15}$ = 54.00, P = 0.011, respectively) and there was a greater percentage on S4 also compared to the S2 and S5 coatings ($U_{15, 16}$ = 52.00, P = 0.011; $U_{15, 15}$ = 55.00, P = 0.008, respectively). For *S. balanoides* there was a much greater percentage on the three fluoropolymers when compared to the silicones S1, S2, S3 and S4 (FP1 vs S2 $U_{14, 15}$ = 41.00, P = 0.005; FP1 vs S3 $U_{14, 16}$ = 33.00, P = 0.001; FP2 vs S1 $U_{14, 16}$ = 67.50, P = 0.036; FP2 vs S2 $U_{14, 15}$ = 43.00, P = 0.006; FP2 vs S3 $U_{14, 16}$ = 38.00, P = 0.001; FP2 vs S4 $U_{14, 15}$ = 63.00, P = 0.037; FP3 vs S1 $U_{16, 16}$ = 62.00, P = 0.021; FP3 vs S2 $U_{16, 15}$ = 58.00, P = 0.013; FP3 vs S3 $U_{16, 16}$ = 50.00, P = 0.003; FP3 vs S4 $U_{16, 15}$ = 69.00, P = 0.041). In addition the silicone coating S5 had a greater percentage covering of *S. balanoides* than the coatings S2 and S3 ($U_{16, 15}$ = 50.00, P = 0.005; $U_{16, 15}$ = 40.00, P = 0.001, respectively).

4.4.1.2. Burnham-on-Crouch, Essex

In 2010 and 2011 with the re-designed racks there was minimum loss of, or damage to, the slides. *E. modestus* was the dominant species present on the microscope slides and the PVC racks; however, there was also *Balanus crenatus*, a calcareous-based barnacle present in small numbers. However, as this was not the target species, percentage cover and CRS were not recorded for this species. The barnacles were covered by dense mats of *Jassa* spp. tubes (Figure 4.7). *Jassa* spp. is an amphipod that builds the tubes from detritus filtered from the water.

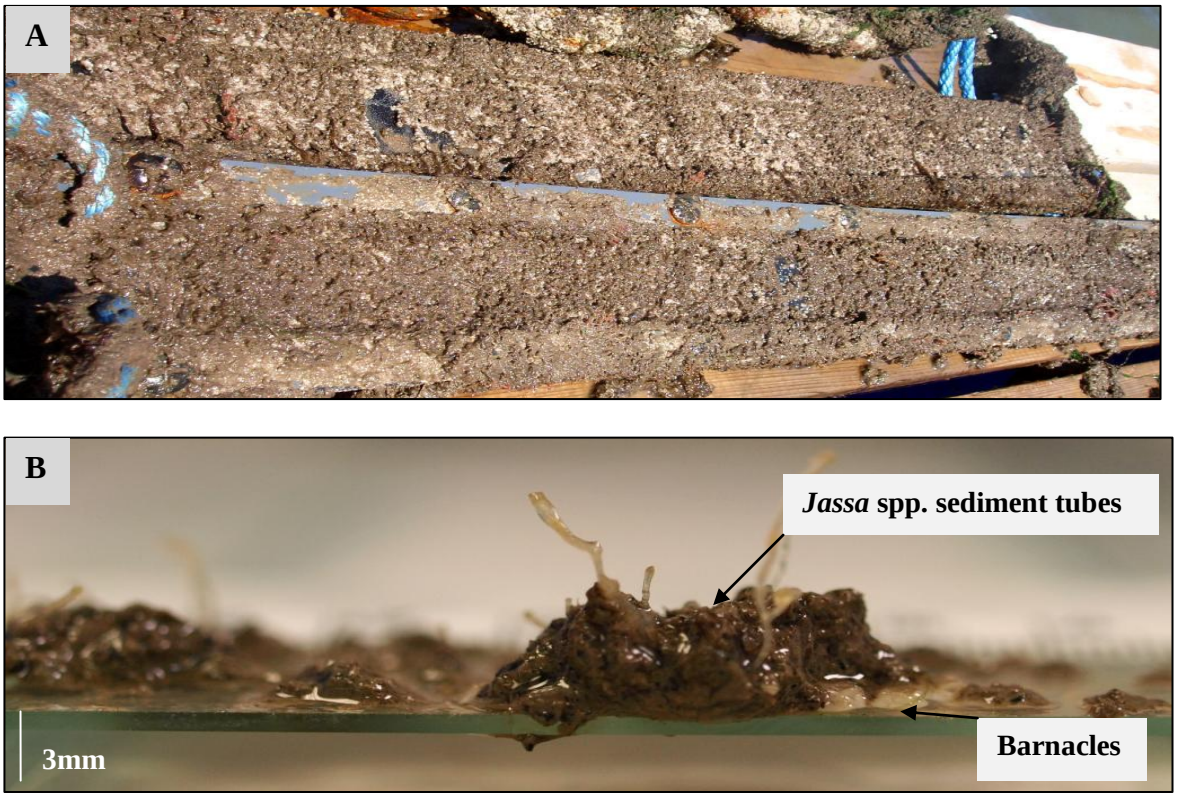

Figure 4.7. Racks from Burnham-on-Crouch immersed in April 2010 after three months immersion time, fouled with *Elminius modestus* which were covered by the sediment tubes of *Jassa* spp. (A). A side view of a silicone coated microscope slide with *Elminius modestus* barnacles covered with a thick layer of *Jassa* spp. tubes (B).

The data were not normally distributed (df = 862, D = 0.262, $P \leq 0.001$) and did not have homogeneous variance (df = 31, $df2$ = 830, F = 39.959, $P \leq 0.001$). The null hypothesis that there was no difference in the percentage cover of *E. modestus* across the four immersion time points (April 2010, June 2010, April 2011 and July 2011), for each of the eight coatings, was only supported by data for one coating. Coating S5 had consistent coverage at each time point (H = 7.209, P = 0.066), whereas the percentage cover for the remaining seven coatings differed across the four immersion periods (S1 H = 31.019, $P < 0.001$; S2 H = 27.590, $P < 0.001$; S3 H = 22.875, $P < 0.001$, S4 H = 27.916, $P < 0.001$; FP1 H = 39.464, $P < 0.001$; FP2 H = 32.954, $P < 0.001$; FP3 H = 32.236, $P < 0.001$) (Figure 4.8). The percentage cover in April 2010 was greater than the coverage of the three remaining immersion times for seven of the eight coatings ($U_{16, 8} \geq 0.000$, $P \leq 0.003$). The percentage coverage during June 2010 was also greater

than the coverage for both the 2011 immersion periods for all three of the fluoropolymers ($U_{12, 8} \geq 4.00$, $P \leq 0.021$). However, for the coatings S1 and S2 the coverage during June 2010 was less than the coverage for the 2011 immersion times ($U_{12, 8} \geq 20.00$, $P \leq 0.045$). Comparisons between the two immersion periods in 2011, only the coating FP1 showed a difference in percentage coverage, with it being higher during the July immersion time than in the April that year ($U_{10, 10} = 7.50$, $P = 0.004$).

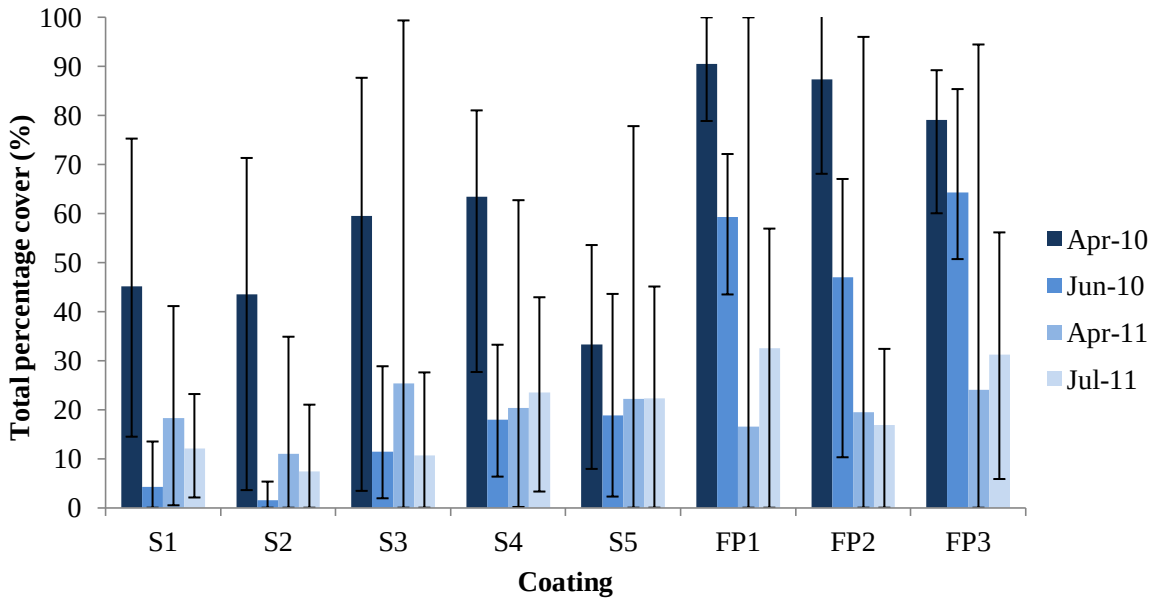

Figure 4.8. The total percentage cover (± range) of *Elminius modestus* on five silicone and three fluoropolymer coatings immersed in Burnham-on-Crouch in April 2010, June 2010, April 2011 and July 2011.

Comparisons between the coatings within a single time period were also completed. The null hypothesis that there was no difference in the percentage cover across the eight coatings within a single immersion period, was confirmed for the April 2011 immersion period ($H = 6.240$, $P = 0.512$). However for the remaining periods (April 10, June 2010 and July 2011), the null hypothesis was not supported - there were significant differences in percentage cover between the eight coatings (April 10; $H = 91.341$, $P < 0.001$, June 2010; $H = 78.063$, $P < 0.001$ and July 2011; $H = 12.931$, $P = 0.002$). The fluoropolymers (FP1, FP2 and FP3) had higher percentage cover than all the silicones during April 2010 and June 2010 (April; $U_{16, 14} \geq 0.00$, $P \leq 0.001$, June; $U_{12, 11} \geq 0.00$, $P < 0.001$). For July 2011, only two of the fluoropolymers (FP1 and

FP3) had a higher coverage than three of the silicones (S1, S2 and S3) ($U_{10, 10} \geq 9.500$, $P \leq 0.040$). The silicones S1 and S2 presented the lowest percentage cover during April 2010, June 2010 and July 2011 (April 2010 $U_{16, 14} \geq 0.001$, $P \leq 0.028$; June 2010 $U_{12, 11} \geq 0.00$, $P \leq 0.033$; July 2011 $U_{10, 10} \geq 9.50$, $P \leq 0.041$), with the coating S5 having the actual lowest percentage cover during April 2010 alone ($U_{14, 16} \geq 0.00$, $P < 0.001$).

4.4.1.3. Comparison in the percentage cover between Fairlie Quay and Burnham-on-Crouch

The null hypothesis that there was no difference in the percentage cover of *E. modestus* at Fairlie Quay during 2010 with the percentage cover of barnacles at Burnham-on-Crouch was confirmed but only for June 2010 for the coatings S1 ($U_{12, 16} = 51.00$, $P = 0.067$) and S2 ($U_{12, 15} = 57.00$, $P = 0.098$), and for April 2011 for the coatings S4 ($U_{9, 15} = 35.00$, $P = 0.053$), FP1 ($U_{8, 14} = 45.00$, $P = 0.450$) and FP2 ($U_{12, 16} = 69.00$, $P = 0.756$). The percentage cover for the remaining coatings and immersion periods from Burnham-on-Crouch were distinctly greater than that from Fairlie 2010 ($U_{16, 16} \leq 38.00$, $P \leq 0.03$) (Figure 4.9).

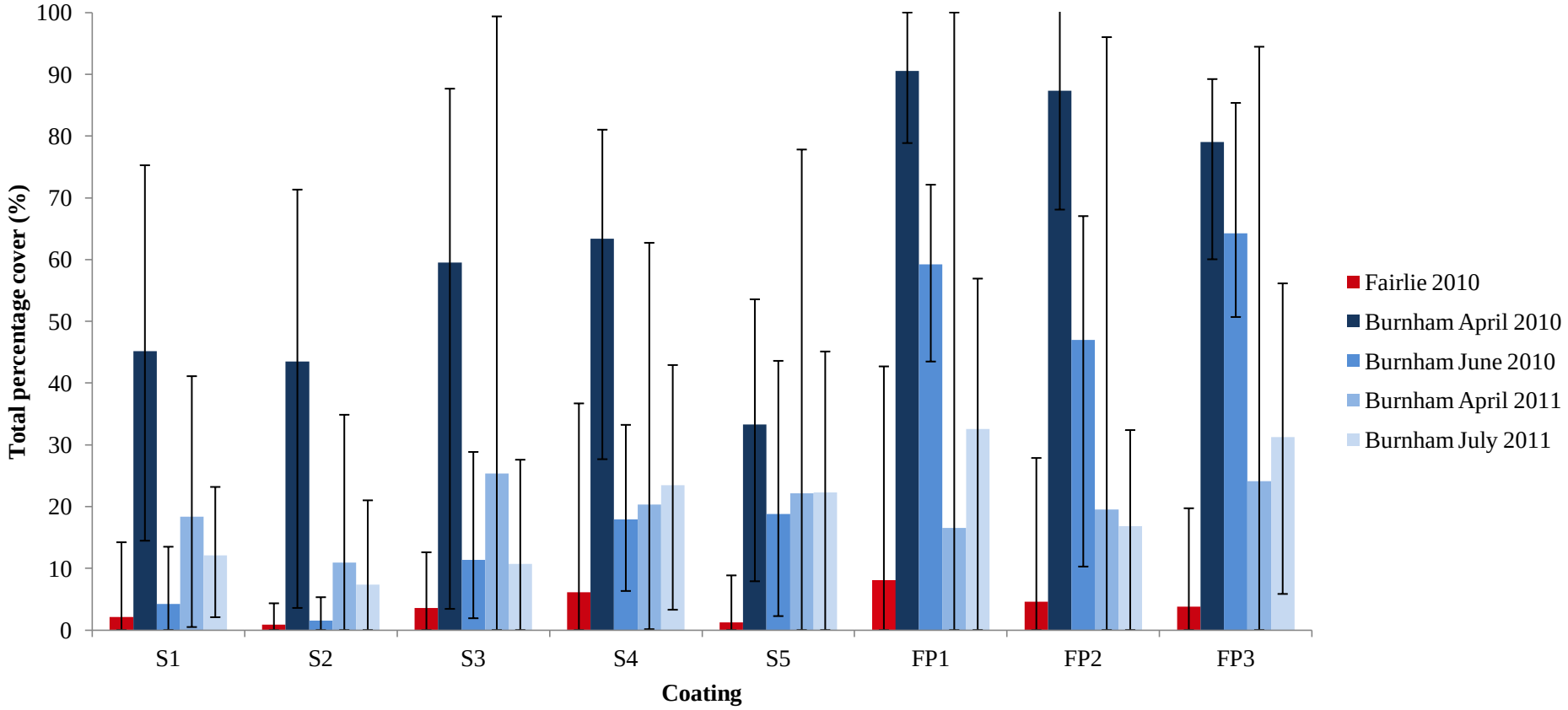

Figure 4.9. The total percentage cover (± range) of *Elminius modestus* on five silicone and three fluoropolymer coatings immersed in Fairlie Quay 2010 and Burnham-on-Crouch in April 2010, June 2010, April 2011 and July 2011.

4.4.2. Laboratory settlement

The data were normally distributed (*df* = 64, *D* = 0.075, *P* = 0.200) with homogeneous variance (*df1* = 7, *df2* = 56, *F* = 2.009, *P* = 0.070). The null hypothesis that there was no difference in the percentage of settled cyprids between the eight coatings was not supported. There was a difference in the percentage settlement of laboratory cultured *E. modestus* between the eight test coatings (*df* = 7, *F* = 16.919, *P* < 0.001) (Figure 4.10 and Table 4.4), in which the silicone coating S5 had less settlement than the other seven coatings (Tukey's $P \leq 0.04$). In addition, the fluoropolymers FP2 and FP3 had a greater percentage settlement than all five silicone coatings (Tukey's $P \leq 0.008$).

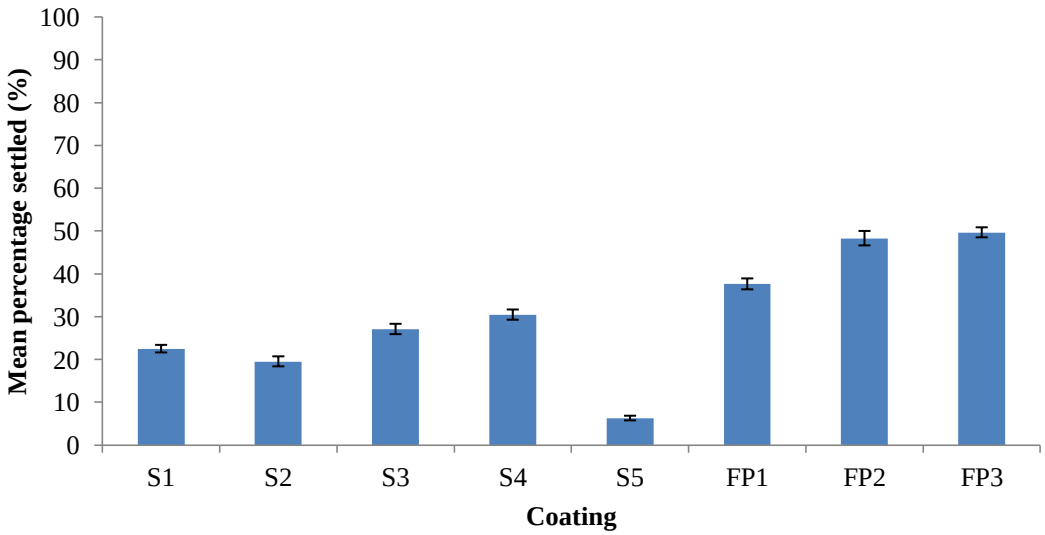

Figure 4.10. The mean percentage settlement (± 1 SE) of laboratory reared *Elminius modestus* on silicone and fluoropolymer coatings.

Table 4.4. ANOVA table of results for the settlement of *Elminius modestus* cyprids on the eight test coatings assayed under laboratory conditions.

	Sum of Squares	*Mean Square*	*df*	*F-value*	*P-value*
Coating	**1.289**	**0.184**	**7**	**16.919**	**< 0.001**

4.4.3. Critical removal stress

4.4.3.1. 2009 Preliminary field trials

The data were normally distributed (df = 338, D = 0.048, P = 0.060) with homogeneous variance ($df1$ = 3, $df2$ =334, F = 2.463, P = 0.117) after transformation by square root. No comparison between the field and laboratory could be made for the coating Intersleek 900, as no barnacles were recruited on the samples immersed in Burnham-on-Crouch during the 2009 preliminary trials. The null hypothesis that there were no differences in the CRS of *E. modestus* settled and grown in the laboratory on Intersleek 700 (IS700) and Intersleek Clear (ISCLR) to the CRS of *E. modestus* that grew in the field at Burnham-on-Crouch in 2009 was not confirmed. The CRS values of barnacles reared in the laboratory were significantly higher than the CRS of barnacles that grew in the field (df = 1, F = 16.852, $P \leq 0.001$) (Figure 4.11 and Table 4.5). There was also a difference in the CRS between the coatings (df = 1, F = 7.911, P = 0.005), and an interaction effect of coating x location (df = 1, F = 11.662, P = 0.001). Hence, the CRS value of *E. modestus* on Intersleek Clear was greater than the CRS of the barnacles on Intersleek 700. This pattern is consistent for both locations, but the CRS values of barnacles from the laboratory culture on the Intersleek coatings were higher than those for barnacles on the corresponding coatings from Burnham-on-Crouch.

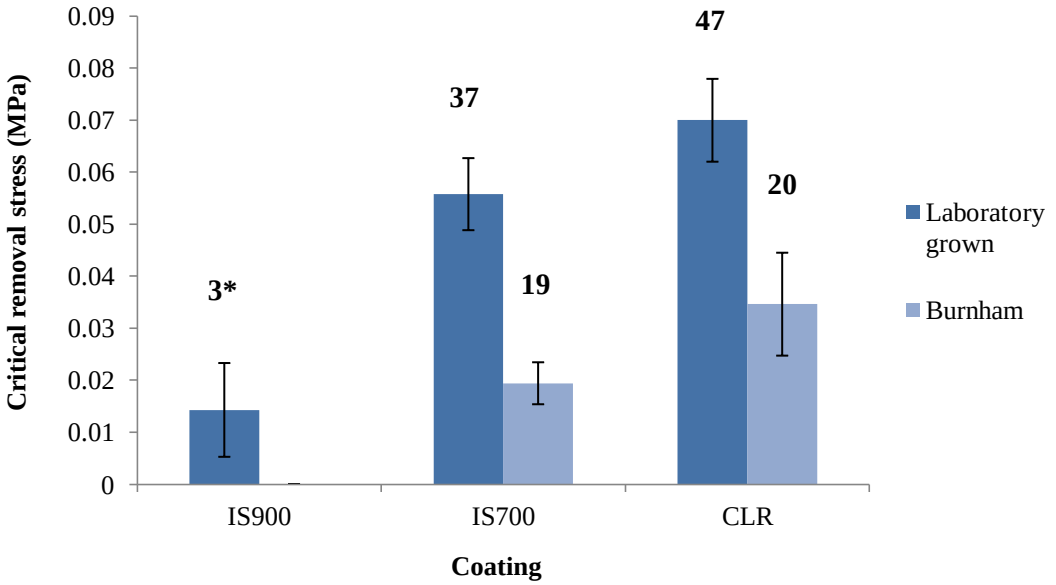

Figure 4.11. The mean critical removal stress (± 95% confidence intervals) of *Elminius modestus* grown on Intersleek 900 (IS900), Intersleek 700 (IS700) and an Intersleek Clear (CLR) in the laboratory and in Burnham-on-Crouch in 2009. The number (n) of barnacles tested is presented above the bars * indicates the samples of individuals that are below the desirable minimum number of replicates as recommended by Conlan et al. (2008) for *Balanus amphitrite.*

Table 4.5. ANOVA table of results for the critical removal stress of *Elminius modestus* from Intersleek 700 and Intersleek Clear from a laboratory culture and from the field population at Burnham-on-Crouch during 2009.

	Sum of Squares	***Mean Square***	***df***	***F-value***	***P-value***
Location	**0.014**	**0.014**	**1**	**16.852**	**≤ 0.001**
Coating	**0.007**	**0.007**	**1**	**7.911**	**0.005**
Coating x location	**0.010**	**0.010**	**1**	**11.662**	**0.001**

4.4.3.2. Fairlie Quay, Ayrshire

The data were normally distributed (df = 156, D = 0.099, P = 0.070) with homogeneous variance ($df1$ = 68, $df2$ = 87, F = 2.720, P = 0.055). The null hypothesis that there was no difference in the CRS of *E. modestus* and the CRS of *S. balanoides* was not confirmed (Figure 4.12 and Table 4.6 for the numbers (n)). The removal stress

for *E. modestus* was greater than that for *S. balanoides* (*df* = 1, *F* = 23.396, *P* = 0.043) (Table 4.7). However, there was no significant interaction effect of species x coating (*df* = 7, *F* = 1.333, *P* = 0.363), which shows that the difference between the two species was not present for all the coatings; this difference was only present for the coatings S4, FP1, FP2 and FP3 (S4 *df* = 1, *F* = 6.692, *P* = 0.017; FP1 *df* = 1, *F* = 11.090, *P* = 0.007; FP2 *df* = 1, *F* = 63.697, *P* < 0.001; FP3 *df* = 1, *F* = 46.239, *P* < 0.001).

The ANOVA results demonstrated that there were significant differences in CRS between the eight coatings (*df* = 7, *F* = 33.287, *P* ≤ 0.001). The *post hoc* Tukey's comparisons showed that the CRS of barnacles removed from the fluoropolymers FP1, FP2 and FP3 were greater than the CRS of barnacles removed from all five of the silicone coatings (Tukey's *P* ≤ 0.001). The CRS of barnacles removed from the coating FP1 was also greater than the CRS of barnacles removed from the coating FP2 (Tukey's *P* = 0.024). Of the silicone coatings only the CRS of the coatings S3 and S5 differed in which S3 was less than that of S5 (Tukey's *P* = 0.005).

There was a nested effect on the barnacle adhesion due to the slide location on the racks. The slides that were positioned on the sheltered side of the rack, those that were closest to the pier leg, had a lower CRS value than slides which were positioned on the non-sheltered, exterior, of the rack (*df* = 1, *F* = 13.801, *P* = 0.045). Although, there was no nested effect due to the depth of the slides on the racks (*df* = 2, *F* = 1.525, *P* = 0.255) or a nested effect due to the different slides (*df* = 15, *F* = 0.123, *P* = 0.974).

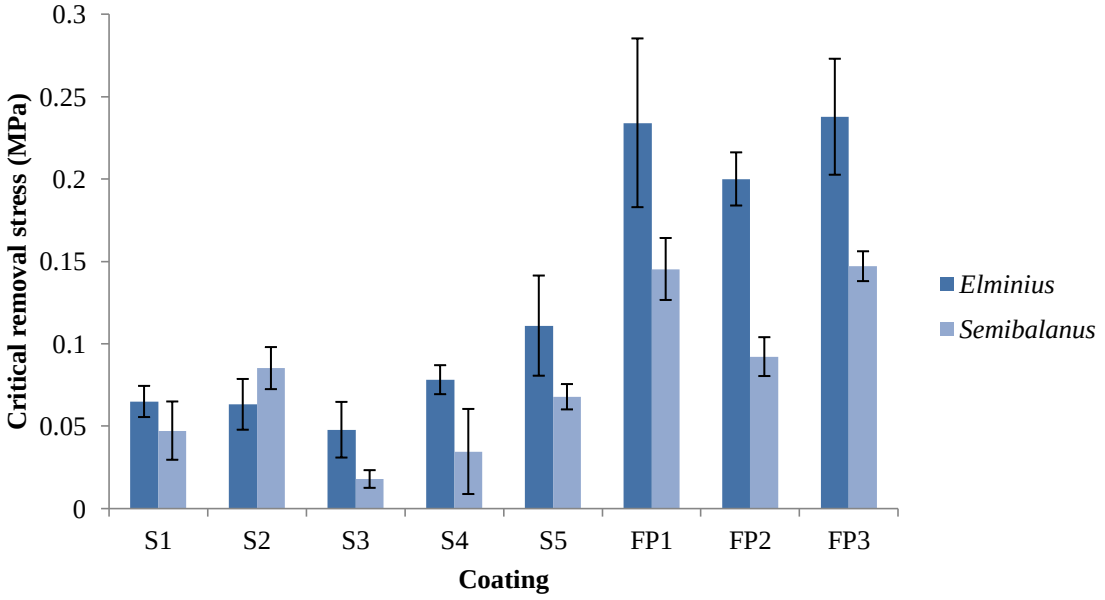

Figure 4.12. The mean critical removal stress (± 95% confidence intervals) of *Elminius modestus* and *Semibalanus balanoides* from silicone and fluoropolymer coatings immersed in Fairlie Quay in 2010.

Table 4.6. The number (n) of *Elminius modestus* and *Semibalanus balanoides* from Fairlie Quay in 2010 used to measure the critical removal stress. * indicates the samples of barnacles that were below the desirable minimum number of replicates as recommended by Conlan et al. (2008) for *Balanus amphitrite*.

Coating	*Number (n) of barnacles*	
	Elminius modestus	*Semibalanus balanoides*
S1	**8***	**8***
S2	**3***	**3***
S3	**6***	**5***
S4	**6***	**6***
S5	**11**	**11**
FP1	**12**	**12**
FP2	**18**	**18**
FP3	**28**	**28**

Table 4.7. ANOVA table of results for the comparison of the critical removal stress of Fairlie Quay *Elminius modestus* and *Semibalanus balanoides* in 2010 (A) and an ANOVA table of results for the comparison of critical removal stress of *Elminius modestus* and *Semibalanus balanoides* per coating (B).

A

	Sum of Squares	***Mean Square***	***df***	***F-value***	***P-value***
Species	**0.055**	**0.055**	**1**	**23.396**	**0.043**
Coating	**0.406**	**0.058**	**7**	**33.287**	**≤ 0.001**
Species x coating	**0.037**	**0.005**	**7**	**1.333**	**0.363**
Side of rack	**0.006**	**0.006**	**1**	**3.801**	**0.045**
Depth	**0.005**	**0.002**	**2**	**1.525**	**0.225**
Slide number	**0.001**	**0.000**	**15**	**0.123**	**0.974**

B

Coating	***Sum of Squares***	***Mean Square***	***df***	***F-value***	***P-value***
S1	**0.002**	**0.002**	**1**	**1.160**	**0.296**
S2	**0.001**	**0.001**	**1**	**2.415**	**0.181**
S3	**0.002**	**0.002**	**1**	**2.919**	**0.107**
S4	**0.005**	**0.005**	**1**	**6.692**	**0.017**
S5	**0.007**	**0.007**	**1**	**3.252**	**0.080**
FP1	**0.039**	**0.039**	**1**	**11.090**	**0.007**
FP2	**0.103**	**0.103**	**1**	**63.697**	**≤ 0.001**
FP3	**0.138**	**0.138**	**1**	**46.239**	**≤ 0.001**

4.4.3.3. Burnham-on-Crouch, Essex

The data were normally distributed (df = 1444, D = 0.056, P = 0.200) with homogeneous variance ($df1$ = 235, $df2$ = 1208, F = 2.601, P = 0.065). The null hypothesis that there was no difference in the CRS of *E. modestus* from the immersion time periods April 2010, June 2010, April 2011 and July 2011 for each coating, was not confirmed (Figure 4.13 and Table 4.8 for the number of barnacles). There were differences in the removal stress of *E. modestus* across the four immersion periods at Burnham-on-Crouch (df = 3, F = 2.838, P = 0.043) (Table 4.9). This is not a repeated measure as different populations of barnacles were measured at each immersion period. The differences between immersion periods were only present for five out of the eight coatings (S1 df = 3, F = 4.479, P = 0.006; S2 df = 3, F = 3.238, P = 0.034; S5 df = 3, F = 35.566, P < 0.001; FP1 df = 3, F = 20.719, P < 0.001; FP3 df = 3, F = 9.617, P <

0.001); however, there was no clear pattern that can be correlated to the month or year of immersion. For instance, for the coating S1, the barnacles from April 2011 had a higher CRS than June 2010 (Tukey's $P = 0.007$). For S2, the CRS was different between April 2010 and June 2010 (Tukey's $P = 0.023$), where the former was higher than the latter. For the S5 coating, the CRS of April 2010 and April 2011 was lower than the CRS of the barnacles that were settled in the later summer months (June 2010, July 2011) (Tukey's $P \leq 0.007$). For the FP1 coating, the barnacles from June 2010 had a higher CRS than the barnacles from July 2011 (Tukey's $P < 0.001$). With FP2 the CRS of barnacles from July 2011 was lower than the CRS values from the three remaining immersion periods (Tukey's $P \leq 0.003$).

There were also significant differences in the CRS values between the coatings ($df = 7$, $F = 108.063$, $P \leq 0.001$). For the coatings FP1 and FP2, the CRS measurements were significantly higher than for the remaining six coatings (Tukey's $P \leq 0.001$). Barnacles removed from coatings FP3 and S5 had significantly higher CRS values than those on the silicone coatings S1, S2, S3 and S4 (Tukey's $P \leq 0.005$). There was a significant interaction effect of immersion period x coating ($df = 21$, $F = 6.027$, $P \leq 0.001$), however interpretation of the results is made difficult by the multiple coatings and immersion periods, as there does not appear to be a consistent trend across the eight coatings and four immersion periods.

There was no nested impact of the slides used to compile the CRS values ($df = 17$, $F = 1.030$, $P = 0.450$) or any nested effect due to the side of the racks ($df = 1$, $F = 1.097$, $P = 0.529$)

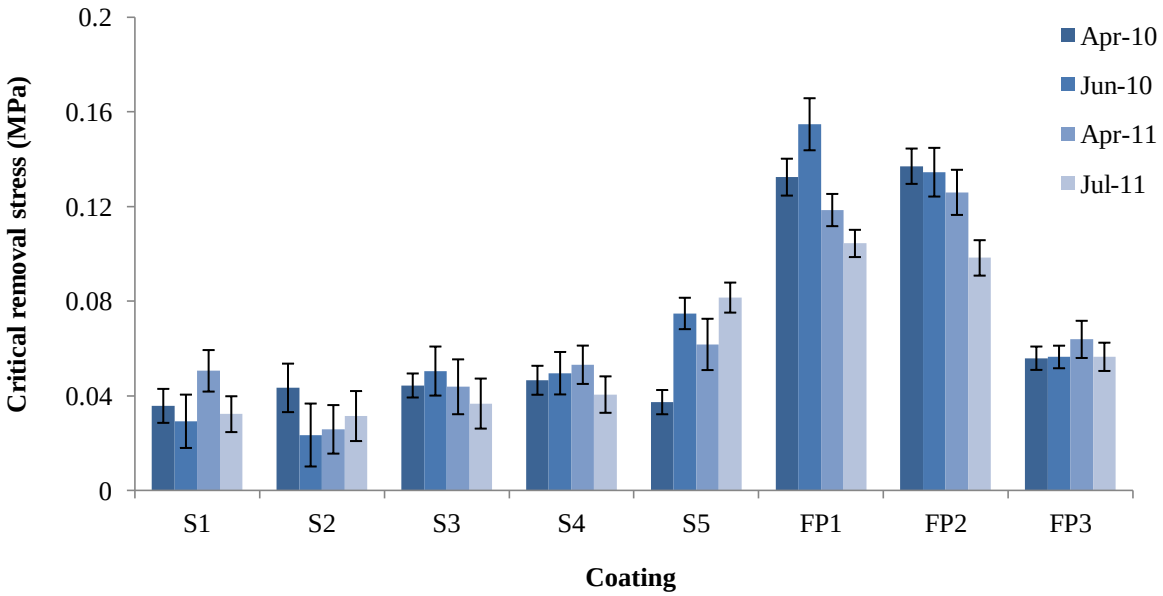

Figure 4.13. The mean critical removal stress (± 95% confidence intervals) of *Elminius modestus* from Burnham-on-Crouch from April 2010, June 2010, April 2011 and July 2011. The number (n) of barnacles presented in Table 4.8.

Table 4.8. The number (n) of *Elminius modestus* used to measure the critical removal stress from Burnham-on-Crouch. * indicates the samples that are below the desirable minimum number of replicates as recommended by Conlan et al. (2008) for *Balanus amphitrite*.

Coating	*Number (n) of barnacles*			
	April 2010	*June 2010*	*April 2011*	*July 2011*
S1	**30**	**35**	**25**	**13**
S2	**16**	**33**	**10***	**21**
S3	**57**	**40**	**25**	**22**
S4	**54**	**52**	**36**	**24**
S5	**70**	**93**	**40**	**56**
FP1	**85**	**103**	**20**	**71**
FP2	**99**	**117**	**32**	**49**
FP3	**56**	**114**	**47**	**58**

Table 4.9. ANOVA table of results for the comparison of the critical removal stress of *Elminius modestus* from Burnham-on-Crouch for the immersion periods April 2010, June 2010, April 2011 and July 2011 (A) and an ANOVA table for the comparison of the critical removal stress of *Elminius modestus* for the four immersion periods per coating (B).

A

	Sum of Squares	***Mean Square***	***df***	***F-value***	***P-value***
Immersion period	**0.010**	**0.003**	**3**	**2.838**	**0.043**
Coating	**0.965**	**0.138**	**7**	**108.063**	**≤ 0.001**
Immersion period x coating	**0.151**	**0.007**	**21**	**6.027**	**≤ 0.001**
Slide number	**0.018**	**0.001**	**14**	**1.030**	**0.450**
Side of rack	**0.016**	**0.001**	**1**	**1.097**	**0.529**

B

Coating	***Sum of Squares***	***Mean Square***	***df***	***F-value***	***P-value***
S1	**0.005**	**0.002**	**3**	**4.479**	**0.006**
S2	**0.003**	**0.001**	**3**	**3.238**	**0.034**
S3	**0.002**	**0.001**	**3**	**1.274**	**0.287**
S4	**0.002**	**0.001**	**3**	**1.171**	**0.287**
S5	**0.076**	**0.025**	**3**	**35.566**	**≤ 0.001**
FP1	**0.111**	**0.037**	**3**	**20.719**	**≤ 0.001**
FP2	**0.056**	**0.002**	**3**	**9.617**	**≤ 0.001**
FP3	**0.002**	**0.001**	**3**	**1.248**	**0.293**

4.4.3.4. Comparison in the critical removal stress between laboratory and field cultured Elminius modestus

Figure 4.14 displays the CRS of *E. modestus* from Fairlie Quay 2010, Burnham-on-Crouch (April 2010, June 2010, April 2011 and July 2011) compared to the barnacles that were grown in the laboratory from a single culture. Table 4.10 displays the number (n) of *E. modestus* barnacles used in the laboratory culture, for the numbers of barnacles tested from Fairlie Quay and Burnham-on-Crouch see Table 4.6 and 4.8.

The data were normally distributed (df = 1933, D = 0.780, P = 0.075) with a homogeneous variance ($df1$ = 23, $df2$ =1909, F = 2.996, P = 0.100). The null hypothesis that there was no difference in the removal stress of *E. modestus* from the three locations, Fairlie Quay, Burnham-on-Crouch and the laboratory was not

supported. There were significant differences in the adhesion strengths of barnacles from the different locations (df = 2, F = 46.076, $P \leq 0.001$) (Table 4.11). The CRS values of barnacles from Burnham-on-Crouch were lower than those from Fairlie Quay and the laboratory culture (Tukey's $P \leq 0.001$). There was also a significant difference between the coatings (df = 7, F = 192.781, $P \leq 0.001$). The *post hoc* Tukey's analysis showed that there were three distinct subsets, namely, the fluoropolymers FP1 and FP2 (Tukey's $P \leq 0.001$), the silicones S1, S2, S3 and S4 (Tukey's $P \leq 0.001$) and the coatings FP3 and S5 (Tukey's $P \leq 0.03$). Within each subset the CRS values of the coatings were similar to each other but distinct from the values of the remaining coatings. However, there was a significant interaction effect of location x coating (df = 14, F = 19.682, $P \leq 0.001$). Interpretation of the results is made difficult by the multiple coatings, locations and immersion periods. Therefore, to better clarify differences between locations ANOVAs were performed for each coating, separately. Seven of the eight coatings showed differences in the CRS values of the barnacles from the three locations (S1 df = 5, F = 4.198, P = 0.002; S2 df = 5, F = 3.283, P = 0.011; S3 df = 5, F = 1.477, P = 0.201; S4 df = 5, F = 2.745, P = 0.021; S5 df = 5, F = 24.180, $P < 0.001$; FP1 df = 5, F = 26.962, $P < 0.001$; FP2 df = 5, F = 38.540, $P < 0.001$; FP3; df = 5, F = 124.016, $P < 0.001$). For five out of these seven coatings (S4, S5, FP1, FP2 and FP3) the CRS from barnacles grown in Fairlie Quay were higher than the CRS of the barnacles from Burnham-on-Crouch across all four of the immersion periods (S4 Tukey's $P \leq 0.039$; S5 Tukey's $P \leq 0.046$; FP1 Tukey's $P \leq 0.003$; FP2 Tukey's $P < 0.001$; FP3 Tukey's $P < 0.001$). For the coatings S1 and S2, CRS from Fairlie Quay was only higher than two (June 2010 and July 2011) and one (June 2010) of the immersion periods from Burnham-on-Crouch, respectively (S1 Tukey's $P \leq 0.046$; S2 Tukey's $P = 0.007$).

The CRS for barnacles that were grown in the laboratory were on average higher than the values for barnacles from Burnham-on-Crouch. For the three fluoropolymers, this difference was present across all four of the immersion periods (FP1 Tukey's $P < 0.001$; FP2 Tukey's $P < 0.001$; FP3 Tukey's $P \leq 0.008$). For S5 this difference was between the laboratory and Burnham-on-Crouch's June 2010 and July 2011 immersion times (Tukey's $P \leq 0.013$).

Comparing the CRS values between barnacles grown in the laboratory and Fairlie Quay barnacles, those from Fairlie Quay had a higher CRS than those settled and grown in the laboratory for the coatings S2, FP1 and FP3 (S2 Tukey's $P = 0.028$; FP1; Tukey's $P < 0.001$; FP3 Tukey's $P < 0.001$).

For the laboratory data, there was no nested impact of the slides ($df = 10$, $F = 1.098$, $P = 0.333$).

Table 4.10. The number (n) of *Elminius modestus* used to measure the critical removal stress from barnacles settled and grown in the laboratory. * indicates the samples that are below the desirable minimum number of replicates as recommended by Conlan et al. (2008) for *Balanus amphitrite*.

Coating	*Number (n) of barnacles*
S1	**31**
S2	**23**
S3	**24**
S4	**32**
S5	**2***
FP1	**44**
FP2	**58**
FP3	**56**

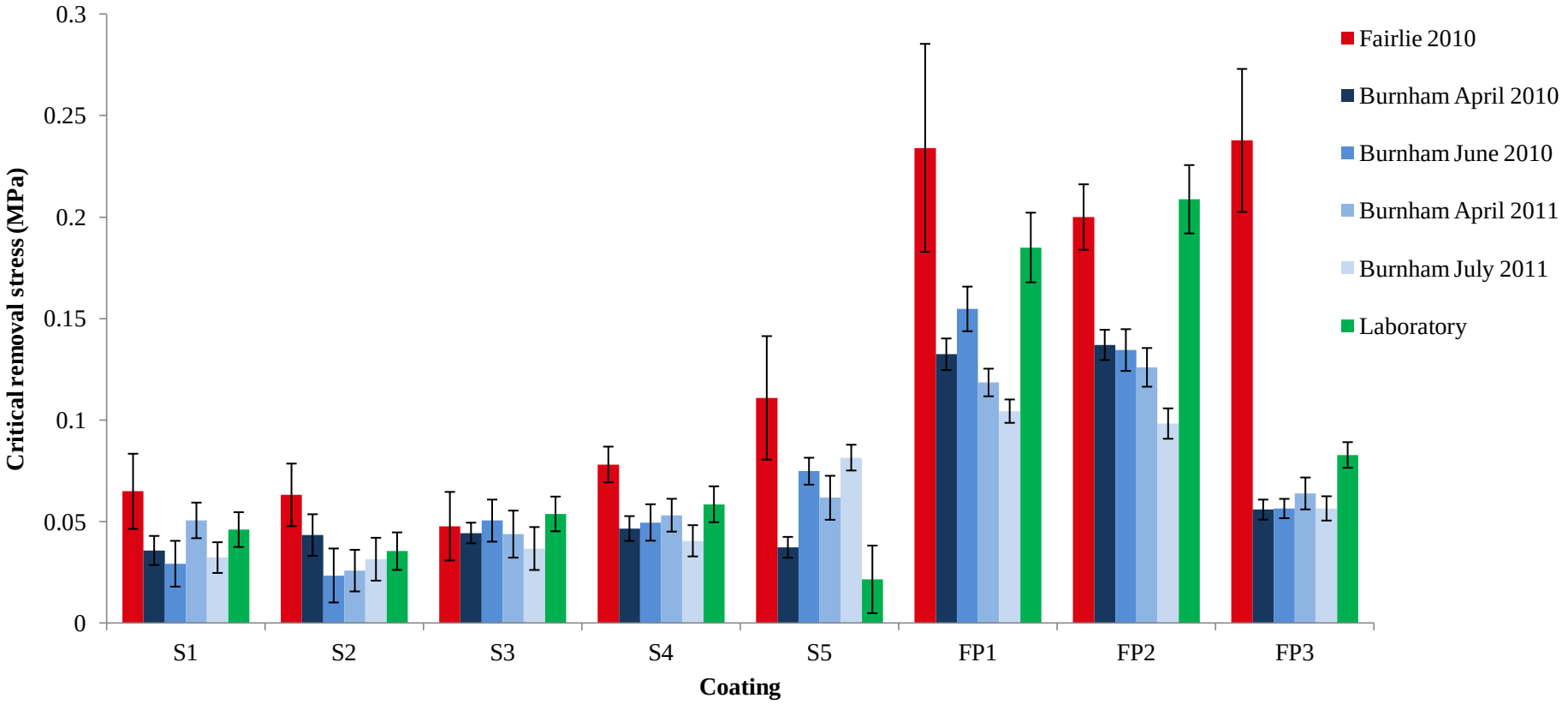

Figure 4.14. The mean critical removal stress (± 95% confidence intervals) of *Elminius modestus* from Fairlie Quay 2010, Burnham-on-Crouch from April 2010, June 2010, April 2011 and July 2011 and barnacles that were cultured in laboratory conditions.

Table 4.11. ANOVA table of results for the comparison of the critical removal stress of *Elminius modestus* from Fairlie Quay (2010), Burnham-on-Crouch (April 2010, June 2010, April 2011 and July 2011) and laboratory (A) and an ANOVA table for the comparison of the critical removal stress of *Elminius modestus* from the three locations per coating (B).

A

	Sum of Squares	***Mean Square***	***df***	***F-value***	***P-value***
Location	**0.144**	**0.072**	**2**	**46.076**	**≤ 0.001**
Coating	**0.114**	**0.302**	**7**	**192.781**	**≤ 0.001**
Location x coating	**0.432**	**0.031**	**14**	**19.682**	**≤ 0.001**
Slide number (Laboratory)	**0.065**	**0.002**	**10**	**1.098**	**0.333**

B

Coating	***Sum of Squares***	***Mean Square***	***df***	***F-value***	***P-value***
S1	**0.009**	**0.002**	**5**	**4.198**	**0.002**
S2	**0.006**	**0.001**	**5**	**3.283**	**0.011**
S3	**0.004**	**0001**	**5**	**1.477**	**0.201**
S4	**0.007**	**0.001**	**5**	**2.745**	**0.021**
S5	**0.106**	**0.021**	**5**	**24.180**	**≤ 0.001**
FP1	**0.277**	**0.055**	**5**	**26.962**	**≤ 0.001**
FP2	**0.440**	**0.008**	**5**	**38.540**	**≤ 0.001**
FP3	**0.476**	**0.095**	**5**	**124.016**	**≤ 0.001**

4.4.4. Influence of biofilm on the critical removal stress of Elminius modestus

The data were normally distributed (df = 80, D = 0.879, P = 0.107) with homogeneous variance ($df1$ = 5, $df2$ =75, F = 2.762, P = 0.114). The null hypothesis that there would be no difference in the CRS of *E. modestus* barnacles grown on surfaces with a 10-day-old biofilm compared to surfaces without a biofilm was supported by the results for all three of the silicone coatings (df = 1, F = 0.083, P = 0.774) (Figure 4.15 and Table 4.11). However, there was a difference in the CRS of the *E. modestus* between the coatings (df = 2, F = 32.257, $P \leq 0.001$), with barnacles grown on Rhodorsil 48V-750 having a significantly lower CRS value than Silastic T-2 and Sylgard 184 (Tukey's, $P \leq 0.001$). There was no significant interaction effect of biofilm x coating (df = 1, F = 0.041, P = 0.959). Finally, there was no nested effect due to the different slides used to grow the barnacles to size (df = 22, F = 0.001, P = 0.996).

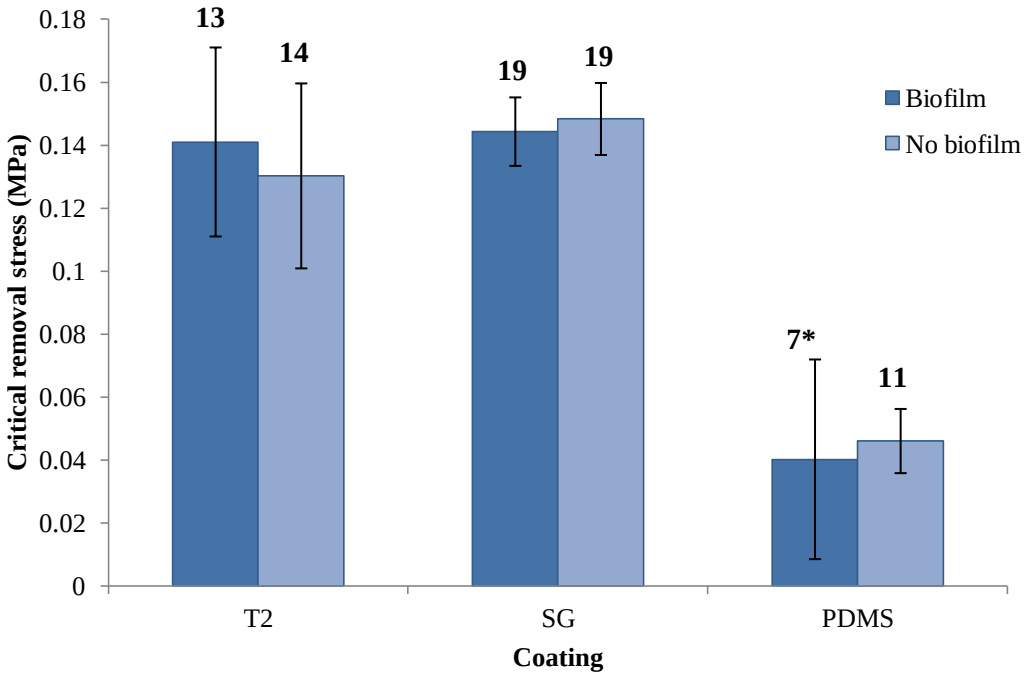

Figure 4.15. The mean critical removal stress (± 95% confidence interval) of *Elminius modestus* grown on Silastic T-2 (T2), Sylgard 184 (SG) and Rhodorsil 48V-750 (PDMS) coatings with and without a 10-day-old laboratory cultured biofilm. The number (n) of barnacles tested is presented above the bars * indicates the samples of individuals that are below the desirable minimum number of replicates as recommended by Conlan et al. (2008) for *Balanus amphitrite.*

Table 4.12. ANOVA table of results for the critical removal stress of *Elminius modestus* barnacles removed from Silastic T-2 and Sylgard 184 coatings with a 10-day-old biofilm and no-biofilm.

	Sum of Squares	*Mean Square*	*df*	*F-value*	*P-value*
Biofilm	**0.000**	**0.000**	**1**	**0.083**	**0.774**
Coating	**0.104**	**0.052**	**2**	**32.257**	**≤ 0.001**
Biofilm x coating	**0.000**	**6.673×10^{-5}**	**1**	**0.041**	**0.959**
Slide number	**3.753×10^{-6}**	**3.753×10^{-6}**	**22**	**0.001**	**0.996**

4.4.5. The influence of temperature on the size and critical removal stress of **Elminius modestus**

The size data were normally distributed (*df* = 83, *D* = 0.080, *P* = 0.200) with homogeneous variance (*df1* = 23, *df2* = 59, *F* = 2.104, *P* = 0.074). The null hypothesis that there would be no difference in the size of the barnacles at the end of the growth period, grown at the four temperatures (22°C, 19°C, 15°C and 12°C), on the coatings Silastic T-2 and Rhodorsil 48V-750, was not supported. There were significant differences in the sizes of the *E. modestus* barnacles grown at the different temperatures across the two coatings (*df* = 3, *F* = 18.715, *P* < 0.001) (Figure 4.16 and Table 4.13). For both Silastic T-2 and Rhodorsil 48V-750 coatings, the barnacles grown at 19°C were smaller than those grown at the three other temperatures (Tukey's, *P* < 0.001). The barnacles grown at 22°C were larger than those grown at 12°C and 15 ºC (Tukey's, *P* = 0.046 and *P* = 0.001, respectively). There was no significant difference in the sizes of *E. modestus* barnacles at the terminus of the growth period between the two coatings (*df* = 1, *F* = 6.664, *P* = 0.055). There was also no interaction effect of temperature x coating (*df* = 1, *F* = 0.380, *P* = 0.771). Finally, there was no nested effect due to the different microscope slides used to grow the barnacles to size (*df* = 5, *F* = 0.355, *P* = 0.851).

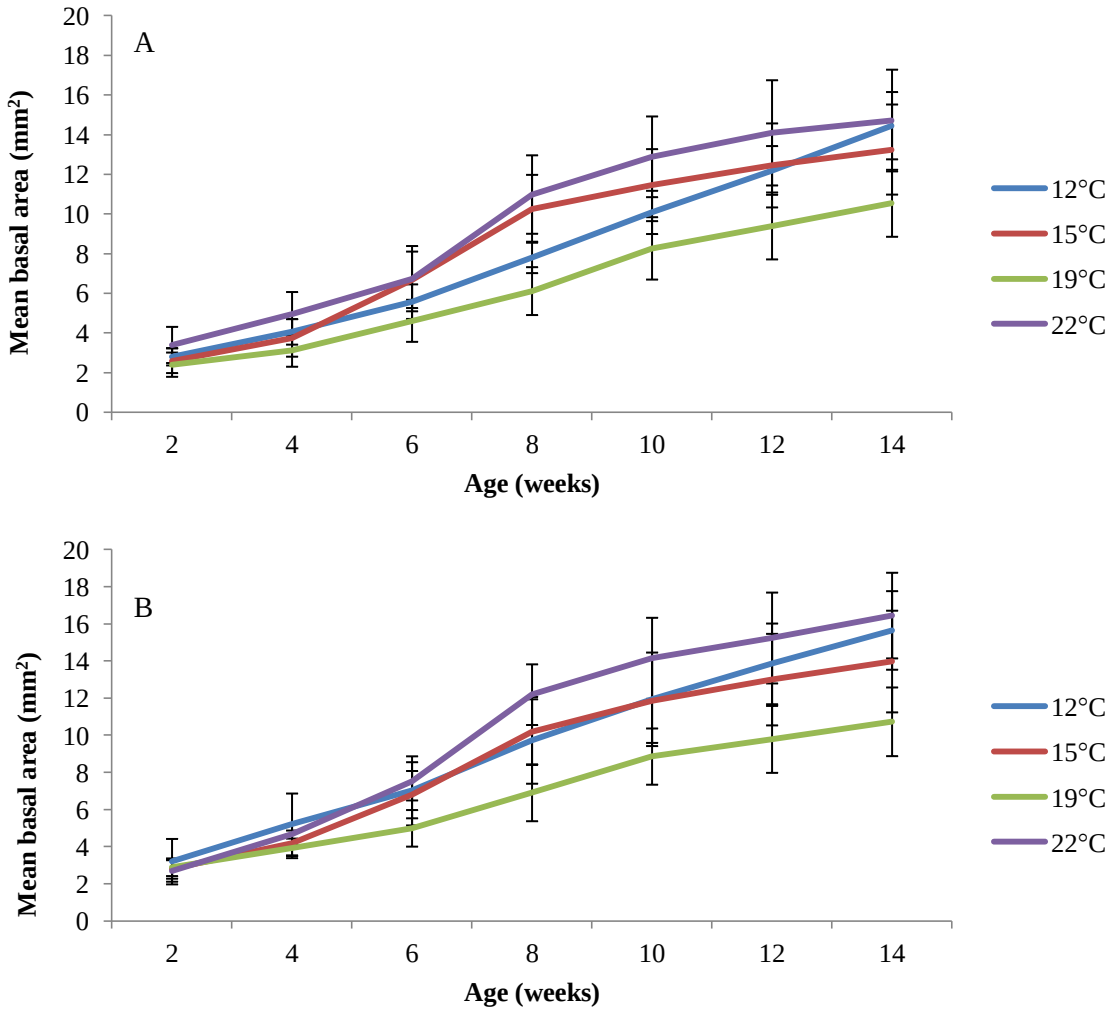

Figure 4.16. The mean basal area (± 1 SD) of *Elminius modestus* on Rhodorsil 48V-750 PDMS (A) and Silastic T-2 (B) grown over a 14 week period at 12°C, 15°C, 19°C and 22°C.

Table 4.13. ANOVA table of results for the size of *Elminius modestus* barnacles grown at four different temperatures (12°C, 15°C, 19°C and 22°C) on Rhodorsil 48V-750 PDMS and Silastic T-2.

	Sum of Squares	*Mean Square*	*df*	*F-value*	*P-value*
Temperature	**386.821**	**128.940**	**3**	**18.715**	**≤ 0.001**
Coating	**31.250**	**31.250**	**1**	**6.664**	**0.055**
Temperature x coating	**7.251**	**2.417**	**3**	**0.380**	**0.771**
Slide number	**9.526**	**1.905**	**5**	**0.355**	**0.851**

The CRS data were normally distributed (*df* = 83, *D* = 0.162, *P* = 0.06) with homogeneous variance (*df1* = 23, *df2* = 59, *F* = 1.910, *P* = 0.088). The null hypothesis that there would be no difference in the CRS of *E. modestus* grown at four temperatures, was confirmed. The temperature did not influence the CRS for barnacles removed from the silicone coatings significantly (*df* = 3, *F* = 1.927, *P* = 0.221) (Figure 4.17 and Table 4.14). However, there was a signicant difference between the coatings, in that the CRS for barnacles removed from Silastic T-2 was greater than those removed from Rhodorsil 48V-750 (*df* = 1, *F* = 102.404, *P* = 0.007). The interaction effect of temperature x coating showed no significant influence on the CRS of the barnacles (*df* = 3, *F* = 5.995, *P* = 0.062). In addition, there was no nested effect of the different microscope slides used to collate the CRS data (*df* = 5, *F* = 1.149, *P* = 0.436).

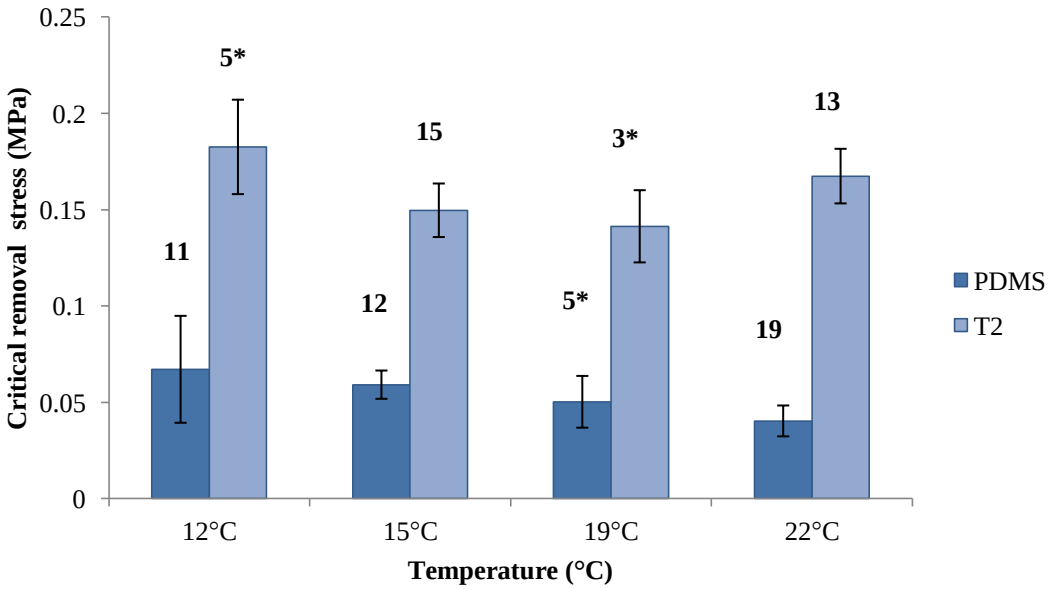

Figure 4.17. The mean critical removal stress (± 95% confidence intervals) of *Elminius modestus* grown on Silastic T-2 and Rhodorsil 48V-750 PDMS at temperatures 12°C, 15°C, 19°C and 22°C. The number (n) of barnacles tested is presented above the bars * indicates the samples of individuals that are below the desirable minimum number of replicates as recommended by Conlan et al. (2008) for *Balanus amphitrite*.

Table 4.14. ANOVA table of results for the critical removal stress of *Elminius modestus* barnacles grown at four different temperatures (12°C, 15°C, 19°C and 22°C) on Rhodorsil 48V-750 PDMS and Silastic T-2.

	Sum of Squares	*Mean Square*	*df*	*F-value*	*P-value*
Temperature	**0.008**	**0.003**	**3**	**1.927**	**0.221**
Coating	**0.140**	**0.140**	**1**	**102.404**	**0.007**
Temperature x coating	**0.004**	**0.001**	**3**	**5.995**	**0.062**
Slide number	**0.012**	**0.002**	**5**	**1.149**	**0.436**

4.5. Discussion

The aim of this chapter was to compare the use of laboratory assays and field immersion trials for evaluating FR coatings. The percentage recruitment from the field at two locations, the percentage settlement from the laboratory and the CRS from both field and laboratory for eight coatings were recorded. There were similarities in the patterns of the field recruitment for each location and immersion period and laboratory settlement across the eight coatings. For example, there were higher percentages on the three fluoropolymers than on the five silicones, with the silicone coating S2 having some of the lowest level of recruitment and settlement. A similar trend was noted for the CRS values, whereby barnacles from the field sites and from the laboratory culture had a greater adhesive strength to the fluoropolymers than the silicones.

In an effort to explain the potential differences between laboratory assays and field immersion trials, the effects of biofilm and temperature on the CRS of laboratory-raised *E. modestus* was investigated. However, in this study the influence of biofilm and temperature was un-determined.

4.5.1. Field recruitment and laboratory settlement

The conclusion that laboratory settlement assays have the potential to be a good representation for field performances could be concluded from the results of the present study. The laboratory cultured cyprids were able to differentiate between the coatings in a similar manner as that for the field. The settlement of *E. modestus* cultured in the

laboratory (Figure 4.10) displayed a trend across the coatings consistent with the recruitment in Burnham-on-Crouch (Figure 4.8) specifically in April 2010. This saw a greater population of barnacles on the fluoropolymers than for the silicones and which saw the silicone coating S5 having the lowest population of all. For the samples that were immersed in June 2010 in Burnham-on-Crouch, the fluoropolymers again had a greater percentage cover than that found on the silicones; S2 had the lowest coverage, whereas S5 had the greatest coverage of the five silicones. It has been demonstrated before with *Balanus amphitrite* (Rittschof & Costlow 1989) and *S. balanoides* (Crisp & Meadows 1962) that results from laboratory trials corresponded well with results from the field. However, when comparing the laboratory settlement in this study to the 2011 immersion periods in Burnham-on-Crouch and to the recruitment of *E. modestus* at Fairlie Quay, there seems to be fewer similarities to draw on. This makes the interpretation of the results complicated. Other previous studies with *B. amphitrite* and *S. balanoides* (Thompson et al. 1998; Matsumura et al. 2000) and *B. improvisus* (O'Connor & Richardson 1996), field recruitment results were found not to be consistent with the laboratory results.

Laboratory assays are often used as a precursor to field immersion trials, in order to down-select the number of coatings for immersion in the field (Swain 1997; Martinelli et al. 2012). Yet the validity of laboratory assays had been called into question as to whether they are truly a good indication of the results likely to be found in the field (Briand 2009). However, in this study even the results from the field differed between locations and over time. When comparing the recruitment between the two field sites (Fairlie Quay and Burnham-on-Crouch) and within an individual field site in the two year period, there are clear differences in the percentage cover. For example the coverage at Fairlie Quay, specifically of *E. modestus,* was substantially less than that seen in Burnham-on-Crouch. In addition, the samples immersed in Burnham-on-Crouch in April 2010 had increased percentage coverage than the samples at the three remaining time periods for this locale.

Spatial and temporal differences in the settlement and recruitment of marine invertebrates in the intertidal zone have been previously documented (Keough 1983; Jeffrey & Underwood 2000; Jenkins et al. 2000; Swain et al. 2000; Wood et al. 2000; Berntsson & Jonsson 2003; Robson et al. 2009). Factors which have been reported to influence the settlement, which is considered to be the permanent transition of

planktonic larvae to the benthic community (Keough & Downes 1982; Pawlik 1992), are often related to larval availability and 'accompanying factors' (Jeffery & Underwood 2000; Jenkins et al. 2000). These factors can include: the size of the adult population affecting the larval abundance (Ramondi 1991); the intensity of the phytoplankton bloom and therefore the availability of food for the larvae (Barnes 1962; Hawkins & Hartnoll 1982); behavioural interactions with adult conspecifics (Keough 1983) and space availability for settling larvae (Pineda 1994). In addition, there are physical factors to consider, such as the local hydrodynamics (e.g. flow and turbulence) transporting and concentrating larvae to specific areas (Hawkins & Hartnoll 1982; Minchinton & Scheibling 1991; Pineda 1994). However, there are then the factors which influence the recruitment of the adult population, the recruitment being defined as when the presence of the 'recruits' have been observed on the substratum (Keough & Downes 1982; Pawlik 1992). This often relates to the post-settlement mortality and therefore the ability of the organisms to survive until observation (Hunt & Scheibling 1997; Jenkins et al. 2000) and can include biological processes such as competition and predation (Paine 1974; Keough & Downs 1982) or physical disturbances such as wave exposure, desiccation and extremes in temperature (Dayton 1971; Harms & Anger 1989).

In this study, the test racks in Burnham-on-Crouch were hung horizontally and were constantly submerged 1m below the level of the water in an estuarine environment where *E. modestus* was the dominant barnacle. Whereas the racks in Fairlie Quay were vertically fixed to a pier piling in the intertidal where *S. balanoides* were more dominant. However, in this study the influence of the tidal height and presence of the pier leg sheltering half the population was discovered to be negligible, only affecting three coatings. The differences in the barnacle community, the level of wave exposure and desiccation between the two locations are just a few of the potential factors influencing the spatial variation in the percentage recruitment. As for the temporal variation seen in Burnham-on-Crouch, one potential factor causing the reduced percentage cover on the samples for the immersion periods in 2011 could be the result of the cold winter during 2010, where the average temperature was 5°C below the average for the month of December (Web reference 2). Harms & Anger (1989) demonstrated that the settlement activity during the spring/summer was drastically reduced following a particularly cold winter as a result of increased adult mortality reducing the larval supply. Supporting this, Gallagher et al. (2015) also attributed the

low abundances of *E. modestus* recorded in 2011 around the coast of the Isle of Cumbrae to the cold winter of 2010.

From Figure 4.6, of the percentage cover of *S. balanoides* and *E. modestus* at Fairlie Quay 2010, there appears to be an interaction effect of species and coatings, but unfortunately due to the nature of the distribution of the data, any interaction effects could not be investigated with the Kruskal Wallis statistical test that were performed. An example of the potential interaction effect in Figure 4.6 of species and coatings can be demonstrated for the coatings S3 and S5, where the percentage cover of *S. balanoides* on S5 was relatively high but the percentage cover of *E. modestus* on the same coating was relatively low. The opposite was that for coating S3, where the percentage cover of *E. modestus* was relatively high and that for *S. balanoides* was low. The two different barnacle species may have different preferences for potential settlement sites with *E. modestus* preferring S3 (along with the coatings S4, FP1 and FP2) and *S. balanoides* preferring S5 (along with the coatings FP1, FP2 and FP3).

In this study the laboratory trials did correspond to the results from the field from Burnham-on-Crouch in 2010. However, for a true representation of the performance of coatings long scale testing involving two or more locations over two or more settlement events and years are necessary as the change in environmental conditions and fouling communities between the different sites and in the two years contributes to the differences in the coverage (Jenkins et al. 2000; Swain et al. 2000; Wood et al. 2000; Robson et al. 2009) and ultimately this can influence the interpretation of the coatings performances.

4.5.2. Critical removal stress

A comparison in the critical removal stress (CRS) of *E. modestus* and *S. balanoides* from 2010 showed that the CRS of *E. modestus* was greater than that of *S. balanoides* for four out of the eight coatings. This may be a factor of the different sizes of the barnacles, with *E. modestus* being a much smaller barnacle compared to *S. balanoides.* The average size of the *E. modestus* (5.32mm in diameter) in this study was approximately half the average size of the *S. balanoides* (10.34mm in diameter). Robson et al. (2009) discovered that with *E. modestus* size was negatively correlated to the CRS, in which the larger the barnacle the lower the adhesive strength. The CRS is

the removal force (N) divided by the basal area (mm^2) of the barnacle. The actual force (N) required to detach *S. balanoides* was greater than the force required to detach *E. modestus,* but the much larger area of the former produces a lower CRS value than the latter. Thus, the larger the barnacle's basal area, the lower the CRS values and therefore the lower the adhesive strength of that barnacle. However, in contradiction to Robson et al. (2009) and the results of this study, Berglin et al. (2001) demonstrated that increasing the basal area of barnacles has produced higher values in the CRS as there is a larger area and quantity of adhesive used by the barnacle to maintain contact with the substratum. Additional factors contributing to the differences in the adhesion may be a result of the difference in the shape and structure of the barnacles' shells. As demonstrated in Chapter 3, the shape and structure of the shell can influence the mode of detachment and potentially the CRS of the barnacles. The shell of *E. modestus* has four non-porous parietal plates with weak butt sutures between the plates, by contrast *S. balanoides* has six non-porous parietal plates joined together with stronger mitred sutures (Barnes et al. 1970). The mechanical strength of the shell of *S. balanoides* is greater than that of *E. modestus*, which Barnes et al. (1970) attributed to it being an intertidal species and therefore offering a better resistance to a higher energy environment. However, *E. modestus* is also present in the intertidal zone, so the explanation provided by Barnes et al. (1970) is confusing! Regardless, the strength and size of the shell may offer some explanation as to why there was only a difference between the CRS of the two barnacles for four of the eight coatings, with the size masking the influence of the shell strength.

S. balanoides was included in this investigation as it has a membranous-basal plate. The intention was to use *S. balanoides* as a second example of a membranous-based barnacle alongside *E. modestus* to compare with the calcareous-based *B. amphitrite*. However, as *S. balanoides* has not been successfully cultured in a laboratory (Kirby 2006) settlement on test coatings depended on field immersion trials. Further, as a result of the low number (n) of barnacles for the CRS measurements and the large error bars, *S. balanoides* did not appear to be a good example of a test species for assessing the performances of fouling-release coatings in this study.

For the *E. modestus* barnacles from Burnham-on-Crouch, the CRS for each coating was compared between all four of the immersion periods (April 2010, June, 2010, April 2011 and July 2011). This was to determine whether there were differences

in the adhesion between the years and seasons. However, the differences that were present in the adhesion between the periods did not follow any distinct pattern that could be attributed to the seasons, i.e. there was no clear increase or decrease in the CRS of the barnacles immersed in June/July over those from April. The fact that there were no distinct trends was made more difficult by the multiple coatings used, as the differences in the CRS between barnacles from the different immersion periods were not consistent for each coating. For example, where there was a difference between June 2010 and April 2011 for coating S1, for coating S2 there was a difference between April 2010 and June 2010 instead. In a study by Swain et al. (2000) which investigated the biofouling community and barnacle adhesion from a selection of FR coatings at multiple field sites, the difference in the removal stress of the barnacles between different field sites was attributed to the length of time of immersion. In this study, the racks for April 2010 were immersed for two months, and then spent ten weeks in a tank in the laboratory, those for June 2010 were immersed for four months with five weeks in a laboratory tank and both April 2011 and July 2011 were out for three months, with only four weeks each in a laboratory tank. The length of time spent in holding tanks in the laboratory varied. The dominant factor for this variation was the minimum size of the barnacles, which needed to be greater than 4.1mm in diameter for the barnacles to be a suitable size for the adhesion measurements (see Chapter 2). The barnacles returning from the April 2010 immersion period were much too small and were therefore held for longer in the laboratory in order to allow for them to grow to this minimum size of 4.1mm in diameter. The subsequent immersion periods in the field were extended to allow for this growth to be in the field as opposed to laboratory holding tanks. Nevertheless, the total length of time allowed for the growth in the field and in the laboratory holding tanks did differ. Berglin et al. (2001) found that barnacles growing for longer periods of time had higher removal stress values than those of equal size which were grown for less time. The difference in the immersion periods in the field and in the laboratory holding tanks is one potential explanation for the difference in CRS between the four time points.

There were differences when comparing removal stress of *E. modestus* from the two field sites, Burnham-on-Crouch and Fairlie Quay, and the removal stress of *E. modestus* reared in the laboratory. The CRS of barnacles from Fairlie Quay were higher than those from the laboratory, which were in turn higher than those from Burnham-on-Crouch, hence the general pattern from highest to lowest is Fairlie Quay > laboratory >

Burnham-on-Crouch. These differences may be a result of different lengths of time allowed for growth; however, genetic variation could also be a factor. Differences in the adhesive plaque of *B. amphitrite* barnacles have been attributed to genetic variations between maternal families (Holm et al. 2005; 2009). Fairlie Quay and Burnham-on-Crouch are two separate populations, of *E. modestus* in the UK; it could be that the differences in CRS in this study, between these two populations is a result of genetic variation.

The difference in the CRS between the two field sites could also be the result of the local environmental conditions. The racks that were immersed in Burnham-on-Crouch were suspended by ropes horizontally 1m below the surface of the water from floating rafts. The tidal flow of the area was strong (max 1.3 – 2.0 knots, Web references 3), but by being suspended and not fixed in place there may be a buffering effect reducing the actual flow across the racks. There is also a population of *Jassa* spp., an amphipod that builds tubes out of sediment. They created a dense mat covering the barnacles, which in turn may have protected the barnacles from any impact and turbulence. The racks in Fairlie Quay were more exposed by being fixed to a pier leg in the intertidal and thus were subjected to tides, currents and waves. It was made clear by the damage caused to the test racks in 2011 of the potential force of the water against the racks.

External biotic and physical factors have been shown to influence the physical attributes of the barnacles including the strength of adhesion. Swain et al. (1998) found that *B. eburneus* barnacles unprotected from predation had increased CRS values than those which were protected. It was concluded that unprotected barnacles would develop a higher resistance to these biological disturbances. Indeed the position of the slides on the racks for the barnacles in Fairlie Quay did influence the adhesive strength of the barnacle; however the barnacles which were positioned on the sheltered side and therefore had more protection from predation and the waves had a higher adhesion than the barnacles on the un-protected exposed side. Nevertheless the combined adhesive strength of the barnacles from Fairlie Quay, that are from a more exposed, more turbulent environment, possess a stronger adhesive in resistance to detachment than the barnacles from Burnham-on-Crouch.

Previous studies have shown positive correlations between the hydrodynamics of the environment and the recruitment and growth of barnacles but not necessarily the

adhesion of specifically adult barnacles (Judge & Craig 1997; Leonard et al. 1998; Jonsson et al. 2004). Yet, to avoid displacement and removal, adhesion theoretically should counterbalance the hydrodynamic force of an environment (Bailly et al. 2009). However, considering this, laboratory-reared barnacles that are not subjected to any flow should possibly have the lowest CRS, and this is not the case. This could indicate that the turbulence of an environment may not have such a significant influence on the adhesion of adult barnacles. Further studies into the CRS of barnacles grown in the laboratory systematically increasing levels of flow or turbulence would help determine the extent to which the wave energy of the coastal environment influences the adhesion and removal of adult barnacles and other potential biofouling organisms.

The CRS results of the barnacles from the two sides of the racks from Fairlie Quay were combined as the number of total barnacles used to provide these results were small. The number of individuals tested does not correlate to the number that had settled. After returning the coated slides to the laboratory a proportion of barnacles had died, but also a larger proportion had to be removed to test the adhesion of a single barnacle. The gregarious nature of barnacles means they settle within close proximity to one another and are often touching and overlapping (Clare & Matsumura 2000). However, only the adhesion of a solitary individual is tested, and therefore the surrounding barnacles need to be removed to isolate just the one individual. This seems to be an inherent problem with field immersion trials as choosing which barnacles to remove is unavoidably selective. This is not often a problem for laboratory cultures as, soon after settlement barnacles can be removed before they begin to grow and overcrowd one another. There is a factor of randomness when selecting a barnacle for removal, but essentially it depends on the position of the barnacles in relation to others on the slide, and specifically, in the case for *S. balanoides*, it also depends on the adhesion the target individual has to the coating as opposed to other barnacles. In some instances, regardless of how careful one was whilst trying to isolate an individual, some *S. balanoides* were better attached to the surrounding barnacles than to the coated slides. This contributed to there being a limited number of samples available for testing the CRS.

The aim of laboratory assays and field immersion trials is to differentiate between the performances of different coatings and this was one of the reasons for using eight coatings. Despite the differences between the two field sites and between the field

and laboratory results it was possible to differentiate between the coatings within a single trial. The fluoropolymers had the higher CRS values than the silicones, with S5 having the highest CRS values of the silicones and S2 having one the lowest values. Therefore it was possible to gauge the performance of the coating under different environmental conditions.

4.5.3. Biofilm

The presence of biofilms on surfaces are important in terms of providing settlement cues to marine larvae, for example, they can inhibit or facilitate settlement of cyprids depending on the age and/or species composition of the film (Maki et al. 1988; 1990; Neal & Yule 1994b; Keough & Ramondi 1995; Wieczorek et al. 1995). With *B. amphitrite* and *B. perforatus*, biofilms have been shown to increase the adhesion of cyprids and partially metamorphosed barnacles to glass surfaces (Neal & Yule 1994a; Zardus et al. 2008). However, *E. modestus* cyprids have been shown to adhere equally to biofilmed and un-biofilmed surfaces (Neal & Yule 1994a). Nevertheless, it was reasonable to question whether the presence of biofilm on the surface of the coatings could influence the adhesion of adult barnacles grown in the field. The racks that were immersed in the field would instantly be covered in a complex film consisting of macromolecules, bacteria and unicellular eukaryotes. Yet, in the laboratory the coatings are typically leached for two weeks in RO water, however, in this experimental section the slides were leached for ten days in RO, after which they were immersed for an hour in ASW prior to settling cyprids. This is not sterile, and a bacterial film could develop, although not to the extent or diversity of the films that would be seen in the field. The control 'un-biofilmed' slides were leached in RO for ten days, because once immersed in water the surface properties of silicone elastomers can change, either through absorbing water or molecular rearrangement of the siloxane chains (Estarlich et al. 2000). This can change the surface energy of the coating and therefore influence adhesion of fouling organisms. Hence it was important to immerse the two groups of slides for an equal amount of time in their respective environments.

The age and method for film development followed the technique used by Zardus et al. (2008) for investigating the adhesion of cyprids and newly metamorphosed *B. amphitrite* barnacles to biofilms. In this study the presence of a 10-day-old biofilm

did not increase the CRS of adult *E. modestus* when compared to those grown on coatings immersed for 10 days in RO. This may be a factor of the size of the organism; larger organisms have a greater surface area of adhesive relative to the thickness of a 10-day-old biofilm. There could also be a factor regarding the change in cement from a cyprid's permanent adhesive to the adult's cement. However, there may be species-specific considerations. In the first instance, *E. modestus* has a preference for settling on un-biofilmed surfaces rather than surfaces with a biofilm (Keough and Ramondi 1995). In addition, Neal & Yule (1994a) found that the adhesion of *E. modestus* cyprids was equal between surfaces with a two-month growth of biofilm and to a clean un-biofilmed surface, whereas *B. perforatus* cyprids adhered much better to the biofilmed surface. Therefore, the presence of a biofilm would seem to have no influence on the CRS of adult barnacles, and does not contribute to the difference seen in the adhesion of field- and laboratory-reared adult barnacles. However, further studies would be beneficial to confirm this, including the use of a more complex series of biofilm cultures (see Keough and Ramondi 1995).

4.5.4. Temperature

Johnston (2010) demonstrated that temperature can influence the CRS of *B. amphitrite*. This was in relation to the rate of growth; individuals that were grown at a colder temperature grew at a slower rate and had a higher adhesion strength than those grown faster at warmer temperatures. That was not the case in this study, the barnacles that were grown at the lowest temperatures did not grow at the slowest rate. The barnacles which grew at the slowest rate were grown at 19°C, yet these barnacles also had the lowest CRS. However, for the barnacles maintained at 19°C and growing at the slowest rate this was believed to be an anomalous result. There was a higher rate of mortality of these barnacles than witnessed with the remaining temperatures. This was thought to be a due to the position of the container within the incubator and that it was not receiving a sufficient amount of light due to a fault with the lighting system, which affected the quality and endurance of the algae that was added as feed. When the algae was added to the containers after one week those at 12, 15 and 22°C, were clear, presumably all of the algae being eaten. However in the container at 19°C, the medium left within that container was more yellow in colour, suggesting that not all the algae had been consumed, and that the algae which remained were deteriorating. Positioning

the container closer to the light source would have prevented such deterioration of the algae and therefore the higher mortality and reduced growth rate of the barnacles at this temperature.

Discounting the growth rate and CRS of barnacles from 19°C, the next slowest growing barnacles and lowest CRS was for barnacles grown at 15°C, whereas the barnacles that had grown at the highest and lowest temperatures had an equal growth rate and CRS. It could be that the range in temperature used in this study was not significant enough to witness an effect in regards to growth as *E. modestus* can tolerate a large range in the temperature, from approximately 6 - 22°C and even higher (Crisp & Davies 1955). Additional studies with a range in temperatures that exceed the range of 6 - 22°C would better examine the influence of temperatures on the correlation between growth and CRS.

The temperature range for the surface waters for Fairlie Quay and Burnham-on-Crouch were not recorded. The water temperature of the Irish Sea at Port Erin which is the closest record station to Fairlie Quay and at Littlebrook in the Thames, the closest station to Burnham-on-Crouch was accessed via the CEFAS website (Website reference 4: see Appendix 2). At Port Erin during the 2010 immersion period the surface water temperature ranged from 7.3 to 14.9°C and from 8.0 to 12.3°C in 2011. At Littlebrook, this ranged from 10.7 to 20.5°C in 2010 and from 12.3 to 17.7°C in 2011. The temperature during the immersion period in Fairlie Quay was cooler than the water temperature in Burnham-on-Crouch. If *E. modestus* was to behave in the same manner as Johnston (2010) reported for *B. amphitrite*, this could be an additional factor to explain why the CRS is so much greater for barnacles from Fairlie Quay than those from Burnham-on-Crouch. The barnacles grown in the laboratory were grown at a constant temperature of 22 ± 1°C which is higher than in the two field sites; this would perhaps suggest that they would have a faster growth rate than in the field. However, the growth rate of barnacles grown in the laboratory is slower than those grown in the field (Costlow & Bookhout 1953). For example, Wiegermann & Watermann (2004) immersed racks coated with Intersleek and Sigma Glide in marinas near Meldorf (along the North Frisian coast) and on the island of Norderney (East Frisian Island), respectively; these are both located in the North Sea along the north coast of Germany. The samples were immersed in these locations both for six weeks from July to August and which had *E. modestus* barnacles 4.5 ± 1.5mm in diameter, which was a much

faster rate of growth than what was shown in Chapter 2. In this study, the *E. modestus* from Burnham-on-Crouch immersed in July 2011 for 13 weeks grew to 6 ± 2mm in diameter. Whereas those immersed in Fairlie Quay in March 2010 were 5 ± 1.5mm in diameter after an immersion period of 17 weeks. The rate of growth of barnacles in both Burnham-on-Crouch and Fairlie Quay was much faster than the growth rate of laboratory cultured barnacles as demonstrated in Chapter 2. If barnacles with a faster growth rate have a CRS that is lower than slower growing barnacles this could explain why barnacles from Burnham-on-Crouch have a lower CRS than the slower growing laboratory barnacles. However, this does not explain why the slower growing laboratory barnacles have a lower CRS than the faster growing barnacles from Fairlie Quay.

One of the criticisms of field immersion trials is that they are supposed to require several months immersion time (Rittschof et al. 2008; Stafslien et al. 2012) and therefore take longer than supposedly rapid laboratory assays. However, this may not necessarily be the case, as laboratory cultured barnacles have a slower growth rate than barnacles from the field. Further investigations regarding the method for growing barnacles in the laboratory would be beneficial to improve the growth rate of barnacles. This could look at the factors such as water currents and flow rates (Crisp 1960; Sanford et al. 1994), dietary regimes of *Artemia* sp. and microalgae e.g. *T. suecica* and lighting treatments (Barnes 1953).

4.6. Conclusion

The aim of this chapter was to compare the use of laboratory assays and field immersion trials for evaluating FR coatings by examining the similarities and differences in the percentage settlement (laboratory) and recruitment (field) and the critical removal stress (CRS) of barnacles from eight coatings. With the addition of investigating the influence of biofilm and temperature on the removal stress of adult barnacles as factors explaining potential differences between the laboratory and field results. From the results of the laboratory assay it was possible to discriminate between the coatings in terms of percentage settlement and CRS, and conclude that the silicone elastomers performed better than the fluoropolymers with S2 having the lowest values for both measurements. Although it must be mentioned that conclusions drawn here are

from one laboratory culture and there is, therefore, a need for caution when interpreting these results. Nevertheless, there were similarities in the pattern of the settlement and CRS of barnacles from the laboratory compared to the recruitment and CRS results from both Fairlie Quay and Burnham-on-Crouch, namely that the silicones performed better than the fluoropolymers with S2 performing the best. S2 had the lowest percentage coverage and the lowest adhesion measurements, and was, therefore, able to resist colonisation by the barnacles better than the fluoropolymers and with those that had settled being removed much more easily. Being able to differentiate between the coatings and decipher which coating has the better FR properties is fundamentally the desired outcome for these tests. However, the actual measurements, specifically of CRS, did differ significantly between the three locations (Fairlie Quay, Burnham-on-Crouch and the laboratory) and over the two years at Burnham-on-Crouch. The general pattern of the CRS between the locations was Fairlie Quay > laboratory > Burnham-on-Crouch.

The influence of a 10-day-biofilm on the adhesion of *E. modestus* and the effect of different temperatures on the growth and adhesion were incorporated in this study in an attempt to explain the differences between laboratory and field environments. However, temperature and the presence of a biofilm did not significantly affect the CRS of adult barnacles and, therefore, are unlikely to have contributed to the differences noted between the adhesion of laboratory-reared and field-grown adult barnacles.

Laboratory assays have their benefits over field trials (Table 4.15). This includes a better control over the population and therefore prevention of overcrowding, and no environmental stresses reducing the level of recruitment for example the adverse weather in Fairlie during 2011 damaging the racks and removing settled barnacles or the cold winter in 2010 which potentially reduced the larval availability and recruitment in Burnham-on-Crouch in 2011. However there can be problems with laboratory assays, for example that seen for the barnacles grown at 19ºC, due to a fault in the equipment limiting the light source, there was deterioration in the algae used as food, which caused the barnacles growth rate to be slow and the CRS value to be low. In addition, one of the specific criticisms of field trials is that they often require several months immersion time for the barnacles to settle and grow to a sufficient size for adhesion testing (Rittschof et al. 2008; Stafslien et al. 2012). However, the barnacles actually grew at a faster rate in the field than under the laboratory conditions in this study.

Table 4.15. Advantages and disadvantages of field immersion trials and laboratory assays for the evaluation of antifouling and fouling-release coatings.

Field Tests		*Laboratory Tests*	
Advantages	***Disadvantages***	***Advantages***	***Disadvantages***

• **Broad spectrum performance of a coating against a wide range of fouling organisms (Stasflien et al. 2012).**
• **Long term durability in a natural environment (Stasflien et al. 2012).**
• **Quicker growth rates (Costlow &Bookhout 1953; Chapter 2 and 4).**
	• **Restricted by seasons for certain species for example *Semibalanus balanoides* (Barnes et al. 1970; Rittschof et al. 2008; Stasflien et al. 2012).**
	• **Can be effected by adverse weather**
	  ○ **Low larval availability (Harms & Anger 1989).**
	  ○ **Damage to the samples (Chapter 4).**
	• **Large volume of samples required (Stasflien et al. 2012).**
	• **Restricted on testing capacity (Stasflien et al. 2012).**
	• **Post recruitment mortality for example through predation (Swain et al. 1998).**
		• **Rapid assessment especially in settlement and toxicity assays (Rittschof et al. 1992).**
		• **Smaller volume of samples (Rittschof et al. 2008; Evariste et al. 2012).**
		• **Lower cost in terms of facilities and resources (Evariste et al. 2012).**
		• **Controlled conditions providing reproducible data (Evariste et al. 2012).**
			• **Slower growth rates (Costlow & Bookhout 1953; Chapter 2 and 4).**
			• **Difficult to mimic the complex interactions in the natural environment (Briand 2009).**

The complexity of the colonisation process and the interactions between physical, chemical and biological components has yet to be fully replicated under laboratory conditions. Briand (2009) may be correct when stating that "*no laboratory bioassay could hope to replicate such a complex process*". Field immersion trials, using multiple locations over several years and settlement events, provide a more accurate measure of a coatings performance. However Evariste et al. (2012) stated that laboratory studies "*are not intended to reflect the complexities of the 'real world'* ". As previously established, laboratory assays are a useful 'tool' to provide an indication of the performance of a coating and can be used to down-select coatings from a larger number to a more manageable collection of coatings as a precursor to field trials (Rittschof et al. 2008; Evariste et al. 2012).

Chapter 5: The Influence of Elastic Modulus of Fouling-Release Coatings on the Adhesion of *Elminius modestus* in Comparison to *Balanus amphitrite*.

5.1. Abstract

The elastic modulus of a coating is an important factor for the detachment of a fouling organism from the coating's surface. In which less force is required to remove fouling from low modulus coatings, in contrast, more force is required to remove fouling from high modulus coatings. This study was to investigate the degree in which the elastic modulus of the coating can influence the critical removal stress (CRS) of the membranous-based *Elminius modestus*, compared to the calcareous-based *Balanus amphitrite*. The CRS of *E. modestus* and *B. amphitrite*, to eight coatings (five polysiloxanes and three fluoropolymers) were measured. The bulk properties of the polysiloxanes and fluoropolymers were modified by changing the polymer chain length and cross-linker density, which provided coatings with a modulus ranging from 0.31 to 19.73 MPa, as determined by a dynamic mechanical analyser (DMA). Regression analysis confirms that increasing the modulus increases the CRS for *E. modestus* and *B. amphitrite*, however the model did not show a strong linear association for either species (R^2 = 0.091 for *E. modestus* and R^2 = 0.089 for *B. amphitrite*). Instead exponential (R^2 = 0.106 for the silicone coatings) and power (R^2 = 0.649 for the silicone and fluoropolymer coatings combined) regression models provided better explanations for the variance in the CRS than a simple linear model for *E. modestus* only. Comparing the CRS of the two barnacle species, there was a significant difference for three out of six the coatings. However, with *B. amphitrite* on the two fluoropolymer coatings with the highest modulus, shell failure occurred before adhesive failure. From this study, it was concluded that *E. modestus* was a suitable test species for future fouling-release research and was able to provide a valuable comparative for studies in adult adhesion.

5.2. Introduction

Fouling-release (FR) coatings function by reducing the adhesion of fouling organisms to the coating so much so that fouling can be removed by its own weight or by the hydrodynamic force of water moving across the surface of the coating (Schultz et al. 1999; Berglin et al. 2003). Silicone polymers (polysiloxanes) and fluoropolymers have been identified as the two groups of material with some of the best FR properties (Brady 2000; Yebra et al. 2004; Finnie & Williams 2010). Of the properties, there are three that have been the main focus of research and these include the surface energy (γ), the elastic modulus (E) and the coating's thickness (Brady & Singer 2000; Singer et al. 2000; Anderson et al. 2003; Berglin et al. 2003; Sun et al. 2004; Yebra et al. 2004; Chaudhury et al. 2005; Wendt et al. 2006). The surface energy is particularly important with regard to the adhesion strength of fouling to the coating; desirably a coating should have a low surface energy value between 20 – 30mJm^{-2}. Whereas the thickness and the elastic modulus are more important with regard to the detachment mechanisms, a thicker coating with a lower modulus improves the release characteristics of the FR coating as demonstrated by Kendall's model (Kendall 1971).

The elastic modulus or Young's modulus refers to the ability of the coating to deform elastically when subjected to an external pressure. The greater the deformation, the lower the modulus. By contrast, when the deformation is reduced and the coating is less flexible, it has a higher modulus. The modulus of a coating can be altered by changing the chain length (molecular weight) of the polymer and the type and quantity of cross-linking agent (cross-linker), without changing the coating's surface energy (Chaudhury et al. 2005). Altering the chain length of a polymer and the cross-linker influences the incidence of cross-linking during the curing process. Polymers with a longer chain length and higher molecular weight have an increased number of monomer units available; this increases the potential of cross-linking that may occur during the curing process. The greater the proportion of cross-linking that occurs, results in a coating that is less flexible and thus has a higher modulus. By contrast, polymers with smaller chain lengths and lower molecular weight may reduce the incidence of cross-linking and result in a coating that is more flexible with a lower modulus (Mark et al. 2005).

When an external force is applied to a fouling organism on a low modulus coating, its adhesive slips on the surface; this slip during the detachment reduces the

energy needed to complete the fracture and to remove the organism from the coating (Brady 1999). As the modulus of the coating increases so too does the removal stress, this reduces the FR ability of the coating. This phenomenon is well documented with pseudobarnacles, barnacles such as *B. eburneus* and *B. amphitrite* as well as fouling algae such as *Ulva* spp. (Brady & Singer 2000; Wynne et al. 2000; Berglin et al. 2003; Stein et al. 2003; Chaudhury et al. 2005; Kim et al. 2007; 2008).

There was evidence, discussed in Chapter 2 and 3 of this study, that suggests the flexible attributes of the membranous-basal plate of *E. modestus* influences its removal from silicone coatings compared to the calcareous-based barnacle *B. amphitrite*. This was with regards to the critical removal stress (CRS) and the time for initial separation and complete removal, which all differed between the two species. The aim of this chapter was to investigate the degree in which the elastic modulus of the coating can influence the removal stress of the membranous-based *E. modestus*, compared to the calcareous-based *B. amphitrite*. The hypotheses to be tested are: 1) that the removal stress of *E. modestus* would increase with increasing modulus, and 2) that the removal stress of *E. modestus* would be lower than that of *B. amphitrite*. In addition, the relationship of elastic modulus (E) and surface energy (γ) using the function ($E\gamma$)½ was investigated (Brady & Singer 2000), to examine how these two coating properties in combination influence the removal stress. The hypothesis to be tested is that the removal stress of *E. modestus* and *B. amphitrite* would increase with increasing the $(E\gamma)^{½}$ value. Finally, to conclude whether *E. modestus* as a test species was capable of discerning between coatings for FR evaluations and whether it was a suitable test species for future FR research.

5.3. Materials and methods

5.3.1. Coating preparation

All coatings in the series were provided and prepared at International Paint Ltd, Felling, UK. The test coatings were coated on to glass microscope slides (76mm x 26mm x 1mm, Fisherbrand). The microscope slides were fixed in rows to adhesive vinyl sheets, which in turn were backed on to plywood boards (750mm x 350mm x

10mm). Prior to being coated the microscope slides were cleaned using Xylene solvent applied with laboratory roll. A tie coat of an acrylic polymer (Valkyrie, International Paint Ltd) (10:1 Polymer:Xylene Solvent) was thinly applied to the slides using an extra smooth gloss paint roller and cured in an environmental cabinet at 23°C and 50% relative humidity for 4 hrs. Once cured, the test coatings were applied to the tie coat using an extra smooth gloss paint roller. These were left to cure at room temperature (RT) for 48 hrs.

5.3.2. Coating formulation

The initial objective was to produce a series of coatings which had:

1) a range of elastic modulus but with a constant surface energy, and

2) coatings with a range of surface energies with a constant modulus.

The silicone and fluoropolymers used to prepare the coatings were provided by International Paint Ltd, UK.

5.3.2.1. Silicones

In the first instance of coating manufacturing the focus was on developing coatings with different modulus and a constant surface energy. Two silicone (PDMS) polymers with different chain lengths (molecular weights) were used, these included:

- Rhodorsil 48V-2000 (High molecular weight)
- Rhodorsil 48V-750 (Low molecular weight)

To alter the modulus of these samples, the percentage of the cross-linking agent Tetraethyl Orthosilicate (TEOS) was changed. The percentages 25%, 50%, 75% and 100% of TEOS were used, and a second cross-linker Methyltrimethoxysilane was included to make up to 100% volume when necessary. The coatings were coded Low 25, Low 50, Low 75, Low 100, High 25, High 50, High 75 and High 100 (Table 5.1). The ‘low’ and ‘high’ refers to the molecular weight of the polymer and the numbers indicate the percentage of the cross-linker TEOS.

Table 5.1. Preliminary silicone coating formulations.

Coating codes	*Functions*	*Components*	*Weights*
Low 25	**Polymer**	**Rhodorsil 48V-750**	**91.45%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.45%**
	Crosslinker	**Tetraethyl Orthosilicate (TEOS)**	**1.02%**
		Methyltrimethoxysilane	**3.08%**
	Solvent	**Xylene**	**3.99%**
Low 50	**Polymer**	**Rhodorsil 48V-750**	**91.45%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.45%**
	Crosslinker	**Tetraethyl Orthosilicate (TEOS)**	**2.05%**
		Methyltrimethoxysilane	**2.05%**
	Solvent	**Xylene**	**3.99%**
Low 75	**Polymer**	**Rhodorsil 48V-750**	**91.45%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.45%**
	Crosslinker	**Tetraethyl Orthosilicate (TEOS)**	**3.08%**
		Methyltrimethoxysilane	**1.02%**
	Solvent	**Xylene**	**3.99%**
Low 100	**Polymer**	**Rhodorsil 48V-750**	**91.45%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.45%**
	Crosslinker	**Tetraethyl Orthosilicate (TEOS)**	**4.11%**
	Solvent	**Xylene**	**3.99%**
High 25	**Polymer**	**Rhodorsil 48V-2000**	**91.45%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.45%**
	Crosslinker	**Tetraethyl Orthosilicate (TEOS)**	**1.02%**
		Methyltrimethoxysilane	**3.08%**
	Solvent	**Xylene**	**3.99%**
High 50	**Polymer**	**Rhodorsil 48V-2000**	**91.45%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.45%**
	Crosslinker	**Tetraethyl Orthosilicate (TEOS)**	**2.05%**
		Methyltrimethoxysilane	**2.05%**
	Solvent	**Xylene**	**3.99%**
High 75	**Polymer**	**Rhodorsil 48V-2000**	**91.45%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.45%**
	Crosslinker	**Tetraethyl Orthosilicate (TEOS)**	**3.08%**
		Methyltrimethoxysilane	**1.02%**
	Solvent	**Xylene**	**3.99%**
High 100	**Polymer**	**Rhodorsil 48V-2000**	**91.45%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.45%**
	Crosslinker	**Tetraethyl Orthosilicate (TEOS)**	**4.11%**
	Solvent	**Xylene**	**3.99%**

The modulus of the preliminary silicone samples (Table 5.2) were measured using a dynamic mechanical analyser (Perkins Elmer PYRIS Diamond DMA) measuring the tensile strength, in which the modulus was calculated by Stress over Strain.

Table 5.2. Young's modulus results of the preliminary silicone test coatings. Modulus was measured using the DMA, testing tensile strength of the silicones.

Coatings	*Modulus (MPa)*
Low 25	**0.329**
Low 50	**0.274**
Low 75	**0.210**
Low 100	**0.186**
High 25	**0.162**
High 50	**0.200**
High 75	**0.132**
High 100	**0.144**

From the initial eight formulations three were selected to produce a coating series with different modulus. Low 25, Low 100 and High 75 were chosen as these mixtures resulted in coatings with relatively high, medium and low modulus values and were coded HMod, MMod and LMod, respectively (H refers to high, M for medium and L for low, the Mod refers to the modulus, i.e. HMod is the high modulus coating). Two additional coatings were prepared to provide coatings with a high and low surface energy whilst having equal modulus and were coded HSE and LSE (see Table 5.5 for the modulus). HSE consisted of a polyether-silicone co-polymer whereas LSE consisted of a PDMS polymer (Dow Corning 3-0213) (formulations of the coatings coded HSE and LSE were provided by International Paint Ltd. per.comms) (H refers to high, L for low, SE refers to the surface energy, i.e. HSE is the coating with the high surface energy) (Table 5.3).

Table 5.3. Final coating formulations.

Coating codes	*Functions*	*Components*	*Weights*
HMod	**Polymer**	**Rhodorsil 48V-750**	**91.43%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.45%**
	Crosslinker	**Tetraethyl Orthosilicate (TEOS)**	**1.02%**
		Methyltrimethoxysilane	**3.08%**
	Solvent	**Xylene**	**3.99%**
MMod	**Polymer**	**Rhodorsil 48V-750**	**91.43%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.45%**
	Crosslinker	**Tetraethyl Orthosilicate (TEOS)**	**4.11%**
	Solvent	**Xylene**	**3.99%**
LMod	**Polymer**	**Rhodorsil 48V-2000**	**91.43%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.45%**
	Crosslinker	**Tetraethyl Orthosilicate (TEOS)**	**3.08%**
		Methyltrimethoxysilane	**1.02%**
	Solvent	**Xylene**	**3.99%**
HSE	**Polymer**	**XX/00843**	**76.6%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.65%**
	Moisture Scavenger	**Triethylorthoformate**	**4.96%**
	Solvent	**1-Methyl-2-propyl acetate**	**17.78%**
LSE	**Polymer**	**Dow Corning 3-0213**	**91.43%**
	Catalyst	**Bis (2-Ethylhexyl) Hydrogen Phosphate**	**0.45%**
	Crosslinker	**Tetraethyl Orthosilicate (TEOS)**	**4.11%**
	Solvent	**Xylene**	**3.99%**

XX/00843 = Polyethyl-silicone co-polymer.

5.3.2.2. Fluoropolymers

The fluoropolymers which were prepared and provided by International Paint Ltd included three modified Perfluoropolyether (PFPE) based moisture cross-linked polymers with different molecular weights and functional groups (Table 5.4). The industry names for these coatings are E10H, D10H and D10, as they were modified by International Paint Ltd, the coatings were prefixed with an ‘m’ and hence are coded mE10H, mD10H and mD10.

Table 5.4. Fluoropolymer coating molecular weight and functional group.

Coating code	*Molecular weight*	*Functional Groups*
mD10	**500**	**CH_2OH**
mD10H	**700**	**CH_2OH**
mE10H	**750**	**$CH_2(OCH_2CH_2)nOH$**

5.3.3. Coating Characterisation

A goniometer (Ramé-hart 250.00 standard goniometer) was used to measure the static contact angles of distilled water and diiodomethane which were then used to measure the surface energy. The contact angle of three droplets per slide for five sample slides for each liquid and coating were measured, the average of these was taken and used to determine the surface energy using the Owens, Wendt, Rabel and Kaelble equation (Eq. 8). This equation calculates the interfacial surface tension (γ_{sl}) or the surface free energy of the polymer by using the surface tensions of the liquid (σ_l) and solid (σ_s) phases, reduced by the geometric mean of the polar (σ_P) and dispersive (σ_D) parts and their interactions between the phases (Owens & Wendt 1969; Kaelble 1970).

$$\gamma sl = \sigma s + \sigma l - 2\left(\sqrt{\sigma_s^D \sigma_l^D} + \sqrt{\sigma_s^P}\sigma_l^P\right) \qquad (8)$$

A dynamic mechanical analyser (The Perkins Elmer Pyris Diamond DMA) measured the elastic modulus of the coatings. Sinusoidal oscillations were applied to a strip of elastomer of a known thickness (measured with a digital calliper). The sample was heated from -140 to 70°C with a heating rate at 4°C /minute, and the strain was measured every 3 seconds (see Appendix 3 for elastic modulus measurements). This measures the glass transition temperature and the modulus reading. The modulus at 22°C for two polymer strips per coating were averaged and provided the modulus presented in this chapter.

Digital callipers were used to measure the thickness of the coatings on the glass slides. The thickness was measured at six points across the slides (Conlan et al. 2008) with ten slides per coating and the average recorded.

5.3.4. Settlement

All eight coatings were used for laboratory settlement. The coatings were leached for two weeks in a static tank of reverse osmosis (RO) water; the water was changed once a week and a carbon filter (Fluval filter) was immersed in the tank to absorb leachate. The coated slides were then immersed for one hour in artificial seawater (ASW) and left to air dry. Once dried they were used immediately for the

settlement of *E. modestus* and *B. amphitrite* cyprids. The method for the laboratory culture of *E. modestus* and *B. amphitrite* was discussed in Chapter 2. Day zero *E. modestus* cyprids and day three *B. amphitrite* cyprids were used for the settlement, in which 20 cyprids were pipetted into a 2ml droplet of 0.2µm filtered ASW centred in each slide. The slides were placed in quadriPERM® culture vessels. After 48 hrs for settlement, 15ml of *T. suecica* was added to the wells of the culture vessels. *E. modestus* were maintained at 22°C and *B. amphitrite* at 28°C, both on a 12:12 L:D cycle and fed 15ml of *T. suecica* at ~3 x 10^5 cells ml^{-1} three times a week. The barnacles were grown for a period of five months in which the average size was 4.4mm (± 0.029 SE) and 4.5mm (± 0.051 SE) in diameter for *E. modestus* and *B. amphitrite*, respectively.

5.3.5. Critical removal stress

The critical removal stress (CRS) was measured using the automated instrument as described in Chapter 2. The CRS values for *E. modestus* barnacles that were smaller than 4.1mm in diameter and for *B. amphitrite* smaller than 3.6mm in diameter (Conlan et al. 2008) were discarded. After removal from the coatings, the basal membrane of ten randomly selected *E. modestus* barnacles, per coating, were inspected using a dissection microscope for any tears that may have occurred in the membrane.

5.3.6. Statistical analysis

The data was checked for normality and an equal variance using a Kolmogorov-Smirnov test (Ennos 2012) and Levene's test (Quinn & Keough 2002), respectively. A linear regression analysis was used to determine how the modulus influences CRS for *E. modestus* and *B. amphitrite* for the silicones, testing the null hypothesis that the slope of the regression was zero and that there was no linear relationship between modulus and CRS for both species. Exponential, power and logarithmic regressions were investigated to determine whether these alternative regressions provided a better fit and higher R^2 values than a linear regression.

The combined data of silicone and fluoropolymers for *E. modestus* was transformed using a square root function. After which a linear regression analysis was

performed to test the null hypothesis that the slope of the regression would equal zero and that there was no linear relationship between modulus and CRS when fluoropolymers are included. Exponential, power, and logarithmic regressions were investigated to determine if these regressions would provide a better fit and higher R^2 values than a linear relationship. It was not possible to perform a regression analysis for *B. amphitrite* for the silicone and fluoropolymers combined, as 100% of the barnacles removed from the fluoropolymers (mD10 and mD10H) had basal failure.

A regression analysis of the CRS against the square root of the surface energy (γ) and the elastic modulus (E) for both species was performed. This was to determine how the surface energy and modulus, when combined, influences the CRS, and how this compared to the elastic modulus alone. The null hypothesis being that the slope of the regression would equal zero and that there is no linear relationship between $(E\gamma)^{1/2}$ and CRS. Exponential, power, and logarithmic regressions were included to investigate to if a better fit and a higher R^2 value than the linear relationship could be attained.

An ANOVA with 0.05 significance level was used to compare the CRS between the barnacle species for each coating testing the null hypothesis that there would be no difference in the CRS between *E. modestus* and *B. amphitrite* barnacles.

5.4. Results

The objective was to produce coatings which had: 1) a range of elastic modulus but with a constant surface energy, and 2) a range of surface energies with a constant modulus. The results of the elastic modulus and coating thickness are presented in Table 5.5 and the surface energy results are presented in Table 5.6. The silicone coatings HMod, MMod, LMod and LSE, provide a range in modulus with minor deviations in the surface energy. The fluoropolymers mD10 and mD10H extended the modulus range up to 19 MPa, and had surface energies with minor deviations from the four silicones (HMod, MMod, LMod and LSE). Unfortunately, the second objective to have coatings with a range of surface energies and a constant modulus was unsuccessful. The coatings HSE and LSE which had different surface energies also had different modulus. The surface energy of mE10H was also higher than the other

fluoropolymers mD10 and mD10H. As the surface energy of HSE and mE10H are higher than the remaining silicone and fluoropolymer coatings, initial analysis will exclude the HSE and mE10H coatings. However they will be present on the graphs represented by a different colour.

Table 5.5. Young's modulus results and thickness of the silicone and fluoropolymer coatings (* highlight the coatings which are fluoropolymers).

Coating code	*Young's Modulus (MPa)*	*Thickness (μm ± 1 SD)*
HMod	**0.66**	**38.8 ± 14.7**
MMod	**0.59**	**46.7 ± 15.0**
LMod	**0.31**	**41.6 ± 11.6**
HSE	**0.96**	**38.3 ± 14.7**
LSE	**0.33**	**33.3 ± 10.3**
mE10H*	**1.88**	**33.3 ± 13.6**
mD10H*	**7.71**	**45.0 ± 10.4**
mD10*	**19.73**	**35.0 ± 10.4**

Table 5.6. Mean water and diiodomethane contact angle measurements of the silicone and fluoropolymers along with calculated surface energies and the polar and dispersive contents (* highlight the coatings which are fluoropolymers).

Coating	*Water contact angle*	*Diiodomethane contact angle*	*Polar*	*Dispersive*	*Total Surface energy*
HMod	**97.64 ± 0.25**	**63.40 ± 0.14**	**1.09 ± 0.05**	**26.62 ± 0.08**	**27.71 ± 0.08**
MMod	**100.04 ± 0.16**	**68.02 ± 0.16**	**1.01 ± 0.03**	**21.00 ± 0.16**	**25.00 ± 0.08**
LMod	**98.23 ± 0.34**	**66.80 ± 0.09**	**1.25 ± 0.07**	**24.68 ± 0.05**	**25.93 ± 0.08**
HSE	**76.21 ± 0.21**	**54.89 ± 0.10**	**6.98 ± 0.10**	**31.51 ± 0.06**	**38.49 ± 0.10**
LSE	**99.31 ± 0.14**	**65.23 ± 0.12**	**0.93 ± 0.03**	**25.57 ± 0.07**	**26.51 ± 0.07**
mE10H*	**61.06 ± 0.37**	**69.51 ± 0.09**	**19.52 ± 0.26**	**23.15 ± 0.05**	**42.67 ± 0.26**
mD10H*	**99.01 ± 0.15**	**75.60 ± 0.29**	**1.93 ± 0.05**	**19.80 ± 0.15**	**21.73 ± 0.13**
mD10*	**94.70 ± 0.24**	**67.63 ± 0.26**	**2.14 ± 0.07**	**24.21 ± 0.15**	**26.35 ± 0.14**

5.4.1. Silicones

The data for the silicones were normally distributed (*df* = 110, *D* = 0.070, *P* = 0.200) with equal variance (*df1* = 3, *df2* = 106, *F* = 0.450, *P* = 0.718). Figure 5.1 shows a positive relationship between the CRS and modulus of *E. modestus* and *B. amphitrite* barnacles for the silicone coatings, where the CRS increases with an increase in the elastic modulus of the coating. The *t*, *F* and *P*-values from the regression analysis (Table 5.7) suggests that the modulus does influence the removal stress for both barnacle species by rejecting the null hypothesis that the slope equals zero and that there is no linear relationship. However the R^2 values for *E. modestus* (0.091) and *B. amphitrite* (0.089) are very low, indicating that the regression equation is not a good linear model for the data and that only 9.1% and 8.9% (*E. modestus* and *B. amphitrite*, respectively) of the variance in the CRS can be explained by the modulus. The exponential (*P* = 0.001, R^2 = 0.106) and power (*P* = 0.001, R^2 = 0.104) regressions for *E. modestus* on the silicones had significant *P*-values and much higher R^2 values than the linear regression, with the exponential regression (Table 5.8) having the highest R^2 value and seemingly the best-fit regression relationship (see Appendix 4 for tables of the power and logarithmic regression results). However, for *B. amphitrite*, the exponential, power and logarithmic regressions did not produce a higher R^2 value than the linear regression analysis (see Appendix 4 for exponential, power and logarithmic regression results). Therefore, the CRS values can be predicted from the modulus of the coating for *E. modestus* by the exponential regression formula CRS = 0.021 x $(1.445)^{\chi}$, and for *B. amphitrite* by the linear formula CRS = 0.024 + (0.064 χ), where χ is the modulus.

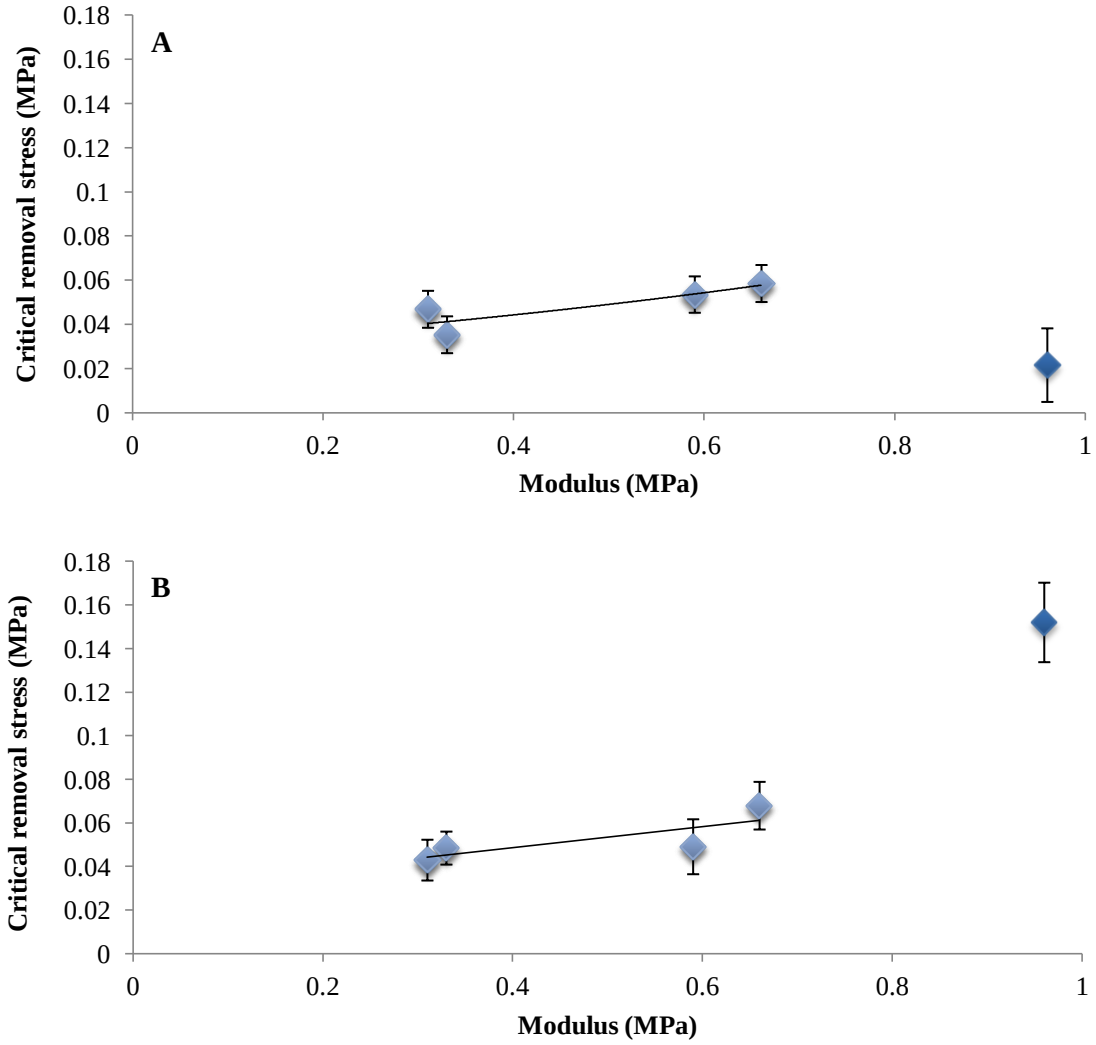

Figure 5.1. The mean critical removal stress (± 95% confidence intervals) of *Elminius modestus* (A) with an exponential regression trend-line and *Balanus amphitrite* (B) with and linear regression trend-line, which were settled and reared in a laboratory from silicone coatings with a range of modulus. The point with a modulus of 0.96 MPa has a higher surface energy to the remaining four. Number (n) of barnacles = A) 37, 26, 26, 34, 2 and B) 34, 28, 24, 49, 55, respectively.

The *E. modestus* settled and grown on HSE (0.96 MPa) suffered an increased mortality rate and there was a considerable decline in the population compared to the remaining silicones. The number of individuals used to calculate the mean CRS of *E. modestus* on this coating was 2, this CRS value was much lower than what was expected. This mortality and population decline was not seen for *B. amphitrite*. The mean CRS of *B. amphitrite* removed from the coating HSE, did increase from the other

four silicone coatings, supporting the hypothesis that the CRS increases with increasing coating modulus (Figure 5.1B). However, the CRS value of the HSE coating is higher than that which would be predicted by projecting the linear trend-line to 0.96 MPa. The surface energy of HSE is also higher than the remaining four silicone coatings. Considering the combination of a higher surface energy and a higher modulus, the CRS measurement of HSE would be expected to be much higher than linear trend-line would predict.

Table 5.7. Linear regression results of the critical removal stress of *Elminius modestus* (A) and *Balanus amphitrite* (B) against the elastic modulus of silicone coatings (modulus range 0.31 to 0.66 MPa).

A	***Coefficient***	***Standard Error***	***Standardized coefficient***	***t***	***P***
Intercept	**0.027**	**0.007**	**0**	**3.663**	**< 0.001**
Slope	**0.047**	**0.014**	**0.301**	**3.285**	**0.001**
Correlation coefficient	**(r) = 0.301 (r^2) = 0.091**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**0.006**	**10.794**		**0.001**
Residual	**108**	**0.001**			

B	***Coefficient***	***Standard Error***	***Standardized coefficient***	***t***	***P***
Intercept	**0.024**	**0.010**	**0**	**2.534**	**0.013**
Slope	**0.062**	**0.019**	**0.299**	**3.315**	**0.001**
Correlation coefficient	**(r) = 0.299 (r^2) = 0.089**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**0.012**	**10.991**		**0.001**
Residual	**112**	**0.001**			

Table 5.8. Exponential regression results of the critical removal stress of *Elminius modestus* against the elastic modulus of silicone coatings (modulus range 0.31 to 0.66 MPa).

	Coefficient	*Standard Error*	*Standardized coefficient*	*t*	*P*
Intercept	**0.021**	**0.004**	**0**	**4.941**	**≤ 0.001**
Slope	**1.445**	**0.403**	**0.326**	**3.587**	**0.001**
Correlation coefficient	**(r) = 0.326 (r^2) = 0.106**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**5.718**	**12.869**		**0.001**
Residual	**108**	**0.444**			

5.4.2. Fluoropolymers

The combined data for silicone and fluoropolymers was transformed using square root function after which the data were normally distributed (*df* = 267, *D* = 0.168, *P* = 0.065) with equal variance (*df1* = 6, *df2* = 260, *F* = 2.675, *P* = 0.090). Figure 5.2 shows a positive relationship between the CRS and modulus of *E. modestus* and *B. amphitrite* barnacles for the silicone and fluoropolymers coatings. In Figure 5.2B the data points for the coatings mD10H (modulus 7.71 MPa) and mD10 (modulus 19.73 MPa) are not present, this was due to failure in the shell upon removal. For 100% of the *B. amphitrite* tested (number (n) of barnacles = 28 and 34 for mD10H and mD10, respectively) the shell failed and greater than ~20% of the basal plate remained on the surface of the coatings, thus the removal stress values were void.

The *t*, *F* and *P*-values from the linear regression analysis when combining the silicone and fluoropolymers (Table 5.9) suggests that the modulus does influence the removal stress for *E. modestus* and that the null hypothesis that the slope equals zero and that there is no linear relationship, can be rejected. However, the R^2 value for *E. modestus* (0.456) is again low. This R^2 value is higher than that from the linear regression analysis of silicones alone, yet the regression is still not a good linear model for the data and suggests that only 45.6% of the variance in the CRS can be explained by the modulus. The logarithmic ($P \leq 0.001$, $R^2 = 0.645$) and power ($P \leq 0.001$, $R^2 = 0.649$) regression analyses provide significant *P*-values with higher R^2 values than the linear regression, with the power regression having the highest R^2 value (Table 5.10)

(see Appendix 4 for logarithmic and exponential regression results). With the power regression, 64.9% of the variance in the removal stress can be explained by the modulus of the coating. Therefore the CRS value for *E. modestus* on the silicone and fluoropolymer coatings can be predicted from the modulus of the coating by the power formula CRS = 0.060 x ($\chi^{0.460}$).

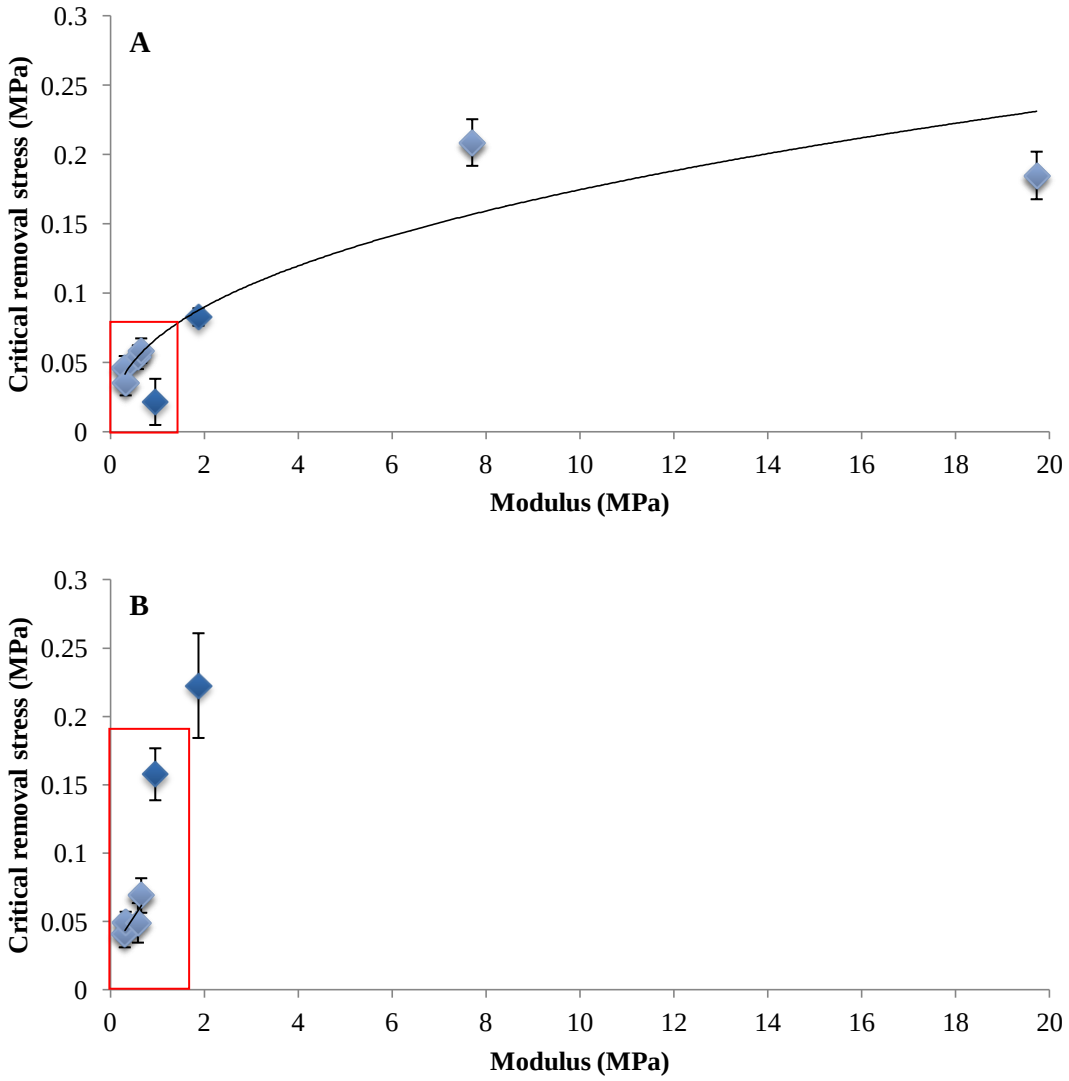

Figure 5.2. The mean critical removal stress (± 95% confidence intervals) of *Elminius modestus* (A) with a power regression trend-line and *Balanus amphitrite* (B) with a linear regression trend-line, reared under laboratory conditions from silicone and fluoropolymer coatings with a range of modulus. The points within the red square are the silicone coatings from Figure 5.1. The points with a modulus 0.96 and 1.88 MPa have a higher surface energy. Number (n) of barnacles = A) 37, 26, 26, 34, 2, 44, 58, 56 and B) 34, 28, 24, 49, 55 and 28 respectively.

Table 5.9. Linear regression results of the critical removal stress of *Elminius modestus* against the modulus of the silicone and fluoropolymers coatings (modulus range 0.31 to 19.73 MPa).

	Coefficient	*Standard Error*	*Standardized coefficient*	*t*	*P*
Intercept	**0.070**	**0.001**	**0**	**14.916**	**< 0.001**
Slope	**0.008**	**0.005**	**0.675**	**14.896**	**< 0.001**
Correlation coefficient	**(r) = 0.338 (r^2) = 0.114**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**0.795**	**221.887**		**< 0.001**
Residual	**265**	**0.004**			

Table 5.10. Power regression results of the critical removal stress of *Elminius modestus* against the modulus of the silicone and fluoropolymers coatings (modulus range 0.31 to 19.73 MPa).

	Coefficient	*Standard Error*	*Standardized coefficient*	*t*	*P*
Intercept	**0.060**	**0.002**	**0**	**29.117**	**< 0.001**
Slope	**0.460**	**0.021**	**0.806**	**22.128**	**< 0.001**
Correlation coefficient	**(r) = 0.806 (r^2) = 0.649**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**124.327**	**489.629**		**< 0.001**
Residual	**265**	**0.254**			

5.4.3. Influence of surface energy and elastic modulus

The CRS was plotted against the square root of the surface energy (γ) and elastic modulus (E) for all coatings ($(E\gamma)^{1/2}$) (Figure 5.3). This shows a positive relationship between the CRS of the barnacles *E. modestus* and *B. amphitrite* and the $(E\gamma)^{1/2}$ of the coatings. The *t*, *F* and *P*-values from the linear regression analysis of $(E\gamma)^{1/2}$ (Table 5.11) shows that the surface energy and elastic modulus together have an impact on the CRS of both *E. modestus* and *B. amphitrite* and that the null hypothesis can be rejected. The R^2 value for *E. modestus* (0.570) and *B. amphitrite* (0.375) was much greater than the R^2 values from linear regressions of silicones, and silicones and fluoropolymers (see Table 5.7 and 5.9), suggesting an improved linear model for the

data. Where a percentage of 57% and 37.5% (*E. modestus* and *B. amphitrite*, respectively) can explain the variance in the CRS due to the $(E\gamma)^{1/2}$. The logarithmic (*E. modestus* $P \leq 0.001$, $R^2 = 0.643$; *B. amphitrite* $P \leq 0.001$, $R^2 = 0.419$) and power (*E. modestus* $P \leq 0.001$, $R^2 = 0.609$; *B. amphitrite* $P \leq 0.001$, $R^2 = 0.389$) regressions improve the model and provides higher R^2 values with significant *P*-values, with the logarithmic regression providing the best fit model for both barnacle species. Using the logarithmic model, 64.3% and 41.9% (*E. modestus* and *B. amphitrite*, respectively) of the variance in the CRS can be explained by $(E\gamma)^{1/2}$. The CRS value can be predicted from $(E\gamma)^{1/2}$ of the coatings by the logarithmic formula CRS = 0.180 + (0.013)$ln\chi$ for *E. modestus* and CRS = 0.122 + (0.034)$ln\chi$ for *B. amphitrite*, where χ equal $(E\gamma)^{1/2}$ and *ln* means natural log.

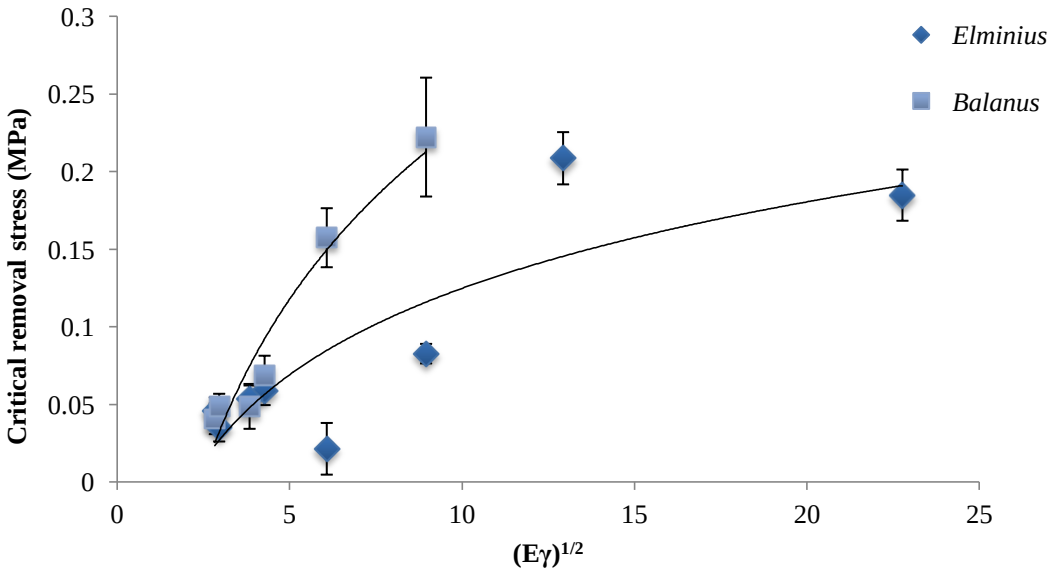

Figure 5.3. The mean critical removal stress (± 95% confidence interval) of *Elminius modestus* and *Balanus amphitrite* against the square root of the surface energy and elastic modulus ($(E\gamma)^{1/2}$) of the silicones and fluoropolymers, with logarithmic regression trend-lines.

Table 5.11. Linear regression results of the critical removal stress of *Elminius modestus* (A) and *Balanus amphitrite* (B) against the square root of the surface energy and elastic modulus ($(E\gamma)^{1/2}$) of the silicones and fluoropolymers.

A	***Coefficient***	***Standard Error***	***Standardized coefficient***	***t***	***P***
Intercept	**0.184**	**0.008**	**0**	**21.873**	**< 0.001**
Slope	**0.013**	**0.001**	**0.755**	**18.821**	**< 0.001**
Correlation coefficient	**(r) = 0.755 (r^2) = 0.570**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**2.175**	**354.213**		**< 0.001**
Residual	**267**	**0.006**			

B	***Coefficient***	***Standard Error***	***Standardized coefficient***	***t***	***P***
Intercept	**0.122**	**0.018**	**0**	**6.682**	**< 0.001**
Slope	**0.034**	**0.003**	**0.6112**	**10.839**	**< 0.001**
Correlation coefficient	**(r) = 0.612 (r^2) = 0.375**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**1.271**	**117.483**		**< 0.001**
Residual	**196**	**0.011**			

Table 5.12. Logarithmic regression results of the critical removal stress of *Elminius modestus* (A) and *Balanus amphitrite* (B) against the square root of the surface energy and elastic modulus ($(E\gamma)^{1/2}$) of the silicones and fluoropolymers.

A	***Coefficient***	***Standard Error***	***Standardized coefficient***	***t***	***P***
Intercept	**0.047**	**0.013**	**0**	**3.682**	**< 0.001**
Slope	**0.131**	**0.006**	**0.802**	**21.935**	**< 0.001**
Correlation coefficient	**(r) = 0.803 (r^2) = 0.645**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**2.453**	**481.125**		**< 0.001**
Residual	**267**	**0.005**			

B	***Coefficient***	***Standard Error***	***Standardized coefficient***	***t***	***P***
Intercept	**-0.004**	**0.027**	**0**	**-0.137**	**< 0.001**
Slope	**0.193**	**0.016**	**0.647**	**11.883**	**< 0.001**
Correlation coefficient	**(r) = 0.612 (r^2) = 0.375**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**1.420**	**141.196**		**< 0.001**
Residual	**196**	**0.010**			

5.4.4. A comparison of the critical removal stress between Elminius modestus *and* Balanus amphitrite

The CRS of *E. modestus* and *B. amphitrite* were different significantly for three of the six coatings compared, these were LSE (df = 1, F = 5.001, P = 0.030), HSE (df = 1, F = 7.417, P = 0.009) and mE10H (df = 1, F = 112.800, P < 0.001). The CRS of *B. amphitrite* for these three coatings was higher than that for *E. modestus.* For the remaining three coatings LMod (df = 1, F = 0.715, P = 0.401), MMod (df = 1, F = 0.374, P = 0.545) and HMod (df = 1, F = 0.792, P = 0.777), there was no difference in the CRS between the two barnacle species. No comparisons were possible for mD10 and mD10H as the basal plates of the *B. amphitrite* tested remained attached to the coatings. For *B. amphitrite* removed from mE10H, 25% of individuals had basal plates remaining on the surface. With the silicone coatings 100% of *B. amphitrite* were cleanly removed with no basal plates remaining on the surface, this was the same for *E. modestus* from all the coatings. With regard to the tearing of the membranous-basal plate, for the silicone there was ≤10% incidence of this occurring, for the fluoropolymers there was 20% incidence of the membrane tearing.

5.5. Discussion

The aim of this chapter was to examine the critical removal stress of *E. modestus* from silicone and fluoropolymer coatings with different bulk properties. This was to measure how the coating's properties influenced the adhesion of this membranous-based barnacle compared to the calcareous-based *B. amphitrite*. The

hypothesis of the study was that increasing the elastic modulus of the coatings (silicones and fluoropolymers) increased the critical removal stress of *E. modestus*, which was established. However, the regression models of the CRS against the modulus of the silicone coatings and of the silicone and fluoropolymer coatings did not show good linear relationships. Instead an exponential model (silicone coatings) and a power model (silicone and fluoropolymer coatings) provided better explanations for the variance in the CRS than the simple linear models for *E. modestus*.

When the surface energy of the coatings were included in the analyses using the square root of the surface energy and modulus ($(E\gamma)^{1/2}$) against the CRS of *E. modestus* and *B. amphitrite* there was a much stronger linear relationship. Although, logarithmic regression analyses for both barnacle species, provided models with higher R^2 values than the linear models.

Comparing the removal stress of *E. modestus* to *B. amphitrite* was only possible for six out of the eight coatings. For the two coatings with the highest modulus (mD10H and mD10), there was basal failure for the *B. amphitrite* removed from this coating and it was thus unable to provide a measure of CRS. For three of the six coatings compared, the CRS of *E. modestus* was significantly less than that of *B. amphitrite*.

5.5.1. Influence of modulus on the critical removal stress of Elminius modestus

The elastic modulus is an important factor influencing the force required for the detachment of fouling organisms such as barnacles from FR coatings (Berglin et al. 2003; Sun et al. 2004; Kim et al. 2007). *E. modestus* and *B. amphitrite* barnacles removed from coatings with a higher modulus were shown to have a greater CRS than coatings with a lower modulus. However, the low R^2 value from the regression analysis indicates that this was not a strong linear association for either species. An exponential model was able to improve the relationship between modulus and CRS for *E. modestus* and provide a higher R^2 value, but it was still a low value of 0.106. None of the alternative regression analyses tested improved on the model for *B. amphitrite*. The modulus range of the four silicones with equal surface energy (LMod, MMod, HMod and LSE) was from 0.31 to 0.66 MPa. Compared to Chaudhury et al. (2005) who used a modulus range from 0.2 to 9.2 MPa and to Kim et al. (2007; 2008) that

used a range from 0.08 to 1.3 MPa, by comparison the modulus range of the silicones used in this study was small.

The fluoropolymers mD10 and mD10H had a similar surface energy to the four silicones, and increased the modulus range of the available test coatings to 19 MPa. The regression with the extended range in modulus when the fluoropolymers were included had a much stronger linear association between the modulus and the removal stress than that with the silicones alone. A power regression considerably improved the R^2 value, from 0.114 for the linear model to 0.649 for the power model. However, fluoropolymers are a different class of polymer to silicones, their different chemical structures results in them achieving effective fouling-release through different mechanisms (Brady 1999; 2001). The differences discussed most often relates to the difference in the surface energy and modulus between the two polymers. Fluoropolymers are said to limit the bonding of adhesives to them by their arrangement of the functional groups, which are closely packed and cross-linked together along the surface. As a result this minimises re-arrangement within the polymer and reduces infiltration of marine adhesives. Therefore, only a weak interface with an adhesive forms, resulting in the coating having a low surface energy, lower than that found for silicones (Brady 2001; Yebra et al. 2006). However, because of the presence of the fluorine atom in fluoropolymers, there is limited rotation of the C – C backbone of the polymer when compared to the rotation of the Si – O backbone of silicones. This means the fluoropolymers have limited flexibility and a much higher elastic modulus than silicones (Brady 2001; Yebra et al. 2006). In summary, the difference is that fluoropolymers generally have a lower surface energy and prevent settlement and adhesion, whereas silicones have a lower modulus and an improved release of adhesives. Although in this study the fluoropolymers mD10 and mD10H had a similar surface energy to that of the silicones (HMod, MMod, LMod and LSE) such that the difference may have only been the modulus. However, additional properties such as the glass transition and molecular porosity of fluoropolymers differ from silicones. What these factors contribute to the removal of adhesive in this instance is unknown. Therefore caution must be taken when interpreting the results presented, these additional properties may have added to the increased removal stress of the barnacles from the fluoropolymers other than modulus.

The surface and bulk properties of FR coatings when considered independently influences the adhesion and removal stress of fouling organisms. However when each physical property is considered in combination with the others there can be a synergistic effect on the removal stress (Brady & Singer 2000; Chaudhury et al. 2005; Kim et al 2007). For example the removal stress correlates better to the product of surface energy and elastic modulus ($(E\gamma)^{1/2}$) than to either the surface energy (γ) or modulus (E) alone (Brady 2000; Brady & Singer 2000; Anderson et al. 2003). The relationship between the CRS and $(E\gamma)^{1/2}$ in this study shows a much stronger linear association than that between CRS and modulus for both *E. modestus* and *B. amphitrite*. However, the linear relationship for *E. modestus* was stronger than that for *B. amphitrite*. The use of an alternative regression model again improves on the R^2 values for both species, this time a logarithmic regression model provided the best fit. The R^2 value for *E. modestus* (0.643) again was higher than the R^2 value for *B. amphitrite* (0.419) in the logarithmic model. In addition, the angle of the regression line for *B. amphitrite* is steeper and quite different than the regression line for *E. modestus*. As the value of $(E\gamma)^{1/2}$ increases the difference between the slopes and therefore the CRS between the two species increases.

5.5.2. A comparison of the critical removal stress of Elminius modestus *and* Balanus amphitrite

The CRS for the membranous-based *E. modestus* was lower than that of *B. amphitrite* for three of the six comparisons. The coatings where the CRS was similar between the species, included the coatings where Rhodorsil was the base polymer. The polydimethylsiloxane (PDMS) coating LSE, the Polyethyl-silicone co-polymer coating HSE, and the fluoropolymer mE10H were the coatings that presented a difference between species. For the coating HSE which showed a lower CRS for *E. modestus*, this difference should be viewed with caution as the sample size of *E. modestus* was two. This sample size is much lower than recommended for being able to discern a difference in CRS (Swain et al. 2000; Conlan et al. 2008). The reason there was a small sample size was that there were only two remaining barnacles larger than 4.1mm in diameter on the coating after the growth period. There was an increase in mortality of *E. modestus* on this coating compared to the remaining coatings. Although this was not the case for *B. amphitrite*. The HSE coating consists of polyethyl-silicone co-

polymer and included a moisture scavenger (to eliminate water) and a solvent that was different from the solvent used for the remaining silicones. It could be that one of the components of this coating was toxic. *E. modestus* may have been more susceptible to this potentially toxic element because its membranous basal plate did not offer the barrier a calcified basal plate would have, thus allowing the toxic element to pass through the membrane into the body of the barnacle. As for the remaining seven coatings, the level of mortality did not reduce the population of barnacles on the slides excessively; there were still enough barnacles for the adhesion tests after the growth period. Any toxicity in these remaining coatings was negligible. The coating HSE, was used as described in Chapter 4 for the field immersion trials with no apparently toxic influences. The volume and flow of water around the coatings in the field may have reduced and/or removed the toxic element of this coating. Increasing the time the coatings were leached for in the laboratory prior to settlement and growing the barnacles in an aerated volume of water could potentially reduce the influence of the toxicity of this coating to the laboratory culture of *E. modestus* in future studies.

The CRS value for the fluoropolymer mE10H of *B. amphitrite* was over twice the value for *E. modestus*. It was not possible to compare the removal stress of the fluoropolymers mD10 and mD10H between species because 100% of the *B. amphitrite* removed suffered shell failure. These fluoropolymers had the highest modulus values. The adhesion of *B. amphitrite* to these coatings was stronger than the integrity of the shell, hence the shell broke and left the calcified basal plate attached to the surface of the coatings before the adhesive failed. During the removal of *E. modestus* from the fluoropolymers and even the silicones, there were no instances of the shell breaking or the basal membrane remaining attached to the surface. The deformation of the shell offered *E. modestus* by the flexibility of it basal membrane better aids removal from coatings with a higher modulus than *B. amphitrite*. Although, as noted in Chapter 3, the membranous-basal plate tore during detachment from the silicone, the incidence of this occurring during the removals in this chapter was less than that which was previously noted, although not every barnacle was inspected. This could be a result of the difference in automated and manual equipment used. With the automated method the speed and angle of applied force is constant, with the manual method there are often difficulties controlling this (Kavanagh et al. 2005; Conlan et al. 2008) and this may have resulted in the increased occurrence of basal tearing noted in Chapter 3.

5.6. Conclusion

The aim of this chapter was to determine how the critical removal stress of *E. modestus* was influenced by the bulk properties of silicone and fluoropolymer coatings in order to conclude whether *E. modestus* was capable of discerning between coatings for FR evaluations. This study demonstrated that increasing the elastic modulus of a coating increased the removal stress of the membranous-based barnacle *E. modestus.* The extent of this increase is consistent with that of *B. amphitrite* for the silicone coatings. Therefore, for the silicones *E. modestus* was capable of discriminating between the silicone test coatings to a similar degree as the model species *B. amphitrite.* However for the fluoropolymers which had the highest values of modulus, it was not possible to compare the influence of the modulus on CRS. This was due to *B. amphitrite* barnacles being so firmly attached to the fluoropolymers with the highest modulus, that the shells failed and the basal plates remained fixed to the surface. To evaluate coatings FR performances the removal stress is a necessary measurement. Although when the basal plate fails and remains on the surface it does provide an insight into the FR properties of that coating, however comparisons between coatings cannot be made. As *E. modestus* was removed from the harder coatings and provided CRS values, a comparison between every coating was possible. Therefore *E. modestus* may be better suited for evaluating FR coatings with a higher range in modulus. *E. modestus* are a suitable test species for future FR research especially if they were used in parallel with *B. amphitrite,* and therefore would provide a more robust evaluation of the coatings FR performance.

Chapter 6: Discussion and Conclusions

6.1. Aims and objectives of the thesis

The main aim of this thesis was to investigate the potential use of the barnacle *Elminius modestus* for evaluating the performance of fouling-release (FR) coatings. A secondary aim was to determine how the membranous-basis of this species affects its fracture mechanics and release from FR coatings, in comparison to barnacles with a calcareous-basis.

The first objective was to ascertain whether *E. modestus* was suitable as a laboratory test species focusing on their settlement, growth and adhesion to two PDMS coatings. For a test species to be suitable essentially it should be an important fouling organism and have a wide geographic distribution. *E. modestus* is an invasive species that has become well established on British and north-west European shorelines (Crisp 1958, Barnes & Barnes 1963). The arrival of *E. modestus* in Britain in the 1940s was attributed to remote dispersal via fouling on the hulls of ships (Bishop 1947) and its distribution along the British and European coasts continues to spread through marginal dispersal (Crisp 1958). Where *E. modestus* is present, it is often the dominant species, especially on new surfaces, created through disturbance and removal of previously settled organisms (Gallagher et al. 2015; 2016) or on artificial structures (Bracewell et al. 2012; 2013). There are, however, increasing concerns that the continuing northerly dispersal is being promoted due to climate change and rising sea temperatures (Witte et al. 2010). As the invasion of Europe has been so successful, the future of other coastlines including Japan (Otani et al. 2007) and the Atlantic coast of North America (Carlton et al. 2011) are considered to be at risk. Therefore *E. modestus* is an important fouling species (Moyse 1960; Buckeridge 1982; Southward 2008) with an expanding distribution.

For a test species to be suitable for *laboratory* trials and to evaluate FR coatings, additional criteria that would be ideal, include:

1) having a short larval development period (Rittschof et al. 1992);

2) being able to provide synchronous mass releases (which would be dependent on the number of adults) to provide adequate numbers of cyprids for multiple samples (Rittschof et al. 1992);
3) being iteroparous and capable of reproducing multiple broods, continuously throughout the year under controlled laboratory conditions (Moyse 1960; Kirby 2006);
4) having larvae capable of settling in static laboratory conditions (Branscomb & Rittschof 1984; Rittschof et al. 1992);
5) providing reproducible data, i.e. reproducible settlement on standard surfaces, such as glass and polystyrene, and reproducible measures of removal stress on standard coatings, such as Silastic T-2 (Evariste et al. 2012);
6) and having a relatively rapid growth rate to reach a size suitable for critical removal stress (CRS) tests (Conlan et al. 2008).

E. modestus has previously been shown to have a short larval development time of 7 days from nauplius stage I to the cyprid stage (Kirby 2006). In addition, *E. modestus* is capable of reproducing throughout the year, synchronously releasing mass numbers of nauplii (Moyse 1960; Tighe-Ford et al. 1970; Billinghurst et al. 2001; Kirby 2006). Within this study the adult brood stock, maintained across two aquarium tanks each containing three polypropylene pipes (3 x 300mm long, 3 x 350mm long, all 40mm in diameter) completely covered in *E. modestus*, was capable of producing approximately an average of 15,000 to 20,000 nauplii every two weeks. Using the method adapted by Kirby (2006) it was possible to produce multiple cultures of viable cyprids which were competent to settle on a standard surface widely used in antifouling studies – polystyrene (Iwaki 24-well plates). There was inter-batch variation in the settlement of *E. modestus* and *B. amphitrite* cyprids, with culture 3 in the comparison between FSW and ASW (section 2.4.1) and culture 1 comparing the two barnacle species (section 2.4.2) presenting very low settlement with less than 20% settled after 48 hours. Omitting these two cultures, the remaining settlement after 48 hours ranged from 25% to 60%. This does appear to be relatively low; however this level of settlement is consistent with the control settlement in previous studies (Branscomb & Rittschof 1984; Clare et al. 1990; Rittschof et al. 1992; Billinghurst et al. 1998; 2001).

B. amphitrite is a species that is used more than any other for evaluating FR coatings in the laboratory. In this study it was used as a standard to directly compare and gauge the performance of *E. modestus*. The percentage settlement of *E. modestus* on the silicone coatings performed to an equal standard as that of *B. amphitrite*, however the percentage settlement on polystyrene surfaces did not, with *E. modestus* having fewer settled cyprids for some of the repeat cultures. This indicates that *E. modestus* does have potential as a test species in settlement assays especially with regard to silicone coating evaluations.

The growth rate of *E. modestus* on the polydimethylsiloxane (PDMS) test coatings, Silastic T-2 and Sylgard 184, when fed a diet of *Tetraselmis suecica*, was faster than the growth rate of *B. amphitrite* when fed the same diet. Although, this is a slower rate of growth than published accounts for *B. amphitrite* in the laboratory (Wendt et al. 2006; Conlan et al. 2008). When fed on a diet of *Artemia* sp. (brine shrimp), *B. amphitrite* can reach a size of 5mm in diameter in 12 weeks in the laboratory. When *E. modestus* were fed *Artemia* sp. in this study there was high mortality. However, this might be resolved by maintaining the cultures of *E. modestus* in an increased volume of aerated water or in flowing water. Further studies to investigate growth using these methods would be beneficial to determine whether the growth rate could be improved. Nevertheless, it was possible to grow *E. modestus* to an average of 4.1mm in diameter of the basis, the minimum size recommended for CRS measurements, in approximately ten weeks. *B. amphitrite* attained an average size of 3.6mm in diameter, the minimum recommended size for this species, in a similar time-frame (Conlan et al. 2008). In Chapter 2 it was concluded that *E. modestus* could be a model test species and be used in laboratory trials to evaluate FR coatings.

There were differences in the CRS between the two species when removed from the PDMS coatings as described in Chapter 2. The second objective of this thesis was to determine to what extent the difference in CRS could be accounted for by differences in the structure of the basis – calcareous for *B. amphitrite* and membranous for *E. modestus* (Chapter 3). The flexibility of the basis was predicted to be important with regard to the fracture mechanics of the release of a barnacle from an elastomeric coating, as a more flexible basis would require less energy for removal (Chung & Chaudhury 2005). With the use of a high-speed camera, positioned underneath the barnacle, it was possible to view the separation process of the two species from the

PDMS coatings. The membranous-basis of *E. modestus* was important in the mechanism of release as it hindered the appearance of fingering instabilities as they propagated across the basis. However, the differences in the patterns of separation, particularly the location of the initial fracture in relation to the direction of force, may have more to do with the differences in the shape and the structure of the shell than the type of basis. Nevertheless, the influences of wetness and coating type on the removal times and the CRS was evident for *E. modestus*, in most cases, but less so for *B. amphitrite*. Suggesting that *E. modestus* is potentially more sensitive to variations in environmental variations such as wetness and substrate type.

Chapter 4 compared the CRS of *E. modestus* grown in the laboratory to those grown at static immersion sites in the field. This was to validate whether laboratory assays are capable of discriminating between coatings in terms of settlement and adhesion, and provide results equivalent to results from the field environment. The present study found differences in the CRS values between laboratory-reared barnacles and those grown in the field across eight test coatings. However, there were also differences in the CRS between barnacles from the two field sites and from the different immersion periods. Nevertheless the rankings of the eight test coatings for *E. modestus* in the laboratory, for example the fluoropolymers having the highest percentage settlement and CRS and the silicone coating S2 having the lowest settlement and CRS, were comparable to the rankings of the coatings obtained in field tests.

The influences of biofilm and temperature on the CRS of *E. modestus* were examined (Chapter 4) to provide a possible explanation for the differences between the field and laboratory results. Biofilms have previously been shown to increase the adhesion of cyprids (Neal & Yule 1994a; Zardus et al. 2008). However, the presence of a 10-day-old biofilm did not influence the CRS of adult barnacles in this study. Temperature has also been shown to influence the rate of growth of *B. amphitrite* and this is inversely correlated to the CRS (Johnston 2010). However, no such relationship was established in this study.

Finally, in Chapter 5, the effect of increasing the elastic modulus of silicone and fluoropolymer coatings on the CRS of *E. modestus* was investigated and compared to the CRS of *B. amphitrite*. Although the range of moduli investigated for silicone

coatings was narrow, there was a positive relationship between the CRS of both species and the modulus; the CRS increased as the modulus increased. However the regression model of the CRS against the modulus of the silicone coatings and of the silicone and fluoropolymer coatings did not show a good linear relationship. Instead an exponential model (silicone coatings) and a power model (silicone and fluoropolymer coatings) provided a better explanation of the variance in the CRS than the simple linear models for *E. modestus*. Although, when the surface energy of the coatings were included in the analyses using the square root of the surface energy and modulus ($(E\gamma)^{1/2}$) against the CRS of *E. modestus* and *B. amphitrite* there was a much stronger linear relationship. However, logarithmic regression analyses for both barnacle species provided models with higher R^2 values than the linear models for $(E\gamma)^{1/2}$ against the CRS.

In the comparison of CRS between the two species, there was only a difference in the removal stress for two out of the five silicone coatings. However, there were differences between the CRS of the two species when removed from the fluoropolymer coatings. All *B. amphitrite* removed from the two fluoropolymers with the highest modulus values (mD10 and mD10H) exhibited failure of the calcareous-basis; where a portion of the basis (greater than 20%) was left on the surface of the coatings. In contrast, when *E. modestus* were removed from the same coatings there was no remnant of the basis on the coatings. Failure of the basis in *B. amphitrite* suggests poor FR performance of the coatings and makes it difficult to compare the coatings. This was the advantage of using *E. modestus* over *B. amphitrite* as performance of every coating could be compared against each other. Using *E. modestus* in addition to *B. amphitrite* in future studies would provide more detailed information regarding the overall performance of a coating.

6.2. Limitations of the study

E. modestus was used in this study as it was assumed that the fracture mechanics and release properties of this species would be characteristic of all membranous-based species. However, results presented in Chapter 3 suggested that the shape and structure of the shell are important to the aforementioned properties. It was the intention to use *Semibalanus balanoides* as a second example of a barnacle with a

membranous-basis, although the objective was to examine how this species responded to the bulk properties of the silicone and fluoropolymer coatings, not examining the detachment process of this species. Nevertheless due to poor post-recruitment survival contributing to a low number of replicates, it was not possible to examine the bulk properties of the coatings with this species nor were there sufficient numbers on the coatings to be used to examine the detachment process. S. *balanoides* is a barnacle with six parietal plates joined together with mitred sutures; the same number and suture type as *B. amphitrite*. However the shell of *S. balanoides* is non-porous and its basis is membranous, like the shell and basis of *E. modestus*. Capturing the detachment process of *S. balanoides* using the high-speed video would have provided additional information on how the structure of the shell and basis influences the fracture process. Using an increased number of coated microscope slides, and therefore an increased surface area for colonisation of barnacles within the field test sites could have provided a greater number of *S. balanoides* and may have prevented the issue of having a small sample size. However, there was a limited amount of space at the immersion sites and therefore only a limited number of panels and slides could be immersed at one time.

The number of coatings used for the field immersion and laboratory trials (Chapter 4) was sufficient to examine the differences between the two environments. However, the research in Chapters 3 and 5 may have been improved by including extra coatings. Two coatings, Silastic T-2 and Sylgard 184 were selected to examine the fracture processes of *E. modestus* and *B. amphitrite* during detachment (Chapter 3). These PDMS coatings are frequently used as a standard to assess the CRS of barnacles (Sun et al. 2004; Kavanagh et al. 2005; Wendt et al. 2006; Ramsay et al. 2008; Larsson et al. 2010). However, the type of coating did not appear to influence the propagation of the fracture or the time for removal, and there was no difference in the CRS of the barnacles between the two coatings. Selecting coatings that are more dissimilar in their bulk and surface properties and result in a difference in the CRS, for example mE10H and LSE (Chapter 5) would provide a more thorough illustration of the fracture processes and the relationship to the type of coating.

The range of moduli of the coatings described in Chapter 5, especially the silicone coatings, was relatively narrow when compared to published works (Chaudhury et al. 2005; Kim et al. 2007; 2008). This limitation may explain the poor linear regression model between modulus and CRS for both *E. modestus* and *B.*

amphitrite. It was unfortunate that the fifth silicone coating (HSE), which improved the modulus range could not be included in the regression analysis as it had a different surface energy.

The influence of surface energy was not thoroughly investigated in this study. It would have been instructive to have a series of coatings which had a constant modulus and a range in different surface energies. This was attempted but was not achieved, as the variation in the modulus of this second coating series was too large and they were not incorporated into this study. When the initial eight coatings described in Chapters 4 and 5 were made, time was a limiting factor. All eight coatings were prepared at the same time. A large number of slides were needed for each coating to provide sufficient numbers for the two field sites, over the two years including the two seasons at Burnham-on-Crouch and laboratory assays of *E. modestus* and *B. amphitrite*.

6.3. Future avenues of research

Nothing is known about the nature of the adhesive of *E. modestus*, how it compares to that of *B. amphitrite*, and if there are differences, and whether these differences are important to the release process (Chapter 3). The research into barnacle adhesive has mostly been investigated using calcareous-based barnacles such as *B. amphitrite* and *Megabalanus rosa*. Calcareous-based barnacle adhesive has been shown to be composed of at least ten different cement proteins (CP), six have been characterised, five of which are unique to barnacles in respect of their primary structure (Kamino 2008; 2013). These six cement proteins have been categorised into four groups 1) six amino-acid biased proteins (CP-68K and CP-19K), 2) a charged amino acid rich protein (CP-20K), 3) a hydrophobic protein (CP-100K and CP-52K) and 4) an enzyme (CP-16K) (Kamino 2008; 2013). However, a recent study did investigate the adhesive of another membranous-based barnacle *Tetraclia japonica formosana* (Lin et al. 2014), in which, *T.j. formosana* was discovered to be lacking the charged amino acid cement protein, CP-20K. An investigation to determine whether this cement protein is present or absent in *E. modestus* would be beneficial and may help to better understand the differences in the adhesion between *E. modestus* and *B. amphitrite* noted in this study.

When barnacles with a calcareous-basis are grown on low modulus, low surface energy coatings in response they produce a thick rubbery or 'gummy' multilayered adhesive as well as a concave basis (Berglin & Gatenholm 2003; Sun et al. 2004; Ramsay et al. 2008). This rubbery multilayered adhesive has different mechanical properties and chemical content to the adhesive produced by barnacles grown on coatings with a higher modulus (Berglin & Gatenholm 2003). Wiegemann & Watermann (2004) commented that *E. modestus*, when grown on PDMS, produces adhesive that is less thick and hydrated than that produced by *Balanus* spp. on the same coatings. However, as stated above there have been no further studies on the adhesive of *E. modestus* or other membranous-based barnacles when grown on FR coatings.

Examples of some of the techniques employed to analyse the structure and components of the cement of *B. amphitrite* when grown on FR coatings include atomic force microscopy (AFM) and scanning electron microscopy (SEM) to image the structure of the adhesive, and X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy to understand the composition of the adhesive (Wiegemann & Watermann 2004; Dickinson et al. 2009; Sullan et al. 2009; Barlow et al. 2010). Some of these analytical techniques would need to be modified before they could be applied to the study of the adhesive of *E. modestus*. For example, images of the adhesive using AFM have been taken directly from the calcareous-basis of living barnacles detached from coatings (Dickinson et al. 2009; Barlow et al. 2010). This was attempted on the membranous-basis of live *E. modestus* (Appendix 5) but was unsuccessful as the vibrations caused by the tapping of the cantilever were sufficient to vibrate the basal membrane and prevent an image of the basis being recorded. It is plausible to image the adhesive remaining on the surface of a coating or a glass cover slip, as was used by Sullan et al. (2009), although this was not attempted at this time. No viable images of the adhesive were obtained during this preliminary trial, which is why it was not included in the study, however, further development of the method is warranted. Investigations on the structure and mechanical properties of the adhesive of *E. modestus* would be beneficial to elucidate the mechanisms of the adhesion of this species in comparison to *B. amphitrite*. Furthermore, the nature of the adhesive has a pivotal role in crack propagation during removal of the barnacle from FR coatings. Examining the adhesive of *E. modestus* would clarify whether there are species-specific differences in the adhesives which influence the detachment processes.

This study focussed on the adhesion of adult *E. modestus* for their potential to evaluate FR coatings. However, *E. modestus* cyprids could also be used for coating evaluations. Through measuring percentage settlement, cyprids of *B. amphitrite* have been and will continue to be used to assess the performances of many antifouling technologies for example microtopographies (Schumacher et al. 2007; Aldred et al. 2010), enzymes (Pettitt et al. 2004; Tasso et al. 2012), natural products (Hellio et al 2004; 2005) and amphiphilic and fluorinated-siloxane technologies (Marabotti et al 2009; Wang et al. 2011; Martinelli et al. 2012). In addition to settlement assays, measurements of cyprid adhesion, and even juvenile barnacle adhesion, through tensile and hydrodynamic testing would provide rapid assessment of the antifouling and FR properties of coatings by reducing the time required to develop the barnacles to a testable size (Berglin et al. 2001; Aldred et al. 2010; Larsson et al. 2010). The adhesion of *E. modestus* cyprids has previously been investigated with regard to their tenacity to bacterial biofilms (Neal & Yule 1994a), however this concept has not been applied to FR testing and should be a topic for future studies.

A relatively new method developed for rapid evaluation of FR coatings involves the use of reattached barnacles (Rittschof et al. 2008: Stafslien et al. 2012; 2016). Barnacles that have been grown on PDMS, such as Silastic T-2, from a laboratory culture are carefully removed and reattached to a new test coating. For reattachment, the barnacles are positioned on the new surface and after 3 hrs are submerged in seawater to be cultured for up to 4 weeks before measuring the CRS following the ASTM D-5618 (1994) method (Rittschof et al. 2008). An improvement to this method used a nylon mesh immobilisation template to hold the barnacles in place to aid reattachment (Stafslien et al. 2012). The reattachment method has been shown to be an effective tool for evaluating FR coatings and has benefits over field immersion trials as it dramatically reduces the time to screen coatings, which is a consideration for industry. Currently only *B. amphitrite* has been used for reattachment. Whether this could be used for the membranous-based *E. modestus* would need investigating. However, there is the issue of the fragile basis of *E. modestus* tearing during detachment, which could suggest it may not be suitable for this method.

Currently the number of organisms used for evaluating antifouling and FR surfaces is limited. The barnacle *B. amphitrite* is one of the most universally used species for these types of evaluations (Aldred & Clare 2008) and the results from this

study suggest that the barnacle *E. modestus* has potential for coating evaluations. However, are these species representative of other barnacle species? From the present study the subtle differences in the shape and structure of the shells of the two barnacles were enough to cause a difference in the release properties. Therefore *B. amphitrite* or *E. modestus* may not be representative of a barnacle with a considerably different shape and structure, for example *Verruca stroemia*. *V. stroemia* is a barnacle from the suborder Verrucimorpha, it has a membranous-basis like *E. modestus* and *S. balanoides* but its four interlocking parietal plates make up an asymmetrical box-like shell (Southward 2008) which has a much weaker structure than the shells of *B. amphitrite*, *E. modestus* and *S. balanoides* (Gubbay 1980). There is also the size of the barnacle to consider; the size of the barnacle being important with regard to the adhesion strength (Berglin et al. 2001; Robson et al. 2009) and could influence the release process. The size of the laboratory grown *B. amphitrite* and *E. modestus* used in this study were similar. Yet the size of the *S. balanoides* (~10mm in diameter) from the field were twice the diameter of the *E. modestus* (~5mm in diameter) from the field at Fairlie Quay and larger specimens of *S. balanoides* with 25mm diameter have been reported (Southward 2008). The largest barnacles, in terms of the size of the barnacle, belong to the genus *Megabalanus* where individual's up to 75mm in diameter are average (Southward 2008).

Barnacles are one of the more dominant species in the fouling community on the hulls of ships (Christie & Dalley 1987; Aldred & Clare 2008; Briand 2009). However, this community is diverse and contains within it many more groups of organisms for example; ascidians, anemones, bryozoans, hydroids, mussels, and tubeworms in addition to the micro-community of bacteria, protozoan and microalgae and also macroalgae assemblages (Callow & Callow 2002; Anderson et al. 2003). Therefore, there is a great diversity of mechanisms for adhesion other than the form utilised by barnacles. A barnacle, once its cyprid form has settled and metamorphosed into a juvenile, is permanently attached directly to the substratum using a complex adhesive that is released from a system of ducts around the edge of the basis forming concentric rings of adhesive (Yule & Walker 1987; Wiegemann 2005). As mentioned above, ten proteins make up the barnacles complex adhesive, six of these have been identified and categorised into four groups: six amino-acid-based proteins, a charged amino acid-rich protein, hydrophobic proteins and an enzyme (Kamino 2006; 2008; 2013). By contrast mussels use byssal threads consisting of a collagenous inner core

surrounded by cured polyphenolic proteins to attach to the substratum (Silverman & Roberto 2007). The byssus are used to attach the post-larva to a substratum while they undergo metamorphosis to the adult form; however, some species such as *Mytilus* spp. and *Dreissena* spp. retain the byssus for attachment throughout the organisms' life (Crisp et al. 1985; Wiegemann 2005; Silverman & Roberto 2007). Mussels are capable of changing the location of their attachment, by breaking the byssal threads using their foot and producing new byssus for attachment elsewhere, although the mussels' ability to do this does decline as the animal ages as the number of threads that are produced increases (Wiegemann 2005). The byssus structure consists of the stem anchored to the root within the muscular tissue of the foot, the proximal thread, the distal thread and the adhesive plaque (Crisp et al. 1985; Wiegemann 2005; Silverman & Roberto 2007). The adhesive, which is produced from the foot of the organism, and is released via the byssal groove down to the adhesive plaque, contains nine unique proteins with high volumes of the modified amino acid 3,4-dihydroxy phenylalanine (DOPA) (Wiegemann 2005; Silverman & Roberto 2007; Kamino 2008; 2013).

The methods of adhesion of barnacles and mussels are distinctly different from one another. To be able to gauge the performance of antifouling and FR coatings and to improve the understanding of the fracture and detachment processes of fouling organisms, investigations into the adhesion and release mechanisms of a greater number of different fouling groups with a more diverse collection of body forms is needed (Kavanagh et al 2001; Holm et al. 2006). However, not all species are suitable for evaluating coatings, *S. balanoides* for example (Chapter 4) and developing standard protocols testing each fouling species would be costly in terms of time and resources. There needs to be a few select species which are representative of other phyla (Callow & Callow 2002) for assessing antifouling and FR coatings.

6.4. Concluding remarks

Any structure immersed in marine and even fresh water environments, be this commercial ships or personal pleasure yachts, oil platforms or marine renewable energy technologies (e.g. wind and wave turbines or tidal barrages), or aquaculture systems, will be colonised by biofouling. The colonisation by a biofouling community has detrimental impacts on the structures, reducing the performance and increasing the

cost of maintenance and repair. Research will continue to strive toward improvements in antifouling and fouling-release technologies, along with methods for evaluating them. These improved evaluation methods should include increasing the number of marine organisms with different adhesive strategies and body morphologies that are used to gauge the antifouling and FR properties of a coating or new technology.

This study has shown that *Elminius modestus*, as a membranous-based barnacle, is suitable as a model laboratory test species and can be used for evaluating new coating formulations. *E. modestus* was found to be more sensitive to the coating type in terms of growth (Chapter 2), removal times (Chapter 3) and CRS (Chapter 2 and 3) than *B. amphitrite*, and therefore *E. modestus* is possibly superior to *B. amphitrite* as a test species for discriminating between the performance of coatings. The different basis, shape and structure of the shell of *E. modestus* influences the release mechanics of the barnacle when compared to *B. amphitrite*. Although the laboratory assay could never replicate the complexity of the colonisation process in the natural environment, settlement and CRS measurements of laboratory-cultured barnacles correlated well with the recruitment and CRS of field-grown barnacles, such that laboratory assays were able predict the FR performance of a coating in the field. Laboratory assays indeed have their benefits over field trials. Which includes a better control over the population and prevention of overcrowding, and no environmental stresses for example cold and adverse weather reducing the level of larval availability and recruitment, and damaging the samples.

Laboratory assays are a valuable tool in FR coating research. *E. modestus* is a good candidate for a laboratory test species, when used in conjunction with *B. amphitrite*, could provide a more robust and thorough assessment of the performance of FR coatings.

References

Abarzua S, Jakubowski S. 1995. Biotechnological investigation for the prevention of biofouling. I. Biological and biochemical principles for the prevention of biofouling. *Marine Ecology Progress Series* **123**: 301 - 312.

Abràmoff MD, Magalhães PJ, Ram SJ. 2004. Image processing with ImageJ. *Biophotonics International* **11** (7): 36 - 42.

Afsar A, de Nys R, Steinberg P. 2003. The effects of foul-release coatings on the settlement and behaviour of cyprid larvae of the barnacle *Balanus amphitrite* Darwin. *Biofouling* **19**: 105 - 110.

Aldred N, Clare AS. 2008. The adhesive strategies of cyprids and development of barnacle-resistant marine coatings. *Biofouling* **24** (5): 351 - 363.

Aldred N, Phang IY, Conlan SL, Clare AS, Vancso GJ. 2008. The effect of a serine protease, Alcalase ®, on the adhesives of barnacle cyprids (*Balanus amphitrite*). *Biofouling* **24** (2): 97 - 107.

Aldred N, Scardino A, Cavaco A, de Nys R, Clare AS. 2010. Attachment strength is a key factor in the selection of surfaces by barnacle cyprids (*Balanus amphitrite*) during settlement. *Biofouling* **26** (3): 287 - 299.

Almeida E, Diamantino TC, Sousa O. 2007. Marine paints: The particular case of antifouling paints. *Progress in Organic Coatings* **59**: 2 - 20.

Alzieu C. 1998. Tributyltin: case study of a chronic contaminant in the coastal environment. *Ocean and Coastal Management* **40**: 23 - 36.

Anderson C, Atlar M, Callow ME, Candries M, Milne A, Townsin RL. 2003. The development of foul release coatings for seagoing vessels. *Proceeding of the institute of marine engineering, science and technology. Part B, Journal of Marine Design and Operations* **4**: 11 - 23.

Armstrong E, Boyd KG, Burgess JG. 2000. Prevention of marine biofouling using natural compounds from marine organisms. *Biotechnology Annual Review* **6**: 221 - 241.

ASTM D-5618. 1994. Standard test method for measurement of barnacle adhesion strength in shear. *American Standard for Testing Materials. Paint Test for formulated products and applied coatings* **06.01**.

Baier RE. 1970. Surface properties influencing biological adhesion. In Manly R (eds) *Adhesion in Biological Systems*. .. Academic Press, Michigan. pp. 15 - 48

Baier RE. 2006. Surface behaviour of biomaterials: The *theta* surface for biocompatibility. *Journal of Material Science: Materials in Medicine* **17**: 1057 - 1062.

Baier RE, Shafrin EG, Zisman WA. 1968. Adhesion: Mechanisms that assist or impede it. *Science* **162**: 1360 - 1368.

Bailly P, Grosjean P, Flammang P. 2009. Measurement of the attachment strength of brachiolaria larvae and metamorphic individuals of the sea star *Asterina gibbosa* by a centrifugation method. *Journal of Experimental Marine Biology and Ecology* **372**: 82 - 90.

Ban S, Burns C, Castel J, Chaudron Y, Christou E, Escribano R, Umani SF, Gasparini S, Ruiz FG, Hoffmeyer M, Ianora A, Kang, HK, Laabir M, Lacoste A, Miralto A, Ning X, Poulet S, Rodriguez V, Runge J, Shi J, Starr M, Uye S, Wang Y. 1997. The paradox of diatom-copepod interactions. *Marine Ecology Progress Series* **157**: 287 - 293.

Barlow DE, Dickinson GH, Orihuela B, Kulp JL, Rittschof D, Wahl KJ. 2010. Characterisation of the adhesion plaque of the barnacle *Balanus amphitrite*: amyloid-like nanofibrils are a major component. *Langmuir* **26** (9): 6549 - 6556.

Barnes H. 1953. The effect of light on the growth rate of two barnacles *Balanus balanoides* (L.) and *B. crenatus* Brug. under conditions of total submergence. *Oikos* **4**: 104 - 111

Barnes H. 1962. Note on variations in the release of nauplii of *Balanus balanoides* with special reference to the spring diatom outburst. *Crustaceana* **4**(2): 118 - 122.

Barnes H, Barnes M. 1963. *Elminius modestus* Darwin: Further European records. *Progress in Oceanography* **3**: 23 - 30.

Barnes H, Barnes M. 1969. *Elminius modestus* Darwin: Records of its present distribution and abundance in the Baie de St Malo and in the region of St Jean-de-Luz. *Journal of Experimental Marine Biology and Ecology* **3**: 156 - 161.

Barnes H, Read R, Topinka JA. 1970. The behaviour on impaction by solids of some common cirrpedes and relation to their normal habitat. *Journal of Experimental Marine Biology and Ecology* **5**: 70 - 87.

Barnes H, Barnes M, Klepal W. 1977. Studies on the reproduction of cirripedes. I. Introduction: Copulation, release of oocytes, and formation of the egg lamellae. *Journal of Experimental Marine Biology and Ecology* **27**: 195 - 218.

Barnes M, Barnes H. 1982. Effect of turbulence on the feeding and moulting of the cirrepede *Balanus balanoides* (L.) given an algal diet. *Journal of Experimental Marine Biology and Ecology* **65**: 163 - 172.

Barnett BE, Crisp DJ. 1979. Laboratory studies of gregarious settlement in *Balanus balanoides* and *Elminius modestus* in relation to competition between these species. *Journal of the Marine Biological Association in the United Kingdom* **59**: 581 -590.

Becka A, Loeb G. 1984. Ease of removal of barnacles from various polymeric materials. *Biotechnology and Bioengineering* **26**: 1245 - 1251.

Beigbeder A, Degee P, Conlan SL, Mutton RJ, Clare AS, Pettit ME, Callow ME, Callow JA, Dubois P. 2008. Preparation and characterisation of silicone-based coatings filled with carbon nanotubes and natural sepiolite and their application as marine fouling-release coatings. *Biofouling* **24** (4): 291 - 302.

Berglin M, Gatenholm P. 1999. The nature of bioadhesive bonding between barnacles and fouling release silicone coatings. *Journal of Adhesion Science and Technology* **13** (6): 713 - 727.

Berglin M, Gatenholm P. 2003. The barnacle adhesive plaque: morphological and chemical differences as a response to substrate properties. *Colloids and Surfaces B-Biointerfaces* **28**:107-117.

Berglin M, Larsson A, Jonsson PR, Gatenholm P. 2001. The adhesion of the barnacle, *Balanus improvisus*, to poly(dimethylsiloxane) fouling release coatings and poly (methyl methylacrylate) panels: The effect of barnacle size on strength and failure mode. *Journal of Adhesion Science and Technology* **15** (12): 1485 - 1502.

Berglin M, Lonn N, Gatenholm P. 2003. Coating modulus and barnacle bioadhesion. *Biofouling* **19** (Supplement): 63 - 69.

Berntsson KE, Jonsson PR. 2003. Temporal and spatial patterns in recruitment and succession of a temperate marine fouling assemblages: a comparison of static panels and boat hulls during the boating season. *Biofouling* **19** (3): 187 - 195.

Bers AV, Wahl M. 2004. The influence of nature surface microtopographies on fouling. *Biofouling* **20** (1): 43 - 51.

Billinghurst Z. Clare AS, Fileman T, Readman MJ. 1998. Inhibition of barnacle settlement by the environmental oestrogen 4-nonylphenol and the natural oestrogen 17β oestradiol. *Marine Pollution Bulletin* **36** (10): 833 - 839.

Billinghurst Z, Clare AS, Depledge MH. 2001. Effects of 4-*n*-nonpylphenol and 17β-oestradiol on development of the barnacle *Elminius modestus*. *Journal of Experimental Marine Biology and Ecology* **257**: 255 - 268.

Bishop MWH. 1947. Establishment of an immigrant barnacle in British coastal waters. *Nature* **159** (4041): 501 - 502.

Bishop MWH. 1951. Distribution of barnacles by ships. *Nature* **167** (4248): 531.

Bourget E. 1988. Barnacle larval settlement: The perception of cues at different spatial scales. In Vannini M. Chelazzi M. (eds), *Behavioural adaptations to the intertidal life.* PlenumPress, NY. pp 53 - 172.

Bracewell SA, Spencer M, Marrs RH, Iles M, Robinson LA. 2012. Cleft, crevice, or the inner thigh: 'Another place' for the establishment of the invasive barnacle *Austrominius modestus* (Darwin, 1854). *PLoS ONE* **7** (11): e48863. https://doi.org/10.1371/journal.pone.0048863.

Bracewell SA, Robinsion LA, Firth LB, Knights AM. 2013. Predicting free-space occupancy on novel artificial structures by an invasive intertidal barnacle using removal experiment. *PLoS ONE* **8** (9): e74457. https://doi.org/10.1371/journal.pone.0074457.

Brady R. 1999. Properties which influence marine fouling resistance in polymers containing silicon and fluorine. *Progress in Organic Coatings* **35**: 31 - 35.

Brady R. 2000. Clean hulls without poisons: Devising and testing nontoxic marine coatings. *Journal of Coating Technology* **72** (900): 45 - 56.

Brady R. 2001. A fracture mechanical analysis of fouling release from nontoxic antifouling coatings. *Progress in Organic Coatings* **43**: 188 - 192.

Brady RF, Singer IL. 2000. Mechanical factors favouring release from fouling release coatings. *Biofouling* **15**: 73 - 81.

Branscomb ES, Rittschof D. 1984. An investigation of low frequency sound waves as a means of inhibiting barnacle settlement. *Journal of Experimental Marine Biology and Ecology* **79**: 149 - 154.

Breitburg DL. 1985. Development of a subtidal epibenthic community: factors affecting species composition and the mechanisms of succession. *Oecologia* **65**: 173 - 184.

Briand J. 2009. Marine antifouling laboratory bioassays: an overview of their diversity. *Biofouling* **25** (4): 297 - 311.

Brooks S, Waldock M. 2009. The use of copper as a biocide in marine antifouling paints. In Hellio C, Yebra DM (eds) *Advances in Marine Antifouling Coatings and Technologies.* Woodhead Publishing Ltd, Cambridge, UK. pp 492 - 521.

Bubel A. 1975. An ultrastructural study of the mantle of the barnacle *Elminius modestus* Darwin In relation to shell formation. *Journal of Experimental Marine Biology and Ecology* **20**: 287 - 324.

Buckeridge JS. 1982. The barnacle subfamily Elminiiae - Two new subgenera and a new Miocene species from Victoria. *Journal of the Royal Society of New Zealand* **12** (4): 353 - 357.

Buckeridge JS, Newman WA. 2010. A review of the subfamily Elimininae (Cirripedia: Thoracica: Austrobalanidae), including a new genus *Protelminius* nov., from the Oligocene of New Zealand. *Zootaxa* **2349**: 39 - 54.

Burgess JG, Boyd KG, Armstrong E, Hang Z, Yan L, Berggren M, May U, Pisacane T, Granmo A, Adams DR. 2003. The development of a marine natural product-based anti-fouling paint. *Biofouling* **19**: 197 - 205.

Caldwell GS, Olive PJW, Bentley MG. 2002. Inhibition of embryonic development and fertilisation in broadcast spawning marine invertebrates by water soluble diatom extracts and the diatom toxins 2-*trans*,4-*trans* decadienal. *Aquatic Toxicology* **60**: 123 - 137.

Caldwell GS, Bentley MG, Olive PJW. 2003. The use of a brine shrimp (*Artemia salina*) bioassays to assess the toxicity of diatom extracts and short chain aldehydes. *Toxicon* **42**: 301 - 306.

Caldwell GS, Lewis C, Olive PJW, Bentley MG. 2005. Exposure to 2, 4-decadienal negatively impacts upon marine invertebrate larval fitness. *Marine Environmental Research* **59**: 405 - 417.

Callow JA, Callow ME. 2011. Trends in the development of the environmentally friendly fouling-resistant marine coatings. *Nature Communications* **2** (244): 1 - 10.

Callow ME, Callow JA. 2002. Marine biofouling: a sticky problem. *Biologist* **49**: 1 - 5.

Callow ME, Fletcher RL. 1994. The influence of low surface energy materials on bioadhesion - a review. *International Biodeterioration & Biodegradation* **1994**: 333 - 348.

Callow ME, Callow JA, Pickett-Heaps JD, Wetherbee R. 1997. Primary adhesion of *Enteromorpha* (Chlorophyta, Ulvales) propagules quantitative settlement studies and video microscopy. *Journal of Phycology* **33**: 938 - 947.

Carl C, Poole AJ, Sexton BA, Glenn FL, Vucko MJ, Williams MR, Whalan S, de Nys R. 2012. Enhancing the settlement and attachment strength of pediveligers of *Mytilus galloprovincialis* by changing surface wettability and microtopography. *Biofouling* **28**(2): 175 - 186.

Carlton J, Newman WA. 2009. Reply to Clare and Høeg 2008. *Balanus amphitrite* or *Amphibalanus amphitrite*? A note on barnacle nomenclature. *Biofouling* **25**(1): 77 - 80.

Carlton JT, Tompson JK, Schemel LE, Nichols FH. 1990. Remarkable invasion of San Francisco Bay (California, USA) by the Asian clam *Potamocorbula amurensis.* I. Introduction and dispersal. *Marine Ecology Progress Series*. **56**: 81 - 94.

Carlton JT, Newman WA, Pitombo FB. 2011. Barnacle invasions: Introduced, cryptogenic and range expanding cirripedia of North and South America. In. Galil BS, Clark PF, Carlton JT (eds) *In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts.* Springer Dordrecht Heidelberg London New York. pp 159 - 213.

Carman Ml, Estes TG, Feinberg AW, Schumacher JF, Wilkerson W, Wilson LH, Callow ME, Callow JA, Brennan AB. 2006. Engineered anti-fouling microtopographies - correlating wettability with cell attachment. *Biofouling* **22**: 11 - 21.

Casellato S, Masiero L, Sichirollo E, Soresi S. 2007. Hidden secrets of the Northern Adriatic: "Tegnúe", peculiar reefs. *Central European Journal of Biology* **2** (1): 122 - 136.

Cassé F, Ribeiro E, Ekin A, Webster DC, Callow JA, Callow ME. 2007. Laboratory screening of coating libraries for algal adhesion. *Biofouling* **23** (3/4): 267 - 276.

Chambers LD, Stokes K, Walsh FC, Wood RJK. 2006. Modern approaches to marine antifouling coatings. *Surface and Coatings Technology* **201**: 3642 - 3652.

Chaudhury MK, Kim KH. 2007. Shear-induced adhesive failure of a rigid slab in contact with a thin confined film. *The European Physical Journal E* **23:** 175 - 183.

Chaudhury MK, Finlay JA, Chung JY, Callow ME, Callow JA. 2005. The influence of elastic modulus and thickness on the release of the soft-fouling green alga *Ulza linza* (syn. *Enteromorpha linza*) from poly(dimethylsiloxane) (PDMS) model networks. *Biofouling* **21** (1): 41 - 48.

Chen M, Yu, Q, Sun H. 2013. Novel strategies for the prevention and treatment of biofilm related infections. I*nternational Journal of Molecular Sciences* **14:** 18488 - 18501.

Chisholm B, Webster DC, Bennet JC, Berry M, Christianson D, Kim J, Mayo B, Gubbins N. 2007. Combinatorial materials research applied to the development of new surface coatings VII: An automated system for adhesion testing. *Review of Scientific Instruments* **78**: 072213.

Christie AO, Dalley R. 1987. Barnacle fouling and its prevention. In Southward AJ (eds) *Barnacle Biology: Crustacean Issue 5*. AA Balkema, Rotterdam. pp. 419 - 434.

Chung JY, Chaudhury MK. 2005. Soft and hard adhesion. *The Journal of Adhesion* **81**: 1119 - 1145.

Clare AS. 1996. Marine natural product antifoulants: Status and potential. *Biofouling* **9** (3): 211 - 229.

Clare AS. 1998. Towards non-toxic anti-fouling. *Journal of Marine Biotechnology* **6**: 3 - 6.

Clare AS, Matsumura K. 2000. Nature and perception of barnacle settlement pheromones. *Biofouling* **15** (1-2): 57 - 71.

Clare AS, Høeg JT. 2008. *Balanus amphitrite* or *Amphibalanus amphitrite*? A note on barnacle nomenclature. *Biofouling* **24** (1): 55 - 57.

Clare AS, Rittschof D, Gerhart DJ, Maki JS. 1992. Molecular approaches to non toxic anti-fouling. *Invertebrate Reproduction and Development* **22**: 67 - 76.

Clare AS, Freet RK, McClary Jr M. 1994. On the antennular secretion of the cyprid of *Balanus amphitrite*, and its role as a settlement pheromone. *Journal of the Marine Biological Association in the United Kingdom* **74**: 243 - 250.

Conlan SL, Mutton RJ, Aldred N, Clare AS. 2008. Evaluation of a fully automated method to measure the critical removal stress of adult barnacles. *Biofouling* **24** (6): 471 - 481.

Costlow JD, Bookhout CG. 1953. Moulting and growth in *Balanus improvisus. Biological Bulletin* **105** (3): 420 - 433.

Crisp DJ. 1958. The spread of *Elminius modestus* Darwin in North-west Europe. *Journal of the Marine Biological Association of the United Kingdom* **37:** 483 - 520.

Crisp DJ. 1960. Factors influencing growth-rate in *Balanus balanoides. Journal of Animal Ecology* **29** (1): 95 - 116.

Crisp DJ, Chipperfield PNJ. 1948. Occurrence of *Elminius modestus* (Darwin) in British waters. *Nature* **161**: 64.

Crisp DJ, Davies PA. 1955. Observations *in vivo* on the breeding of *Elminius modestus* grown on glass slides. *Journal of the Marine Biological Association in the United Kingdom.* **34**: 357 - 380.

Crisp DJ, Meadows PS. 1962. The chemical basis of gregariousness in cirripedes. *Proceedings of the Royal Society London, Series B, Biological Sciences* **156** (965): 500 - 520.

Crisp DJ, Walker G, Young GA, Yule A. 1985. Adhesion and substrate choice in mussels and barnacles. *Journal of Colloid and Interface Science* **104**: 40 - 50.

Darwin C. 1854. A monograph of the sub-class cirrepedia with figures of all species: the Balanidae, the Verrucidae. Ray Society, London.

Davidson I, Scianni C, Hewitt C, Everett R, Holm E, Tamburri M, Ruiz G. 2016. Mini-review: Assessing the drivers of ship biofouling management - aligning industry and biosecurity goals. *Biofouling* **32** (4): 411 - 428.

Dayton PK. 1971. Competition, disturbance and community organisation: The provision and subsequent utilisation of space in a rocky intertidal community. *Ecological Monographs* **14** (4): 351 - 389.

Di Fino A, Petrone L, Aldred N, Ederth T, Liedberg B, Clare, AS. 2014. Correlation between surface chemistry and settlement behaviour in barnacle cyprids (*Balanus improvisus*). *Biofouling* **30** (2): 143 - 152.

Dickinson GH, Vega IE, Wahl KJ, Orihuela B, Beyley V, Rodriguez EN, Everett RK, Bonaventura J, Rittschof D. 2005. Barnacle cement: a polymerisation model

based on evolutionary concepts. *The Journal of Experimental Biology* **212**: 3499 - 3510.

Dobretsov S, Dahms HU, Qian PY. 2006. Inhibition of biofouling by marine microorganisms and their metabolites. *Biofouling* **22** (1): 43 - 54.

Eckman JE, Savidge WB, Gross TF. 1990. Relationship between duration of cyprid attachment and drag forces associated with detachment of *Balanus amphitrite* cyprids. *Marine Biology* **107**: 111 - 118.

Ekin A, Webster DC, Daniels JW, Stafslien S, Cassé F, Callow JA, Callow ME. 2007. Synthesis, formulation, and characterisation of siloxane-polyurethane coatings for underwater marine applications using combinatorial high-throughput experimentation. *Journal of Coating Technology and Research* **4** (4): 435 - 451.

Elbourne PD, Veater RA, Clare AS. 2008. Interaction of conspecific cues in *Balanus amphitrite* Darwin (Cirripedia) settlement assays: Continued argument for the single-larva assay. *Biofouling* **24** (2): 87 - 96.

Ennos R. 2012. Statistical and data handling skills in Biology. 3rd Edition, Pearson Education Ltd, Essex, UK.

Estarlich FF, Lewey SA, Nevell TG, Thorpe AA, Tsibouklis J, Upton AC. 2000. The surface properties of some silicone and fluorosilicone coatings materials immersed in seawater. *Biofouling* **16** (2-4): 263 - 275.

Evariste E, Gachon CMM, Callow ME, Callow JA. 2012. Development and characteristics of an adhesion bioassay for ectocarpoid algae. *Biofouling* **28** (1): 15 - 27.

Fang J, Kelarakis A, Wang D, Giannelis EP, Finlay JA, Callow ME, Callow JA. 2010. Fouling release nanostructured coatings based on PDMS-polyurea segmented copolymers. *Polymer* **51**: 2636 - 2642.

Finlay J, Callow ME, Schultz MP, Swain GW, Callow JA. 2002. Adhesion strength of settled spores of the green alga *Enteromorpha*. *Biofouling* **18** (4): 251 - 256.

Finnie AA, Williams DN. 2010. Paint and coatings technology for the control of marine fouling. In Durr S, Thomason JC (eds) *Biofouling* Wiley Blackwell, Oxford UK. pp. 185 - 206.

Flowerdew MW. 1984. Electrophoretic comparison of the Antipodean cirripede *Elminius modestus*, with immigrant European populations. *Journal of the Marine Biological Association in the United Kingdom* **64**: 625 - 635.

Foster BA. 1982. Two new intertidal balanoid barnacles from eastern Australia. *Proceedings of the Linnean Society of New South Wales* **106**: 21 - 32.

Franco SC, Aldred N, Cruz T, Clare AS. 2016. Modulation of gregarious settlement of the stalked barnacles *Pollicipes pollicipes*: a laboratory study. *Scientia Marina* **80** (2): 217 - 228.

Gallagher MC, Davenport J, Gregory S, McAllen R, O'Riordan R. 2015. The invasive barnacle species, *Austrominius modestus:* Its status and competition with indigenous barnacles on the Isle of Cumbrae, Scotland. *Estuarine, Coastal and Shelf Science* **152**: 134 - 141.

Gallagher MC, Culloty S, McAllen R, O'Riordan R. 2016. Room for one more? Coexistence of native and non-indigenous barnacle species. *Biological Invasions* **18** (10): 3033 - 3046. doi:10.1007/s10530-016-1198-y.

Gao G, Clare AS, Rose C, Caldwell GS. 2016. Non-cryogenic preservation of thalli, germlings, and gametes of the green seaweed *Ulva rigida*. *Aquaculture* **473**: 246 - 250.

Gollasch S. 2002. The importance of ship hull fouling as a vector of species introductions into the North sea. *Biofouling* **18**: 105 - 121.

Grenon J, Elias J, Moorcroft J, Crisp DJ. 1979. A new apparatus for force measurement in marine bioadhesion. *Marine Biology* **5** (4): 381 - 388.

Griffith AA. 1921. The phenomena of rupture and flow in solids. P*hilosophical Transactions of the Royal Society of London Series A* **221**: 163 - 193.

Gubbay S. 1983. Compressive and adhesive strengths of a variety of British barnacles. *The Journal of the Marine Biological Association in the United Kingdom* **63**: 541 - 555.

Gudipati CS, Greenlief CM, Johnson JA, Prayongpan P, Wooley KL. 2004. Hyperbranched fluoropolymer and linear poly(ethylene glycol) based amphiphilic crosslinked networks as efficient antifouling coatings: An insight into the surface compositions, topographies, and morphologies. *Journal of Polymer Science Part A: Polymer Chemistry* **42** (24): 6193 - 6208.

Harms J, Anger K. 1989. Settlement of the *Elminius modestus* Darwin on tests panels at Hegoland (North Sea): A ten year study. *Topics in Marine Biology Sci. Mar.* **53** (2-3): 417 - 421.

Hawkins SJ. Hartnoll RG. 1982. Settlement patterns of *Semibalanus balanoides* (L.) in the Isle of Man (1977 - 1981). *Journal of Experimental Marine Biology and Ecology* **62**: 271 - 283.

Hellio C, de La Brois D, Dufossé L, Le Gal Y, Bourgougnon N. 2001. Inhibition of marine bacteria by extracts of macroalgae: potential use for environmentally friendly antifouling paints. *Marine Environmental Research* **52**: 231 - 247.

Hellio C, Marechal JP. Véron B, Bremer G, Clare AS, Gal YL. 2004. Seasonal variation of antifouling activities of marine algae from the Brittany coast (France). *Marine Biotechnology* **6**: 67 - 82.

Hellio C, Tsoukatou M, Maréchal JP, Aldred N, Beaupoil C, Clare AS, Vagias C, Roussis V. 2005. Inhibitory effects of Mediterranean sponge extracts and metabolites on larval settlement of the barnacle *Balanus amphitrite*. *Marine Biotechnology* **7**: 297 - 305.

Hills JM, Thomason JC. 1998. On the effect of tile size and surface texture on recruitment pattern and density of the barnacle *Semibalanus balanoides*. *Biofouling* **13**(1): 31 - 50.

Hiscock K, Hiscock S, Baker JM. 1978. The occurrence of the barnacle *Elminius modestus* in Shetland. *Journal of the Marine Biological Association in the United Kingdom.* **58:** 627 - 629.

Holland R, Dugdale TM, Wetherbee R, Brennan AB, Finlay JA, Callow JA, Callow ME. 2004. Adhesion and motility of fouling diatoms on a silicone elastomer. *Biofouling* **20** (6): 323 - 329.

Holm ER, Orihuela B, Kavanagh CJ, Risttschof D. 2005. Variation among families for characteristics of the adhesive plaque in the barnacle *Balanus amphitrite*. *Biofouling* **21** (2): 121 - 126.

Holm ER, Kavanagh CJ, Meyer AE, Wiebe D, Nedved BT, Wendt D, Smith CM, Hadfield MG, Swain G, Wood CD, Truby K, Stein J, Montemarano J. 2006. Interspecific variation in patterns of adhesion of marine fouling to silicone surfaces. *Biofouling* **22**: 233 - 243.

Holm ER, Kavanagh CJ, Orihuela B, Rittschof D. 2009. Phenotyptic variation for adhesive tenacity in the barnacle *Balanus amphitrite*. *Journal of Experimental Marine Biology and Ecology* **380**: 61 - 67.

Hui E, Moyse J. 1982 Settlement of *Elminius modestus* cyprids in contact with adult barnacles in the field. *Journal of the Marine Biological Association in the United Kingdom* **62:** 477 - 482

Hui CY, Long R, Wahl KJ, Everett RK. 2011. Barnacles resist removal by crack trapping. *Journal of the Royal Society Interface* **8**: 868 - 879.

Hunt HL. Scheibling RE. 1997. Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Marine Ecology Progress Series* **155**: 269 - 301.

International Marine Organisation (IMO). 2002. Focus on IMO. Anti-fouling systems. http://www.imo.org/ accessed on the 28th October 2006.

International Marine Organisation (IMO). 2010. Prevention of air pollution from ships. *Marine Environmental Protection Committee* 60th session. 22nd - 26th March 2010. http://www.imo.org/MediaCentre/MeetingSummaries/MEPC/Pages/MEPC-60th-Session.aspx. accessed on 10th October 2012.

International Marine Organisation (IMO). 2012. Energy efficiency measures for ships. *Marine Environmental Protection Committee* 64th session 1st - 5th October 2012. http://www.imo.org/MediaCentre/MeetingSummaries/MEPC/Pages/MEPC-60th-Session.aspx Accessed on 5th May 2013.

Jeffrey CJ, Underwood AJ. 2000. Consistent spatial patterns of arrival of larvae of the honeycomb barnacle *Chamaesipho tasmanica* Foster and Anderson in New South Wales. *Journal of Experimental Marine Biology and Ecology*. **252**: 109 - 127.

Jenkins SR. Martins GM. 2010. Succession on hard substrata. In Durr S, Thomason JC (eds) *Biofouling*. Wiley Blackwell, Oxford UK. pp 60 - 69.

Jenkins SR, Åberg P, Cervin G, Coleman RA, Delany J, Della Santina P, Hawkins SJ, LaCroix E, Myers AA, Lindegarth M, Power AM, Roberts MF, Hartnoll RG. 2000. Spatial and temporal variation in settlement and recruitment of the intertidal barnacle *Semibalanus balanoides* (L.) (Crustacea: Cirripedia) over a European scale. J*ournal of Experimental Marine Biology and Ecology* **243**: 209 - 225.

Johnston LA. 2010. Temperature affects adhesion in the acorn barnacle (*Balanus amphitrite*). MS in Biological Sciences California Polytechnic State University.

Jonsson PR, Berntsson KM, Larsson AN. 2004. Linking larval supply to recruitment: Flow-mediated control of initial adhesion of barnacle larvae. *Ecology* **85** (10): 2850 - 2859.

Judge ML, Craig SF. 1997. Positive flow dependence in the initial colonization of a fouling community: results from in situ water current manipulations. *Journal of Experimental Marine Biology and Ecology* **210**: 209 - 222.

Kaelble DH. 1970. Dispersion - polar surface tension properties of organic solids. *The Journal of Adhesion* **2** (2): 66 - 81.

Kaffashi A, Jannesari A, Ranjbar Z. 2012. Silicone fouling-release coatings: effects of the molecular weight of poly(dimethylsiloxane) and tetraethyl orthosilicate on the magnitude of pseudobarnacle adhesion strength. *Biofouling* **28** (7): 729 - 741.

Kamino K. 2006. Barnacle underwater attachment. In Smith AM, Callow JA (eds) *Biological Adhesives* Springer-Verlag, Berlin. pp. 145 - 166.

Kamino K. 2008. Underwater adhesive of marine organisms as the vital link between biological science and material science. *Marine Biotechnology* **10**: 111 - 121.

Kamino K. 2013. Mini-review: Barnacle adhesives and adhesion. *Biofouling* **29** (6): 735 - 746.

Kavanagh C, Schultz MP, Swain GW, Stein J, Truby K, Darkangelo Wood C. 2001. Variation in adhesion strength of *Balanus eburneus, Crassostrea virginica* and *Hydroides dianthus* to fouling-release coatings. *Biofouling* **17**(2): 155 - 167.

Kavanagh C, Swain GW, Kovach BS, Stein J, Darkangelo Wood C, Truby K, Holm E, Montemarano J, Meyer A, Wiebe D. 2003. The effects of silicone fluid additives and silicone elastomer matrices on barnacle adhesion strength. *Biofouling* **19**(6): 381 - 390.

Kavanagh CJ, Quinn RD, Swain GW. 2005. Observations of barnacle detachment from silicones using high-speed video. *The Journal of Adhesion* **81**: 843 - 868.

Kendall K. 1971. The adhesion and surface energy of elastic solids. *Journal of Physics D: Applied Physics* **4**: 1186 - 1195.

Keough MJ. 1983. Patterns of recruitment of sessile invertebrates in two subtidal habitats. *Journal of Experimental Marine Biology and Ecology* **66**: 213 - 245.

Keough MJ, Downes BJ. 1982. Recruitment of marine invertebrates: the role of active larval choices and early mortality. *Oecologia* **54**: 348 - 352.

Keough MJ, Raimondi PT. 1995. Responses of settling invertebrates larvae to bioorganic films: effects of different types of films. *Journal of Experimental Marine Biology and Ecology* **185**: 235 - 253.

Kerr A, Cowling MJ. 2003. The effects of surface topography on the accumulation of biofouling. *Philosophical Magazine* **83** (24): 2779 - 2795.

Khandeparker L, Anil AC. 2007. Underwater adhesion: The barnacle way. *International Journal of Adhesion and Adhesives* **27**: 165 - 172

Kim J, Chisholm BJ, Bahr J. 2007. Adhesion study of silicone coatings: the interaction of thickness, modulus and shear rate on adhesion force. *Biofouling* **23** (2): 113 - 120.

Kim J, Nyren-Erickson E, Stafslien S, Daniels J, Bahr J, Chisholm BJ. 2008. Release characteristics of reattached barnacles to non-toxic silicone coatings. *Biofouling* **24** (4): 313 - 319.

Kirby M. 2006. Barnacle settlement behaviour in response to con- and allo-specific cues. PhD Thesis. Newcastle University UK.

Knight-Jones EW. 1948. *Elminius modestus*: Another imported pest of East coast oyster beds. *Nature* **161**: 201 - 202.

Knight-Jones EW, Crisp DJ. 1953. Gregariousness in barnacles in relation to the fouling of ships and to anti-fouling research. *Nature* **171**: 1109 - 1110.

Knight-Jones EW, Stevenson JP. 1950. Gregariousness during settlement in the barnacle *Elminius modestus* Darwin. *Journal of the Marine Biological Association in the United Kingdom* **29**: 281 - 297.

Kohl J, Singer IL. 1999. Pull-off behaviour of epoxy bonded to silicone duplex coatings. *Progress in Organic Coatings* **36**: 15 - 20.

Krishnan S, Ayothi R, Hexemer A, Finlay JA, Sohn KE, Perry R, Ober CK, Kramer EJ, Callow ME, Callow JA, Fischer DA. 2006. Anti-biofouling properties of comblike block copolymers with amphiphilic side chains. *Langmuir* **22**: 5075 - 5086.

Larman VN, Gabbot PA. 1975. Settlement of cyprid larvae of *Balanus balanoides* and *Elminius modestus* induced by extracts of adult barnacle and other marine animals. *Journal of the Marine Biological Association in the United Kingdom* **55***:*183 - 190.

Larsson AI, Mattsson-Thorngren L, Granhag LM, Berglin M. 2010. Fouling-release of barnacles from a boat hull with comparison to laboratory attachment strength. *Journal of Experimental Marine Biology and Ecology* **392**: 17 - 114.

Lawson J, Davenport J, Whitaker A. 2004. Barnacle distribution in Lough Hyne Marine Nature Reserve: a baseline and an account of invasion by the introduced Australasian species *Elminius modestus* Darwin. *Estuarine, Coastal and Shelf Science* **60**: 729 - 735.

Lemaire E, Levitz P, Daccord G, Van Damme H. 1991. From viscous fingering to viscoelastic fracturing in colloidal fluids. *Physical Review Letters* **67** (15): 2009 - 2012.

Leonard GH, Levine JM, Schmidt PR, Bertness MD. 1998. Flow-driven variation in intertidal community structure in a marine estuary. *Ecology* **79** (4): 1395 - 1411.

Li J, Jiang G, Du Q, Guo S. 2010. Adhesion and delamination mechanisms in alternating layered copolymers. *Society of Plastics Engineers: Plastic Research Online*. doi: 10.1002/spepro.002585.

Lin HC, Wong YH, Tsang LM, Chu KH, Qian PY, Chan BKK. 2014. First study on gene expression of cement proteins and potential adhesion-related genes of a membranous-based barnacle as revealed from Next-Generation Sequencing technology. *Biofouling* **30** (2): 169 - 181.

Löschau M, Krätke R. 2005. Efficacy and toxicity of self polishing biocide-free antifouling paints. *Environmental Pollution* **138**: 260 - 267.

Lunn I. 1974. Antifouling. Thame, BCA Publications.

Magin CM, Cooper SP, Brennan AB. 2010. Non-toxic antifouling strategies. *Materials today* **13** (4) 36 - 44.

Maki JS, Rittschof D, Costlow JD, Mitchell R. 1988. Inhibition of attachment of larval barnacles, *Balanus amphitrite*, by bacterial surface films. *Marine Biology* **97:** 199 - 206.

Maki JS, Rittschof D, Samuelsson MO, Szewzyk U, Yule AB, Kjelleberg S, Costlow JD, Mitchell R. 1990. Effect of marine bacteria and their exopolymers on the attachment of barnacle cypris larvae. *Bulletin of Marine Science* **46** (2): 499 - 511.

Marabotti I, Morelli A, Orsini LM, Martinelli E, Galli G, Chiellini E, Lien EM, Pettit ME, Callow ME, Callow JA, Conlan SL, Mutton RJ, Clare AC, Kocijan A,

Donik C, Jenko M. 2009. Fluorinated/siloxane copolymer blends for fouling release: chemical characterisation and biological evaluation with algae and barnacles. *Biofouling* **25** (6): 481 - 493.

Maréchal JP, Matsumura K, Conlan S, Hellio C. 2012. Competence and discrimination during cyprid settlement in *Amphibalanus amphitrite*. *International Biodeterioration and Biodegradation* **72**: 59 - 66.

Mark JE. Allock HR, West R. 2005. Polysiloxanes and related polymers. In 2nd Edition *Inorganic Polymers*. Oxford University Press. pp 154 - 199.

Martinelli E, Suffredini M, Galli G, Glistenti A, Pettitt M, Callow ME, Callow JA, Williams D, Lyall G. 2011. Amphiphilic block copolymer/poly (dimethylsiloxane) (PDMS) blends and nanocomposites for improved fouling-release. *Biofouling* **27** (5): 529 - 541.

Martinelli E, Sarvothaman MK, Gallo G, Pettitt ME, Callow ME, Callow JA, Conlan SL, Clare AS, Sugiharto AB, Davies C, Williams D. 2012. Poly(dimethyl siloxane) (PDMS) netwerk blends of amphiphillic acrylic copolymers with poly(ethylene glycol)-fluoroalkyl side chains for fouling-release coatings. II. Laboratory assays and field immersion trials. *Biofouling* **28** (6): 571 - 582.

Matsumura K, Hills JM, Thomason PO, Thomason JC, Clare AS. 2000. Discrimination at settlement in barnacles: Laboratory and field experiments on settlement behaviour in response to settlement-inducing protein complexes. *Biofouling* **16** (2-4): 181 - 190.

Milne A. 1977. Patent GB1470465A International Paint Ltd.

Milne A, Hails G. 1976. Patent GB1457590A International Paint Ltd.

Minchin D, Gollasch S. 2003. Fouling and ship's hulls: How changing circumstances and spawning events may result in the spread of exotic species. *Biofouling* **19**: 111 - 122.

Minchinton TE, Scheibling RE. 1991. The influence of larval supply and settlement on the population structure of barnacles. *Ecology* **72** (5): 1867 - 1879.

Miralto A, Barone G, Romano G, Poulet SA, Ianora A, Russo GL, Buttino I, Mazzarella G, Laabir M, Cabrini M, Giacobbe MG. 1999. The insidious effect of diatoms on copepod reproduction. *Nature* **402**: 173 - 176.

Molnar JL. Gamboa RL. Revenga C. Spalding MD. 2008. Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment* **6** (9): 485 - 492.

Moore LB. 1944. Some intertidal sessile barnacles of New Zealand. *Transactions and Proceedings of the Royal Society of New Zealand* **73**: 315 - 334.

Moyse J. 1960. Mass rearing of barnacle cyprids in the laboratory. *Nature* **185**: 120.

Moyse J. 1963. A comparison of the value of various flagelletes and diatoms as food for barnacle larvae. *ICES Journal of Marine Science* **28** (2): 175 - 187.

Neal AL, Yule AB. 1994a. The tenacity of *Elminius modestus* and *Balanus perforatus* cyprids to bacterial films grown under different shear regimes. *Journal of the Marine Biological Association of the United Kingdom* **74:** 251 - 257.

Neal AL, Yule AB. 1994b. The interaction between *Elminius modestus* Darwin cyprids and biofilms of *Deleya marina* NCMB1877. *Journal of Experimental marine Biology and Ecology* **176**: 127 - 139.

Newman WA. 1987. Evolution of cirripedes and their major groups. In Southward AJ (eds) *Barnacle Biology: Crustacean Issue 5*. AA Balkema, Rotterdam. pp. 3 - 42.

Newman WA, Ross A. 1976. Revision of the balanomorpha barnacles; including a catalog of the species. *San Diego Society of Natural History Memoir* **9**: 1 - 108.

O'Connor NJ, Richardson DL. 1996. Effects of bacterial films on attachment of barnacles (*Balanus improvisus* Darwin) larvae: laboratory and field studies. *Journal of Experimental Marine Biology and Ecology* **206**: 69 - 81.

O'Riordan RM, Ramsay NF. 1999. The current distribution and abundance of the Australasian barnacle *Elminius modestus* in Portugal. *Journal of the Marine Biological Association of the United Kingdom* **79**: 937 - 939.

O'Riordan RM, Ramsay NF. 2013. Two new location records in the Algarve, Portugal for the non-indigenous barnacle *Austromininus modestus*. *Marine Biodiversity Records* **6**: doi:10.1017/S1755267213000985.

Olivier F, Tremblay R, Bourget E, Rittschof D. 2000. Barnacle settlement: field experiments on the influence of larval supply, tidal level, biofilm quality and age on *Balanus amphitrite* cyprids. *Marine Ecology Progress Series* **199**: 185 - 204

Olsen SM, Pedersen LT, Laursen MH, Kiil S, Dam-Johansem K. 2007. Enzyme-based antifouling coatings: a review. *Biofouling* **23** (5): 369 - 383.

Otani M, Oumi T, Uwai S, Hanyuda T, Prabowo RE, Yamaguchi T, Kawai H. 2007. Occurrence and diversity of barnacles on international ships visiting Osaka Bay, Japan and the risk of their introduction. *Biofouling* **23** (3/4): 277 - 286.

Owens DK, Wendt RC. 1969. Estimation of the surface free energy of polymers. *Journal of Applied Polymer Science* **13** (8): 1741 - 1747.

Packham D. 2003. Surface energy, surface topography and adhesion. *International Journal of Adhesion and Adhesives* **23**: 437 - 448.

Palmer AR. 1982. Predation and parallel evolution: recurrent parietal plate reduction in balanomorph barnacles. *Paleobiology* **8** (1): 31 - 44.

Pawlik JR. 1992. Chemical ecology of the settlement of benthic marine invertebrates. *Oceanography and Marine Biology. An Annual Review* **30**: 273 - 335.

Petrone L, Di Fino A, Aldred N, Sukkaew P, Ederth T, Clare AS, Liedberg B. 2011. Effects of surface charge and Gibbs surface energy on the settlement behaviour of barnacle cyprids *(Balanus amphitrite). Biofouling* **27** (9): 1043 - 1055.

Pettitt ME, Henry SL, Callow ME, Clare AS. 2004. Activity of commercial enzymes on settlement and adhesion of cypris larvae of the barnacles *Balanus amphitrite*, spores of the green alga *Ulza linza* and the diatom *Navicula perminuta. Biofouling* **20**(6): 299 - 311.

Phang IY, Aldred N, Clare AS, Callow JA, Vancso GJ. 2006. An in situ study of the nanomechanical properties of barnacles (*Balanus amphitrite*) cyprid cement using atomic force microscopy (AFM). *Biofouling* **22**: 245 - 250.

Pieper RJ, Ekin A, Webster DC, Cassé F, Callow JA, Callow ME. 2007. Combinational approach to study the effect of acrylic polyol composition on the properties of crosslinked siloxane-polyurethane fouling-release coatings. *Journal of Coatings Technology and Research* **4** (4): 453 - 461.

Pike JK, Ho T, Wynne K. 1996. Water-induced surface rearrangements of poly(dimethylsiloxane-urea-urethane) segmented block copolymer. *Chemical Materials* **8**: 856 - 860.

Pineda J. 1994. Spatial and temporal patterns in barnacle settlement rate along a southern California rocky shore. *Marine Ecology Progress Series* **107**: 125 - 138.

Piola RF, Dafforn KA, Johnston EL. 2009. The influence of antifouling practices on marine invasions. *Biofouling* **25**(7): 633 - 644.

Prendergast GS, Zurn CM, Bers AV, Head RM, Hansson LJ, Thomason JC. 2008. Field-based video observations of wild cyprid behaviour in response to textural and chemical settlement cues. *Biofouling* **24** (6): 449 - 459.

Qian PY, Xu Y, Fusetani N. 2010. Natural products as antifouling compounds: recent progress and future perspectives. *Biofouling* **26** (2): 223 - 234.

Qiu JW, Qian PY. 1997. Effects of food availability, larval source and culture method on larval development of *Balanus amphitrite amphitrite* Darwin: implications for experimental design. *Journal of Experimental Marine Biology and Ecology* **217**: 47 - 61.

Qiu JW, Hung OS, Qian PY. 2008. An improved barnacle attachment inhibition assy. *Biofouling* **24** (4): 259 - 266.

Quinn GP, Keough MJ. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge UK.

Raimondi PT. 1991. Settlement behaviour of *Chthamalus anisopoma* larvae largely determines the adult distribution. *Oecologia* **85**: 349 - 360.

Rainbow PS. 1984. An introduction to the biology of British littoral barnacles. *Field Studies* **6**: 1 - 51.

Ramsay D, Dickinson GH, Orihuela B, Rittschof D, Wahl KJ. 2008. Base plate mechanics of the barnacle *Balanus amphitrite* (= *Amphibalanus amphitrite*). *Biofouling* **24**(2): 109 - 118.

Rasband WS. 1997 - 2013. ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/

Reise K, Gollasch S, Wolff WJ. 1999. Introduced marine species of the North Sea coasts. *Helgolander Meeresunters* **52**: 219 - 234.

Ricciardi A, Whoriskey FG, Rasmussen JB. 1997. The role of the zebra mussel (*Dreissena polymorpha*) in structuring macroinvertebrate communities on hard substrata. *Canadian Journal of Fisheries and Aquatic Science* **54** (11): 2596 - 2608.

Rittschof D. 2000. Natural product antifoulants: One perspective on the challenges related to coatings development. *Biofouling* **15**(1-3): 119 - 127.

Rittschof D, Costlow JD. 1989. Bryozoan and barnacle settlement in relation to initial surface wettability: A comparison of laboratory and field studies. *Topics in Marine Biology Sci. Mar*. **53** (2-3): 411 - 416.

Rittschof D, Branscomb S, Costlow JD. 1984. Settlement and behaviour in relation to flow and surface in larval barnacles, *Balanus amphitrite* Darwin. *Journal of Experimental Marine Biology and Ecology* **82** (2-3): 131 - 146.

Rittschof D, Clare AS, Gerhart DJ, Avelin Mary S, Bonadventura J. 1992. Barnacle *in vitro* assays for biologically active substances: Toxicity and settlement inhibition assays using mass cultured *Balanus amphitrite amphitrite* Darwin. *Biofouling* **6**: 115 - 122.

Rittschof D, Orihuela B, Stafslien S, Daniels J, Christianson D, Chisholm B, Holm E. 2008. Barnacle reattachment: a tool for studying barnacle adhesion. *Biofouling* **24** (1): 1 - 9.

Roberts D, Rittshof D, Holm E, Schmidt AR. 1991. Factors influencing initial larval settlement: temporal, spatial and surface molecular components. *Journal of Experimental Marine Biology and Ecology* **150**: 203 - 211.

Robson MA, Williams D, Wolff K, Thomason JC. 2009. The effect of surface colour on the adhesion strength of *Elminius modestus* Darwin on a commercial non-biocidal antifouling coating at two locations in the UK. *Biofouling* **25** (3): 215 - 227.

Ruiz GM, Carlton JT, Grosholz ED, Hines AH. 1997. Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent and consequences. *American Zoologist* **37**: 621 - 632.

Saffman PG, Taylor G. 1958. The penetration of a fluid into a porous medium or Hele-Shaw cell containing a more viscous liquid. *Proceedings of The Royal Society of London, Series A, mathematical and Physical Sciences.* **245**: 312 - 329.

Sandison EE. 1950. Appearance of *Elminius modestus* Darwin in South Africa. *Nature*. **165** (4185): 79 - 80.

Sanford E, Bernudez D, Bertness MD, Gaines SD. 1994. Flow, food supply and acorn barnacle population dynamics. *Marine Ecology Progress Series* **104**: 49 - 62.

Scardino AJ, de Nys R. 2011. Mini review: Biomimetic models and bioinspired surfaces for fouling control. *Biofouling* **27**(1): 73-86.

Scardino AJ, Guenther J, de Nys R. 2008. Attachment point theory revisited: the fouling response to microtextured matrix. *Biofouling* **24** (1): 45 - 53.

Schultz MP 2007. Effects of coating roughness and biofouling on ship resistance and powering. *Biofouling* **23** (5): 331 - 341.

Schultz MP, Kavanagh CJ, Swain GW. 1999. Hydrodynamic forces on barnacles: implication on detachment from fouling-release surfaces. *Biofouling* **13**(4): 323 - 335.

Schultz MP, Finlay JA, Callow ME, Callow JA. 2000. A turbulent channel flow apparatus for the determination of the adhesion strength of microfouling organisms. *Biofouling* **15** (4): 243 - 251.

Schultz MP, Bendick JA, Holm ER, Hertel WM. 2011. Economic impact of biofouling on a naval surface ship. *Biofouling* **27** (1): 87 - 98.

Schumacher J, Aldred N, Callow ME, Finlay JA, Callow JA, Clare AS, Brennan AB. 2007. Species-specific engineered antifouling topographies: correlations between the settlement of algal zoospores and barnacle cyprids. *Biofouling* **23**(5): 307 - 317.

Silverman HG, Roberto FF. 2007. Understanding marine mussel adhesion. *Marine Biotechnology* **9**: 661 - 681.

Singer IL, Kohl JG, Patterson M. 2000. Mechanical aspects of silicone coatings for hard foulant control. *Biofouling* **16**: 301 - 309.

Sommer S, Ekin A, Webster DC, Stafslien SJ, Daniels J, VanderWal LJ, Thompson SEM, Callow ME, Callow JA. 2010. A preliminary study on the properties and fouling-release performance of siloxane-polyurethane coatings prepared from poly(dimethylsiloxane) (PDMS) macromers. *Biofouling* **26** (8): 961 - 972.

Southward AJ. 2008. Barnacles, keys and notes for the identification of British species. *Synopses of the British Fauna (New Series).* Crothers JH, Hayward PJ (eds). The Linnean Society of London.

Stafslien S, Daniels J, Bahr J, Chisholm B, Ekin A, Webster D, Orihuela B, Rittschof D. 2012. An improved laboratory reattachment method for the rapid assessment of adult barnacle adhesion strength to fouling-release marine coatings. *Journal of Coating Technology and Research* **9** (6): 651 - 665.

Stafslien S, Christianson D, Daniels J, VanderWal L, Chernykh A, Chisholm BJ. 2015. Combinatorial materials research applied to the development of new surface coatings XVI: fouling-release properties of amphiphilic polysiloxane coatings. *Biofouling* **31** (2): 135 - 149.

Stafslien S, Sommer S, Webster DC, Bodkhe R, Pieper R, Daniels J, Vander Wal L, Callow MC, Callow JA, Ralston E, Swain G, Brewer L, Wendt D, Dickinson GH, Lim CS, Lay-Ming Teo S. 2016. Comparison of laboratory and field testing performance evaluations of siloxane-polyurethane fouling-release marine coatings. *Biofouling* **32** (8): 949 - 968.

Statz A, Finlay J, Dalsin J, Callow ME, Callow JA, Messersmith PB. 2006. Algal antifouling and fouling-release properties of metal surfaces coated with polymer inspired by marine mussels. *Biofouling* **22** (6): 391 - 399.

Stein J, Truby K, Darkangelo-Wood C, Takemori M, Vallance M, Swain G, Kavanagh C, Kovach B, Schultz M, Wiebe D, Holm E, Montemarano J, Wendt D, Smith C, Meyer A. 2003. Structure - property relationships of silicone biofouling - release coatings: Effect of silicone network architecture on pseudobarnacle attachment strengths. *Biofouling* **19**(2): 87 - 94.

Stone CJ. 1988. Test of sequential feeding regimes for larvae of *Elminius modestus* Darwin (Cirripedia: Balanomorpha). *Journal of Experimental Marine Biology and Ecology* **115**: 41 - 51.

Stubbings HG. 1950. Earlier records of *Elminius modestus* Darwin in British waters. *Nature* **166**: 277 - 278.

Sullan RMA, Gunari N, Tanur AE, Chan Y, Dickinson GH, Orihuela B, Rittschof A, Walker GC. 2009. Nanoscale structures and mechanics of barnacle cement. *Biofouling* **25** (3): 263 - 275.

Sun Y, Guo S, Walker GC, Kavanagh CJ, Swain G. 2004. Surface elastic modulus of barnacle adhesive and release characteristics from silicone surfaces. *Biofouling* **20**: 279 - 289.

Swain G. 1997. Field evaluations of non-toxic antifouling coatings: New field technologies and performance criteria. *Naval Research Review* **49**: 46 - 50.

Swain G, Schultz MP. 1996. The testing and evaluation of non-toxic antifouling coatings. *Biofouling* **10** (1 - 3): 187 - 197.

Swain G, Griffith JR, Bultman JD, Vincent HL. 1992. The use of barnacle adhesion measurements for the field evaluation of non-toxic foul release surfaces. *Biofouling* **6**: 105 - 114.

Swain GW, Nelson WG, Preedeekant S. 1998. The influence of biofouling adhesion and biotic disturbance on the development of fouling communities on non-toxic surfaces. *Biofouling* **12** (1-3): 257 - 269.

Swain G, Anil AC, Baier RE, Chia F, Conte E, Cook A, Hadfield M, Haslbeck E, Holm E, Kavanagh C, Kohrs D, Kovach B, Lee C, Mazzella L, Meyer AE, Qian PY, Sawant SS, Schultz M, Sigurdsson J, Smith C, Soo L, Terlissi A, Wagh A, Zimmerman R, Zupo V. 2000. Biofouling and barnacle adhesion data for

fouling-release coatings subjected to static immersion at seven marine sites. *Biofouling* **16**(2-4): 331 - 344.

Tasso M, Conlan SL, Clare AS, Werner C. 2012. Active enzyme nanocoatings affect settlement of *Balanus amphitrite* barnacle cyprids. *Advanced Functional Materials* **22** (1): 39 - 47.

Thiyagarajan V, Nair KVK, Subramoniam T, Venugoplan. 2002. Larval settlement barhaviour of the barnacle *Balanus reticulatus* in the laboratory. *Journal of the Marine Biological Association in the United Kingdom.* **82**: 579 - 582.

Thomas KV. 2001. The environmental fate and behaviour of anti-fouling paint booster biocides: a review. *Biofouling* **17**: 73 - 86.

Thomason JC. 2014. Sampling and experiments with biofilms in the environment; Section 1. Field trials with biofilms. In Dobretsov S, Williams DN, Thomason JC (eds) *Biofouling Method*s. Wiley Blackwell, Oxford UK. pp. 168 - 173.

Thomason JC, Hills, JM, Clare AS, Neville A, Richardson M. 1998. Hydrodynamic consequences of barnacle colonization. *Hydrobiologia* **375/376**: 191 - 201.

Thomason J, Hills J, Thomason PO. 2002a. Field-based behavioural bioassays for testing the efficacy of antifouling coatings. *Biofouling* **18** (4): 285 - 292.

Thomason J, Letissier MDA, Thomason PO, Field SN. 2002b. Optimising settlement tiles: the effects of surface texture and energy, orientation and deployment duration upon the fouling community. *Biofouling* **18** (4): 293 - 304.

Thompson RC, Norton TA. Hawkins SJ. 1998. The influence of epilithic microbial films on the settlement of *Semibalanus balanoides* cyprids - a comparison between laboratory and field experiments. *Hydrobiologia* **375/376**: 203 - 216.

Tighe-Ford DJ, Power MJD, Vaile DC. 1970. Laboratory rearing of barnacle larvae for antifouling research. *Helgoländer wiss. Meeresunters*. **20**: 393 - 405.

Todd CD, Keough MJ. 1994. Larval settlement in hard substratum epifaunal assemblages: a manipulative field study of the effects of substratum filming and the presence of incumbents. *Journal of Experimental Marine Biology and Ecology*. **181**: 159 - 187.

Todd JS, Zimmerman RC, Crews P, Alberte RS. 1993. The antifouling activity of natural and synthetic phenol acid sulphate esters. *Phytochemistry* **34** (2): 401 - 404.

Townsin RL. 2003. The ship hull fouling penalty. *Biofouling* **19**: 9 - 15.

Wahl M. 1989. Marine epibiosis. I. Fouling and antifouling: some basic aspects. *Marine Ecology Progress Series* **58**: 175 - 189.

Walker G, Yule AB. 1984. Temporary adhesion of the barnacle cyprid: The existence of an antennular adhesive secretion. *Journal of the Marine Biological Association in the United Kingdom* **64**: 679 - 686.

Wang Y, Pitet LM, Finlay JA, Brewer LH, Cone G, Betts DE, Callow ME, Callow JA, Wendt DE, Hillmyer MA, DeSimone JM. 2011. Investigation of the role of hydrophobic chain length in amphiphilic perfluoropoyether/poly(ethylene glycol) networks: towards high-performance antifouling coatings. *Biofouling* **27** (10): 1139 - 1150.

Watermann B, Berger HD, Sönnichsen H, Willemsen P. 1997. Performance and effectiveness of non-stick coatings in seawater. *Biofouling* **11** (2): 101 - 118.

Webster DC, Chisholm BJ. 2010 New directions in antifouling technology. In Durr S, Thomason JC (eds) *Biofouling*. Wiley-Blackwell, Oxford UK. pp. 366 - 387.

Webster DC, Chisholm BJ, Stafslien SJ. 2007. Min-review: Combinatorial approaches for the design of novel coating systems. *Biofouling* **23** (3/4):179-192.

Wendt DE, Kowalke GL, Kim J, Singer IJ. 2006. Factors that influence elastomeric coating performance: the effect of coating thickness on basal plate morphology, growth and critical removal stress of the barnacle *Balanus amphitrite*. *Biofouling* **22**: 1 - 9.

Wethey D. 1986. Ranking of settlement cues by barnacle larvae: Influence of surface contour. *Bulletin of Marine Science* **39** (2): 393 - 400.

Wieczorek SK, Todd CD. 1998. Inhibition and facilitation of settlement of epifaunal marine invertebrate larvae by microbial biofilm cues. *Biofouling* **12** (1-3): 81 - 118.

Wieczorek SK, Clare AS, Todd CD. 1995. Inhibitory and facilitatory effects of microbial films on settlement of *Balanus amphitrite amphitrite* larvae. *Marine Ecology Progress Series* **119**: 221 - 228

Wiegemann M. 2005. Adhesion in blue mussels (*Mytilus edulis*) and barnacles (genus *Balanus*): Mechanisms and technical applications. *Aquatic Sciences* **67**: 166 - 176.

Wiegemann M, Watermann B. 2003. Peculiarities of barnacle adhesive cured on non-stick surfaces. J*ournal of Adhesion Science and Technology*. **17** (14): 1957 - 1977.

Wiegemann M, Watermann B. 2004. The impact of desiccation on the adhesion of barnacles attached to non stick coatings. *Biofouling* **20**: 147 - 153.

Wisely B. 1960. Experiments on rearing the barnacle *Elminius modestus* Darwin to the settling stage in the laboratory. *Australian Journal of Marine and Freshwater Research* **11** (1): 42 - 54.

Witte S, Buschbaum C, van Beusekom JEE, Reise K. 2010. Does climatic warming explain why an introduced barnacle finally takes over after a lag of more than 50 years? *Biological Invasions* **12**: 3579 - 3589.

Wood CD. Truby K, Stein J, Wiebe D, Holm E, Wendt D, Smith C, Kavanagh C, Montemrano J, Swain G, Meyer A. 2000. Temporal and spatial variations in macrofouling of silicone fouling-release coatings. *Biofouling* **16** (2): 311-322.

Woods Hole Oceanographic Institution (WHOI). 1952. *Marine Fouling and its Prevention.* US Naval Institute, Annapolis.

Wynne KJ, Swain GW, Fox RB, Bullock S, Uilk J. 2000. Two silicone nontoxic fouling release coatings: hydrosilation cured PDMS and $CaCO_3$ filled, ethoxysiloxane cured RTV11. *Biofouling* **16**: 277 - 288.

Yebra DM, Kiil S, Dam-Johansen K. 2004. Anti-fouling technology - past, present and future steps towards efficient and environmentally friendly anti-fouling coatings. *Progress in Organic Coatings* **50**: 75 - 104.

Yebra DM, Kiil S, Weinell CE, Dam-Johansen K. 2006. Presence and effects of marine microbial biofilms on biocide-based anti-fouling paints. *Biofouling* **22**: 33 - 41.

Yule AB, Walker G. 1984a. The temporary adhesion of barnacle cyprids: Effects of some differing surface characteristics. *Journal of the Marine Biological Association in the United Kingdom* **64**: 429 - 439.

Yule AB, Walker G. 1984b. The adhesion of the barnacle, *Balanus balanoides*, to slate surfaces. *Journal of the Marine Biological Association of the United Kingdom.* **64**: 147 - 156.

Yule AB, Walker G. 1987. Adhesion in barnacles. In. Southward AJ. (eds) *Crustacean Issue 5, Barnacle Biology*. AA Balkema, Rotterdam. pp 389 - 402.

Zardus JD, Nedved BT, Huang Y, Tran C, Hadfield MG. 2008. Microbial biofilms facilitate adhesion in biofouling invertebrates. *Biological Bulletins* **214**: 91 - 98.

Zhou Z, Calabrese DR, Taylor W, Finlay JA, Callow ME, Callow JA, Fischer D, Kramer EJ, Ober CK. 2014. Amphiphilic triblock copolymers with PEGylated hydrocarbon structures as environmentally friendly marine antifouling and fouling-release coatings. *Biofouling* **30** (5): 589 - 604.

Web references

Web reference 1: www.random.org

Web reference 2: http://www.metoffice.gov.uk/climate/uk/2011/winter.html

Web references 3: http://www.yachtpilot.net/crouch.html

Web reference 4: http://www.cefas.defra.gov.uk/our-science/observing-and-modelling/monitoring-programmes/sea-temperature-and-salinity-trends/presentation-of-results.aspx

Appendix 1: The Total Number of Barnacles Recruited in the Field on Silicone and Fluoropolymer Coatings.

Table A1.1. Total number of barnacles and number of adult barnacles recorded from the field in Burnham-on Crouch from four immersion periods, and in Fairlie Quay from 2010 on five silicone and three fluoropolymer coatings. Adult barnacles refer to barnacles over 3mm in diameter (Crisp & Davies 1955). No data available from Burnham-on-Crouch April 2010, images were taken from above, viewing the barnacles from the top, and therefore individual barnacles were not discernible.

Coating	*Total Number*	**Burnham-on-Crouch**				**Fairlie Quay**	
		April 2010	*June 2010*	*April 2011*	*July 2011*	*Elminius modestus*	*Semibalanus balanoides*
	Number of Adults						
S1	***Total***	**-**	**93**	**793**	**171**	**104**	**69**
	Adults	**-**	**67**	**207**	**144**	**79**	**69**
S2	***Total***	**-**	**78**	**568**	**215**	**41**	**34**
	Adults	**-**	**53**	**159**	**186**	**32**	**34**
S3	***Total***	**-**	**220**	**786**	**441**	**84**	**23**
	Adults	**-**	**189**	**282**	**371**	**71**	**23**
S4	***Total***	**-**	**415**	**732**	**361**	**119**	**48**
	Adults	**-**	**342**	**278**	**284**	**92**	**48**
S5	***Total***	**-**	**332**	**452**	**214**	**38**	**164**
	Adults	**-**	**274**	**307**	**188**	**29**	**164**
FP1	***Total***	**-**	**571**	**212**	**444**	**103**	**153**
	Adults	**-**	**475**	**135**	**273**	**84**	**153**
FP2	***Total***	**-**	**393**	**479**	**250**	**82**	**136**
	Adults	**-**	**256**	**293**	**185**	**58**	**136**
FP3	***Total***	**-**	**690**	**619**	**488**	**77**	**182**
	Adults	**-**	**441**	**315**	**412**	**57**	**182**

Appendix 2: The Monthly Surface Water Temperatures for the Irish Sea and the Thames.

The mean monthly temperature of the surface waters of two recording stations were accessed from CEFAS website (http://www.cefas.defra.gov.uk/our-science/observing-and-modelling/monitoring-programmes/sea-temperature-and-salinity-trends/presentation-of-results.aspx). The recording station Port Erin on the Isle of Man and Littlebrook in Kent were selected as these stations were the closest to the two field sites, Fairlie Quay and Burnham-on-Crouch, respectively, and had a complete record of the monthly temperature from 2009 to 2011.

Figure A2.1. Location of the recording stations Port Erin, Isle of Man and Littlebrook, Kent, in relation to the two field sites 1) Fairlie Quay, Ayrshire and 2) Burnham-on-Crouch, Essex.

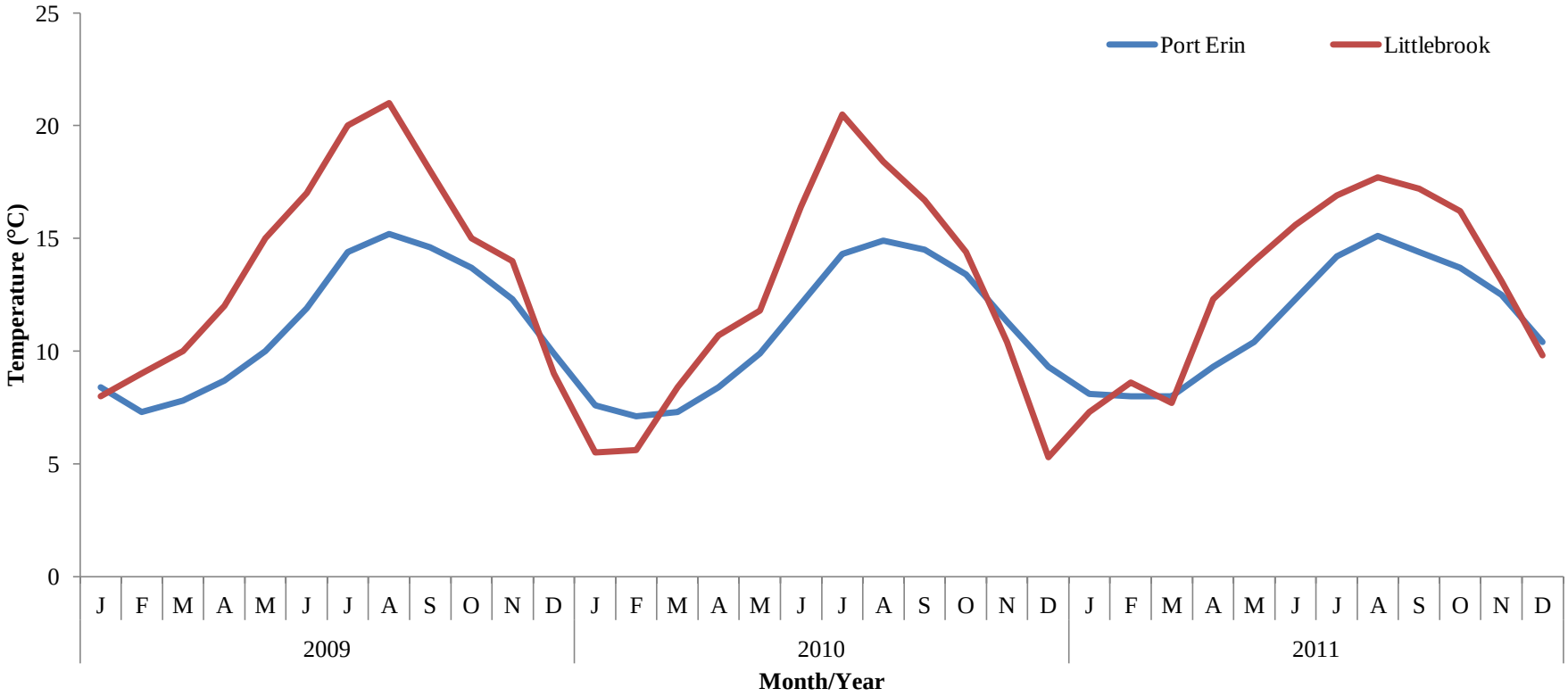

Figure A2.2. Mean monthly surface water temperatures from 2009 to 2011 for Port Erin, Isle of Man and Littlebrook, Kent. Source: CEFAS, Web reference 4.

Appendix 3. The Dynamic Mechanical Analysis of Silicone and Fluoropolymer Coatings.

A dynamic mechanical analyser (The Perkins Elmer Pyris Diamond DMA) was used to measure the modulus of the silicone and fluoropolymer coatings in Chapter 5. Sinusoidal oscillations were applied to a strip of the polymer which was heated from -140 to 70°C with a heating rate of 4°C/minute. The modulus at 22°C for two polymers strips per coating were averaged and provided the modulus presented in Chapter 5.

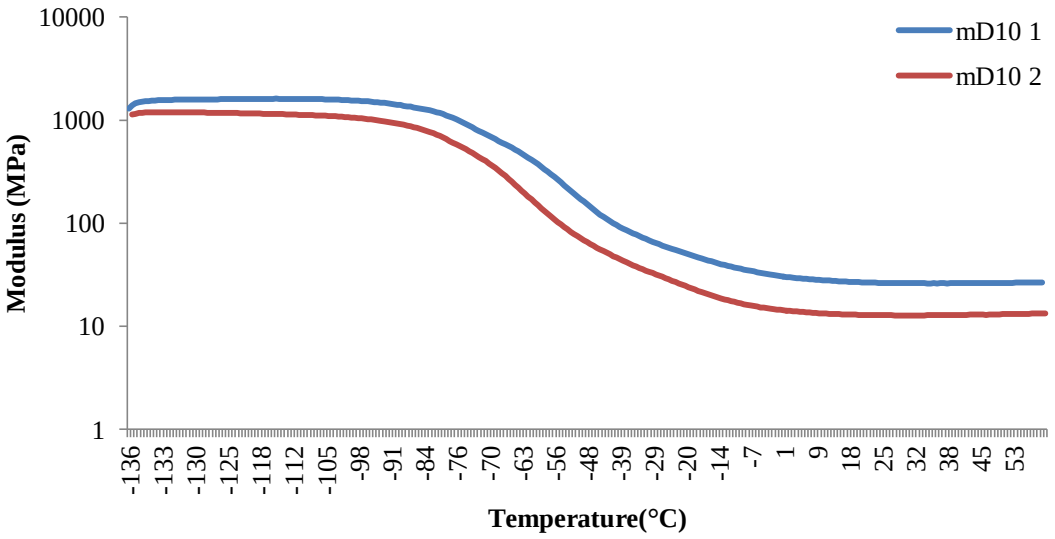

Figure A3.1. The elastic modulus of two mD10 polymer strips heated from -140 to 70°C with a heating rate of 4°C/minute measure using a dynamic mechanical analyser.

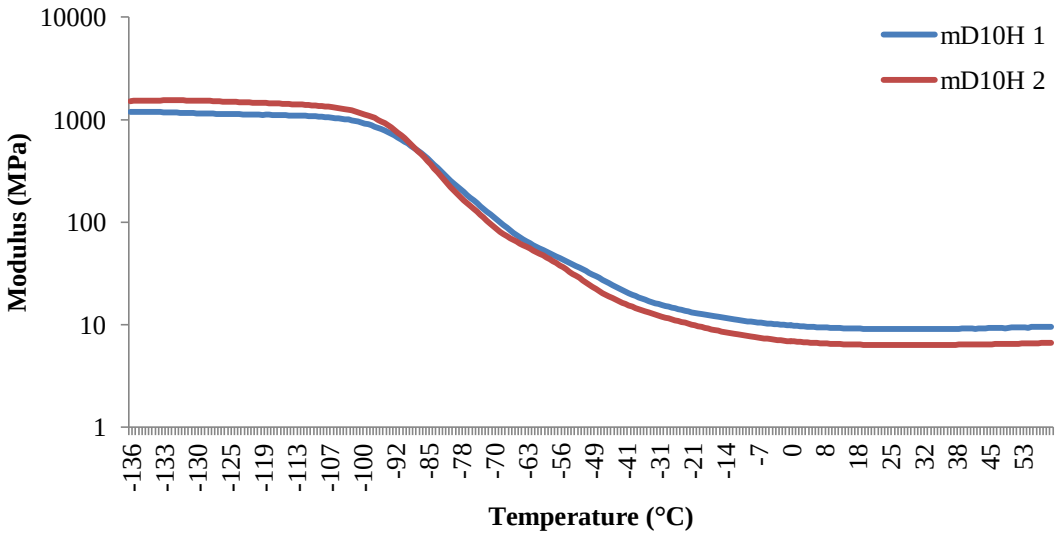

Figure A3.2. The elastic modulus of two mD10H polymer strips heated from -140 to 70°C with a heating rate of 4°C/minute measure using a dynamic mechanical analyser.

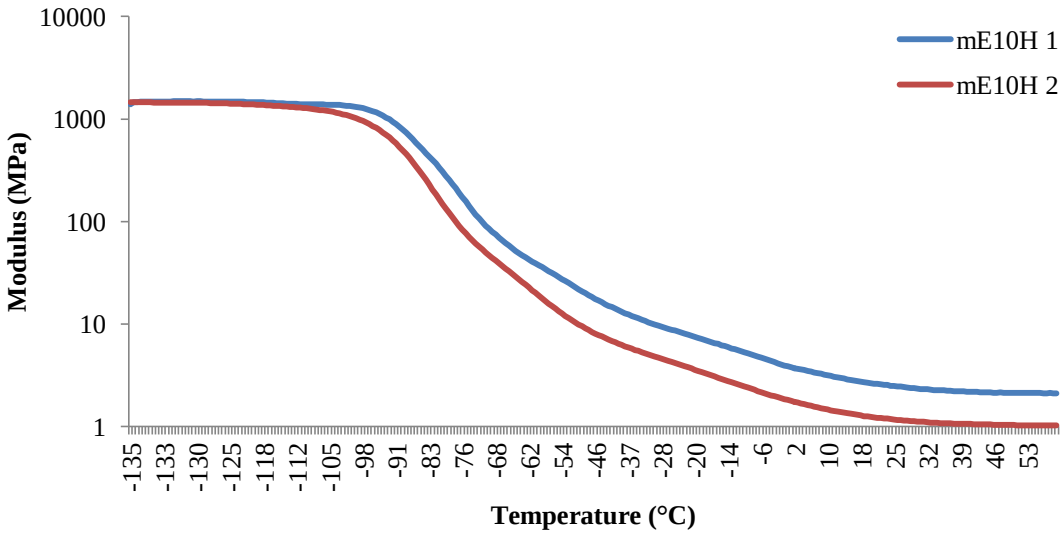

Figure A3.3. The elastic modulus of two mE10H polymer strips heated from -140 to 70°C with a heating rate of 4°C/minute measure using a dynamic mechanical analyser.

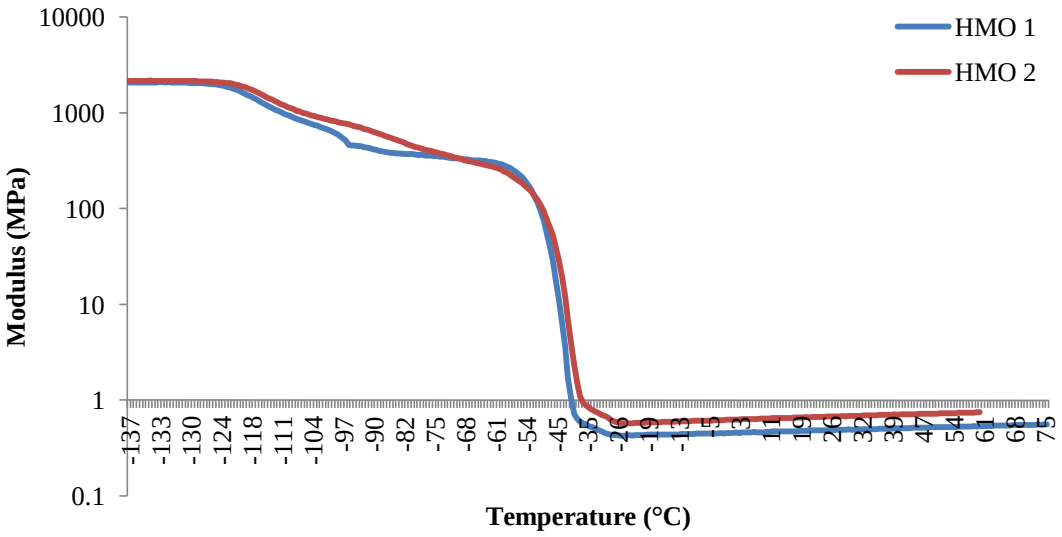

Figure A3.4. The elastic modulus of two HMod (HMO) polymer strips heated from -140 to 70°C with a heating rate of 4°C/minute measure using a dynamic mechanical analyser.

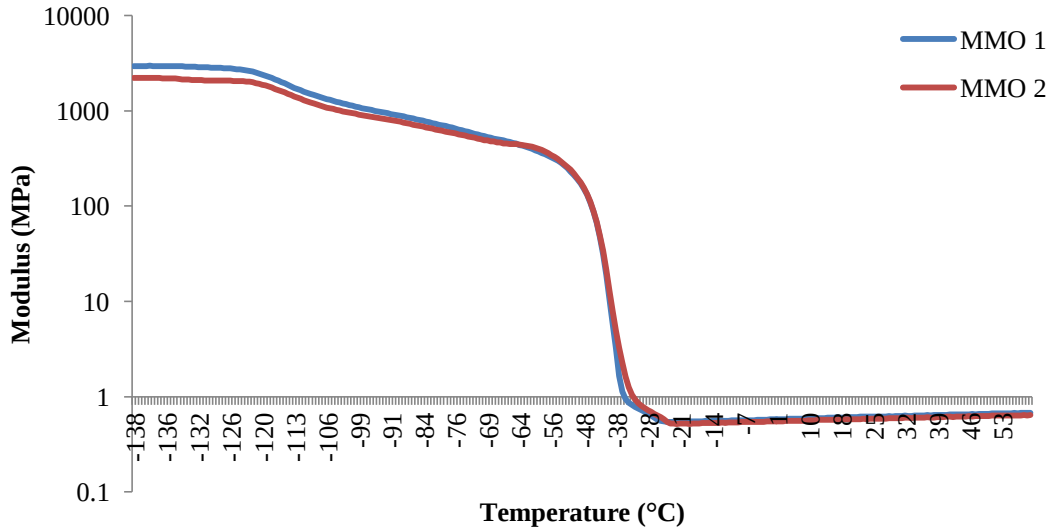

Figure A3.5. The elastic modulus of two MMod (MMO) polymer strips heated from -140 to 70°C with a heating rate of 4°C/minute measure using a dynamic mechanical analyser.

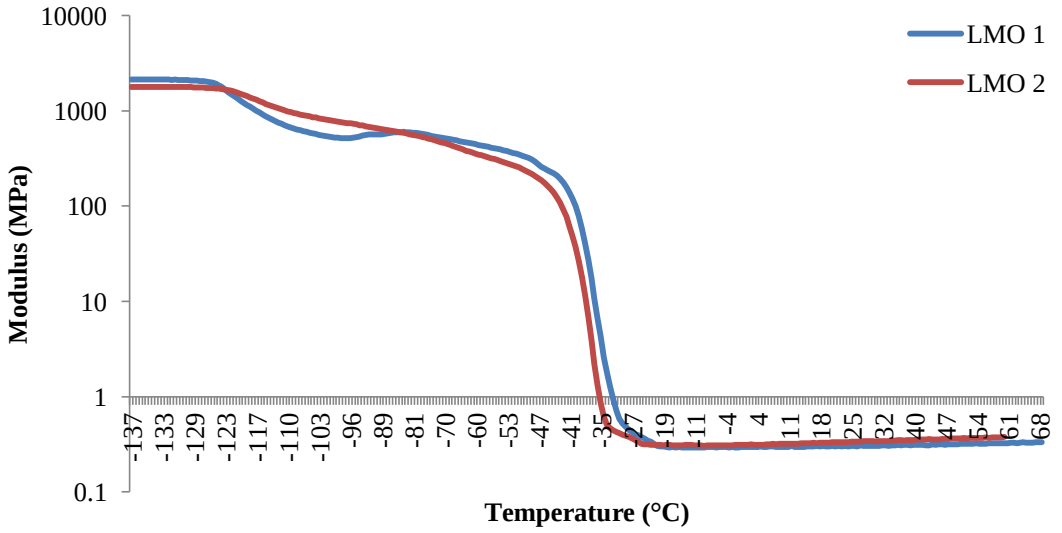

Figure A3.6. The elastic modulus of two LMod (LMO) polymer strips heated from -140 to 70°C with a heating rate of 4°C/minute measure using a dynamic mechanical analyser.

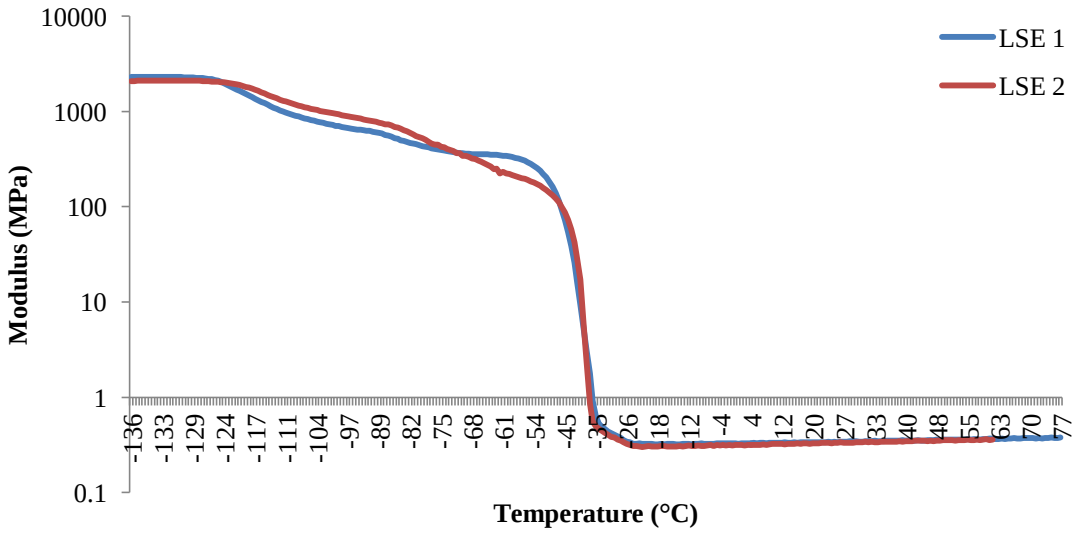

Figure A3.7. The elastic modulus of two LSE polymer strips heated from -140 to 70°Cwith a heating rate of 4°C/minute measure using a dynamic mechanical analyser.

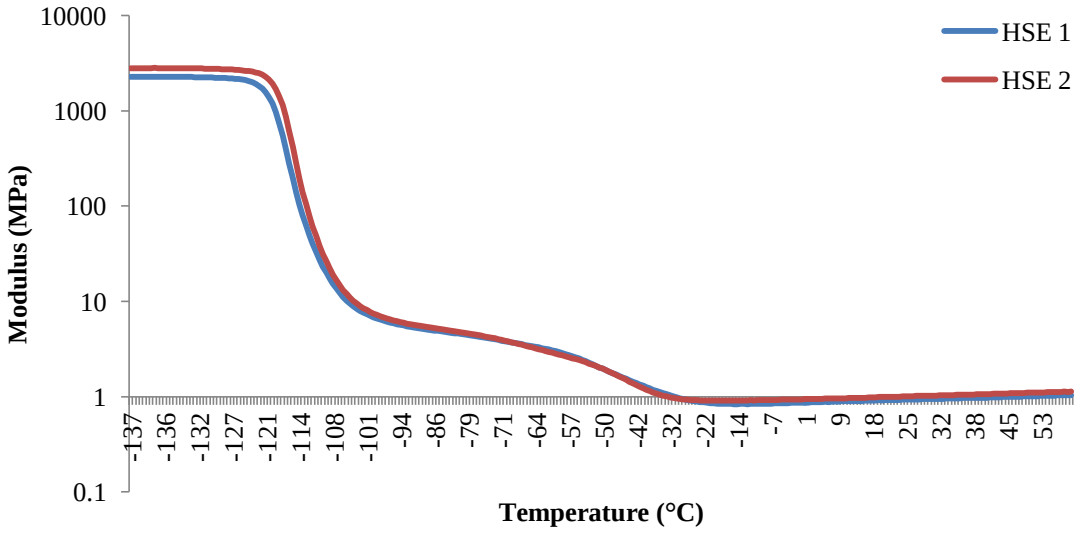

Figure A3.8. The elastic modulus of two HSE polymer strips heated from -140 to 70°C with a heating rate of 4°C/minute measure using a dynamic mechanical analyser.

Appendix 4. Exponential, Power, and Logarithmic Regression Results of the Critical Removal Stress Against the Elastic Modulus and $(E\gamma)^{1/2}$ of the Coatings from Chapter 5.

Table A4.1. Exponential regression results of the critical removal stress of *Balanus amphitrite* against the elastic modulus of silicone coatings (modulus range 0.31 to 0.66 MPa).

	Coefficient	*Standard Error*	*Standardized coefficient*	*t*	*P*
Intercept	0.024	0.005	0	4.473	0.008
Slope	1.198	0.441	0.248	2.714	< 0.001
Correlation coefficient	(r) = 0.248 (r^2) = 0.062				
Source	*df*	*MS*	*F*		*P*
Regression	1	4.299	7.364		0.008
Residual	112	0.584			

Table A4.2. Power regression results of the critical removal stress of *Elminius modestus* (A) and *Balanus amphitrite* (B) against the elastic modulus of silicone coatings (modulus range 0.31 to 0.66 MPa).

A	*Coefficient*	*Standard Error*	*Standardized coefficient*	*t*	*P*
Intercept	0.069	0.011	0	6.213	< 0.001
Slope	0.657	0.185	0.323	3.548	0.001
Correlation coefficient	(r) = 0.323 (r^2) = 0.104				
Source	*df*	*MS*	*F*		*P*
Regression	1	5.605	12.585		0.001
Residual	108	0.445			

B	*Coefficient*	*Standard Error*	*Standardized coefficient*	*t*	*P*
Intercept	0.066	0.012	0	5.628	0.008
Slope	0.549	0.205	0.246	2.681	< 0.001
Correlation coefficient	(r) = 0.246 (r^2) = 0.060				
Source	*df*	*MS*	*F*		*P*
Regression	1	0.012	10.991		0.001
Residual	112	0.001			

Table A4.3. Logarithmic regression results of the critical removal stress of *Elminius modestus* (A) and *Balanus amphitrite* (B) against the elastic modulus of silicone coatings (modulus range 0.31 to 0.66 MPa).

A	***Coefficient***	***Standard Error***	***Standardized coefficient***	***t***	***P***
Intercept	**0.066**	**0.006**	**0**	**11.497**	**< 0.001**
Slope	**0.021**	**0.007**	**0.297**	**3.235**	**0.002**
Correlation coefficient	**(r) = 0.297 (r^2) = 0.088**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**0.006**	**10.463**		**0.002**
Residual	**108**	**0.001**			

B	***Coefficient***	***Standard Error***	***Standardized coefficient***	***t***	***P***
Intercept	**0.077**	**0.008**	**0**	**10.125**	**<0.001**
Slope	**0.028**	**0.009**	**0.295**	**3.265**	**0.001**
Correlation coefficient	**(r) = 0.295 (r^2) = 0.087**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**0.012**	**10.991**		**0.001**
Residual	**112**	**0.001**			

Table A4.4. Exponential regression results of the critical removal stress of *Elminius modestus* against the elastic modulus of silicone and fluoropolymer coatings (modulus range 0.31 to 19.73 MPa).

	Coefficient	***Standard Error***	***Standardized coefficient***	***t***	***P***
Intercept	**0.055**	**0.003**	**0**	**19.629**	**< 0.001**
Slope	**0.080**	**0.006**	**0.643**	**13.656**	**< 0.001**
Correlation coefficient	**(r) = 0.643 (r^2) = 0.413**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**79.150**	**186.497**		**< 0.001**
Residual	**265**	**0.424**			

Table A4.5. Logarithmic regression results of the critical removal stress of *Elminius modestus* against the elastic modulus of silicone and fluoropolymer coatings (modulus range 0.31 to 19.73 MPa).

	Coefficient	*Standard Error*	*Standardized coefficient*	*t*	*P*
Intercept	0.081	0.003	0	24.706	< 0.001
Slope	0.044	0.002	0.803	21.943	< 0.001
Correlation coefficient	(r) = 0.803 (r^2) = 0.645				
Source	*df*	*MS*	*F*		*P*
Regression	1	1.125	481.488		< 0.001
Residual	265	0.002			

Table A4.6. Exponential regression results of the critical removal stress of *Elminius modestus* (A) and *Balanus amphitrite* (B) against the square root of the surface energy and elastic modulus ($(E\gamma)^{1/2}$) of the silicones and fluoropolymers.

A	*Coefficient*	*Standard Error*	*Standardized coefficient*	*t*	*P*
Intercept	0.186	0.006	0	31.289	< 0.001
Slope	0.045	0.003	0.718	16.864	< 0.001
Correlation coefficient	(r) = 0.718 (r^2) = 0.516				
Source	*df*	*MS*	*F*		*P*
Regression	1	25.293	284.407		< 0.001
Residual	267	0.089			

B	*Coefficient*	*Standard Error*	*Standardized coefficient*	*t*	*P*
Intercept	0.144	0.010	0	14.648	< 0.001
Slope	0.119	0.012	0.591	10254	< 0.001
Correlation coefficient	(r) = 0.591 (r^2) = 0.349				
Source	*df*	*MS*	*F*		*P*
Regression	1	15.983	105.147		< 0.001
Residual	196	0.152			

Table A4.7. Power regression results of the critical removal stress of *Elminius modestus* (A) and *Balanus amphitrite* (B) against the square root of the surface energy and elastic modulus ($(E\gamma)^{1/2}$) of the silicones and fluoropolymers.

A	***Coefficient***	***Standard Error***	***Standardized coefficient***	***t***	***P***
Intercept	**0.114**	**0.006**	**0**	**20.661**	**< 0.001**
Slope	**0.458**	**0.022**	**0.780**	**20.384**	**< 0.001**
Correlation coefficient	**(r) = 0.780 (r^2) = 0.609**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**29.854**	**415.507**		**< 0.001**
Residual	**267**	**0.072**			

B	***Coefficient***	***Standard Error***	***Standardized coefficient***	***t***	***P***
Intercept	**0.092**	**0.009**	**0**	**9.943**	**< 0.001**
Slope	**0.684**	**0.061**	**0.624**	**11.173**	**< 0.001**
Correlation coefficient	**(r) = 0.624 (r^2) = 0.389**				
Source	***df***	***MS***	***F***		***P***
Regression	**1**	**17.811**	**124.825**		**< 0.001**
Residual	**196**	**0.143**			

Appendix 5. Atomic Force Microscopy of the Basal Membrane of *Elminius modestus*.

Atomic force microscopy (AFM) (Department of Material Science and Metallurgy, University of Cambridge) in tapping mode was used to image the adhesive morphology of *Elminius modestus*. The barnacles were grown on Rhodorsil 48V-750 silicone elastomers. The silicone coated microscope slides were immersed in Fairlie, Ayrshire, in 2010 for 5 months. After this time they were transferred to the laboratory and maintained in 20L of aerated ASW fed *Tetraselmis* sp. 3 times a week for 6 weeks. Live and freeze dried specimens were tested. Freeze dried specimens were removed from the silicone coatings, only barnacles which had intact basal plates were used, these were rinsed in Milli-Q ultra pure water, and then frozen at -80 ºC and dried for 24hrs in a freeze dryer. Live specimens were removed and were rinsed in Milli-Q water immediately prior to AFM imaging.

Imaging the live barnacles proved unsuccessful, the movement of the barnacle inside the shell moved the basal membrane too much for a clear image to be recorded. Dissecting the body of the barnacle from the shell leaving the basis intact was also unsuccessful and did not produce a clear image. The tapping of the cantilever was sufficient to vibrate the basis and prevent an image being recorded. Other attempts included dissecting the membrane from the shell immediately prior to test run, however this desiccated at a very rapid pace. Immersing the samples in water would avoid desiccation, however, this was not attempted at this juncture.

Freeze dried samples did successfully produce images. However, due to the freeze drying the state of the basis and adhesive would have been altered and may not provide a true image of the basis. Nevertheless the following are examples of the images from the AFM of the freeze dried adhesive from two *E. modestus* barnacles. Although no further analysis was completed due to limited time and resources.

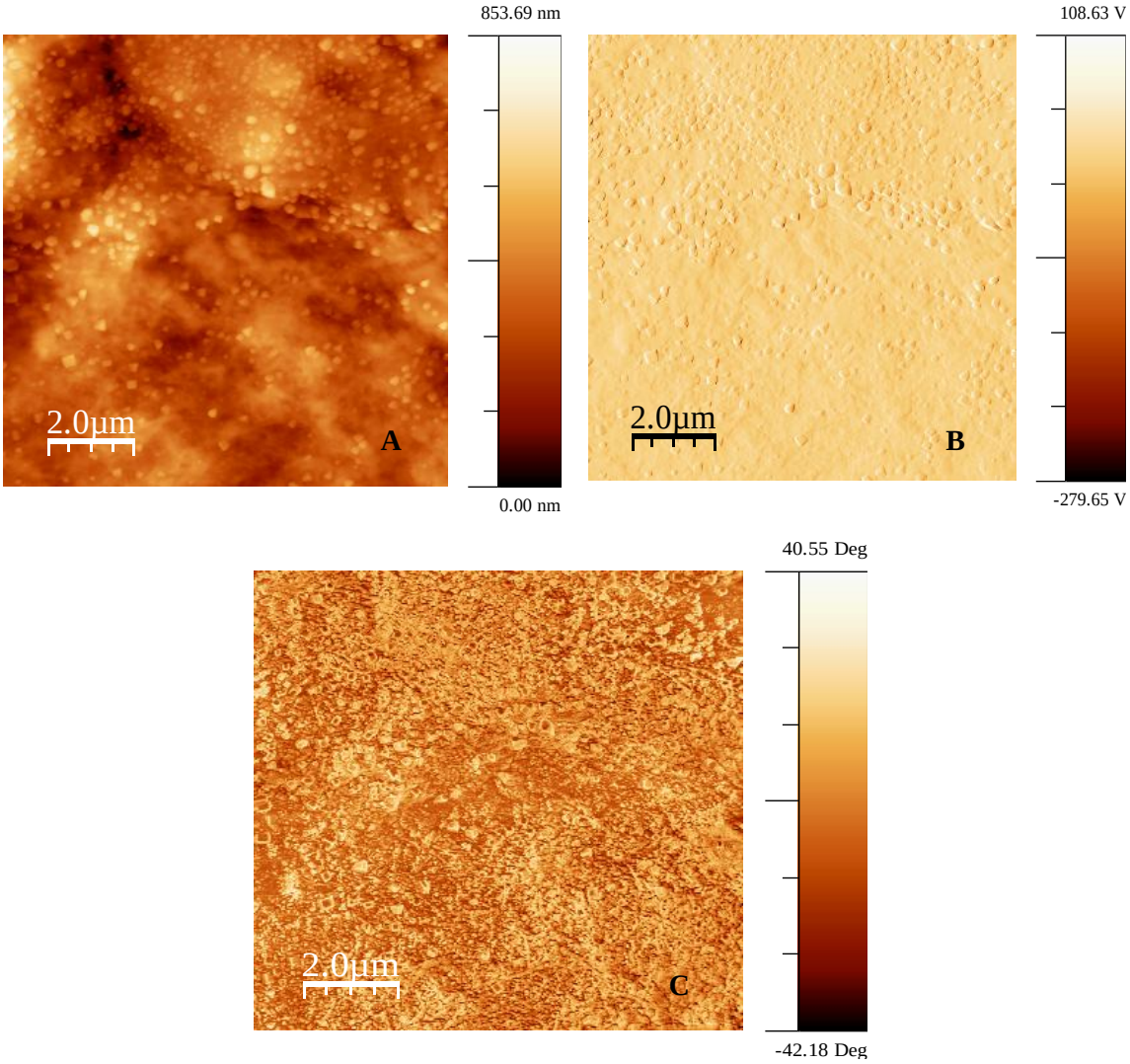

Figure A5.1. AFM images of the adhesive of a freeze dried *Elminius modestus* at 10µm^2. A) topographic image, B) amplitude image and C) phase image.

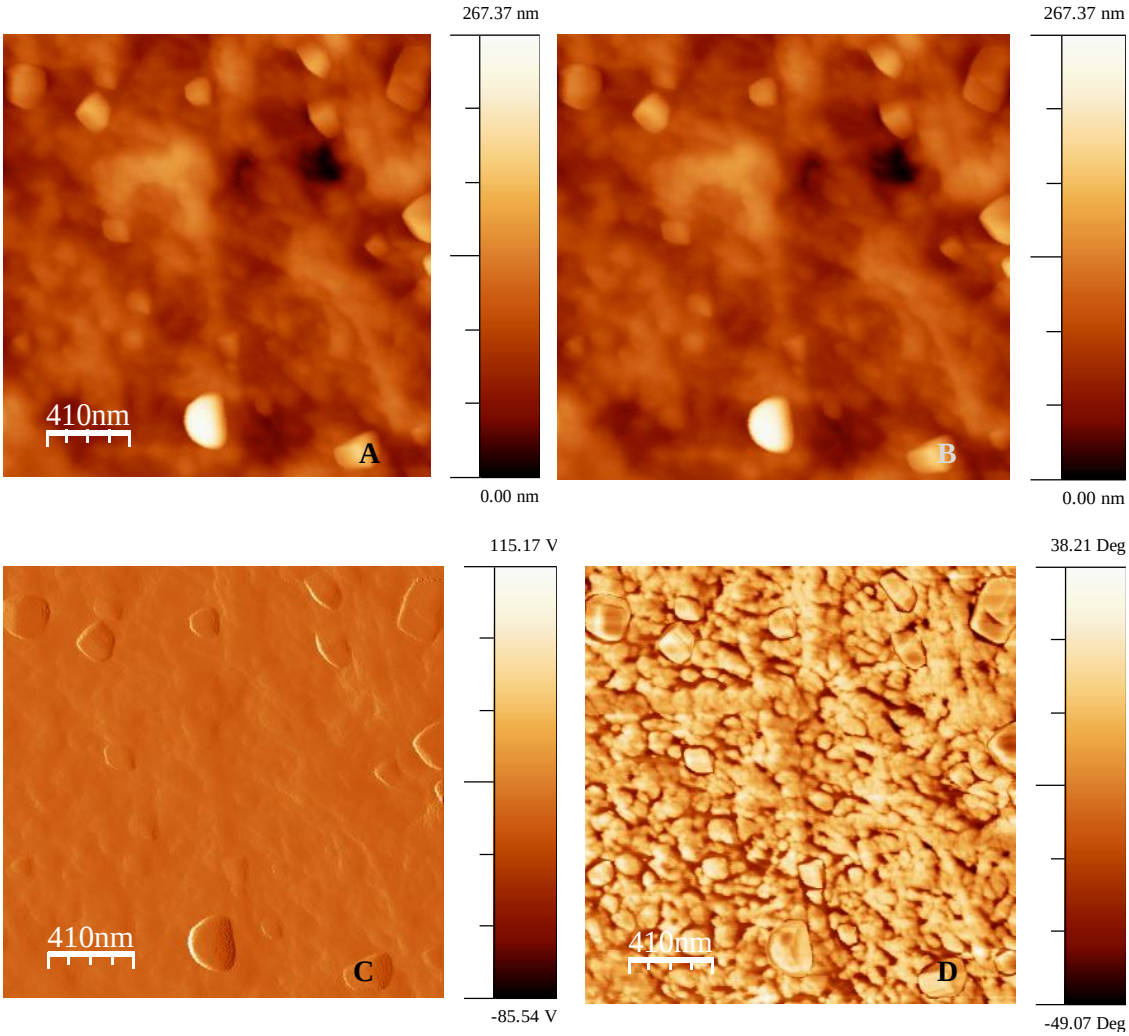

Figure A5.2. AFM images of the adhesive of a freeze dried *Elminius modestus* 2µm^2, A) topographic image, B) 3d topographic image C) amplitude and D) phase image.

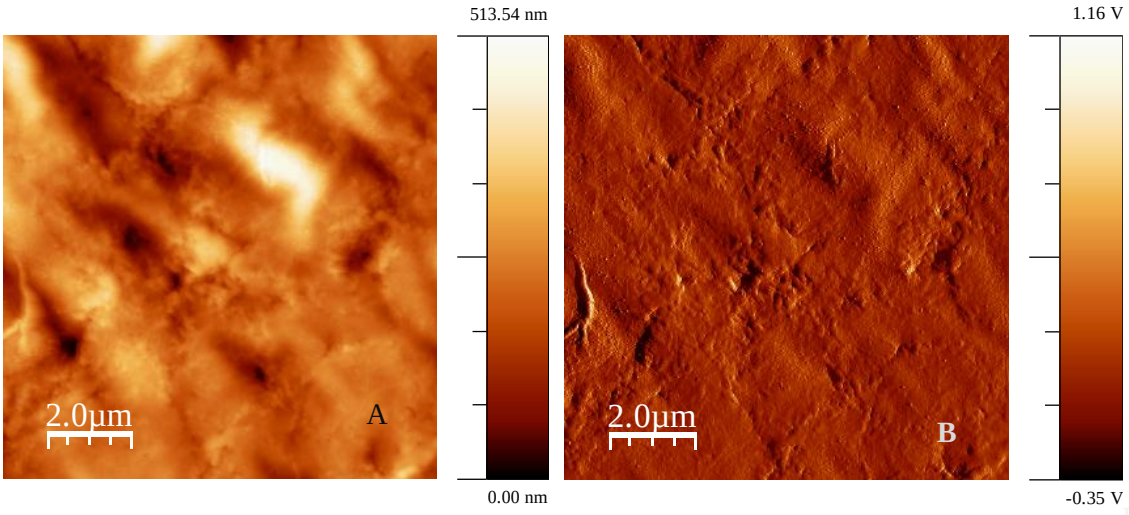

Figure A5.3. AFM images of the adhesive of a freeze dried *Elminius modestus* at 10µm^2. A) topographic image, and B) amplitude image.