Risk taking in *Semibalanus balanoides*

By Amanda Tomas

A thesis submitted for the degree of Doctor of Philosophy

School of Biology
Newcastle University.
August 2014

Abstract

This thesis examined factors influencing the success of the larval cyprid phase, and the distribution of adult *Semibalanus balanoides* in the Clyde Sea. Experiments were conducted in the Clyde Sea area (2004 – 2007) because it is effectively both a closed system in its whole extent for release of nauplii and cypris settlement, and at a larger scale it is an open system for the actual settlement on individual rock types. The effect of geology, exposure and position on the shore is examined in relation to barnacle fecundity (number of eggs produced) with metamorphic rock, exposed shores and mid shore settlement of *Semibalanus balanoides* having higher barnacle fecundity than igneous and sedimentary rock types, sheltered shores and upper or lower shore height areas. Frustum volume was found to be a reliable non-destructive way to estimate fecundity, and egg size did not alter significantly from year to year.

Supply was most reliably measured using pumps or adhesive panels, and artificial rock panels as an alternative to natural rock; the majority of cypris left after four seconds investigating artificial panels. Cannibalism takes place in the Clyde Sea with 44 % of adults investigated on the shore showing signs of this, frustum size and height didn’t affect propensity to cannibalise. Video recording demonstrated a mean of nine cypris and / or nauplii consumed per adult per hour. Settlement occurred on all major rock types in the Clyde Sea. Cypris preferentially settled on metamorphic and igneous rock types. There was no significant effect of north or south orientation on vertical panels for settlement intensification. Settlement levels were higher on natural rock substratum than artificial panels, but were not significantly different; and showed larger per area settlement on smaller areas as 1 cm^2 had a mean settlement of 49.40 cypris, 5 cm^2 18.40, 10 cm^2 22.42, 50 cm^2 13.92, 100 cm^2 9.34 and 500 cm^2 5.65. On natural rock around the Clyde Sea (as opposed to areas cleared for experiments), areas of < 1 cm^2 and 1 cm^2 are more common as potential settlement sites, comprising of 51.99 % and 23.98 %, respectively of available settlement area.

The mean number of barnacles in the Clyde Sea area was calculated to be 9.454×10^{11}, which could produce a mean number of 1.444×10^{15} eggs.

Acknowledgements

I would like to thank my initial supervisor, Dr Jeremy Thomason for his constant support even when he left the university. He encouraged, inspired and advised me throughout. His patience in teaching statistics was truly amazing as was his broad knowledge of the marine environment. Dr Gordon Port was extremely generous with his time, advice and patience as first of all my second supervisor then my only supervisor; I couldn't have had better supervisors. This thesis would not have been possible without the support of my husband Neil who believed in me and advised me on the mapping; the patience, fortitude and understanding of my daughter Cara, son Adam and Jessica the cat (who would cuddle into me). The School of Biology was incredibly supportive and understanding of my illness (ME) beyond the call of duty, and I really appreciated it.

The work would not have been possible without the assistance in field work, Dr Phil Cowie deploying a frame from the pier at the University Biological Station, Millport and collecting adult *Semibalanus balanoides* when I was unable to get to Cumbrae. Colleagues in the Research Group helped in both field work and advice especially H. E. Sugden, G. S. Prendergast, H. Naser and C. M. Zurn. I would also like to thank the staff at the University Biological Station, and Fairlie Pier who both allowed access and experiments to be deployed from their piers.

I would like to thank the examiners Dr Phil Cowie (External) and Professor Matt Bentley (Internal) who provided much helpful advice during my viva.

Contents

1 Introduction 1

1.1 Characteristics of rocky shores and threats to rocky shore communities 1

1.2 Barnacles fouling boats and installations such as power stations 7

1.2.1 Barnacle adhesion 8

1.3 *Semibalanus balanoides* 10

1.3.1 Why choose this species? 10

1.3.2 Life history and Morphology 11

1.3.3 Distribution and abiotic factors 16

1.3.4 Supply side ecology 17

1.3.5 Predators 21

1.3.6 Gregariousness 22

1.3.7 Population (intraspecific competition) 22

1.3.8 Community (interspecific competition) 23

1.3.9 Post settlement mortality 24

1.3.10 Genetic effects 24

1.4 Why choose the Clyde Sea area? 25

1.5 Overview of thesis 27

1.5.1 Aims and objectives of thesis 27

2 Mapping, geology, bathymetric data and development of exposure ratings 30

2.1 Introduction 30

2.1.1 Clyde Sea area 30

2.2 Aims of chapter 33

2.3 Methods and materials 34

2.3.1 Mapping of the foreshore and its features 34

2.3.2 Geological mapping 36

2.3.3 Bathymetric mapping 39

2.3.4 Development of shore exposure ratings 46

2.3.5 Data analysis 48

2.4 Results 48

2.4.1 Mapping of the foreshore and its features 48

2.4.2 Geological mapping 54

2.4.3 Bathymetric mapping 57

2.4.4 Combined mapping to OSGB 36 59

2.4.5 Exposure rating of different shores 61
2.5 Discussion 68
2.6 Conclusions 72
3 Fecundity 73
3.1 Introduction 73
3.2 Aims of chapter 79
3.3 Methods and materials 80
3.3.1 Sample collection 80
3.3.2 Non-destructive estimation of fecundity 85
3.3.3 Determination of fecundity 87
3.3.4 Data analysis 89
3.4 Results 90
3.4.1 Fecundity estimates 90
3.4.2 Relationship between rock type, exposure, density, shore height and barnacle density 97
3.4.3 Fecundity, egg size and somatic tissue for 2004 and 2005, fecundity and egg size for 2006 105
3.5 Discussion 109
3.6 Conclusions 115
4 Cyprid supply and the influence of substratum 116
4.1 Introduction 116
4.2 Aims of chapter 124
4.3 Methods and materials 125
4.3.1 Cyprid supply and settlement frame 125
4.3.2 Pumps 125
4.3.3 Traps 128
4.3.4 Plain settlement panels 128
4.3.5 Adhesive settlement panels 128
4.3.6 Deployment 128
4.3.7 Filming of cypris arrival, departure and settlement on artificial rock panels 129
4.3.8 Cypris settlement on natural rock versus artificial rock panels 131
4.3.9 Data recording and analysis 132

4.4 Results 134
  4.4.1 Cyprid supply and settlement frame 134
  4.4.2 Filmed settlement on artificial rock panels 139
  4.4.3 Natural versus artificial rock panels 143
4.5 Discussion 144
4.6 Conclusions 149
5 Cannibalism 150
5.1 Introduction 150
5.2 Aims of chapter 153
5.3 Methods and materials 153
  5.3.1 Gut contents analysis 154
  5.3.2 Filming of cannibalism 154
  5.3.3 Data analysis 155
5.4 Results 157
  5.4.1 Is *Semibalanus balanoides* cannibalistic? 157
  5.4.2 Does the size of the adult affect cannibalistic tendancies 160
  5.4.3 Encounter and capture rates 161
  5.4.4 Is increased height of adult an indicator to cannibalism? 163
  5.4.5 Does state of the tide affect cannibalism? 164
5.5 Discussion 167
5.6 Conclusions 172
6 Effect of rock type on settlement 173
6.1 Introduction 173
6.2 Aims of chapter 178
6.3 Methods and materials 179
  6.3.1 Data analysis 187
6.4 Results 187
  6.4.1 Effect of rock type on settlement 187
6.5 Discussion 189
6.6 Conclusions 192
7 Effect of available substratum area on settlement intensification 193
7.1 Introduction 193
7.2 Aims of chapter 195

- 7.3 Methods and materials 196
  - 7.3.1 Settlement on artificial panels 196
  - 7.3.2 Settlement on natural (rock) substratum 198
  - 7.3.3 Space available for settlement 202
  - 7.3.4 Data analysis 202
- 7.4 Results 203
  - 7.4.1 Settlement on artificial panels 203
  - 7.4.2 Settlement on natural (rock) substratum 205
  - 7.4.3 Space available for settlement 209
- 7.5 Discussion 210
- 7.6 Conclusions 214

8 Final Discussion 215

9 References 230

List of figures

Figure 1-1 *Semibalanus balanoides a*dults, newly metamorphosed juveniles and cypris 11
Figure 1-2 Configuration of the cirri (numbers 1-6 which are altered thoracic limbs) of *Semibalanus balanoides*. 13
Figure 1-3 Drawing of the United Kingdom with the Clyde Sea Area in the small box 26
Figure 1-4 Diagram of thesis overview and how chapters are linked together 29
Figure 2-1 Illustration of Gnomonic projection 40
Figure 2-2 Ordnance Survey data tidied 49
Figure 2-3 Mean Low Water Springs (MLWS in green) and Mean High Water Springs (MHWS in red) of the Clyde Sea area 51
Figure 2-4 MHWS with places shown for data collection and experiments in Clyde Sea area 53
Figure 2-5 Geology of the Clyde Sea area with MHWS to show the foreshore 55
Figure 2-6 Bathymetric map of Clyde Sea area, of all charts to OS36 projection and MHWS and MLWS 58
Figure 2-7 Combined map of Clyde Sea area, of Ordnance Survey data, geology and bathymetric data (OS36 projection) 60
Figure 2-8 Rose diagram or compass rose divided into 16 segments used to estimate fetch. 63
Figure 2-9 Sites split into sheltered and exposed by the Rose method 65
Figure 2-10 Sites split using Rose method as a whisker diagram 66
Figure 3-1 Photograph of quadrat taken at Carradale 86
Figure 3-2 Screen shot of ImageJ programme for counting areas of alive and dead barnacles, and area of rock and fucoid cover 86
Figure 3-3 The relationship between morphological index (MI) and the number of eggs per barnacle (data from 2004, 2005 and 2006). 92
Figure 3-4 The relationship between frustum volume (mm^3) and the number of eggs per barnacle (data from 2004, 2005 and 2006). 93
Figure 3-5 Regression fitted line plot to show the relationship between frustum volume and the number of eggs produced for 2004. 94
Figure 3-6 Regression fitted line plot to show the relationship between frustum volume and the number of eggs produced for 2005. 94

Figure 3-7 Regression fitted line plot to show the relationship between frustum volume and the number of eggs produced for 2006. 94

Figure 3-8 Regression fitted line plot of the internal measured volume of the 2004 barnacles (using dental impression material). 95

Figure 3-9 Linear regression between the dry weight of somatic tissue (mg^{-1}) and the dry weight of the eggs(mg^{-1}) for the corresponding barnacles for 2004 and 2005, showing 95% confidence intervals. 96

Figure 3-10 Grouped error bar plot (95% confidence interval) for the mean density of adult barnacles (cm^2) on different rock types for 2004, 2005 and 2006. 98

Figure 3-11 Scatterplot for the number of eggs produced (all years, all heights of shore and both exposures) against adult barnacle density (cm^2). 99

Figure 3-12 Error bar plot (95% confidence interval) showing the mean number of eggs produced in 2006 against exposure, ($p < 0.001$). 101

Figure 3-13 Error bar plot (95% confidence interval) showing the mean number of eggs produced in 2006 against position on the shore. 102

Figure 3-14 Error bar plot (95% confidence interval) showing the mean number of eggs produced in 2006 against rock type. 103

Figure 3-15 Error bar plot for mean number of eggs (95% confidence interval) produced each year. All rock types, exposures and positions on shore. 106

Figure 3-16 Error bar plot for mean number of eggs per mg^{-1} of dried somatic tissue. 108

Figure 3-17 Error bar plot for mean egg size for 2004, 2005 and 2006. 109

Figure 4-1 Diagram of pumps, traps, settlement panels and adhesive panels in Latin square design for deployment. 126

Figure 4-2 Photograph of Cyprid supply and settlement frame in Latin square design. 127

Figure 4-3 Diagram of natural and artificial rock frames 132

Figure 4-4 *Semibalanus balanoides*. Ordinary least squares regression of the number of cyprids (cm^{-2}) on the settlement panels verses the number of cyprids (l^{-1}) caught in the pumps for all depths and deployments. 135

Figure 4-5 *Semibalanus balanoides*. Ordinary least squares linear regression of larval supply. 136

Figure 4-6. *Semibalanus balanoides*. Interaction plot of transformed fitted means from the ANOVA of cyprids densities on plain settlement panels for cohort vs. experiment number. 137

Figure 4-7 *Semibalanus balanoides*, 3D surface plot of planktonic cyprid densities (cyprids l^{-1}) by depth and experiment number. 138

Figure 4-8 Raw data showing the total number of cyprids (880) that arrived on all artificial rock panels against the time in minutes when they arrived. 139

Figure 4-9 Bar graph for the total number of cypris (880) on all artificial rock types against how long the cypris stayed in seconds. 142

Figure 4-10 Settlement on artificial rock panels (substrate type 1), and natural rock (substrate type 2) showing 95 % Confidence limits. 143

Figure 5-1 Number of larvae eaten by date using remains of gut contents from *Semibalanus balanoides* from the shore of Isle of Cumbrae in March and April of 2007.. 159

Figure 5-2 Scatterplot of frustum (mm^3) volume against number of larvae consumed by *Semibalanus balanoides* collected from the Isle of Cumbrae shore in March and April of 2007. 160

Figure 5-3 Encounter rates per minute of larvae by adults of *Semibalanus balanoides* on the Isle of Cumbrae shore in April of 2007. 161

Figure 5-4 Capture rates of larvae by adults of *Semibalanus balanoides* on the Isle of Cumbrae shore in April 2007 for all tides. 162

Figure 5-5 Regression heights (mm) of adult barnacles *Semibalanus balanoides* against encounter rate per minute. 163

Figure 5-6 Grouped error bar plot (95% confidence interval) for the mean encounter rate (per minute) of cypris and adult *Semibalanus balanoides* and the state of the tide. . 164

Figure 5-7 Grouped error bar plot (95% confidence interval) of univariate GLM ANOVA for the mean capture rate by adult *Semibalanus balanoides* percentage per hour and the state of the tide. 166

Figure 6-1 Fairlie Commercial Pier where the effect of rock type on

settlement experiment was conducted. 179
Figure 6-2 Frame 1 front showing igneous, metamorphic from Carradale, red Sandstone, Loch Fyne metamorphic and yellow sandstone. 180
Figure 6-3 Igneous rock. 180
Figure 6-4 Loch Fyne Metamorphic. 182
Figure 6-5 Metamorphic from Carradale. 182
Figure 6-6 Yellow sandstone. 184
Figure 6-7 Red sandstone. 185
Figure 6-8 Mean cumulative cyprid settlement per tile over a day and night high tide (classed as a day). 188
Figure 7-1 Fairlie Sands (OS ref. NS2080 5620), Clyde Sea, showing site of the settlement panels experiment. 196
Figure 7-2 Schematic example of arrangement of settling panels for combined A = area settlement and for B = a single area of settlement. 198
Figure 7-3 Natural rock settlement arrangement to be read as one line. 200
Figure 7-4 Parameter b from Table 7-3 showing 95% confidence interval for settlement intensification (areas). 206
Figure 7-5 Graph showing settlement intensification from the transformed data using Estimate marginal means for settlement (area in cm^2). 208

List of tables

Table 2-1 Classes of rock shown in geology layer. 37
Table 2-2 Charts used for bathymetric map. 46
Table 2-3 Areas of rock types available for settlement. 56
Table 2-4 Modified maximum fetch determined by Wright (1981) and Baker and Crothers (2009). 61
Table 2-5 Results of univariate GLM ANOVAs using a variant of Modified Maximum Fetch. 62
Table 2-6 Fetch using the Rose system for calculation of exposure. 64
Table 2-7 Site, grid references, years used, exposure and rock type. 68
Table 3-1 Summary table of sampling locations, years, methods used and the number of replicates. 85
Table 3-2 Best sub-set regression with predictor as eggs for December 2004, 2005 and 2006. 91
Table 3-3 Adult barnacle density (adults cm^2) on different rock types for 2004, 2005 and 2006 data combined. 97
Table 3-4 *Post-hoc* test using transformed fecundity to find significant contributors to fecundity in the Clyde Sea for 2006. 100
Table 3-5 Position on shore sub-sets to find effects of shore height on fecundity for 2006. 102
Table 3-6 Custom table of mean and 95 % confidence interval values of eggs produced (cm^2) from barnacles on different rock types, different tidal heights on the shore, and different exposure using 2006 data set. 104
Table 3-7 Mean egg sizes and the number of eggs per barnacle per year with 95 % confidence intervals. 105
Table 3-8 Sub-sets for the mean number of eggs produced for 2004, 2005 and 2006. 107
Table 4-1 The numbers of cypris collected using pumps, adhesive panels, traps and settlement panels at Fairlie Commercial Pier, Clyde Sea. 134
Table 4-2 Number of cyprids that stayed or left from each type of artificial rock panel during the filming period. 140
Table 5-1 Gut contents of 100 *Semibalanus balanoides* collected from the Isle of Cumbrae shore in March and April of 2007. 157

Table 5-2 Number of larvae (nauplii and / or cyprids) in gut contents of 100 *Semibalanus balanoides* collected from the Isle of Cumbrae shore in March and April of 2007. 158
Table 5-3 Sub-sets for the mean number of encounters per minute and the state of the tide. 165
Table 5-4 Sub-sets for the mean capture rate percentage per hour and the state of the tide. 167
Table 6-1 Ryan-Einot-Gabriel-Welsch Range. 189
Table 7-1 Ryan-Einot-Gabriel-Welsch Range. 204
Table 7-2 Mean raw data for settlement panels. 204
Table 7-3 Area results of raw data for settlement intensification. 205
Table 7-4 Ryan-Einot-Gabriel-Welsch Range. 207
Table 7-5 Raw data of natural rock percentages per area (cm^2), available for settlement (rounded to two decimal places from areas). 209
Table 8-1 *Calanus finmarchicus and Semibalanus balanoides* larval stage areas for comparison of size. 223

Chapter 1. Introduction

1.1 Characteristics of rocky shores and threats to rocky shore communities

One type of coastal border to the sea is the rocky shore. The rocky shore has a spatial and temporal heterogeneity (Cervin et al. 2004). Organisms living on the rocky shore can be subjected to a large range of physical conditions, from occupying a large flat rock directly in the Sun's path, to living deep in cracks and crevices, or under boulders and overhangs of rock (Sousa 1985, Miron et al. 1999, Menge & Branch 2001, Little & Kitching 2005, Baker & Crothers 2009). The habitat can change due to the height on the shore within the intertidal, to deep below the sea's surface and the direction of the compass the individual rock faces (Little & Kitching 2005). The extent of exposure to waves, their swell, and splash zone can also alter the habitat of the rocky shore (Miron et al. 1999, Little & Kitching 2005). Many shores may be composed of smaller boulders (> 256 mm) or cobbles (64 – 256 mm) as well as outcrops of rock, and barnacles may be found on all of these (Wentworth 1922, McClintock et al. 2007). Cobbles and small boulders encrusted with barnacles may indicate that the shore is very sheltered, and that physical disturbance events only occur rarely at a temporal span greater than a year (McClintock et al. 2007).

Below the sea level, generally the annual temperature will fluctuate by less than 10^{o} C, however, on the rocky shore when the tide has gone out the more variable air temperature can easily change over the tidal cycle by 10 – 20^{o} C, and subsequently the year by 40^{o} C depending on where on the planet the rocky shore is (Raffaelli & Hawkins 1999). Many animals can only forage (as many are filter feeders or predators of filter feeders) and take in oxygen through the water while they are immersed. Emersion (time in the air) can cause stresses to the fauna and flora due to exposure, as can humidity and fresh water due to precipitation, and in rock pools this causes changes in salinity (Raffaelli & Hawkins 1999, Bernhardt & Leslie 2013, Kuklinski et al. 2013). The higher temperatures can cause possible desiccation to tissues, with enzyme breakdown causing permanent damage and inefficient respiration (Bertness & Gaines 1993, Begon et al. 1999, Menge & Branch 2001, Chan & Williams 2003, Little & Kitching 2005, Jones et al. 2012). Many organisms such as limpet (*Patella vulgate*), dog whelks (*Nucella lapillus*), periwinkles (*Littorina littorea*) and barnacles attach firmly to the substratum or close any breathing holes to help prevent desiccation; this however does not eliminate heat stress from the heating rock to which they are attached (Bernhardt & Leslie 2013, Cahill et al. 2013). Low temperatures in the sea or air can cause tissues to break due to ice forming in cells, and ice scour removing whole organisms, or even whole communities from the rocks that they occupy (Little & Kitching 2005, Macpherson et al. 2008, Cahill et al. 2013). The action of the waves especially during storms, especially if they contain flotsam can damage animals and plants on the intertidal areas (Menge & Branch 2001, Little & Kitching 2005).

The intertidal gradient on the rocky shore causes a vertical stress slope and zonation, where organisms higher on the shore are more tolerant of the heat stress (Connell 1961a, b, Connell 2003). Lower down the shore, animals and plants are less tolerant but may out-compete the animals and plants higher up the intertidal for example *Chthamalus* spp. and *Semibalanus balanoides* (Connell 1961a, b, Connell 2003).

Encrusting lichens and cyanobacteria are found at the top of the intertidal rocky shore in the splash zone, littoral fringe or supralittoral fringe, and support small littorinid snails (Raffaelli & Hawkins 1999). Lower down a dark area of lichen (*Verrucaria* spp.) and cyanobacteria supports small invertebrates such as isopods, mites and nematodes which are then predated by small crabs (Raffaelli & Hawkins 1999). In temperate zones ephemeral algae are also found here, but in high latitudes this layer may be removed by ice scouring during the winter (Raffaelli & Hawkins 1999, Macpherson et al. 2008).

The midlittoral (intertidal), or eulittoral zone of the rocky shore has algae, sessile and mobile animals such as limpets (*Patella, Acmaea, Cellana* spp.), mussels (*Aulacomya* spp., *Mytilus* spp., and *Perna* spp.) and acorn barnacles such as *Balanus, Semibalanus, Elminius*, *Chthamalus* and *Tetraclita* spp. (Raffaelli & Hawkins 1999). Lower down, eulittoral barnacles including *Balanus* spp. (acorn barnacles) and *Pollicipes* spp. (goose barnacles); mobile herbivores (that are able to move over the whole zone when there is a high tide) such as true limpets (*Patella* spp., *Acmaea* spp., and *Cellana* spp.), keyhole limpets such as *Fissurella* spp., pulmonate limpets (*Siphonaria* spp.), predatory whelks such as dog whelks (*Nucella* spp.), and topshells (*Trochids* spp.) (Raffaelli & Hawkins 1999). Starfish (such as *Asterias* spp. and *Pisaster* spp.), crabs (for instance *Cancer* spp. and *Carcinus* spp.), and shanny's (*Lipophrys* spp.) may move up the intertidal at high tide to feed on fauna and flora; but during low tides birds such as seagulls may predate intertidal animals (Little & Kitching 2005). In temperate regions large algae may offer cover for the animals, for example *Laminaria* spp. *Fucus vesiculosus, Fucus serratus*, and *Ascophyllum nodosum* can cause a blanket cover on the mid shore in the northern hemisphere (Raffaelli & Hawkins 1999, Little & Kitching 2005). Around Australia *Macrocystis* spp., are more evident in sheltered coasts and are patchy in moderately exposed shores of the intertidal (Raffaelli & Hawkins 1999, Little & Kitching 2005). Fucoids are mainly absent from exposed shores due to propagules being dislodged before they are able to attach, or after attachment due to wave action and / or the effect of drift wood and other detritus in the waves. Herbivores such as limpets graze algae allowing space for sessile animals to attach to the substratum. In warmer regions Ascidians may be found, as may the upper edge of corals and Tunicates; and sea urchins (Echinoidea) may move up to graze especially in the North - East Pacific (Raffaelli & Hawkins 1999).

Many of the animals in the intertidal have a pelagic larval form which transports them away from their natal home shore (Gaines & Roughgarden 1985). These propagules and pelagic larvae may also act as food for larger zooplankton and fish.

The sublittoral is the area above the continental shelf and below the intertidal; in this rocky area competition and constant predation may prevent species from the intertidal colonising these lower shores. However, modern structures that are permanently covered by the sea such as ship's hulls, oil rigs and newer quays, breakwaters or well-designed coastal defences may be colonised by intertidal fouling organisms.

The upper limits of the intertidal fauna and flora are set by physical factors (abiotic factors) such as heat, desiccation and ice, while the lower limits are set by biotic factors such as competition from grazing and predation (Jenkins 2005, Helmuth et al. 2006a, b, Porri et al. 2007, Macpherson et al. 2008).

Global warming may have an effect on all shores, causing raising sea levels by thermal expansion of the upper levels of the sea, melting of glaciers around the world and the reduction or total thawing of ice caps (Hawkins et al. 2008, Hawkins et al. 2009, Kuklinski et al. 2013). Although the effect on a shore depends on where it is, for example the rocky shore in the north and west of Scotland is rising due to the isostatic rebound from the last ice age (the melting of the glacial ice mass there) (Raffaelli & Hawkins 1999, Root et al. 2002, Hawkins et al. 2008, Hawkins et al. 2009, Jones et al. 2012). Bleaching of corals on areas of the eulittoral caused by warmer waters can cause the destruction of this important habitat, and the loss of breeding and nursery grounds for fish. Increased temperature of the substratum may mean fauna that had previously been within its thermal stress limits, now experience lethal conditions (Bertness et al. 1999, Root et al. 2002, Wethey et al. 2011, Jones et al. 2012, Bartolini et al. 2013, Michaelidis et al. 2014). Increased temperatures may cause desiccation of shore life, but this will alter from area to area around the globe (Raffaelli & Hawkins 1999, Bernhardt & Leslie 2013).

More energy in the system may cause stronger waves on sheltered shores, and their biota will change to species more tolerant of exposure; there may be increased turbidity reducing the feeding ability of filter feeders, possibly reducing fitness and the input of eggs and zooplankton for higher trophic levels (Przeslawski et al. 2005, Helmuth et al. 2006a, Helmuth et al. 2006b, Wethey et al. 2011, Bernhardt & Leslie 2013).

Cold water upwellings in some areas may bring food supplies (Bernhardt & Leslie 2013, Eerkes-Medrano et al. 2013), but if these alter due to global warming, then this again may alter the fauna and flora of that particular rocky shore.

Cooling water from power stations is usually warmer than the ambient water temperature around the installation, therefore the water temperature may alter the composition of the community, i.e. *Semibalanus balanoides* do not reproduce in warmer water therefore warmer areas are more amenable to *Chthamalus* spp. or *Elminius modestus* (Lawson et al. 2004).

Fishing in coastal waters with poisons such as cyanide and bleach, and explosives, damage fish stocks that are not sought after and corals, as well as other fauna and flora of that ecosystem. Damage is also caused by anchors of fishing boats and / or boats that take snorkelers and scuba divers to look at the substratum and its inhabitants (Raffaelli & Hawkins 1999, Nybakkon & Bertness 2005, Castro & Huber 2008). Scuba divers may help distribute bacteria and toxins between corals as they move over a reef, or even between reefs if they dive them in succession and do not dry out or sterilise equipment and wet suits between contaminated areas and other reefs. Fishing from the shore may damage organisms from lost fishing hooks and lead weights. In Southern Europe stalked barnacles, large acorn barnacles, sea urchins, crabs and octopus are collected for food as are some algae both in Europe and other parts of the world (Raffaelli & Hawkins 1999). Inappropriate and over fishing of mussels and oysters could reduce these biota beds due to the inhabitants being immediately reduced to the point of local extinction of the colony, or it being reduced so that reproduction is no longer affective within the larger area (Raffaelli & Hawkins 1999, Castro & Huber 2008).

The collection of shells will kill the inhabitants, if the collector is not careful to make sure that the shell is not occupied and could reduce predator and / or prey species; those remaining organisms may not reproduce effectively due to ‘recruitment overfishing’ where some animals have the sex ratio of populations altered (Raffaelli & Hawkins 1999). Shore animals dried for curios and sessile fauna such as mussels used for bait, may alter the wider community or make the organism’s locally extinct (Raffaelli & Hawkins 1999).

Sewage nutrients can smother rocky shore species that cannot move if there is discharge into the sea near the shore, and the nutrients may stimulate macro algae (for example *Enteromorpha, Cladophora* and *Ulva*) to grow and cover the animals (Raffaelli & Hawkins 1999). If algae trap silt or sand this may be good for burrowing animals, but the animals that affix to rock may be smothered and die, and may not be replaced by larval stages brought to the substratum by the action of the sea currents, also those animals that predate them may die due to their food source being removed.

Blooms of toxic dinoflagellates (red tides) are natural events that affect the animals on the shore, for example barnacles and mussels that filter feed, and grazers such as limpets. However, plant life such as fucoids appear to be less affected (Raffaelli & Hawkins 1999). Some red tides are caused by humans through eutrophication of the shore and coastal waters due to fertiliser run off and sewage outfalls causing blooms of *Gyrodinium aureolum*, *Chrysochromulina polylepis* and *Ceratium* spp. reducing fish stocks (Raffaelli & Hawkins 1999).

Oil pollution by the now illegal practise of cleaning out tanks at sea, by oil and diesel seeping from wrecks on the sea floor, a grounded ship or oil spill at a refinery may smother the animals and plants on the rocky shore moved by the action of currents, waves and wind; exposed rocky shores are less affected than sheltered rocky shores due to the break-up of the oil by the action of the waves (Raffaelli & Hawkins 1999). If the oil is trapped by vegetation or sediment it takes longer for the shore to recover. Dispersants for the oil also has toxic effects on shore animals. A rocky shore can take fifteen years to recover through natural recolonization (Raffaelli & Hawkins 1999, Castro & Huber 2008).

Chemicals that have spilled by industrial processes, runoff, or been illegally dumped in rivers can affect the rocky shore communities. This can include heavy metal pollution from current mining activities, or past activities where mining has stopped. However, it has been found that some barnacles store zinc and copper, as granules segregated from the main cells and systems (Pullen & Rainbow 1991, Raffaelli & Hawkins 1999, Clare 2013).

Introductions of alien species can also affect rocky shore communities. Examples include: *Elminius modestus* (a barnacle from Australia to North West Europe that replaces the native barnacles such as *Semibalanus balanoides* by out competing the local fauna) (Lawson et al. 2004); *Littorina littorea* (a mollusc from Europe to Eastern USA); and *Sargassum muticum* (a macrophyte from Japan to Europe). These species may be transported by ships on their hulls, ballast waters and air boats (Raffaelli & Hawkins 1999). Canals, such as the Suez Canal transporting organisms from the Red Sea to the Mediterranean, and the Panama Canal between the Caribbean and the Pacific (although fewer organisms have managed to use this route), are known to increase the spread of alien species (Raffaelli & Hawkins 1999).

Coastal development by infilling lagoons, salt marshes or the rocky foreshore covered by housing or coastal defences (if not designed correctly), mean the loss of important habitats (Raffaelli & Hawkins 1999). For example, the dykes of the Netherlands are coastal defences that stop erosion of reclaimed land (Raffaelli & Hawkins 1999).

Antifouling paints containing Tributyltin TBT, were found to slough off very small amounts of these chemicals causing ‘imposex’ (penis like outgrowths), in dog whelks (*Nucella lapillus*) on the rocky shore, sterilising the females and also blocking egg release which caused the death of females (Raffaelli & Hawkins 1999, Finnie & Williams 2010).

Rocky shore communities are thus exposed to a wide range of natural and man-made stresses which will affect the component species in a number of ways.

1.2 Barnacles fouling boats and instillations such as power stations

Barnacle larvae have been shown to be the second most abundant larvae in samples of zooplankton from Southampton Water, averaging 13 % but sometimes representing up to 60 % and are therefore an important food supply for plankton eating fish (Muxagata et al. 2004). On the North East coast of North America, *S. balanoides* can represent up to 15 % of zooplankton (Gyory & Pineda 2011). Nauplii and then cyprids are meroplanktonic (in the plankton for a restricted length of time), before settling on substratum (Chan & Williams 2003, Svensson et al. 2004). Barnacles foul both natural and man-made structures in the sea from hydrothermal vents to the top extent of the intertidal zone, whales, ships (resulting in reduced efficiency contributing to global warming), piers, slipways and cooling water systems of power stations and desalination plants where they reduce the diameter of intake pipes (Schultz et al. 1999, Berglin et al. 2005, People 2006, Aldred & Clare 2008, Southward 2008, Polman et al. 2013). In 1997 the world’s commercial fleet used 700 mt of fuel oil costing $2.16 billion, but barnacle fouling (light to heavy) increased the costs to between $2.19 - $2.77 billion, and a decrease of speed for wind powered yachts of up to 28 % (Thomason et al. 1998).

1.2.1 Barnacle adhesion

Since legislation against the use of metal-based antifouling coatings, especially organotins, interest in barnacle settlement has increased in order to ultimately develop nontoxic antifoulants and better forms of gauging their adhesive strength (Clare 1998, Conlan et al. 2008). Until recently there have been few investigations of cyprid adhesion compared to adult barnacle adhesive. In time synthetic temporary reversible underwater adhesives may also be developed from cypris temporary adhesive (Aldred et al. 2013b). It is thought that there are three types of adhesive: cypris temporary adhesive, cypris permanent adhesive (or cement) and adult adhesive or cement.

When a cypris is exploring a substratum for settlement, it leaves behind antennular secretions (‘footprints’) that are known as cypris temporary adhesive, and can be detected by the protein dye reagent Coomassie Brilliant Blue (Dreanno et al. 2006, Phang et al. 2009). These ‘footprints’ are oval in shape, and for *Balanus amphitrite* measure 34.8 ± 6.5 µm and approximately 50 µm for *Semibalanus balanoides* (Dreanno et al. 2006, Phang et al. 2008). This proteinaceous secretion acts as a settlement cue similar (related immunologically) to the adult settlement inducing protein complex (SIPC), for subsequent exploring cyprids and a temporary adhesive or ‘wet adhesion’ (Dreanno et al. 2006, Phang et al. 2006, 2008). This secretion allows the cypris to explore the substratum fully without being ‘washed off’ by currents and waves, therefore making them one of the few marine animals that are able to use reversible adhesion (Aldred et al. 2010, Aldred et al. 2013b). However, it has been shown that the cypris constantly moves its ambulatory antennules if it needs a weak attachment, and flattens them out onto the surface it is exploring if it needs a strong attachment, using strength and a flicking motion to remove itself (Aldred et al. 2013b). The temporary adhesive is secreted onto the two attachment discs of the ambulatory antennules by modified tegumentary glands (hypodermal glands) in the second antennular segment, allowing the bi-pedal ‘walking’ behaviour of up to two body lengths a second (Dreanno et al. 2006, Aldred and Clare 2008). As the attachment discs have cuticular villi like structures producing a nanotextured surface, it is proposed that this may help adhesion by van der Waals forces (‘dry adhesion’), if the temporary adhesive also works as a water displacer (Dreanno et al. 2006, Aldred and Clare 2008, Phang et al. 2008). It has been found with *Semibalanus balanoides*, that the cypris on smooth glass have a removal strength of 0.068 – 0.076 MPa, and produce approximately three times as much secretion on glass surfaces that are R-NH_2 terminated, than on R-CH_3 suggesting that a cypris would be able to ‘walk’ 530 ± 20 footprints on the R- NH_2 and 1600 ± 40 R-CH_3 glass (Aldred and Clare 2008, Phang et al. 2008).

Cypris permanent cement or cyprid cement of *B. amphitrite* is thought to be a distinct proteinaceous cement unrelated to either the cyprid temporary adhesive or the adult cement, as genes coding the adult cement are not expressed in cypris (Aldred and Clare 2008). The cement is thought to originate from two different cell types in glands within the cypris body itself (Phang et al. 2006, Aldred and Clare 2008, Aldred et al. 2013a). In *B. amphitrite*, the cement is known to be extruded through antennular cement ducts forming a disc < 100 µm in diameter, which covers the third and fourth segments of the antennules, permanently attaching the cypris for metamorphosis into the adult form (Phang et al. 2006, Aldred and Clare 2008). Phang et al. (2006) suggest that this is a dual – component cement, not completely mixed as it is expressed, and it cures by molecular chain cross – linking (possibly quinine cross – links between phenolic amino acids) which significantly changes the protein conformation. Aldred et al. (2013a) also found that the adhesive was not homogeneously mixed; sometimes this led to the adhesive remaining as two different deposits depending on the substratum that the cypris was attaching to. It is estimated that the cement takes 116 minutes for the surface layer proteins, and possibly 15 hours for the entire cement disc to cure (Phang et al. 2006, Aldred and Clare 2008). The force to pull off the cypris from the substratum would be approximately 2.5 nN for the cements cured outer layer (Phang et al. 2006).

Adult permanent cement or adult permanent adhesive, is produced throughout the barnacles life, is formed in glands or giant cells closely associated with ovarian tissue that measure 200 – 300 µm in diameter in *S. balanoides*, and is secreted through ducts that transect the basis (basal membrane) (Aldred and Clare 2008). It has been suggested that there are two types of adult adhesive, primary and secondary, but this might be due to the curing of the protein composition (Aldred and Clare 2008). The primary cement forms a thin layer between the basis of the barnacle and the substratum, which when cured is translucent and stiff (Aldred and Clare 2008). In *Megabalanus rosa* it comprises of two hydrophobic and insoluble polypeptides, with molecular masses of 52 kDa and 100 kDa, that are structural proteins to which other adhesive polypeptides attach such as 19 kDa and 68 kDa which are more soluble (Aldred and Clare 2008). A 20 kDa polypeptide is thought to be involved in calcite bonding, with the 68 kDa a surface primer (Aldred and Clare 2008). The secondary cement is secreted after a barnacle has been partially removed or wounded, is opaque and has a 'filler' role, although barnacles grown in the laboratory on low – modulus silicone coatings produce more of this cement suggesting that this type of cement may be better to adhere on that type of substratum (Aldred and Clare 2008).

1.3 *Semibalanus balanoides*

1.3.1 Why choose this species?

Semibalanus balanoides has a well-defined hermaphrodite reproductive stage (Gaines & Bertness 1992, Hyder et al. 1998, Thomason et al. 2000) and was the only barnacle species recruiting intertidally at the time of the experiments, therefore the nauplii and cyprids caught were known to be of this species (Thomason et al. 2002a). This reduced the time that would have been used to identify every cypris and nauplii to species level, and allowed ease of plankton counts, counts of cypris on the shore (where they prefer to settle), and to demonstrate cannibalism.

Semibalanus balanoides fouls most man-made objects within the intertidal zone and shallow sublittoral (although it has been found down to 63 m), if there is little competition or predation (both at the shore and out to sea), including power station in-flows, quays, ship's and boat's hulls (Stubbings 1975, Southward 2008, Polman et al. 2013). This can reduce the smooth flow of water, causing increased fuel consumption and time in dry dock for ships to have the barnacles and other fouling organisms removed (Aldred & Clare 2008). *S. balanoides* is found below *Chthamalus montagui* in the Clyde Sea, as it can out compete *Chthamalus* spp. in the mid – intertidal by overgrowing, crushing or undercutting, but *Chthamalus* has a greater tolerance to desiccation so can live higher up the shore (Connell 1961a, Connell 2003).

1.3.2 Life history and morphology

Semibalanus balanoides (Linnaeus, 1767) is a boreo-arctic barnacle with a range extending from Spitzbergen in the north, to the north of Spain at the southern extent of its European range (Darwin 1854, Jenkins et al. 2000, Thomason et al. 2000, Southward 2008). It also has populations on the east coast of north America in the Atlantic and on the west coast of north America in the Pacific (Barnes 1963, Barnes & Barnes 1967, Stubbings 1975, King et al. 1993, Holm & Bourget 1994, Begon et al.1999, Jenkins et al 2000, Schmidt et al. 2000, Thomason et al. 2000, Brind'Amour et al. 2002, Pineda et al. 2002, Hills & Thomason 2003b, Lawson et al. 2004, Svensson et al. 2005, Burrows et al. 2010, Gyory & Pineda 2011, Jones et al. 2012, Gyory et al. 2013). *S. balanoides* larvae require a temperature of 6 – 12 ^{o}C for their development (Lawson et al. 2004). It has been suggested by Barnes (1953a) that there were two distinct populations relating to size of nauplii in the Clyde Sea area from different points on the shore, but in another population in Sweden this was not the case.

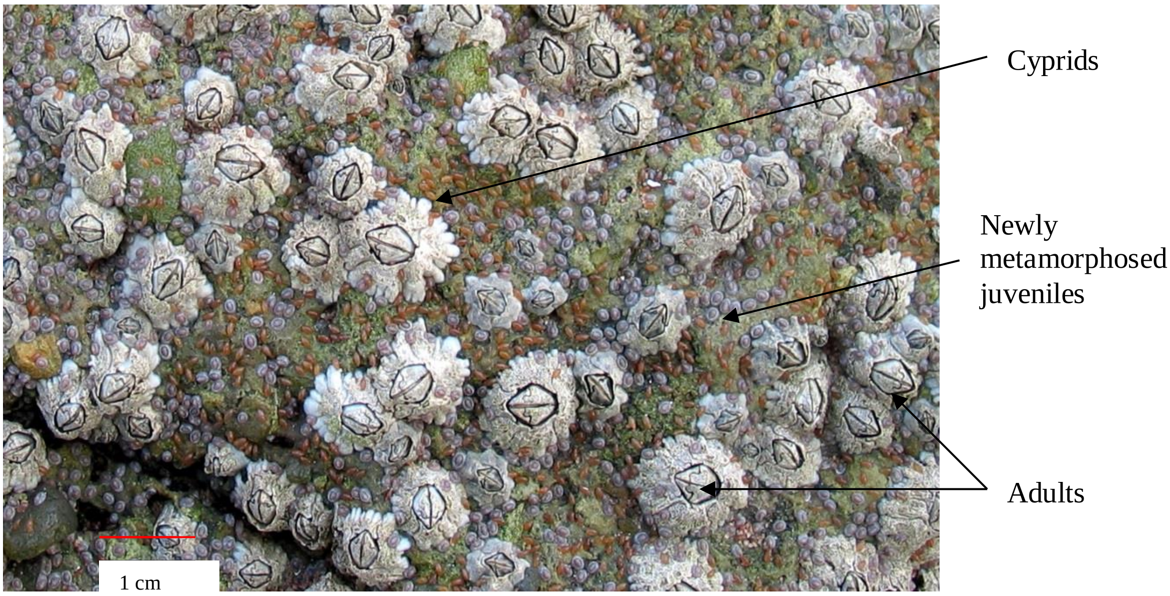


Figure 1-1 ***Semibalanus balanoides a*****dults, newly metamorphosed juveniles and cypris (© A. Tomas, April 2006)**

This species of barnacle has six plates, a membranous basis and a broad rostral plate that overlaps the two lateral plates which distinguish it from *Chthamalus* species, with a diamond shaped operculum where the sutures of the terga and scuta are sloped down towards the carinal end (Stubbings 1975, Southward 2008). An uncrowded adult (Figure 1-1) can easily reach 10 mm in diameter (although 25 mm has been known) and 8 mm high, if the barnacle is crowded on all sides, trumpet shaped specimens can occur that have a small 'foot-print' on the substratum to which it is attached, whilst being attached vertically to all its neighbours reaching recorded heights of 50 mm (Stubbings 1975, Southward 2008). Juvenile (Figure 1-1) *S. balanoides* can grow 1 mm a week, and in its first year the newly metamorphosed barnacle can grow to a diameter of 10 mm (Crisp 1961). If a growing barnacle touches another, it will grow in a different direction, and therefore for groups of barnacles, all available spaces are filled up (Crisp 1961). When they touch each other in all directions, the barnacles will grow upwards forming 'hummocks' (Crisp, 1961, Lopez & Gonzalez 2003). The position in a 'hummock' can have an effect on prey capture rates as those that are higher in the mound capture more than solitary barnacles, and barnacles lowest in a 'hummock' capture fewer prey than solitary barnacles (Mauck & Harkless 2001, Lopez & Gonzalez 2003).

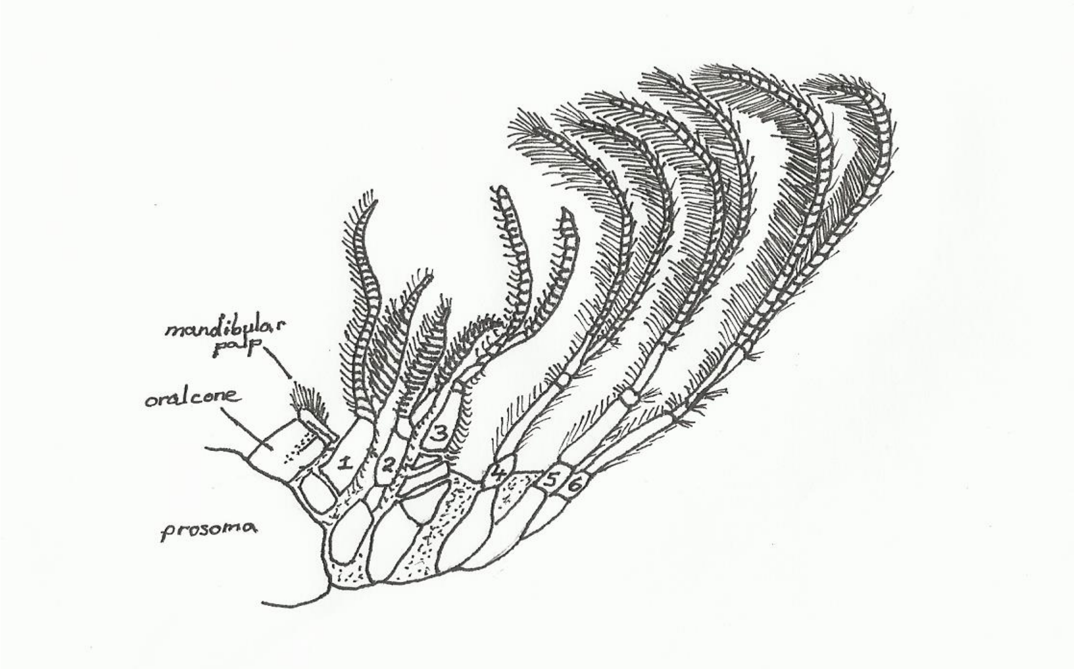


Figure 1-2 Configuration of the cirri (numbers 1-6 which are altered thoracic limbs) of *Semibalanus balanoides*, that the barnacle uses for food capture from the water, before passing to the mandibular palp, oral cone and digested (drawn from Southward (2008) and Stubbings (1975))

Semibalanus balanoides requires the water currents to bring it food, which it captures using its cirri that are thoracic limbs that have altered because the animal is sessile (Starr et al. 1991, Southward 2008). The six pairs of cirri differ slightly (Figure 1-2), with pairs 4 – 6 very similar although increasing in length to 6, pairs 1 – 3 are smaller and stouter and placed nearer the mouth, but all cirri have setae which are about 2 µm apart although the gaps in the cirral net are about 30 µm wide and are used as a food gathering net by extending them in the water column via increasing the haemocoel pressure (Stubbings 1975, Southward 2008).

There are five recognised activities of the cirri cited by Stubbings (1975):
1: testing - where the cirri are within the shell but the operculum is open slightly
2: pumping – slight protrusion of the cirri but no extension, and the operculum valves move strongly. Respiration occurs due to the water current through the mantle cavity.
3: normal beat – the cirri are extended and withdrawn in rhythm with the operculum valves. Respiration occurs due to the water current through the mantle cavity. Micro-

feeding of bacteria and very small organisms (down to 2 µm) also occurs, caught by the smaller cirri of 1 and 2.
4: fast beat – strong and fast cirral beat but reduced opercular movement. Cirri 3 – 6 are used for the capture of organisms 1mm and smaller in relatively weak flows of the sea.
5: extension – where the cirri are held outside fully extended without regular movement (usually in response to a strong movement of the water), capturing organisms up to 1 mm.

Organisms are removed by either the maxillipedes or anterior cirri then swallowed, and a current of water is also moved through the mantle from the rostral end to the carinal end which serves as a respiratory mechanism but any food found in this, is filtered off and passed to the mouth parts (Southward 2008).

The setae of the cirri pierce the organisms caught in the net; transfer of the organisms to the mouth is triggered by the presence of L – glutamic acid, L – proline and potassium ions in the food (Stubbings 1975). The faecal pellets produced are cylindrical with blunt ends (Stubbings 1975). While the embryos are in the mantle cavity, fast beat gathering of food stops and food gathering reduces, it is possible that this activity poses a threat to the embryos retention (Stubbings 1975).

The outer calcareous plates (six in this species) are increased as the animal inside grows, but ecdysis occurs for the exoskeleton of the cirri, thorax and prosoma every 10 – 15 days, which ceases when the eggs are being brooded (Stubbings 1975, Southward 2008).

In Britain and in particular the Clyde Sea area, *S. balanoides* copulates internally (as all crustaceans do), in late November / early December (Barnes 1956, Stubbings 1975, Evans 1987, Giangrande et al. 1994, Southward 2008, Burrows et al. 2010). The embryos develop within the mantle cavity to be released in the Clyde Sea area in February and March (as nauplii) after the adults have fed (therefore there will be food for the nauplii), with the cypris settling in April or early May (Barnes 1956, Stubbings 1975). There is a clockwise release of nauplii by *S. balanoides* around Britain from the south–west of England past Scotland, to the north–east of England over a 2 - 2½ month period (Evans 1987). Sometimes there is an early release of nauplii, stage I and II have been found as early as the last week of February in the Clyde Sea (Evans 1987). A possible explanation for early release is damage through storms or predation, as the embryos are viable weeks before the phytoplankton bloom and adult damaged tissue would release the hatching substance (Evans 1987, Gyory & Pineda 2011). All stages of the process are food dependent (Barnes 1956, Stubbings 1975, Evans 1987, Giangrande et al. 1994, Southward 2008). Where the fertilised embryo membranes touch they adhere (unfertilised eggs don’t adhere to one another), and are oval in shape (Stubbings 1975). The embryos hatch after the adult has fed in the spring and has released a hatching substance (which may be prostaglandin or similar), which stimulates the nauplii to secrete dopamine producing muscular movements, and hatching may be on expulsion into the sea or within the mantle cavity, this ensures that the nauplii have an adequate food supply (Stubbings 1975, Starr et al. 1991). Small egg size gives rise to planktotrophic larvae, and reduces the amount of energy costs for the parents (Giangrande et al. 1994). Planktotrophy encourages high fecundity in the parents, but also results in high mortality for the larvae with dispersal, which allows recolonization of extinct populations and a large geographic range (Giangrande et al. 1994).

Semibalanus balanoides has six naupliar stages that are planktotrophic and the final cyprid stage which doesn’t feed (lecithotrophic), but is solely involved in finding a suitable settlement place in which to metamorphose into the sessile state (Darwin 1854, Stubbings 1975, Pechenik 1999, Southward 2008). Release into the plankton is stimulated by the spring phytoplankton bloom, the composition of the bloom relating to size of diatoms (for example *Skeletonema costatum* has a stronger effect than *Phaeodactylum tricornutum*), and is also dependent on the adult moulting (Evans 1987, Starr et al. 1991, Thomason et al. 2000). On the east coast of North America, release of nauplii was correlated with winter storms, suggesting that water turbulence and the associated increase in feeding may lessen the consumption of nauplii by adults consuming re-suspended particles (Gyory & Pineda 2011, Gyory et al. 2013). The first two naupliar stages are passed though relatively quickly, and then each of the naupliar stages takes about three to four days before moulting into the cyprid stage depending on food availability (Barnes 1956). The cypris are in the water column three to four weeks after nauplii I are released from the adults (Barnes 1956, Bertness & Gaines 1993), so that the whole planktonic stage lasts for up to six weeks (Thomason et al. 2000).

Barnes (1956) suggests that in the Clyde Sea there are ‘normal years’ where there is development as above, but in some years the nauplii stages failed at some point, and this he suggests is coincident with diatom irregularities or failure. Certainly it was found that in 1950 (a ‘normal’ year) there was high numbers (3000 cells / ml) of *Skeletonema*, but in subsequent ‘failure’ years of 1951 and 1952 this was virtually absent or late in April (Barnes 1956).

Nauplii I are photopositive, but nauplii II are phototactic when they are well fed and in strong sunlight which may be a survival adaptation as at the surface more phytoplankton are to be found for new nauplii (Stubbings 1975, Hills & Thomason 1998a, b, Gyory & Pineda 2011). It is also possible that at the surface there may be more predators that may find the nauplii just another zooplankton to be consumed (Muxagata et al. 2004). Cypris are initially photopositive, but then become photonegative to swim or sink down to possible settlement sites. While looking for a suitable settlement site the cypris appear indifferent to light (Stubbings 1975). Cyprids prefer grooves and indentations for settlement sites, and in the final settlement stages orientate themselves with their anterior end pointing towards the light (Stubbings 1975, Jenkins 2005). The metamorphosed individual changes its orientation to the currents and flow so that its carinal or anterior end is downstream, allowing for the cirral net to be deployed upstream (Crisp 1955, Stubbings 1975). If the adults are not predated or die due to exposure, they can live up to five or six years if they are higher on the intertidal zone; barnacles from lower sites on the intertidal usually die younger, possibly due to higher predation (Stubbings 1975). However, Barnes (1956) suggests that the life span in the Clyde Sea is four years at the most.

1.3.3 Distribution and abiotic factors

Semibalanus balanoides is generally found on the intertidal rocky shore from just above MLWS (mean low water spring), which is usually determined by interspecific competition or predation, to just below MHWS (mean high water spring) with the upper limit determined by heat and desiccation (Stubbings 1975, Little & Kitching 2005, Southward 2008). In Arctic waters it is usually found sublittorally occurring at depths up to 30 – 45 m in order to avoid ice scouring, and has been found down to 63 m (Stubbings 1975, Little & Kitching 2005, Southward 2008). Where *S. balanoides* is found at shallower depths in the Gulf of St. Lawrence, Canada; the ice scour contributes to low abundance of adults over-riding recruitment, resulting in a regional pattern of population structure (Cole et al. 2011). Marfenin et al. (2013) also found no correlation between recruitment and adult population of *S. balanoides* in the Arctic White Sea intertidal, suggesting population patchiness isn't spatially stable. *S. balanoides* requires air temperatures that are consistently (for at least 20 days) below 10 ^{o}C for gonad maturation, and cannot survive for very long in air temperatures about 25 ^{o}C and above (Begon et al. 1999).

S. balanoides is able to acclimatise to salinities 12 – 50, and can therefore take advantage of this wide range of salinities to settle in most areas of the Clyde Sea; although most of Clyde Sea salinity is in the region of 31-33 and well within this species tolerance levels (Foster 1970, 1971, Stubbings 1975, Hall-Spencer & Atkinson 1999, Albessard & Mayzaud 2003, White 2008).

1.3.4 Supply side ecology

Geomorphological factors such as upwellings, the surface Ekman layer, winds, and Langmuir surface foam lines (or surface slicks) can be an advantage to barnacle larvae being transported to the coast but a disadvantage if transported away from settlement sites (Barnes 1956, Raimondi 1988, Alexander & Roughgarden 1996, Bertness et al. 1996, Eckman 1996, Lagos et al. 2008, Burrows et al. 2010). Spatial variation can account for changes of recruitment site during differing settlement years or seasons, influencing population and community dynamics in coastal ecosystems (Crisp 1955, Barnes 1956, Bertness et al. 1996, Delany et al. 2003, Broitman et al. 2008, Lagos et al. 2008, Rilov et al. 2008, Burrows et al. 2010). However, larvae may be able to mitigate some of the disadvantages by behavioural responses, such as sinking into a different layer of water travelling in another direction, thereby remaining close to the shore (Porch 1998, Jenkins 2005, Mace & Morgan 2006, Broitman et al. 2008, Lagos et al. 2008). Cyprids and general zooplankton have been found to be more abundant in the lee of small headlands, bays and inlets due to eddies and slower tidal currents or flows

(Jenkins & Hawkins 2003, Mace & Morgan 2006). Jeffery (2002) indicates that cyprids accumulate together in the plankton, and gregarious settlement may be a result of this aggregation (Jeffery 2003, McQuaid & Miller 2010).

It is known that cyprids do not settle randomly (Grosberg 1982, McQuaid & Miller 2010). Cypris have a poor swimming ability in comparison to tides and local hydrodynamics (Olson 1985, Hills & Thomason 1996, 1998b). Cypris can travel a great distance in a current, for example Hughes (1990), suggests that a current speed of 1 m sec^{-1} would result in 84 km in 24 hours (Minchinton 1997). At the range of kilometres, sea and ocean currents, wind and tides are overwhelmingly important to cypris settlement, whereas at distances of less than a few tens of metres cypris selection becomes important (Walters 1992, Bertness et al. 1996, Hills & Thomason 1996, Miron et al. 1999, Jenkins 2005, Broitman et al. 2008). At distances of centimetres and millimetres it may be possible for the cypris to act actively, and to detect settlement substances excreted by other settling cypris and thereby encourage gregarious settlement (Bertness et al. 1996, Hills & Thomason 1996, 1998a, b, Miron et al. 1999, Jeffery 2002). Where *S. balanoides* is found in a river-fed bay or close to rivers, flushing due to high rainfall may reduce larval retention and settlement (Bertness et al. 1996).

The planktonic dispersal phase of barnacles has both advantages of being transported away from parents and therefore not competing with them, and disadvantages such as being transported away from a suitable settlement site (Eckman 1996, Todd 1998, Pechenik 1999, Jenkins & Hawkins 2003, Bullard et al. 2004, Jenkins 2005, McQuaid & Miller 2010). Therefore, in many places recruitment to a population is independent of that populations fecundity (being able to produce offspring and how fertile the organism is), and is described as ‘open’ (Roughgarden et al. 1985, Hughes 1990, Largier 2003, Svensson et al. 2004). Hyder et al. (2001) describe an ‘open’ population as a population where recruitment rates do not increase with a higher local population, and *S. balanoides* is a good model organism as the adults are sessile. Settlement rate depends on larval encounter rate with that populations substratum, which can be influenced by cues the cypris encounter, and can alter by several orders of magnitude from year to year (Gaines et al. 1985, Bertness et al. 1992, Delany et al. 2003, Berntsson et al. 2004, Prendergast 2010). Over 90 % mortality is encountered by pelagic *Balanus amphitrite* larvae, including being advected away from the coast, predation, starvation, UV, high / low temperatures and differences in salinity, this might be similar for *S. balanoides* (Eckman 1996, Pechenik 1999, Tremblay et al. 2007).

Fucus spiralis (L.), *Ascophyllum nodosum* (L.) and *Fucus serratus* (L.) are all fucoids that are canopy algae, and have a negative effect on the settlement of *S. balanoides* due to their sweeping action knocking cypris off the substratum; or possibly by the fronds limiting the movement of water when they are immersed, and acting as a barrier to the cyprids and zooplankton (Jenkins et al. 1999b, Hancock & Petraitis 2001, Jenkins & Hawkins 2003, Cervin et al. 2004, Burrows et al. 2010). However, in the Gulf of Maine on the East coast of North America, a non – trophic effect was found with *S. balanoides* on newly settled seedlings of *Ascophyllum nodosum;* where metamorphosed young barnacles overgrow the fucoid, but adult barnacles (≥ 1 year) provide a refuge from grazers therefore facilitating growth of the macro algae (Kordas & Dudgeon 2011). Fucoids also provide predators with a damp place to hide during low tides; however for any barnacles under the canopy it provides an area of reduced desiccation (Jenkins et al. 1999b, Hancock & Petraitis 2001, Jenkins & Hawkins 2003). If a cypris settles among adult barnacles under the fucoid cover, then the cypris' survival is increased (Jenkins et al. 1999b). Adults also appear to limit the damage done to cypris by the grazing action of the common periwinkle *Littorina littorea* and common limpets *Patella vulgata*, however the likelihood of being damaged or dislodged decreases with the increasing age of juveniles (Jenkins et al. 1999b, Hancock & Petraitis 2001, Chan & Williams 2003, Holmes et al. 2005).

Metamorphosis of the cypris into juveniles is a very energy intensive process requiring up to 30 % of the cypris organic carbon reserves (Tremblay et al. 2007). If the cypris has delayed settling for too long, it may not have enough Cypris Major Protein (CMP) and lipids to build cell structures and metamorphose (Tremblay et al. 2007). It is known that *S. balanoides* requires a carbon level (from lipids, proteins and carbohydrates) of 13 µg for successful metamorphoses (Jarrett & Pechenik 1997, Harder et al. 2001, Jarrett 2003, Tremblay et al. 2007). Previous work on *S. balanoides* and *Balanus amphitrite* has shown that if nauplii don't feed well in the plankton then slow growth results, which may result in poor competition and possibly increased predation there (Jarrett & Pechenik 1997, Hyder et al. 2001). The poor nauplii state then can be carried onto a poor cyprid stage, and even into settlement and growth as a juvenile (Jarrett & Pechenik 1997, Harder et al. 2001, Burrows et al. 2010). It is known from laboratory experiments on *Balanus glandula*, that in this species a cypris lipid concentration only results from nauplii VI food concentrations; however, food concentration between nauplii IV – V alters the nauplii period to longer and smaller size of cypris (Hentschel & Emlet 2000).

Triacylglycerol (TAG) is a neutral lipid used as an energy source by the non-feeding but swimming and exploring cypris (Miron et al. 1999). This lipid cannot be used on its own to estimate the physiological condition of cypris, as it alters with larva size (Miron et al. 1999). Triacylglycerol can be used in ratio with cholesterol (TAG / CHOL), to estimate how ‘desperate’ a cypris is to settle; the ratio decreases as the lipid is used up by metabolism, and has been found to be higher in cypris settling in the optimal sites of the lower intertidal (Toonen & Pawlik 1994, Jarrett & Pechenik 1997, Miron et al. 1999, Matsumura et al. 2000, Tremblay et al. 2007). It is known that cypris become increasingly less selective of their settlement sites as their reserves diminish, this is known as the ‘desperate larvae hypothesis’, and are more likely to settle on less than perfect substratum (Knight-Jones 1953, Toonen & Pawlik 1994, Jarrett & Pechenik 1997, Holm et al. 2000, Matsumura et al. 2000, Marshall & Keough 2003, Tremblay et al. 2007).

Settlement and metamorphosis is very energy intensive, therefore there is an optimal settlement time where the cypris has had time to select a suitable site, and still has enough energy to metamorphose (Miron et al. 1999, Jarrett 2000, Phillips 2006, Tremblay et al. 2007). Miron et al (1999) also discovered that in Canada, *S. balanoides,* the newly settled cypris in the best condition were those that had settled in the lower intertidal, where they encountered less desiccation (Bertness et al. 1992). After metamorphosis, the juvenile barnacle has a new set of conditions to cope with, mortality of *S. balanoides* of up to 30 days old has been shown to differ from 29.9 to 70.2 %, and adult abundance on the intertidal varied spatially due to dislodgement, predation, desiccation and varies with cohorts (Jarrett 2000, Bullard et al. 2004).

1.3.5 Predators

A shadow (which might signify a predator) can cause a barnacle to withdraw (Mauck & Harkless 2001). Groups of *S. balanoides* may reduce predation by withdrawing into their shells where they cannot feed; however, groups spend less time hiding than solitary individuals (Mauck & Harkless 2001). 'Collective detection' of predators may reduce predation, allowing productive feeding, due to rather than hiding for longer than required a barnacle may commence feeding more quickly, thus increasing their body weight and therefore fecundity. For example when fish such as the blenny *Blennius pholis* (L) predate and bite the cirri, they will soon move off to another group therefore diluting the effect of predation over the group (Mauck & Harkless 2001). As the barnacles resume feeding, this movement is detected by others, and they emerge, something a solitary barnacle cannot do, and therefore resume feeding quicker (Mauck & Harkless 2001). Barnacles are known to have chemoreceptors and mechanoreceptors which help the barnacle to sense what is going on in the rest of the group (Mauck & Harkless 2001).

Whilst in the plankton a wide range of larger organisms feed on *S. balanoides* larvae, including: ctenophores, jelly fish, large copepods, chaetognatha (arrow worms), other larval and adult plankton, and feeding fish, for example herring (Barnes 1956).

The common periwinkle *Littorina littorea* has been shown to have a negative effect on *S. balanoides* cypris recruitment through biological disturbance, and *Patella vulgata* has been found to be a positive influence for *S. balanoides* settlement by grazing the algae (Holmes et al. 2005). However, *Patella vulgata* is also known to 'bulldoze' new cypris and consume them with algae, but only for the first 24 hours after settlement until they metamorphose (Sousa 1985, Hyder et al. 2001). Around the shore of north-west Europe and especially Britain, the prosobranch mollusc *Nucella lapillus* (dog whelk) is a voracious predator of *Semibalanus balanoides* (Stubbings 1975, Thomason et al. 2000, Little & Kitching 2005). The presence of *Nucella lapillus* in the vicinity of *S. balanoides* results in the barnacle using fewer cirri swipes during feeding, however, this was not found in juvenile barnacles (Matassa & Trussell 2011, Johnston et al. 2012). The isopod *Hemioniscus balani* parasitizes this barnacle by living inside the mantle cavity where it castrates and reduces fecundity of the host (Stubbings 1975, Southward 2008). At Millport on the Isle of Great Cumbrae in the Clyde Sea, the nudibranch *Onchidoris fusca* has been observed feeding on *S. balanoides*, the polychaete green leaf worm *Eulalia viridis* consumes barnacles, the blenny *Blennius pholis* and the three – spined stickleback *Gasterosteus aculoetus* consume barnacles by biting off the extended cirri as the barnacle feeds (Stubbings 1975, Mauck & Harkless 2001). Also the crab *Carcinus maenas* is known to consume this species of barnacle (Thomason et al. 2000, Mauck & Harkless 2001). However, on the East coast of North America, the effect of *Carcinus maenas* on *S. balanoides* is positive as the crab predates *Nucella lapillus* (Matassa & Trussell 2011). Birds may also predate barnacles on the shore by breaking open the calcareous plates (Little & Kitching 2005). Predators can have a large impact on local populations as predator – prey interactions are of more importance to barnacles and their communities at lower latitudes (Sutherland 1990).

1.3.6 Gregariousness

S. balanoides must settle within a penis length of a conspecific in order to reproduce, and is therefore by nature a gregarious barnacle, for more information see the Fecundity chapter (Chapter 3) (Crisp 1985, Toonen & Pawlik 1994, Havenhand & Styan 2010). Gregariousness is caused by settling close to other barnacles of the same species. Cypris will settle preferentially near conspecific adults and newly metamorphosed juveniles using a variety of cues including chemical ones from the barnacles, and from biofilms of bacteria and algae living on the substratum (Crisp 1961, Stubbings 1975, Toonen & Pawlik 1994, Osman & Whitlatch 1995, Miron et al. 1999, Thomason et al. 2000, Jeffery 2002, Berntsson et al. 2004, Porri et al. 2007).

1.3.7 Population (intraspecific) competition

Semibalanus balanoides is to be found throughout the eulittoral zone where individual animals compete reducing their densities on the shore, and even the existing population can alter larval settlement rates by cannibalism of larvae, alluded to by Osman and Whitlatch (1995), and others (Eckman 1996, Begon et al. 1999, Jenkins & Hawkins 2003, Bullard et al. 2004, Little & Kitching 2005, Todd et al. 2006). However, Osman and Whitlatch (1995) suggest that recruitment was ‘less affected’ by the residents than by changes in the physical environment such as desiccation, predation in the mid and low intertidal, and silting up of an area (Menge 2000). Sutherland (1990) with *Chthamalus fissus* in the Pacific found that initial recruitment to the substratum was a good indicator of final settlement. Individuals may cluster too close producing hummocks where the animals' shells are touching each other (Gaines & Roughgarden 1985, Sousa 1985, Todd 1998, Todd et al. 1998, Begon et al. 1999, Hyder et al. 2001, Lopez & Gonzalez 2003). This competition results in reduced fecundity and possible survival, due to morphological changes which may affect resistance to desiccation known to affect *S. balanoides* (Gaines & Roughgarden 1985, Sousa 1985, Todd 1998, Begon et al. 1999, Hyder et al. 2001, Lopez & Gonzalez 2003). Hummocks of barnacles usually have a very small imprint on the rocks surface, resulting in the danger of the whole colony being broken away if individuals die or are damaged in storms (Gaines & Roughgarden 1985, Sousa 1985, Todd 1998, Begon et al. 1999, Hyder et al. 2001, Lopez & Gonzalez 2003). Thomason et al. (1998) have shown that the density of barnacles has an effect on the flow over the population, affecting capture rates by the cirri, removal of waste, removal of larvae and delivery of oxygen. This can result in lower rates of capture due to slower velocities of downstream individuals, than those upstream or mid-hummock (Thomason et al. 1998). However, small barnacles in troughs of taller barnacles may obtain enough food and oxygen due to particles being trapped by vortices (Thomason et al. 1998). Gaines and Roughgarden (1985) suggest that crowded barnacles were more vulnerable to predation.

1.3.8 Community (interspecific) competition

Semibalanus balanoides competes with *Chthamalus stellatus* and *Chthamalus montagui* on many shores of north-west Europe (Eckman 1996, Begon et al. 1999, Little & Kitching 2005). Generally *S. balanoides* out competes *Chthamalus* spp. lower down the intertidal by growing over or undercutting *Chthamalus*, but higher up the intertidal near MHWS, *Chthamalus* is able to survive due to increased desiccation of *S. balanoides* which *Chthamalus* spp. is more able to tolerate (Gaines & Roughgarden 1985, Begon et al. 1999, Little & Kitching 2005). However, *S. balanoides* is known to have higher survival rates in exposed areas when there is high recruitment (Jeffery 2003). As *S. balanoides* only reproduces annually, if it has successive 'failure years' where very few cypris settle, then *Chthamalus* will be able to fill any free space that is the result of storms, excessive wave action or by *S. balanoides* dying of old age (Barnes 1956).

1.3.9 Post settlement mortality

Smothering of existing barnacles by either their own or another species can be an altering effect for a community killing older specimens and replacing them with a younger cohort, as can the effect of storms that may scour areas of the intertidal (Crisp 1961, Little & Kitching 2005). Uncrowded acorn barnacles have a relatively large surface area attaching to the substratum compared to their external shell, therefore their body temperature is close to the rock temperature by about 1 ^{o}C, therefore when exposed on a hot day they may suffer from desiccation (Bertness & Gaines 1993). Work on the barnacle *Tesseropora rosea* and settling cypris, found that rock temperature (using infrared imagery – IR), decreased significantly within 15 mm of adults due to adults shading the rock, compared to 16 – 30 mm away (Lathlean et al. 2013).

1.3.10 Genetic effects

A dispersing larval stage has both advantages and disadvantages for *S. balanoides* including a reduced likelihood of inbreeding between adults and offspring, and the recolonization of other local assemblages that have become extinct for some reason (Pechenik 1999). This allows gene flow over a large distance, which might reduce any genetic complications and differentiations (Bertness & Gaines 1993, Pechenik 1999, Bell 2012). However, oceanographic factors such as upwellings, wind driven currents, and larval behaviour may not disperse the cyprids and nauplii; therefore genetic differentiation may occur due to selection on the shore (Bertness & Gaines 1993). Bell (2012) found in the Strangford sea lough, Northern Ireland, that there was no significant difference in genetic diversity between *S. balanoides* found in the lough to those outside it. Therefore, it is suggested that S. *balanoides* should not be taken into account when suggesting Marine Protected Areas (MPA), or Marine Reserve networks which are important to fisheries (Bell 2012).

1.4 Why choose the Clyde Sea area?

The Clyde Sea area is situated between 55^{o} N and 56^{o} N, which is equivalent to Copenhagen, Labrador and southern Alaska (see Figure 1 – 3), but it is ice free due to the North Atlantic Drift allowing an average winter sea temperature of 3 ^{o}C (Tivy 1986, Albessard & Mayzaud 2003). The Highland Boundary Fault crosses the area so that there are igneous, metamorphic and sedimentary rock types (Tivy 1986). The Clyde Sea is the largest fjordal system in Britain with sea lochs of Fyne, Striven, Goil, Long and Gareloch, all having sills into the 'Firth', and a sill 'The Great Plateau' divides the Clyde Sea (which extends between the Ayrshire coast and southern Kintyre), and The North Channel (Tivy 1986, Thomason et al. 1997, Matthews et al. 1999, Young & Holt 2007), for more information see Chapter 2. As a result, this is a relatively sheltered sea (Edwards et al. 1986). The inner fjords of Loch Fyne, Striven, Goil, Long and Gareloch have fjordic circulation due to the influence of their freshwater inflow; the outer firth is a partially enclosed coastal sea due to the effect of the Great Plateau, but it has tides and is influenced by the wind, rain, and fresh water from the inner fjords (Matthews et al. 1999). The Clyde Sea has deep water anchorages for the Royal Navy, as well as a large tourist sailing area (Edwards et al. 1986). It has a residence time for the sea surface water of two months with weak tidal currents and wind driven flows, and an estimated return period for rainwater of over 500 years (Edwards et al. 1986, Thomason et al. 1997). There is an average freshwater flow into the Clyde Sea of 156 m^{3} s^{-1} from its six major rivers: Clyde, Leven, Kelvin, Eachaig, White Cart Water and Black Cart Water (Thomason et al. 1997).

Figure 1-3 Drawing of the United Kingdom with the Clyde Sea Area in the small box

This makes the Clyde Sea a microcosm of the Boreo arctic seas, which can be monitored relatively easily as there are roads around the fjords, mainly close to the shore. Being ice free, this allows the easy collection of barnacles containing embryos during November and December, and as there is no ice scouring, the barnacles are on all rock surfaces they can inhabit within the intertidal area. As the three main rock types are represented here, experiments can be conducted on them, and barnacles harvested from them. The long residence time for sea water, allows the assumption that released nauplii will remain in the Clyde Sea throughout their stages to cypris and metamorphosed juveniles. Therefore, adults can be assumed to have resulted from nauplii released into the Clyde Sea and not washed out over the Great Plateau. There is the potential for small numbers of nauplii being washed into the Clyde Sea by the action of wind driven water and tides, but there is no documented evidence; and it has been suggested that fish stocks are separate from those outside the Great Plateau (McIntyre et al. 2012).

1.5 Overview of thesis

This thesis concentrates on *Semibalanus balanoides* and its interrelationship with the Clyde Sea environment. In order to couple the larval cyprid phase and the benthic phase of the barnacles a closed system is required (Eckman 1996). A closed system (or closed populations within the system), is where gene flow is not possible from outside the immediate area (Eckman 1996). Therefore recruitment from outside a bay or fjord is either none existent, or there is very little exchange of recruitment for the animals and plants; as a result gene mixing is severely restricted, which may lead eventually to separate species (Eckman 1996). The Clyde Sea can be considered a closed system, as when the benthic adults release the nauplii into the sea, their six stages and the cyprid settlement stage, fit easily into the turn over time for the sea water over the Great Plateau and into the North Channel (see Chapter 2). However, for the populations within the various intertidal areas, the populations could be described as open because the nauplii are transferred away from their natal area to other settlement sites within the Clyde Sea (Svensson et al. 2004). Open systems (or open populations within the system), are where gene flow is freely possible; therefore recruitment from other populations outside the immediate area, (for example outside a certain shore or bay) is possible.

1.5.1 Aims and objectives of thesis

The aims of this thesis, are to try and estimate the risks that the barnacle *Semibalanus balanoides* takes through its life cycle; and to ultimately work out the number of adults and their possible input of nauplii into the Clyde Sea area. In order to do this the chapters are organised as follows.

In order to accurately estimate the number of adult *Semibalanus balanoides,* the geology, bathymetric information, intertidal and exposure of the area had to be recorded (Chapter 2). Different rock types may support more adults, or the adults on the different rock types have a greater fecundity (Chapter 3). Fecundity is coupled to the supply of cypris to the shore, and the differences in geology allowed an evaluation of differences in settlement of cypris on each rock type (Chapter 4). Artificial rocks and real rocks were compared, in order to find out if there were differences in settlement between them

(Chapter 4). With information on rock types and their exposure, overall numbers of adults and their possible input into the sea, may be calculated, and then to the risk of cannibalism (Chapter 5) from adults. Cypris supply and the type of rock that the cypris were landing on were investigated, and the available space for settlement (Chapters 6 and 7), incase there were differences in the Clyde Sea area. As there are many *Semibalanus balanoides* around the Clyde Sea, it would be useful to find out at what area cypris most commonly settle in, (i.e. whether cypris prefer to settle in areas 1cm^2 or 10 cm^2 (Chapter 7). All this culminates in the risk for settlement of a cypris in Chapter 8, and if the first site is rejected, then the inherent risk involved in finding a new site. Figure 1-4 is a schematic overview of the thesis which shows the inter-relationship between the chapters.

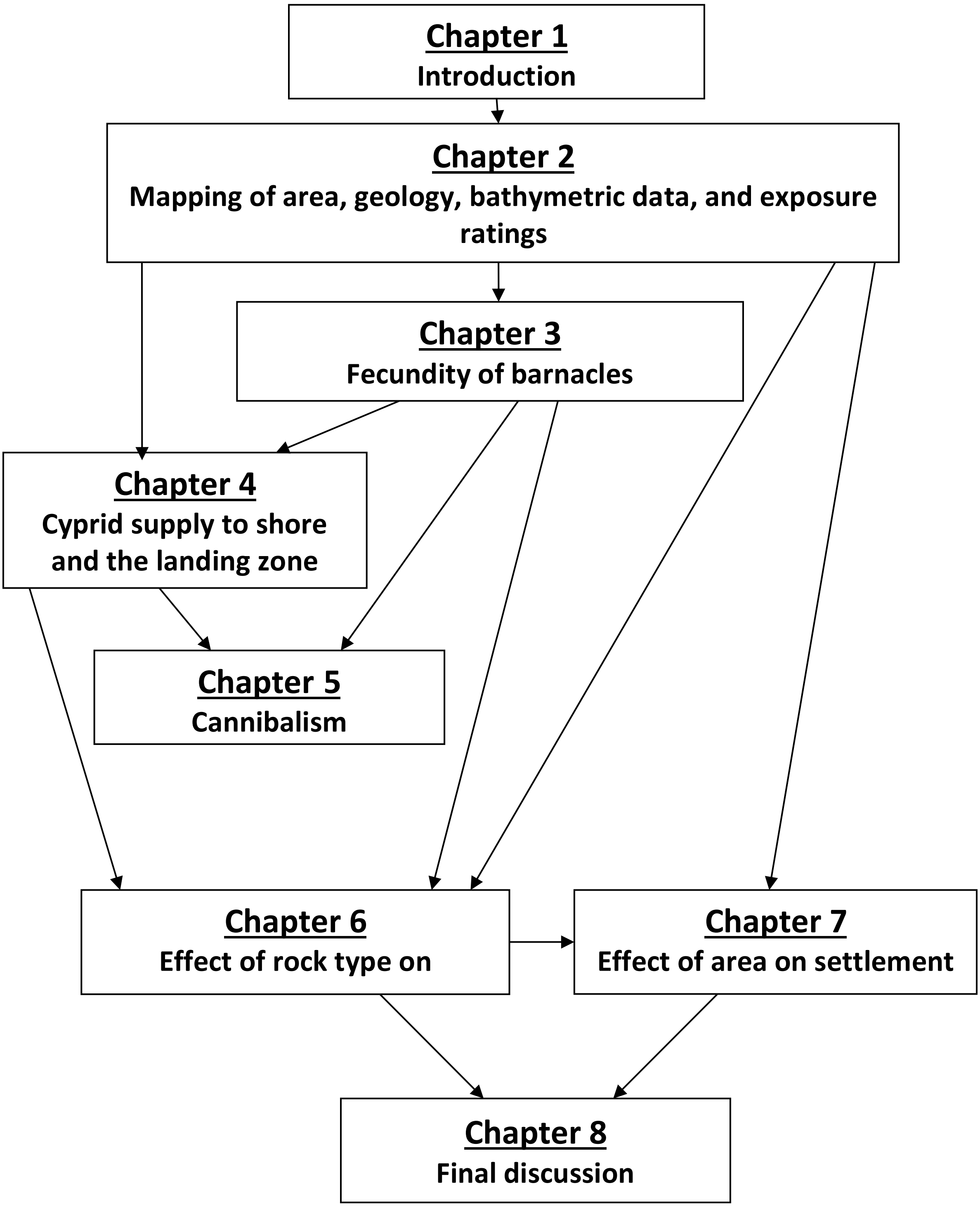


Figure 1-4 Diagram of thesis overview and how chapters are linked together

Chapter 2. Mapping, geology, bathymetric data and development of exposure ratings

2.1 Introduction

2.1.1 Clyde Sea area

All the plants and animals in the Clyde Sea system are ruled by the waters that they call home (Tett 1992), therefore the area has to be understood, by way of ground features such as rock, boulders, the underlying geology, the bathymetric data (to give the height of the waves coming ashore), and how exposed each shore is (which determines the rocky shore community). The geology and bathymetric conditions may affect distribution of both adult *Semibalanus balanoides*, and where cypris may settle. It is assumed that such a large area may contain both sheltered and exposed areas; however, examining every shore using biological ratings would be far too time consuming. Initial examination of Ordnance Survey maps suggested shores that were easily accessible, but it was unknown if the local rock type was a contributing factor for settlement.

The Clyde Sea is the largest fjord system in Britain with a surface area of 3671 km^2, and a volume of 179 km^3 (Tivy 1986, Thomason et al. 1997, Matthews et al. 1999, Young & Holt 2007, McIntyre et al. 2012), and is found on the west coast of Scotland (Simpson & Rippeth 1993, Kasai et al. 1999, McIntyre et al. 2012). Fjords have been carved out by glaciers, and are dominated by inlets and basins that are typically separated from the sea by a shallow sill that has been a more erosion resistant rock (Elliott et al. 2003, Wilding et al. 2005). The sill that separates the Clyde Sea from the North Channel is known as the Great Plateau with a minimum depth of 45 m (Simpson & Rippeth 1993, Kasai et al. 1999, Matthews et al. 1999, Wilding et al. 2005). This sill restricts the flow into the North Channel and the salinity in the Clyde basin is reduced up to 1.5 compared to the North Channel (Simpson & Rippeth 1993, Kasai et al. 1999, McIntyre et al. 2012).

The resulting hydrography of the Clyde Sea is a consequence of the bathymetry (McIntyre et al. 2012). Freshwater in the Clyde Sea comes from the surrounding rivers, principally the Clyde River and the adjoining sea lochs at a rate of 60 - 700 $m^3 s^{-1}$ (Simpson & Rippeth 1993, Kasai et al. 1999), or 156 $m^3 s^{-1}$ from its six major rivers: Clyde, Leven, Kelvin, Eachaig, White Cart Water and Black Cart Water (Thomason et al. 1997) depending on how the authors have measured the inflow. This produces a Region of Fresh Water Influence (ROFI) which is typical of fjords (Simpson & Rippeth 1993, Huthnance 1997, Matthews et al. 1999). The hydrography is complex and water in the basin is stratified with the lighter fresh water on the surface (0 – 10m) and the denser more saline water deep (40m – sea bed) in the basins with only about 20 % below 70 m (Simpson & Rippeth 1993, Kasai et al. 1999, Matthews et al. 1999, Wilding et al. 2005, McIntyre et al. 2012); which is maintained by water entering over the sill from the North Channel. As tidal stirring is limited by generally weak tides (less than 0.5 ms^{-1}) in the basins (Simpson & Rippeth 1993, Huthnance 1997), the stratification is stable for most of the year, with mixing between layers usually occurring in November and February with winter storms (Huthnance 1997, Matthews et al. 1999, McIntyre et al. 2012). The currents in the North Channel predominantly run parallel to the sill (the Scottish Coastal Current) flowing northwards (Inall et al. 2009); water leaving the Clyde Sea is quickly removed and mixed (Kasai et al. 1999). There is a strong front on the sill where the different densities of the water in the Clyde Sea and the North Channel cause an exchange which is influenced by the earth’s rotation due to the sill being approximately 20 km long, and the internal Rossby radius which is usually less than 5 km (Simpson & Rippeth 1993, Kasai et al. 1999); that is the length of scale where the rotational effects of the earth become as important as buoyancy or wave effects in the flow around the disturbance (Kasai et al. 1999). The Clyde Sea is warmer (maximum ~ 13 ^oC in August) than the North Channel in the summer, and cooler (minimum ~ 6 ^oC March and April) in the winter, with occasional surface ice at the head of some lochs (McIntyre et al. 2012). Chlorophyll-a peaks (4 – 5 µg/l) in the Clyde Sea during March and July; and nitrate is higher than adjacent coastal waters in winter (10 -12 µg-at / l), but falls in the summer due to phytoplankton production (McIntyre et al. 2012). Oxygen saturation is lowest in August at about 80 % in the surface waters (0 – 10 m), however, for the rest of the year oxygen saturation is close to 100 %, and it is only in the deep basins where it falls below this although it is sufficiently high enough for *Nephrops norvegicus* to proliferate (McIntyre et al. 2012).

The inflow of water into the Clyde Sea has been estimated to be 10^4 m^3 s^{-1} (Simpson & Rippeth 1993), dependent on rivers and / or inflow from the sea lochs and annual precipitation levels. Therefore the flushing time is thought to be about 4 months (assuming that all the inflowing water exchanges with all the water that was currently there) into the North Channel which is comparable with isotope measurements of 3 – 4.5 months (Simpson & Rippeth 1993). However, a report completed for the Department of Trade and Industry suggests a residence time for the waters of the Outer Firth of the Clyde Sea of about two months (Wilding et al. 2005). The time between release of nauplii and cyprid settlement is approximately 3 - 4 weeks, therefore it is probable that most if not all nauplii released will be retained in the Clyde Sea system due to the geology of the fjord retaining its waters. If the number of larvae released can be calculated (assuming no loss over the sill), potential settlement can also be calculated (Hansson et al. 2003).

It is important to know how exposed a shore is, if it is very sheltered there may be more *Semibalanus balanoides* living there, or conversely they may prefer exposed sites as exposure does have implications for penis morphology (Hoch 2008, Hoch 2010), there is more food available and there may be lower numbers of predators (Little & Kitching 2005, Baker & Crothers 2009). In the Clyde Sea area, the winds come predominantly from the SSW to WNW rose quarter (with 44 % of the wind coming from these directions between October 1996 to December 2005 at Prestwick), 16 % (the largest percentage) coming from WSW and the next largest (10.5 %) was from the west (Meteorological-office 2012). Therefore due to the predominant winds, it was thought that west facing shores would be more exposed than the east facing shores with the hills of the Kintyre peninsular behind them. In order to construct an easy exposure index that can be used with maps, rather than from in-situ monitoring on an actual site over a long time, the wave action due to the prevailing wind has to be determined.

When waves come ashore from deep water, the water particles start to move differently in a more backwards and forwards motion rather than in the circular orbits found in deepwater (Little & Kitching 2005), and the wave length decreases but its height increases until it breaks. The mechanical effect of the water particles and pressures, together with sand and shingle suspended in the water column, can cause scouring effects and bombardment on creatures living on rocks of the shore and intertidal in exposed areas (Little & Kitching 2005). Fetch is the distance that the wind blows uninterrupted towards a shore (Little & Kitching 2005, Baker & Crothers 2009), and this has an immense influence on the intensity of wave action against a specific shore (Baker & Crothers 2009). The bathymetric layer was important to map in order to calculate fetch for different geological areas. It was hypothesised that different rock types may be more or less favourable to settling larvae. Consequently, an evaluation of the geology of the Clyde Sea was required. Including a determination of the extent of the main differing rock types (igneous, metamorphic and sedimentary), and the number of settling larvae per rock type was calculated. If we know on average how many adult barnacles there are per square metre, then the number of larvae input into the system can be calculated.

2.2 Aims of chapter

One single projection was required to define the Clyde Sea; as working with different projections for the bathymetric sheets and O.S. 36 for the intertidal and geology was difficult, and time consuming. From this it was hoped to use the one projection to highlight possible sites (sheltered and exposed) for field work. This could then be incorporated into one scalable map, which could be used on a laptop and zoomed into or printed out.

It was hypothesised that a map based system could be used to suggest or highlight sheltered and exposed shores, rather than necessitate going to all shores and using Ballantines biological exposure scale (Ballantine 1961, Little & Kitching 2005), which would be both expensive and time consuming. This would provide the possibility to scale up future findings from separate shores to a possible whole area aggregate.

2.3 Methods and materials

2.3.1 Mapping of the foreshore and its features

Digital maps were used from the Ordnance Survey under licence, in the form of MasterMap™ data which was loaded onto a laptop into the ArcView programme. However this data was not restricted to the foreshore and its features, but included all features on every tile that covered the Clyde Sea area (including houses, roads etc). These large amounts of unwanted data not only detracted from the overall map, but took a long time to load on a laptop. Therefore much of the extra unwanted data was removed using the mapping programme ArcView version 8.0, but the first few houses back from the shore, roads and large structures (such as lighthouses and churches) were left in, as they were required when adding the bathymetric and geology data. Ordnance Survey data uses OSGB 36, the official datum in Great Britain (except for Northern Ireland); and all its maps (both digital and paper copies) are based on this system (Iliffe 2005). OSGB 36 is a version of the Transverse mercator projection that places the spheroid at Greenwich (since the nineteenth century survey of Great Britain) and coincides with the geoid at the Greenwich meridian (Iliffe 2005):-

$\varphi = 51^0\ 28'\ 40''$ N $\quad \lambda = 00^0\ 00'\ 00''$

Much of the required data was either duplicated, had many joins in or was not jointed (slightly missing two ends), known as “spaghetti data”; this had to be tidied up to give complete lines for example of the mean high water springs MHWS. This was done by transferring the data from ArcView to the cartographic package MapMaker™ that is more user friendly and is quicker to edit in. Mean High Water Springs and Mean Low Water Springs were required separately coloured so that it was easy to see where shore features are located. The sites used for this thesis were selected initially using information from McQuaid et al. (2000); where the sites were classified as igneous, metamorphic, sedimentary and exposed or sheltered without referring to the substratum’s existing biota, but by using the data developed in this chapter. Access to the shore from roads and car parks to allow ease of sampling was required, and these criteria were partially used to select the final sites (McQuaid et al. 2000). Suitable sites were also investigated to discern if they were in the correct wave exposure classification as the ‘Ballantine’s scale’, which uses biologically defined scales, and had a large population of *S. balanoides* on them (Ballantine 1961, Little & Kitching 2005). At each site random quadrats were used on the lower, mid and upper settlement area of the intertidal for *Semibalanus balanoides* (McQuaid et al. 2000).

In order to show all the mapping of the different elements together, another map was constructed. The Ordnance Survey datum is set at Newlyn (Iliffe 2005). The tide gauge at Millport (on the Isle of Cumbrae) is -1.62 m below this, therefore the Clyde Sea digital maps are set at chart datum which is the approximate lowest level of the tides due to astronomical effects, but not metrological effects and to which all depths are published on navigational charts (Pol 2007). In order to remove the redundant line data and tidy the map, the data was put into a function of the software called live layer, and live layer actions, tidying, tidy line junctions, were used to remove the redundant line junctions. The manual for MapMaker is a PDF (www.mapmaker.com/v3/download.htm) supplied with the software.

The foreshore annotations included: rock, rock scattered, boulders, boulders scattered, scree, shingle, sand, mussel beds and mud. However, these definitions are not given sizes or how they are defined on either the MasterMap™ data or on OS sheets. General information (below) was obtained from Vanessa Lawrence (Director General for Ordnance Survey) in a personal communication. The surface features for large scale mapping are only represented when the area is at least 0.1 ha and has a minimum width of 5 m. Rock to scree features have their limits shown by a pecked line. Shingles to mud features are annotated, and areas of mixed cover are represented by annotations and / or area labels with the predominant feature appearing first. The definition of rock is an aggregation of mineral particles that are hard, consolidated and massive. Rocks that are large may be named and conspicuous rock will be described as rock. Further classifications are applicable to rock above MHWS and down to MLWS are:
25 – 100 % of rock and less than 25 % is classified as scattered rock; it is presumed that the surveyors use this definition on the foreshore. Boulders are defined as a large rounded rock > 0.2 m in diameter. Where boulders are up to 20 m apart they are classified as boulders, greater than this they are described as boulders scattered. Sand

separated or combined with shingle is shown where it extends 20 m or more from a water feature such as a river or loch, and it is shown if it extends over 1 ha. Shingle is defined as areas of water worn pebbles where it extends 20 m from a water feature, or is over 1 ha. Where shingle forms a mussel bed this is described on the map.

2.3.2 Geological mapping

The geology of the Clyde Sea foreshore was digitised into MapMaker using hard copy British Geological Survey 1: 250 000 scale maps of the Clyde and Argyll (McAdam et al. 1985, Highton & Hall 1987), showing the solid geology in conjunction with a wireless scroll tablet (TTrust 1200-V2). This was because at the time they were required, the digital maps would have had to have been purchased at high expense. The geology maps were on the same projection as the MasterMap data (OSGB 36) and therefore the maps didn't require transforming to fit the Ordnance Survey layer. The hard copy maps were photographed and saved as Tagged Image File Format (TIFs) in order to make the digitising easier. The TIFF was taken into MapMaker as a layer then a new live layer was added named geology which was shown on top of the TIF. The polygon tool was used to draw each area, and nine different rock types in each of the three main types of igneous, sedimentary and metamorphic were assigned colours and names (see Table 2-1 for rock types and their abbreviations). Labels were assigned to each rock type using edit, the insertion point and dragging to the centre of each rock type.

Main class of rock	**Abbreviation**	**Actual rock class**
Igneous	Iep	Extrusive, basic lava, Permo-carboniferous
Igneous	Ied	Extrusive, intermediate lava, Devonian
Igneous	Ieo	Extrusive, basic lava, Ordovician
Igneous	Iia	Intrusive, fine grained, acid
Igneous	Iib	Intrusive, fine grained, basic
Igneous	Ibm	Intrusive, fine grained, metamorphosed, basic
Igneous	Iic	Intrusive, course grained, metamorphosed, basic
Igneous	Iiu	Intrusive, ultrabasic
Igneous	Iii	Intrusive, course grained, acid
Sedimentary	Sps	Sandstone, Permian
Sedimentary	Sc	Undivided, westphalian, namurian and dinantian, carboniferous
Sedimentary	Sdcs	Sandstone, Devono-carboniferous
Sedimentary	Sds	Undivided, upper and lower; sandstone, Devonian
Sedimentary	Sdc	Undivided, upper and lower; conglomerate, Devonian
Sedimentary	Sog	Greywacke, undivided, Ashgill, Ordovician
Sedimentary	Soc	Conglomerate, undivided, Ashgill, Ordovician
Sedimentary	Stu	Undivided, Triassic
Sedimentary	Spt	Triassic and Permian
Metamorphic	Msm	Southern highland group, dalradian
Metamorphic	Msp	Southern highland group, dalradian
Metamorphic	Msg	Southern highland group, dalradian
Metamorphic	Mal	Tayvallich subgroup, Argyll group, dalradian
Metamorphic	Mac	Crinian subgroup, Argyll group, dalradian
Metamorphic	Mae	Easdale subgroup, Argyll group, dalradian
Metamorphic	Maq	DBCN Crinian subgroup, Argyll group, dalradian
Metamorphic	Mdc	Undifferentiated, Southern Highland group, dalradian
Metamorphic	Mdb	Undifferentiated, Argyll group, dalradian

Table 2-1 Classes of rock shown in geology layer

Transferring data from MapMaker into ArcMap requires you to be in MapMaker. The workflow for this is: MapMaker > utilities > vector utilities > export files from MapMaker selection manager e.g. with a display of S* the wildcard (*) brings anything with 'S' including MSM not just sedimentary rock information. The workflow for transferring data from ArcView to MapMaker is: go into MapMaker > utilities > vector utilities > import files (the data should be made into shape files in Arc via catalogue > toolbox > put into geodatabase > convert geodatabase to shape file). The shape files are instant and permanent in a map.

In ArcView queries can easily be run using the table of contents > attribute table > options and make a selection. This was used to find any duplicates in areas. To find areas, for example, metamorphic boulders,

- ArcView was used with tools
- GeoProcessing Wizard, intersecting two layers with metamorphic tidal area on the top and boulders second
- Specify shape file.
- Open the attribute table, (checking the right hand side shape area and sort these in order to find any doubles), highlight anything that is duplicated and remove these to obtain area by options,
- Switch selection, right click on layer part and export data to a new layer removing original selection layer.
- When the information is in its own layer, then:
- Open attribute table,
- Then in shape_Area, right click, statistics to get sum of area, count, minimum size and maximum size.

This was used to obtain tidal areas of rock, rock etc (the etc can be anything, for example sand or shingle, that is not defined as major rock type), etc and rock, scattered rock, boulders, boulders etc, etc and boulders and scattered boulders for each major rock type (areas where *Semibalanus balanoides* may be found). Having conducted this intensive process, the amount of intertidal area covered by the different features was calculated (see results section).

The different rock types were then investigated in order to determine which were suitable settlement sites for a *S. balanoides* cypris to settle, and the area of rock available as sand, shingle or mud would be unsuitable for settlement. Assumptions had to be made on the area of substratum that was suitable for settlement. This was made by gauging the amount of substratum that might be suitable from visiting various shores around the Clyde Sea. For rock 100 % of the area was assumed available, and for boulders it was also assumed to be 100 % although there may be spaces between the boulders on the shore they usually have a large 3D shape that increases the available area for settlement.

2.3.3 Bathymetric mapping

The bathymetric layer was digitised into ArcView using lighthouses, churches and road junctions to fit the charts to OS36 projection by ‘pulling’ the other projections to fit; as the digital information from SeaZone was prohibitively expensive, and their information had been gained from Admiralty charts. However, the charts were not photographed as there were 13 of them, would require the use of a fixed horizontal camera and they are protected under copyright laws that restricts the area of each chart that can be copied in this way (under data protection). Instead they were digitized using a wireless scroll tablet (TTrust 1200-V2) with the paper charts placed individually on it. The problems with the Admiralty charts were that they used various projections including the Gnomonic, Transverse Mercator and Mercator. These had to be first transformed into OSGB 36 (a version of the Transverse Mercator projection), so that the overall map used one projection, and was therefore more accurate for the purpose of mapping fetch.

The Gnomonic projection is a type of azimuthal projection and is appropriate for small countries or islands (Iliffe 2005). This projection isn’t used by modern applications and is formed by projecting its points from the centre of the earth; as a result the distortion becomes greater the further from the centre of the projection (Iliffe 2005). However this projection allows for straight circles giving the shortest route between two points on a sphere (Iliffe 2005), see Figure 2-1.

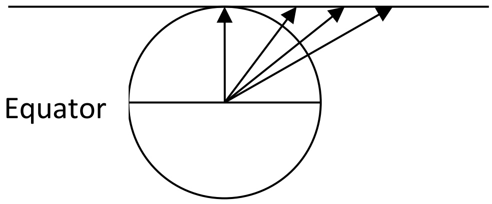


Figure 2-1 Illustration of Gnomonic projection

The Mercator projection is a type of cylindrical projection (that appears to stand up like a can). This projection gives north south meridians that are parallel and the poles have an infinite size and therefore they cannot be represented accurately (Iliffe 2005). Each map or navigation chart shows only a small part of the Earth and the scale factor varies with the latitudes (smaller towards the equator and longer distances towards the poles), this projection is optimised for the equatorial region (Iliffe 2005).

The Transverse Mercator projection is a type of cylindrical projection, but with the cylinder on its side, so that the line of contact is a particular meridian - a sideways version of latitude (Iliffe 2005). The meridians are not parallel to each other and are not straight lines except the central meridian which is straight. It is usually used for a narrow band of ± 3^o on either side of the central meridian, and is used primarily as a survey projection and is widely used for regions with a large north – south extent, but a narrow east – west extent (Iliffe 2005). It is used by the Ordnance Survey and is used by a worldwide projection (the Universal Transverse Mercator UTM) that divides the world up into 60 zones of longitude (each 6^0 wide), for example the UK ‘lies in UTM zones 30 and 31’ (Iliffe 2005).

Table 2-2 shows which charts were used in order to incorporate bathymetric data into the map of the Clyde Sea area (all charts were for Scotland – West coast, published at Taunton and depths shown were in metres).

Chart number	Chart name	Scale	Projection	Printed	Source data
1403	Loch Ryan	Loch Ryan 1:25000 Stranraer 1:10000	Transverse Mercator	July 1999	British Government Surveys 1987 = 1:12500 1987 = 1:1000 1972 = 1:20000 1972 = 1:7500 Commercial Surveys 1996-99 = 1:500 – 1:1000 1998-99 = Digital 1987 = 1:1000 – 1:2500
1867	Plans in the Firth of Clyde	Rothesay Bay and approaches 1:10000 Rothesay Harbour 1:2500 Hunterston and approaches 1:12500 Nato Pier, Fairlie 1:5000 Hunterston Jetty 1:5000	Transverse Mercator	May 1988	Admiralty Surveys 1977 = 1:2500 1977 = 1:10000 1964 = 1:10000 1940-42 = 1:10560 1949 = 1:25000 1904 = 1:10560 (lead-line survey) Other Surveys (not specified) 1972-82 = 1:500 – 1:1000 1974-76 = 1:10000
1906	Kyles of Bute	1:25000	Gnomonic	Dec 1991	Admiralty Surveys 1985 = 1:12500 – 1:25000 1977-79 = 1:10000 – 1:12500 1977 = 1:2500 – 1:5000

					1964-75 = 1:10000 – 1:25000 1947-52 = 1:10560 Department of Environment Surveys 1989 = 1:500 Other Surveys (not specified) 1972-88 = 1:500 – 1:1000
1907	Little Cumbrae Island to Cloch Point	Little Cumbrae Island to Cloch Point 1:25000 Loch Striven Nato Fuel Jetty 1:10000	Transverse Mercator	Sept 1983	Admiralty Surveys 1960-77 = 1:10000 – 1:36000 1940-52 = 1:10000 – 1:25000 1904 = 1:10000 (lead-line surveys) Other surveys (not specified) 1974-76 = 1:10000 1972-79 = 1:500 – 1:1000
1994	Approaches to the River Clyde	Approaches to the River Clyde 1:15000 Greenock Great Harbour 1:5000	Transverse Mercator	June 1999	British Government Surveys 1996-97 = 1:10000 – 1:12500 1993 = 1:12500 1986 = 1:12500 1964-66 = 1:12500 1935-43 = 1:2500 – 1:15300 Clydeport Operations Ltd Surveys 1996 = 1:2500 1985-87 = 1:500 – 1:2500 Department of

					Environment Surveys 1984-92 = 1:500 – 1:12500 1972-80 = 1:500 – 1:1000 Commercial Surveys 1998 = 1:2000 1997 = 100% bottom search !963 = 1:15500 1933 = 1:2500
2000	Gareloch	Gareloch 1:12500 Faslane Base 1:6250 Rhu narrows 1:6250	Gnomonic	Jan 1994	Admiralty Surveys 1990-93 = 1:10000 – 1:12500 1984 = 1:12500 1964-66 = 1:6000 – 1:12500 Admiralty Surveys (lead-line) 1911-19 = 1:10560 – 1:10580 Department of Environment Surveys 1980-93 = 1:500 – 1:1500 Other surveys (not specified) 1993 = 1:1000
2007	River Clyde	1:15000	Gnomonic	May 1995	Detail in the docks and the maintained channel from information supplied by Clydeport Ltd to 1991. Remainder

					from numerous Admiralty Surveys from 1860 to 1964.
2126	Approaches to the Firth of Clyde	1:75000 at latitude 56^{0}00’	Mercator	Nov 1992	Admiralty Surveys 1984-85 = 1:25000 – 1:50000 1980-83 = 1:5000 – 1:18000 1980 = 1:36000 1974-77 = 1:6250 – 1:25000 1960-67 = 1:18000 – 1:25000 1968 = 1:50000 Admiralty Surveys (lead-line) 1840-1913 = 1:6000 – 1:64000 Other surveys (not specified) 1989 = 1:10000 1951 = 1:6000
2131	Firth of Clyde and Loch Fyne	1:75000 at latitude 56^{0}00’	Mercator	Jan 1999	Admiralty Surveys 1986-93 = 1:12500 1986 = 1:12500 1981-85 = 1:25000 1960-80 = 1:5000 – 1:25000 1968 = 1:75000 1941-58 = 1:10000 – 1:25000 Admiralty Surveys (lead-line) 1908-32 = 1:10600 – 1:25000 Department of Environment Surveys 1980-93 = 1:500 –

					1:1500
2199	North Channel Northern Part	1:75000 at latitude 55^{0}15’	Mercator	July 1990	Admiralty Surveys 1972-87 = 1:2500 – 1:20000 1972-85 = 1:25000 – 1:50000 1964-68 = 1:50000 1944 = 1:25000 Admiralty Surveys (lead-line) 1836-56 = 1:10560 – 1:24344 1887 = 1:63800 1839-40 = 1:24344
2383	Inchmarnoch Water	1:25000	Gnomonic	May 1989	Admiralty Surveys 1985 = 1:12500 1984-85 = 1:25000 1975 = 1:12500 1975 = 1:25000
2491	Ardrossan to Largs	1:25000	Gnomonic	July 1990	Admiralty Surveys 1985 = 1:12500 1984-85 = 1:25000 1980 = 1:5000 1968 = 1:75000 1940 = 1:10560 Admiralty Surveys (lead-line) 1846 = 1:24300 Other Surveys (not specified) 1985-88 = 1:500 – 1:1000 1972-83 = 1:500 – 1:1000 1976 = 1:10000
3746	Loch Long and Loch Goil	Loch Long and Loch Goil 1:25000 Upper Loch	Gnomonic	July 1990	Admiralty Surveys 1983-86 = 1:12500 1954-68 = 1:5000 – 1:12500

		Long 1:25000 Approaches to Finnart 1:12500			Admiralty Surveys (lead-line) 1924-32 = 1:10600 1908 = 1:10558 Department of Environment Surveys 1981-89 = 1:500

Table 2-2 Charts used for bathymetric map

The largest scale for each area was used to digitise the digital map layer.

2.3.4 Development of shore exposure ratings

Two exposure indexes were investigated to find out if they were suitable to use on the Clyde Sea. The first uses fetch and the angle of the shore to the sea which is open - the modified maximum fetch (Wright 1981, Baker & Crothers 2009), and a variation based on Thomas (1986) using a rose divided into 22.5^{o} sectors. All shores used were easily accessible from a road or car park.

The modified maximum fetch was devised for Shetland where there was a significant correlation between this method and biological estimates of exposure (Wright 1981, Baker & Crothers 2009). This system uses the effect of fetch, undersea characteristics and open angle of the shore to the sea. The following information was required:-

maximum fetch from shore to next shore in a line in km	(MF)
the angle subtended by the sea horizon	(SH)
minimum width of the channel to the outer coast in km	(MW)
the distance to the outer coast in km if in an inlet	(DIST)
the maximum depth within 1 mile perpendicular offshore in fathoms	(D)
the maximum depth in the whole study area in fathoms	(D_{max})

This gives the modified maximum fetch equation as:-

Formula = $MF * (SH + 180) / 180 * MW / (DIST + MW) * D / D_{max}$

This results in a number, the higher the number the more exposed the shore is. However, this model uses the imperial measurement of fathoms (1 fathom = 1.8288 m) and miles (1 mile = 1.6093 km), and therefore mixes imperial and metric measurements. Variations on this model were used (as all the charts for the Clyde Sea use metres instead of fathoms for depths). For example spot depths (in metres) were used at 1 km and at 5 km for maximum depth instead of 1 mile (D) and maximum depth in study area (D_{max}), and contour depths (in metres) were used at 1 km and 5 km for D and D_{max}.

This model was used on nine known sites (Carradale, Cloch Point, Culzean, Inverkip, Lendalfood, Strachur, and Tighnabruaich – see Figure 2-3 and Table 2-7), that had been used for winter collection of *S. balanoides* during 2004 and 2005; for which their exposure could be estimated. Spot depths and contour depths were as close as possible to fathoms and mile sites as could be estimated on metric charts.

A variation on Thomas (1986) was used to obtain the exposure of a shore, using an acetate overlay that is divided into 16 sections of 22.5^{o}. This was placed on the MHWS mark in the centre of the shore that was investigated (initially the 9 original sites, then later all sites – see Table 2-7). The centre of the north section was aligned with north on the chart or map with the shore under investigation. The centre of each section was used to obtain the fetch distance. For each section the fetch was calculated if less than 50% of the section contained land, where more than 50% of the section was land it was not used in the overall calculation for fetch. Each area with a fetch in was added together for the shore and then the mean fetch (in metres) for that shore was calculated. This was completed for all shores used in this thesis (see Table 2-6). This system had been tested on a variety of locations in Bermuda and the Bay of Fundy in Canada. Another variation of this method was used by Burrows et al (2010) in order to work out wave fetch around Scotland as Burrows et al (2008), using 16 sections of 22.5^{o} but with angular sectors, giving a maximum value of 3200 km per cell. Burrows et al (2008) indicated that shores that were ‘sheltered’ tended to have communities comprising of ‘sheltered’ animals and macro algae; whereas ‘exposed’ shores had ‘exposed’ communities irrespective of the prevailing wind, but bathymetric data close to the shore and geology should be taken into consideration.

2.3.5 Data analysis

In order to verify how accurate the modified maximum fetch was, the following statistical analysis was used. Univariate GLM ANOVAs were run on variations of the modified maximum fetch with spot heights, contour heights, 1 km spot heights, 1 km contour heights, 5 km spot heights and 5 km contour heights as dependent variables and the exposure ratings assigned them split between their larger and smaller arbitrary numbers at each shore.

A univariate GLM ANOVA was run with rose Distance as the fixed factor and Exposure rating the dependent variable for all shores investigated over the three years that barnacle samples were taken for the fecundity and rock type chapters.

2.4 Results

2.4.1 Mapping the foreshore and its features

The Ordnance Survey data tidied is shown (Figure 2-2). It is shown in the figure with the Ordnance Survey (OSGB 36) grid, and latitude and longitude positions annotated to aid positioning. It appears to have angular edges to the mapping due to taking the features back to the last part of the tile to show foreshore features, and features such as churches were kept even if they were more than a street back from the foreshore to construct the rest of the mapping. In some areas the features on the original MasterMapTM data were continuous from the foreshore to the whole tile; therefore these features could not be removed.

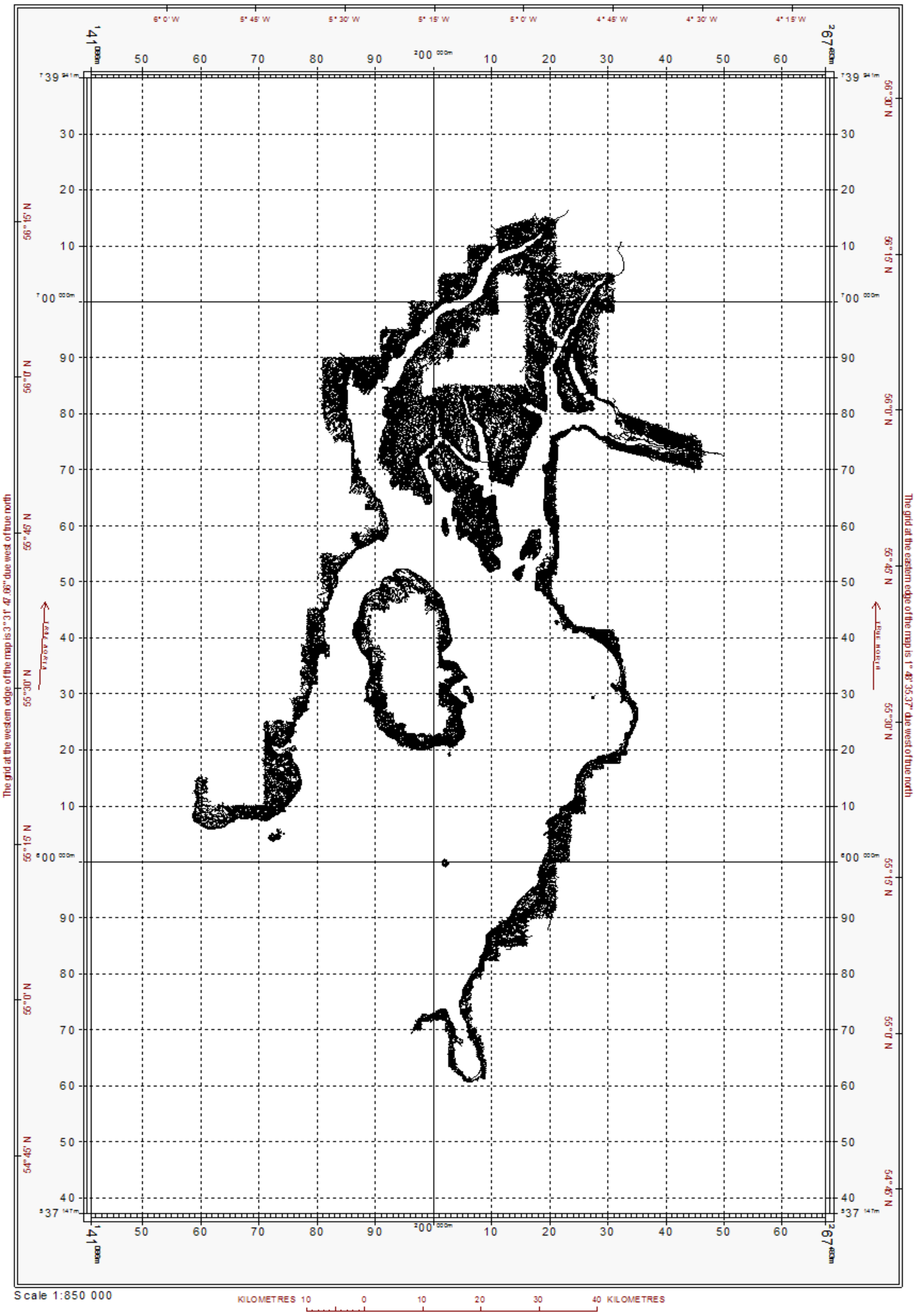


Figure 2-2 Ordnance Survey data tidied - showing the foreshore mapping and tiles with lighthouses, churches and roads that were linked into other features that could not be deleted

The Mean High Water Springs and Mean Low Water Springs are shown (Figure 2-3), as an individual map so that it was easy to see where different foreshore features are. Mean High Water Springs are shown in red, and Mean Low Water Springs are shown in green. From this information the area of foreshore with rock (its varying amounts) and boulders could be estimated.

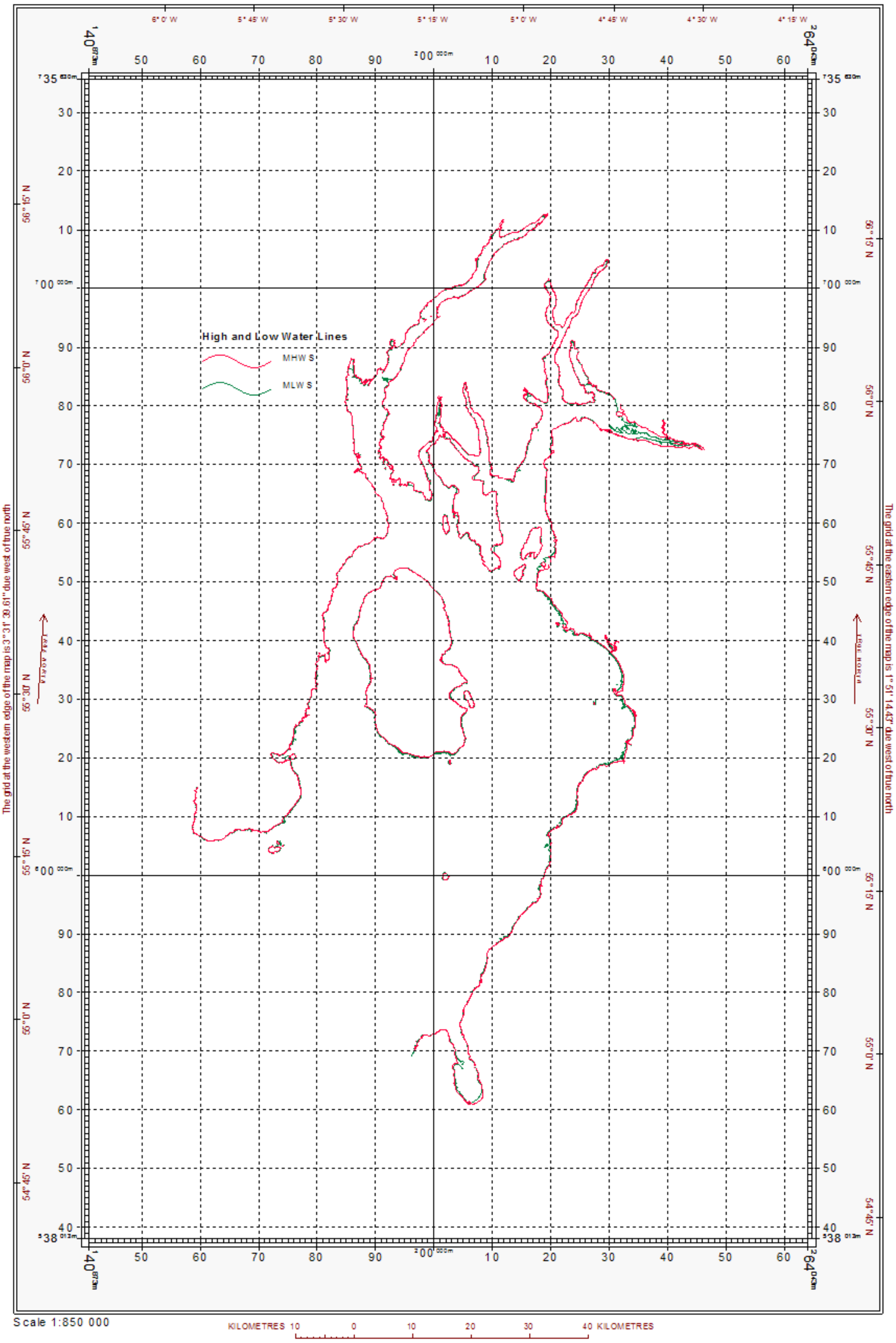


Figure 2-3 Mean Low Water Springs (MLWS in green) and Mean High Water Springs (MHWS in red) of the Clyde Sea area

An individual map of the Mean High Water Springs with the sites shown where data collection and experiments took place was constructed (Figure 2-4). The colours represent the underlying geology that the sites were on (red denotes igneous, orange is sedimentary and green are metamorphic sites). Rocks were taken from some of these areas to make artificial rock panels for later experiments. The black filled circles denote where experiments took place.

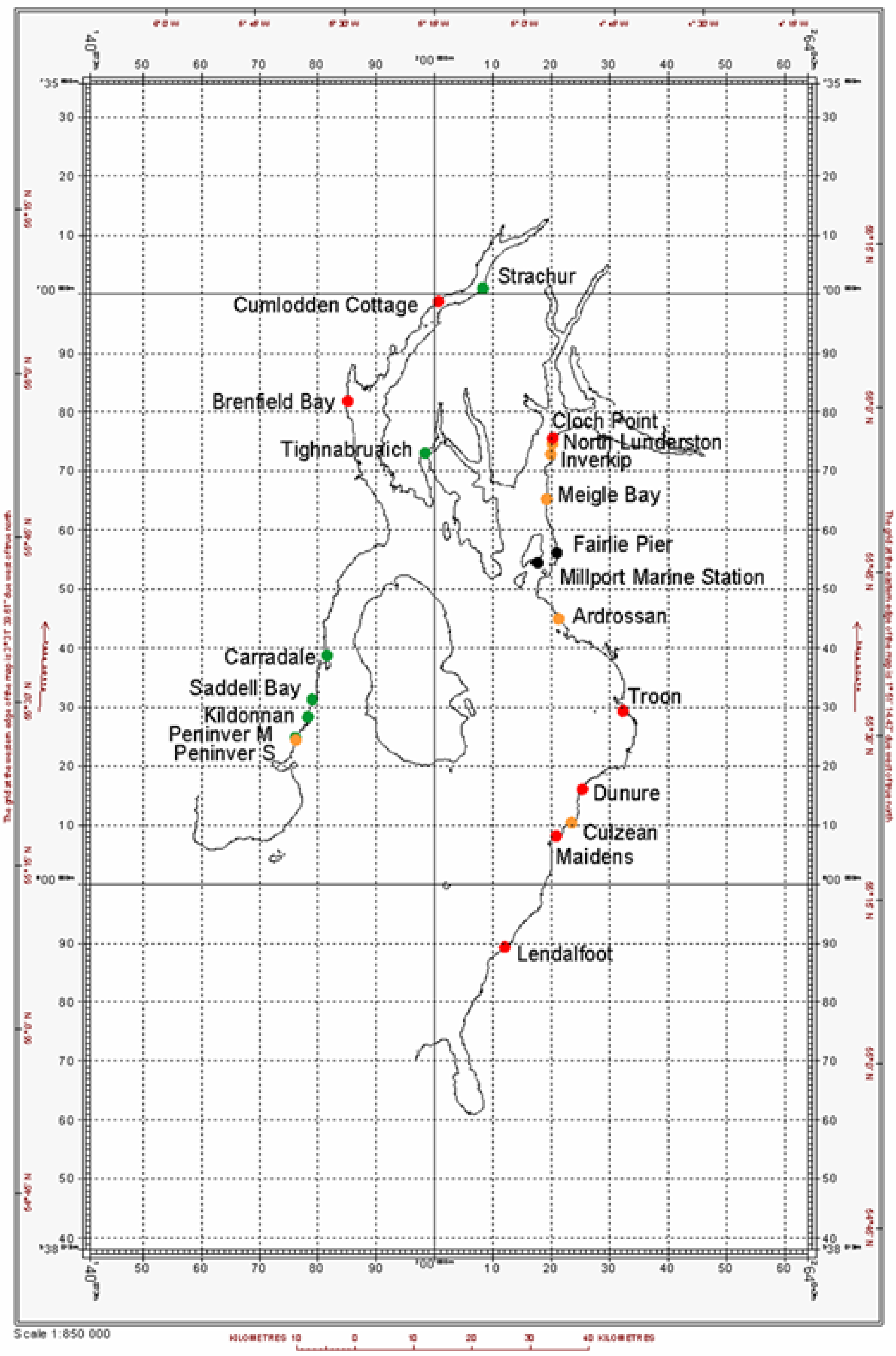


Figure 2-4 MHWS with places shown for data collection and experiments in Clyde Sea area (red denotes igneous, orange is sedimentary and green are metamorphic sites)

2.4.2 Geological mapping

A map was then configured showing the geology (Figure 2-5) from the digitised British Geological Survey (solid geology) maps. The geology maps were the same projection (OSGB 36) as the Ordnance Survey MasterMap^{TM} data, therefore this did not need to be altered. This would help selection of future sites, for the same number of geology sites.

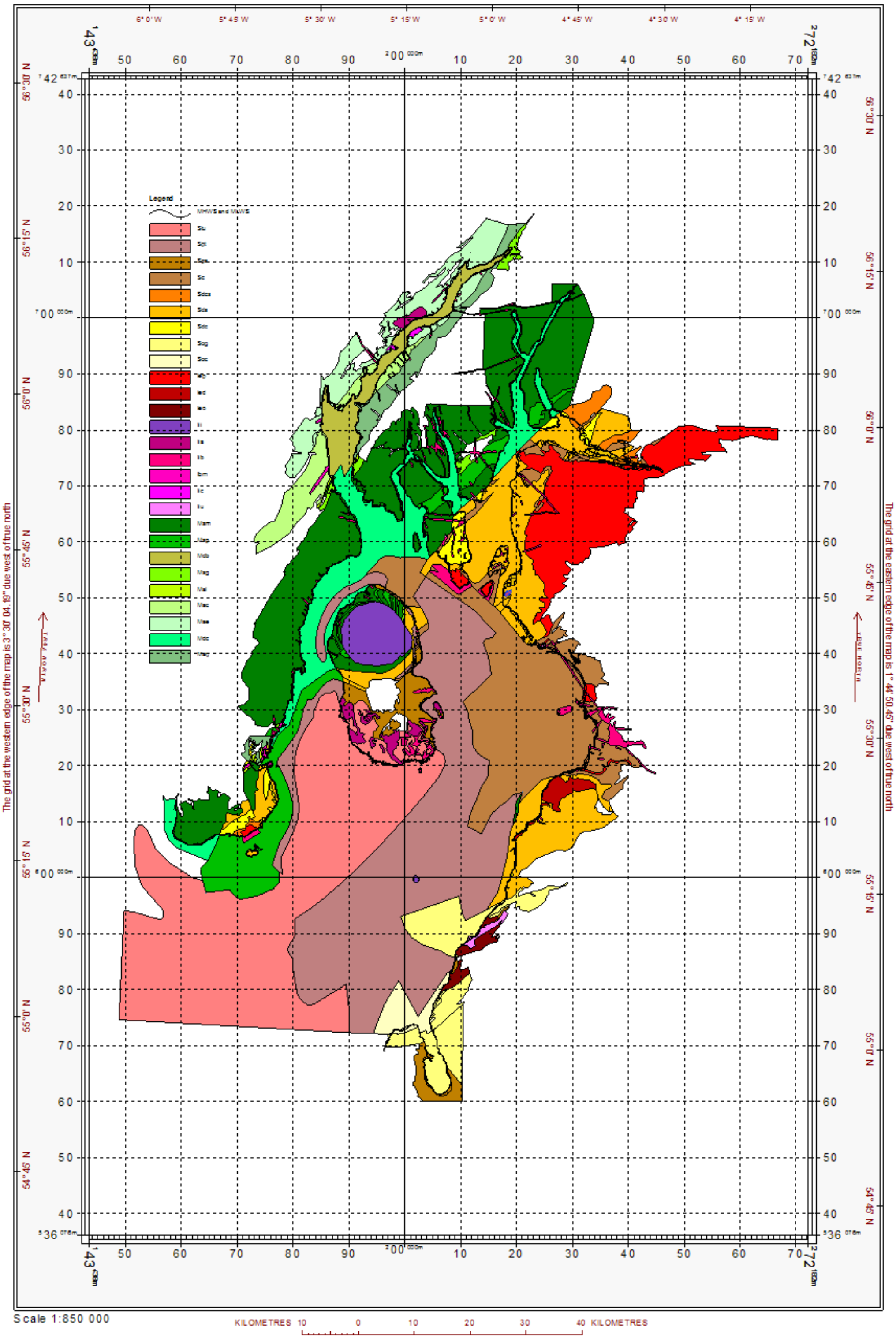


Figure 2-5 Geology of the Clyde Sea area with MHWS to show the foreshore (greens indicate metamorphic, reds are igneous and yellows are sedimentary rock)

The intertidal foreshore totalled 10732.322 ha in which :-

Igneous intertidal area = 1199.256 ha
Sedimentary intertidal area = 4737.819 ha
Metamorphic intertidal area = 2613.153 ha
Other areas e.g. river banks, ramps and quays = 2182.094 ha

From this, the area suitable for cypris to settle could be estimated, using rocks and boulders (Table 2-3).

		Igneous		**Sedimentary**		**Metamorphic**	
Geological feature	**% available for settlemet**	**Area on map (ha)**	**Area available for settlement (ha)**	**Area on map (ha)**	**Area available for settlement (ha)**	**Area on map (ha)**	**Area available for settlement (ha)**
Rock	100	343.305	343.305	567.363	567.363	374.649	374.649
rock etc	75	107.182	80.386	201.879	151.409	142.142	106.607
etc and rock	40	16.852	6.741	29.812	11.925	13.631	5.452
scattered rock	20	125.856	25.171	119.744	23.949	8.431	1.686
boulders	100	175.813	175.813	232.217	232.217	142.675	142.675
boulders etc	75	101.109	75.832	190.135	142.601	129.502	97.127
etc and boulders	40	208.928	83.571	411.044	164.418	573.032	229.213
scattered boulders	20	6.657	1.331	31.271	6.254	5.157	1.031
Area available to settle (ha)			**792.150**		**1300.136**		**958.440**

Table 2-3 Areas of rock types available for settlement

The total area for settlement on the rock of the Clyde Sea for *S. balanoides* is 3050.7262 ha, which is considerably less than the total area of foreshore when sand, mud and shingle habitats are removed. Igneous rock makes up 26%, sedimentary 42.6% and metamorphic 31.4%.

2.4.3 Bathymetric mapping

The bathymetric map (Figure 2-6) was made using Admiralty charts of varying projections. These were digitised and 'pulled' into an OSGB 36 projection. This shows the depths of the Clyde Sea which may influence how the waves arrive at the shore, together with Mean High Water Springs and Mean Low Water Springs.

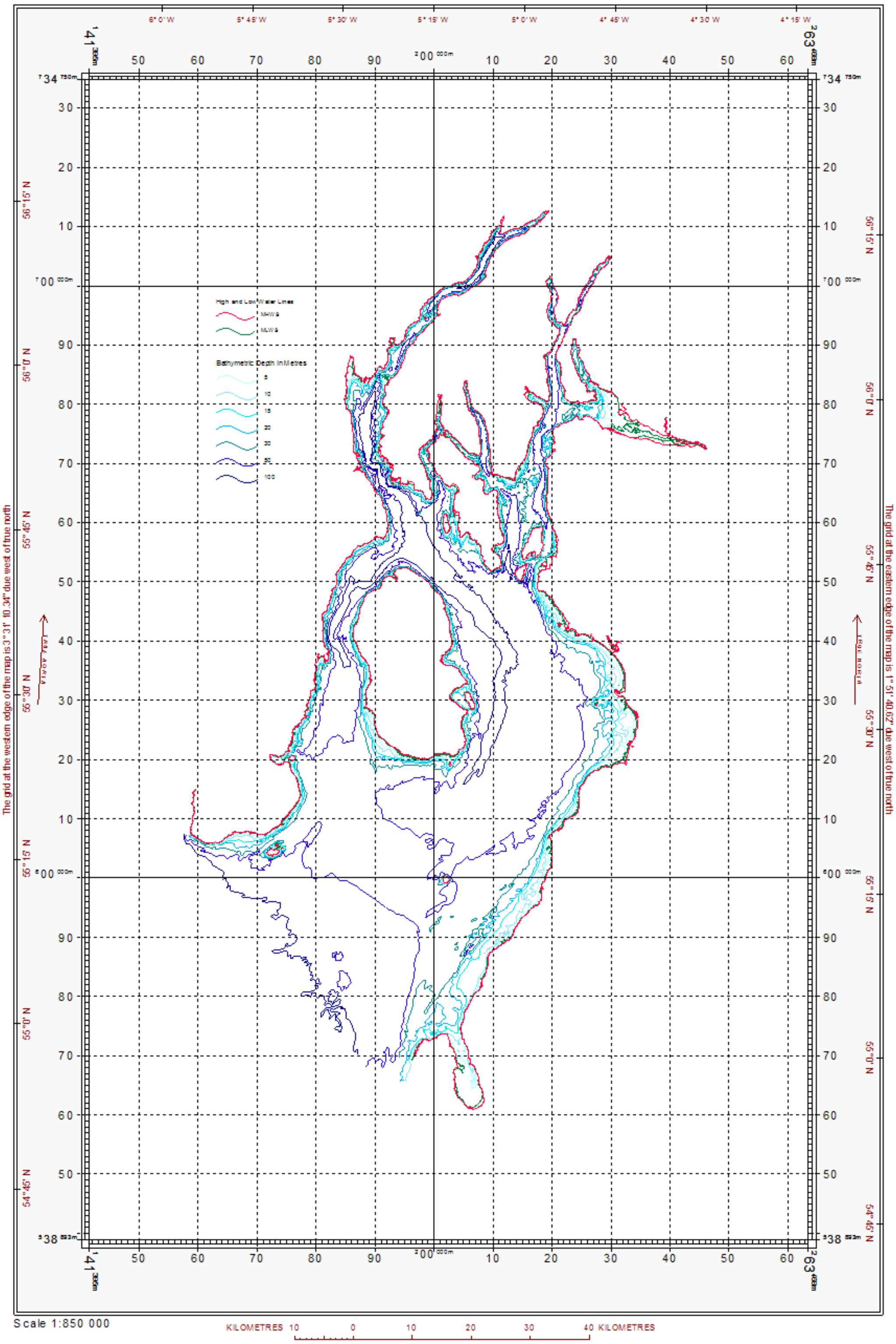


Figure 2-6 Bathymetric map of Clyde Sea area, of all charts to OS36 projection and MHWS and MLWS

2.4.4 Combined mapping to OSGB 36

The separate maps of OSGB 36 projections were then all incorporated into one map (Figure 2-7), which allows the Ordnance Survey data with the coloured Mean High Water Springs and Mean Low Water Springs, the geology and the bathymetric data to be consulted, and examined at once.

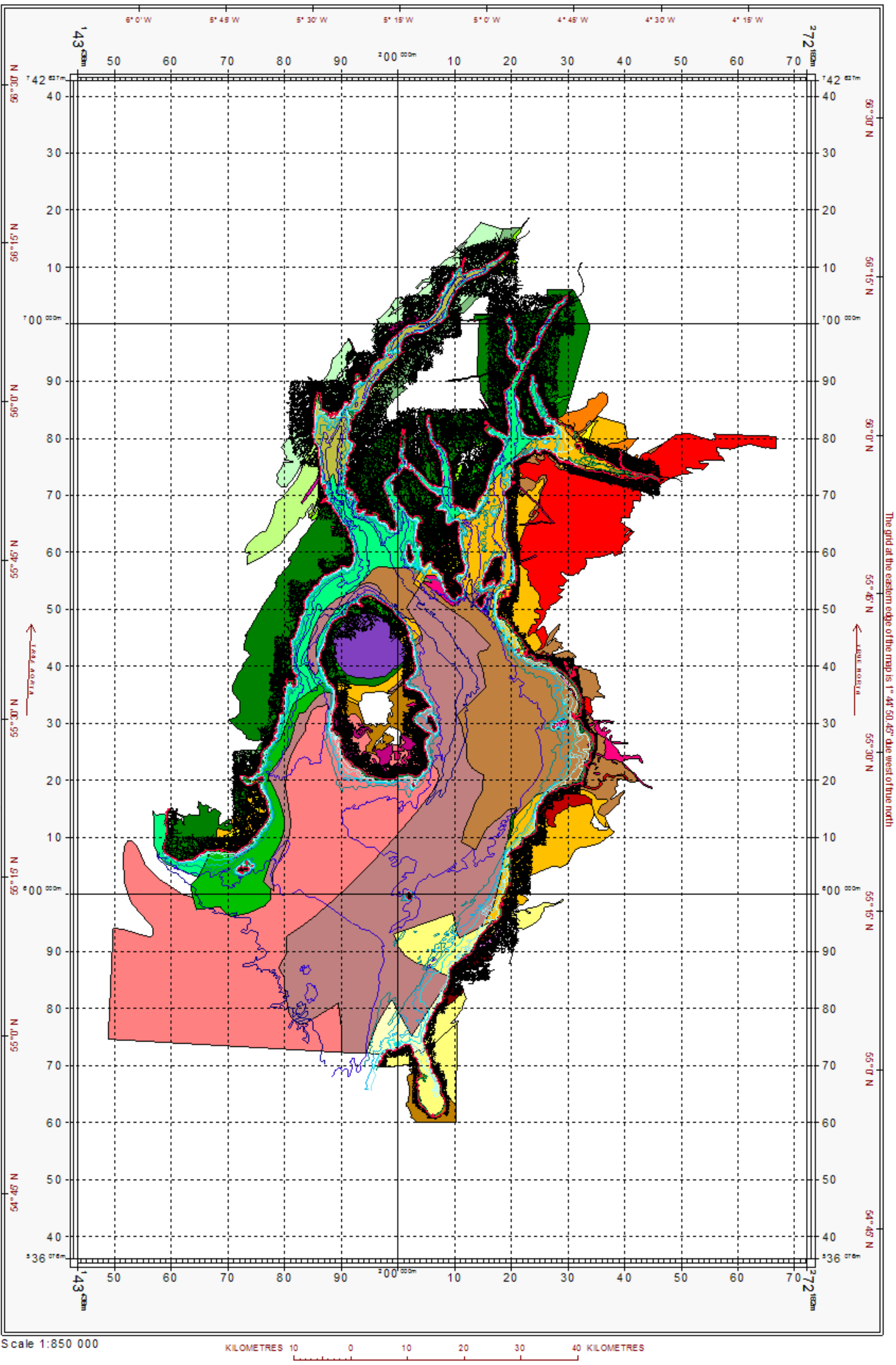


Figure 2-7 Combined map of Clyde Sea area, of Ordnance Survey data, geology and bathymetric data (OS36 projection)

2.4.5 Exposure rating of different shores

Although the modified maximum fetch system appeared to work it was a time consuming method, using the bathymetric data (Figure 2-6), to find the spot depths or the contour depth, with each of these giving a modified maximum fetch.

Site	**Spot depth (1 mile)**	**Contours (1 mile)**	**Spot depth at 1 km**	**Spot depth at 5 km**	**Contours at 1 km**	**Contours at 5 km**
Lendalfoot	4.94	7.05	3.53	16.22	3.53	21.16
Culzean	3.93	4.91	2.45	10.31	2.45	14.72
Ardrossan	1.93	2.41	1.21	9.16	1.21	12.05
Inverkip	0.04	0.05	0.03	0.14	0.05	0.13
North Lunderston Bay	0.23	0.23	0.10	0.24	0.14	0.23
Cloch Point	0.21	0.21	0.12	0.22	0.20	0.20
Strachur	0.07	0.10	0.07	0.07	0.10	0.10
Tighnabruaich	0.04	0.05	0.03	0.05	0.05	0.08
Carradale	1.65	1.94	0.91	1.65	0.97	1.94

Table 2-4 Modified maximum fetch determined by Wright (1981) and Baker and Crothers (2009)

Using the fetch values (Table 2-4), the spot depths and contours at both 1 km and 5 km suggest that an exposed site was any value over 0.99. However, the exception to this was Carradale where the spot depth at 1 km and contours at 1 km showed the shore to be sheltered, but the spot depth and contour at 1 mile and the spot depth and contour at 5 km suggested that the shore was exposed. When the shore was visually inspected it suggested that the shore was sheltered with plenty of fucoids, but also with *S. balanoides.* Carradale is protected from the West by Kintyre and Arran to the North East.

Univariate GLM ANOVAs for spot heights, contour heights, spot heights at 1 km and 5 km and contour heights at 1 km and 5 km showed that the exposure ratings were all significant for all the depths shown on the bathymetric charts (see Table 2-5).

Type of Modified Maximum Fetch	**P - value**	**F – value**	**R^2**
Spots (1 mile)	0.001	30.884	0.740
Contours (1 mile)	0.001	28.148	0.737
1 km spots	0.001	28.931	0.744
1 km contours	0.001	29.478	0.734
5 km spots	<0.001	69.085	0.896
5 km contours	<0.001	81.107	0.912

Table 2-5 Results of univariate GLM ANOVAs using a variant of Modified Maximum Fetch

Any of these types of Modified Maximum Fetch could be used in conjunction with the relevant chart to find an exposure rating for a shore.

The Rose system (Figure 2-8) with its exposure rating in metres was investigated. The exposure rating resulting from using the Rose overlay was used with the shores (Table 2-6).

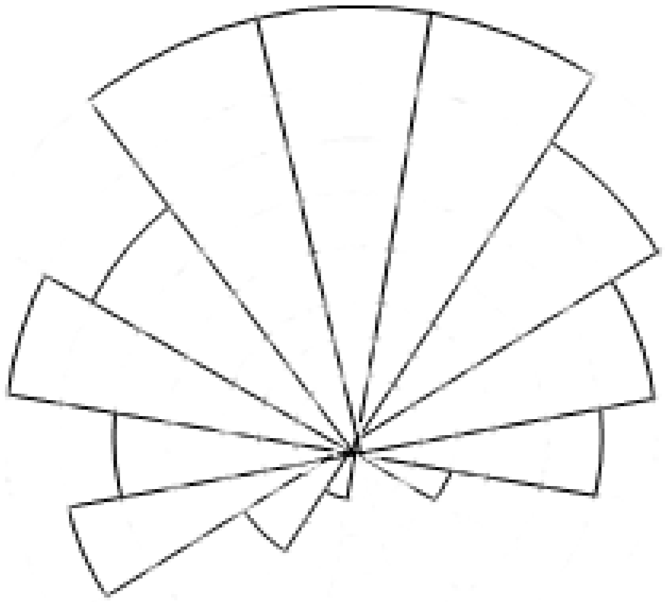


Figure 2-8 Rose diagram or compass rose divided into 16 segments used to estimate fetch. For example the north and each segment either side would be the furthest to the next land mass, the east and west segments are of an intermediate distance to land and the south and south south - east would have land within these segments, and the south and south south – east would have more than 50 % land.

The furthest distance to the next landfall from a shore in the Clyde Sea area was Northern Ireland over the Great Plateau. All other landfalls were within the Clyde Sea area.

Shore	**Exposure rating (m)**	**Rock type**	**Exposure**
Cumlodden Cottage	3068.08	I	S
Strachur	3548.50	M	S
Tighnabruaich	4133.42	M	S
Inverkip	4795.86	S	S
North Lunderston Bay	6423.88	S	S
Carradale	7345.23	M	S
Cloch Point	7514.00	I	S
Brenfield Bay	8852.16	I	S
Kildonan	12728.49	M	E
Saddell Bay	13837.86	M	E
Peninver M	20968.08	M	E
Peninver S	22313.98	S	E
Maidens	22508.90	I	E
Ardrossan	28859.13	S	E
Troon	32200.81	I	E
Dunure	32299.66	I	E
Lendalfoot	36252.91	I	E
Culzean	38558.90	S	E
Meigle Bay	3777.17	S	S

Table 2-6 Fetch using the Rose system for calculation of exposure

Rock type: I = Igneous, M = Metamorphic and S = Sedimentary. Exposure: S = sheltered and E = exposed. Exposure rating = mean fetch in metres.

It was decided that a sheltered shore was less than 10,000 m and an exposed shore over 10,000 m. Rock type was added onto the table so that it can be referred back to when calculating which sites to use (See Table 2-6).

The univariate GLM ANOVA using the method modified from Thomas (1986) showed that Rose distance was significant ($p = < 0.001$, $F = 102.774$, $R^2 = 0.720$), and could be grouped into two distinct groups (sheltered and exposed). Because a standard acetate overlay was aligned and used each time, this method could be used on maps or charts of a shore and was quicker than the other method used. The difference between sheltered and exposed shores was larger using the Rose method, and therefore easier to differentiate. Graphs were obtained showing how the sites split into exposed and sheltered (Figures 2-9 and 2-10).

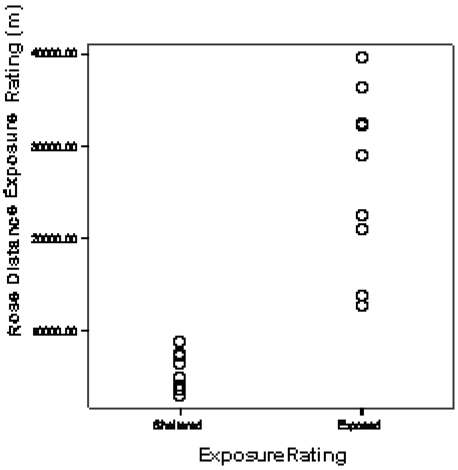


Figure 2-9 Sites split into sheltered and exposed by the Rose method

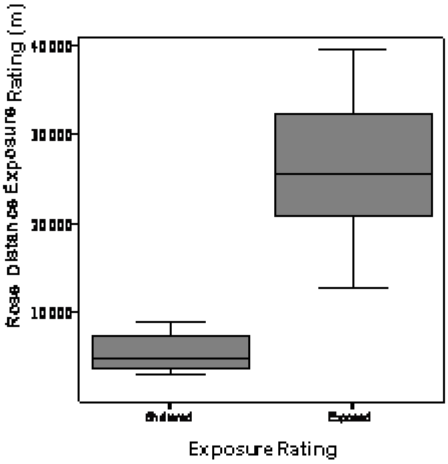


Figure 2-10 Sites split using Rose method as a whisker diagram showing median line and spread of Rose distance exposure

From the mapping and exposure ratings Table 2-7 was constructed using the site, grid reference for the shore used, the years that each site was used, its exposure and the rock type; this table of information could be referred to subsequently.

Site	**Grid Reference**	**Year used**	**Exposure**	**Rock type**	**Chapter used**	**Experiment**
Ardrossan	NS213 450	Dec 2005 Dec 2006	Exposed	Sedimentary	3, 7	ES, ST, EES & D; BNo.
Brenfield Bay	NR853 819	Dec 2006	Sheltered	Igneous	3, 7	ES, ST, EES & D; BNo.
Carradale	NR816 388	Dec 2004 Dec 2006	Sheltered	Metamorphic	3, 7	ES, ST, EES & D; BNo.
Cloch Point	NS203 757	Dec 2004 Dec 2005 Dec 2006	Sheltered	Igneous	3, 7	ES, ST, EES & D; BNo.
Culzean	NS235 105	Dec 2004 Dec 2005 Dec 2006	Exposed	Sedimentary	3, 7	ES, ST, EES & D; BNo.
Cumlodden Cottage	NS008 989	Dec 2006	Sheltered	Igneous	3, 7	ES, ST, EES & D; BNo.
Dunure	NS254 161	Dec 2006	Exposed	Igneous	3, 7	ES, ST, EES & D; BNo.
Fairlie Pier	NS209 562	April 2005	Sheltered	Sedimentary	4, 6, 7	F, FAD, RA, R, O, A
Inverkip	NS199 729	Dec 2004 Dec 2006	Sheltered	Sedimentary	3, 7	ES, ST, EES & D; BNo.
Keppel Pier	NS177 544	April 2007	Sheltered	Igneous	4, 5, 7,	C; A
Kildonan	NR783 283	Dec 2006	Exposed	Metamorphic	3, 7	ES, ST, EES & D; BNo.
Lendalfoot	NX121 894	Dec 2004	Exposed	Igneous	3, 7	ES, ST, EES & D; BNo.
Maidens	NS208 082	Dec 2006	Exposed	Igneous	3, 7	ES, ST, EES & D; BNo.
Meigle Bay	NS192 653	Dec 2006	Sheltered	Sedimentary	3, 7	ES, ST, EES & D; BNo.
North Lunderston Bay	NS202 747	Dec 2005 Dec 2006	Sheltered	Sedimentary	3, 7	ES, ST, EES & D; BNo.
Peninver M	NR761 249	Dec 2006	Exposed	Metamorphic	3, 7	ES, ST, EES & D; BNo.

Peninver S	NR762 245	Dec 2006	Exposed	Sedimentary	3, 7	ES, ST, EES & D; BNo.
Saddell Bay	NR790 314	Dec 2006	Exposed	Metamorphic	3, 7	ES, ST, EES & D; BNo.
Strachur	NN083 011	Dec 2004 Dec 2005 Dec 2006	Sheltered	Metamorphic	3, 7	ES, ST, EES & D; BNo.
Tighnabruaich	NR983 731	Dec 2004 Dec 2006	Sheltered	Metamorphic	3, 7	ES, ST, EES & D; BNo.
Troon	NS324 294	Dec 2006	Exposed	Igneous	3, 7	ES, ST, EES & D; BNo.

Table 2-7 Site, grid references, years used, exposure and rock type

Chapter 3: ES = Egg Size, ST = Somatic Tissue, EES = External Egg shell Size, D = Density of barnacles
Chapter 4: F = Frame experiment, FAD = Filming Arrival, Departing and settlement, Rock versus Artificial rock
Chapter 5: C = Cannibalism (gut contents and filming)
Chapter 6: R = Rock type settlement, Orientation
Chapter 7: BNo. = Barnacle Numbers (Alive, Dead, Rock space), A = Area available for settlement

2.5 Discussion

The mapping using the Ordnance Survey MasterMap$^{\text{TM}}$ data did require much tidying of its ‘spaghetti’ data (removal of two plus lines on top of each other, and the joining up of data lines for example MHWS); also the cropping of data that was more than about 100 m from the foreshore. Once this was completed the other data for the geology layer and bathymetric layer could be attended to. The geology and bathymetric data could have been purchased in digital format and quickly added to the base map of the Ordnance Survey. However, both data sets were very expensive when they were required (the bathymetric data being at least four times more expensive than first orally quoted by SeaZone of ~ £200), therefore digitising from paper copies was undertaken. Digitising does take a long time but it was required for future chapters such as the

Fecundity chapter, and the Rock type chapter. Bathymetric charts use data obtained from the 1800's to modern times and as these charts were current then it must be assumed that they are still accurate (see Table 2-3), as the Admiralty only updates those charts where there are known problems, for example moving sandbanks and new wrecks.

Pulling or reshaping the bathymetric data into OSGB 36, was required to have all information in one projection. The SeaZone bathymetric mapping may have been in the Admiralty chart projections or have been transferred into OSGB 36 by that company. All the information on one map allowed depths, geology, the foreshore and fetch to be taken into account when deciding on sites to be used. For example, in the fecundity chapter all this information was required in order that the adult barnacles harvested could be separated into rock types and exposure, to deduce whether either of these influences had an effect on the size or number of eggs produced. The geology information was used in the rock type chapter and the number of adults calculated for different rock types to see if there was a statistical difference.

Obviously when the mapping was complete there may be slight errors of where each rock type changed in relation to the OS data, especially when using 50,000 mapping scales. However, when the sites were visited, all sites were of the main rock type expected from consulting the combined data on the computer. This mapping data allows an overall estimate of the number of adult *Semibalanus balanoides* in the Clyde Sea to be made, and their potential larval input from the exposure and rock type they are from.

Technology within the mapping industry has increased, allowing handheld Global Positioning Systems (GPS) to be used for 100 mm accuracy. However, this accuracy is dependent on the satellite GPS systems, at the minute relying on American satellites which have a built in data shift (for security purposes), and will be much more accurate when the new European Galileo is in service (OS personal communication).

Data files produced by ArcView and ArcMap (9.0 and above), are difficult to export to different mapping programmes, which require larger processing power. The MasterMap^{TM} data of later versions omits many intertidal features such as differentiating between rocks and boulders (OS personal communication), which would be a problem for working out areas of these features. Google Earth satellite photographs are increasing in definition all the time and rocks can usually be discerned by this approach; however, when the mapping was completed each pixel represented 15 m and was therefore unusable. Similarly waterproof tablets are now produced (for example by Husky Hunter) that can be loaded with a base map of the area and amendments can be made whilst at a site, especially if it incorporates a GPS system. A Leica total station was trialled on land, but was found to be very time consuming, although it gave accuracy to mm levels – both in elevation and horizontal information only one site could have been surveyed per low water tide, taking too long to survey the shores used in the time available.

It has been stated that there is no one approach to quantitatively measure wave amplitude and fetch (Burrows et al. 2008), then relating this to a shores exposure. Wave amplitude can be measured over a period of time, but the equipment is usually very expensive requiring deployment, monitoring, and retrieval (Palumbi 1984). In the United Kingdom most shores can be frequented by the general public, therefore vigilance must be used to prevent equipment being stolen or tampered with.

Burrows et al (2008) used a Geographical Information System (GIS) on the west coast of Scotland, in order to investigate fetch determined to the nearest land (up to a maximum distance of 200 km) in 16 angular sections, and the average wind speed with its occurrence. The maximum fetch corresponded to grid boundaries and the transition point (TP), where the fetch is a suitable distance to fully develop the wave amplitude (Burrows et al. 2008). Burrows et al (2008) found that their wind data had only a small exposure effect on the west coast of Scotland, but that the nearshore bathymetry played a part in how exposed a shore was, and suggested that geology may play a part in recruitment. Burrows et al (2010) investigated 259 sites around Scotland, and used photographs of three shore heights, recording adult and juvenile *Semibalanus balanoides* finding that shore heights were similar, but survey year had the largest effect on this species. However, density of barnacles (adults and juveniles) increased with fetch (Burrows et al. 2010). von der Meden et al (2012) working on South Africa's coast with the mussel *Perna perna*, found that habitat availability differed with the scale investigated (broad scales of 1000 and 100 m compared to a scale of 10 m), and suggested that residence time (between open coast and bays) allowed increased settlement. Therefore scale and global position should be taken into consideration.

Biological ratings (Lewis 1955, Ballantine 1961, Little & Kitching 2005) are not usually quantifiable and are informal, requiring a shore to be investigated first, rather than a few sites highlighted to subsequently match with exposure levels (Burrows et al. 2008). If the wave fetch and subsequent exposure can be calculated, it may be possible to predict communities and approximate numbers of a specific species over a large area. Once this has been completed for a shore, unless there are large installations built in the adjacent coastal waters, the data will not require updating. Quantitative measures also allow for smaller or larger classifications as required, rather than a costly and time consuming trip to the shore.

The exposure ratings showed that both the Modified Maximum Fetch after Wright (1981), and a variation on Thomas (1986) from the MHWS mark on the shore could be used to assess the exposure of shores in the Clyde Sea area (see Table 2-6 and Figures 2-9 and 2-10). This information could be used in the fecundity chapter, as exposures are evaluated to find out if exposure alters the number or size of eggs produced by *S. balanoides* living on these shores.

2.6 Conclusions

- It was possible to develop a mapping system comprising OS MasterMap^{TM} data, geology data from paper maps, and bathymetric data from Admiralty charts.

- Shore exposures can be calculated using a combined mapping system in the office, rather than relying on going to the shores to use a field based system. The exposures were best calculated using the Rose method as it was quicker, and there was a significant difference between exposed and sheltered results.

- There are plenty of exposed and sheltered shores in the three major rock types within the Clyde Sea area giving a very varied coastline.

Chapter 3. Fecundity

3.1 Introduction

Fecundity has been defined as the eggs produced in relation to the weight of the barnacle (Barnes & Barnes 1968). It is important to understand the breeding cycle of *Semibalanus balanoides* and the potential input into the Clyde Sea area of nauplii, in order to collate a complete understanding of the ecology of this barnacle within the fjordal system. The information then allows an appreciation of future generations of the species in this environment. In the rocky shore community, barnacles (both adults and their progeny) are an important food source for invertebrates and vertebrates (see Chapter 1).

Populations of *S. balanoides* may fall in response to climate change, either by reduced reproduction, heat stress or competition from alien species with eggs and larvae stages possibly the most vulnerable (Przeslawski et al. 2005, Mieszkowska et al. 2006, Cheung et al. 2009, Harley 2011). Climate change affects abundance and geographical distribution with global temperatures averaging an increase of 0.6^{o}C over the last 100 years, and up to 1^{o}C increase in areas of the North East Atlantic (Root et al. 2002, Hawkins et al. 2008). The increased temperatures may lead to a change in community structure, for example shores that were dominated by *S. balanoide*s may alter to *Chthamalus* which may decrease algal 'escapes' reducing fucus cover on those rocky shores (Hawkins et al. 2008, Hawkins et al. 2009, Cahill et al. 2013). The increased temperature may also alter predator (dog whelks, blennys, three – spined sticklebacks, green crabs, ctenophores, jelly fish, large copepods, arrow worms, limpets, nudibranch, polychaete green leaf worms) numbers of *S. balanoides,* as replacing barnacle species are unlikely to fit exactly into the niche (Connell 1961a, b, Muxagata et al. 2004). Fecundity may vary due to external factors which may have an effect on the number of nauplii released into the Clyde, increasing or decreasing the risks that the nauplii have due to intraspecific and interspecific competition for food and the risk of being eaten themselves, by other species in the food chain. This would have implications for the number of nauplii that became cypris, and their chances of settlement and producing their own offspring as adult barnacles. Knowing the fecundity allows for the input to the system to be noted, so that the numbers of nauplii lost can be estimated before the cyprid state develops in order to advance a mathematical model.

S. balanoides has an annual reproductive cycle that is adapted to the northern boreal conditions of its range (Barnes 1963, Barnes & Barnes 1968, Wethey 1984a, Charnov 1987, Hills & Thomason 2003a, b). There is a generalized north, south reproductive gradient such that mating occurs in Spitzbergen during August to September, at Tromso it is the mid to end of October, in early November it is in the Clyde Sea area (Barnes & Barnes 1967), and in northern Spain it is the end of November (King et al. 1993).

Some describe *S. balanoides* as an obligate cross-breeding hermaphrodite (Stubbings 1975, King et al. 1993, Schmidt et al. 2000, Dufresne et al. 2002, Pineda et al. 2002, Veliz et al. 2006a, b), whilst others describe it as a protandrous hermaphrodite (Charnov 1987), Thomason et al. 2000, Hills & Thomason 2003a) producing both male and female gonads during the breeding season (Charnov 1987). The two definitions are not contradictory statements, but different ways to describe *S. balanoides* reproductive process which is described in detail below.

Copulation is not simultaneously mutual, as only one of a pair is a ‘female’ at a time, and if it has more than one mate, sperm competition may take place (Charnov 1987). The ‘female’ receptive barnacle may signal chemically to other barnacles, although ‘male’ acting barnacles also search for ‘females’. When a ‘female’ barnacle is found, the ‘male’ then copulates with it, and often more than one functional ‘male’ will copulate with a receptive ‘female’. Although a single male / female pair undertaking copulation is the most common, up to six functional ‘males’ have been reported, with two to three fairly common (Stubbings 1975, Charnov 1987). After fertilization the eggs form two ovigerous lamellae (secreted by the oviducal gland) in the mantle cavity which may take up to a few hours, and only takes place after at least two insertions of a penis (Barnes & Barnes 1959, Hurley 1973, Stubbings 1975, Charnov 1987). The eggs are oxygenated by the water passing through the mantle cavity, and apart from the eggs and sperm there is no parent to offspring nutrient transfer (Charnov 1987). After mating the penis is lost through programmed cell death during the moult, suggesting it is very expensive to the barnacle as it is non-existent for a large part of the year and only grows rapidly in September and October, reaching its full size for the mating season (Barnes 1962, Stubbings 1975, Hoch 2008, Klepal et al. 2008, Klepal et al. 2010). An electron microscope investigation on the cuticle of the penis, showed elongated structures that added to the width, and probably provided strength and flexibility (Klepal et al. 2010). The eggs are incubated over the winter while the barnacle is in an anecdysis state (Barnes 1962, Charnov 1987, King et al. 1993). The release of nauplii occurs when the adult barnacles start feeding on the spring algal bloom of *Skeletonema costatum* (Barnes 1956, Charnov 1987, Starr et al. 1991, King et al. 1993).

There are large changes in an individual's body weight throughout the year corresponding with a large variation in food intake. Weight increases in spring and summer when reserves are laid down, but is then lost during the breeding season when semen is ejaculated during copulation, and very little feeding occurs during the winter (Barnes 1963, Barnes & Barnes 1968, Hurley 1973, Bertness et al. 1991). However, *S. balanoides* requires a constant food supply through the summer in order to develop its sexual organs (Charnov 1987, Thomason et al. 2000, Hills & Thomason 2003a).

Starvation of the adult during ovarian development in the late summer may lead to regression of the ovaries, but if feeding is resumed then development of the eggs takes place (Barnes 1956, 1963, Barnes & Barnes 1967). It is also thought that temperature plays an important role during the later stages of development. Some authors suggest the critical temperature is around 10^{o}C (Barnes 1963, Barnes & Barnes 1967, Stubbings 1975, Svennson et al. 2005), others 3^{o}C – 9^{o}C (King et al. 1993) or 6^{o}C – 12^{o}C (Lawson et al. 2004) and that above these temperatures the later reproductive stages do not take place.

Resources used for reproduction, for example to make the large penis, are unavailable for other functions (Wethey 1984a, b, Charnov 1987) and it may affect the barnacle's overall reproduction due to the large reserves required to produce it, leaving an adult less competent for the reproductive season (Hoch 2008). Some barnacles may require a larger penis if they are in an exposed site where wave motion may damage a more diminutive penis, or where mates are a large distance apart (Hoch 2008, 2010).

However a large penis may impede feeding more than a smaller one where barnacles are close together, as it is positioned between the cirri (Hoch 2008).

It has been suggested that fecundity of barnacles can be affected by crowding (Wu 1980, Wu 1981, Wethey 1984a, Lopez & Gonzalez 2003, Leslie 2005), position on the shore (Bertness et al. 1991, Burrows et al. 1992, Jeffery & Underwood 2001), exposure (Burrows et al. 1992, Hoch 2008, Hoch 2010), genetics (Brind'Amour et al. 2002, Jarrett 2003, Veliz et al. 2004, Veliz et al. 2006a, b), pollution (Wu & Levings 1980), climate change (Svensson et al. 2005), age (Hurley 1973), size (Barnes & Barnes 1968, Hurley 1973, Burrows et al. 1992, Leslie 2005), intraspecific competition (Wethey 1984a, b, Kendall & Bedford 1987, Hills & Thomason 2003a, b, Leslie 2005), starvation (Barnes & Barnes 1967), or even being on live or dead mussels (Buschbaum 2001).

Hansson et al (2003) suggests that the fecundity of *S. balanoides* within the Clyde Sea area is the highest in Europe, with up to 5333 eggs mg^{-1} of dried adult somatic tissue and approximately 5000 eggs per individual. Hills and Thomason (2003b) estimated the number of eggs as 1333 eggs mg^{-1} of dried egg mass for barnacles also from the Clyde Sea, and Barnes and Barnes (1968) working at Millport in the Clyde Sea found 3667 – 5334 eggs per mg^{-1} of dried somatic tissue. King et al (1993) in Eire found 562 -7120 embryos per animal, and Brind'Amour et al (2002) found 2455 to 6532 eggs per individual in North America or 69 eggs per mg^{-1} of wet egg tissue. It has been suggested that the dried weight of an egg is approximately 2.7 µg (Wethey 1984a). These vastly different figures suggest that the number of eggs per mg^{-1} of dried somatic tissue varies considerably for this species over different years, even when the counts have been made using barnacles from the Clyde Sea area.

Gregariousness of conspecifics in barnacles is a requirement for reproductive success in *S. balanoides* (Knight-Jones 1953, Hills & Thomason 2003a), and this tendency may also benefit individuals in other ways such as a refuge from predation and increased cirral activity increasing food supply (Hills & Thomason 2003b). Thomason et al (2000) and Hills and Thomason (2003b) expected to find a proportional relationship between egg weight and density of adults in *S. balanoides*, but could not find one. However, Hills and Thomason (2003a) found from their experiments with *S. balanoides*, that the mass of eggs produced by the barnacles was negatively related to the morphological index, which suggested more eggs were to be found in conical barnacles than columnar barnacles, and the heaviest egg masses, with the most eggs were found to be from conical barnacles that had the heaviest shells. Work on *Austromegabalanus psittacus* in Chile (Lopez et al. 2007) found that there were no energetic differences for barnacles living in groups or independently, and that high densities would reduce the density-dependent effects such as food acquisition. *Jehlius cirratus*, also from Chile, showed higher fecundity in solitary rather than intermediate and high density living individuals (Lopez & Gonzalez 2003). Some barnacles of *J. cirratus* in crowded aggregations had larger egg masses, and smaller than expected egg masses were found in some solitary individuals suggesting that energy allocation may be responsible for this if less energy is required for mechanical support then that energy may be available for reproduction (Lopez & Gonzalez 2003). It had been suggested by Wethey (1984a) that the sharing of shells in high density aggregations of either *S. balanoides* or *Chthamalus dalli* may benefit individuals, by an increase of habitable volume, rather than thicker shelled solitary conical individuals who have to reinforce their shells by themselves against the hydrodynamic forces. Both *S. balanoides* and *C. dalli* produced larger egg masses per unit somatic tissue in crowded and columnar barnacles rather than uncrowded colonies (Wethey 1984a). Wethey (1984a) suggested that this was due to energy allocation with a greater proportion allocated to reproduction and a reduction to maintenance, competition, predator avoidance and damage from waves due to living in an aggregation. As Wethey (1984a) points out, the widespread occurrence of gregariousness and high densities of *S. balanoides* on the shore, suggest that crowding at some level must be beneficial to the species. However, very high densities where trumpet shaped barnacles appear in hummocks reduced fecundity. The conical body shape is favoured in *S. balanoides* possibly due to its lower hydrodynamic drag (Denny 1994), and in aggregations where shells are shared with neighbours lower energy expenditure may favour this trait than individuals reinforcing their shells in the region of contact (Wethey 1984a). In *Balanus glandula* Leslie (2005) found that larger barnacles grew in low density areas and these larger barnacles produced more eggs, however at higher densities there was enhanced survival for barnacles (but they were smaller in size) suggesting more eggs were produced per original recruit to these areas.

Many different methods have been used to estimate fecundity: from eggs per dried somatic tissue (Barnes & Barnes 1968, King et al. 1993), morphological index (MI) (Thomason et al. 2000, Hills & Thomason 2003a, b), basal diameter (Hurley 1973), aperture length ‘from the tip of the tergum to the scutum edge’ (King et al. 1993), and egg mass (Brind’Amour et al. 2002, Hills & Thomason 2003b). In the present work a reliable method of estimating fecundity was required that would allow fecundity to be measured accurately, without destruction to individual organisms. Many methods used in the past to estimate fecundity, have caused the destruction of individual animals. Although this is an accepted procedure for invertebrates, destruction of individuals may alter the ecosystem of that small area where this has occurred and may be ethically unacceptable to some people; therefore a non-destructive and less time consuming way of estimating fecundity should be adopted. A quick and effective technique was required allowing external measurements of the barnacle to be taken in order to estimate potential fecundity. The information would be collected over three years to allow for temporal variability, as Svensson et al. (2005) suggests that every fourth year there is lower recruitment of this species.

The Clyde Sea intertidal area is a very diverse environment (see Chapter 2). It has been previously suggested that barnacle fecundity alters in this area with shore height (Barnes & Barnes 1968); therefore it is also possible that fecundity may vary due to exposure of the shore or its rock type. If the fecundity of the barnacles in the Clyde Sea alters with exposure, rock type or position on the shore, this will alter the overall input into this system, and affect the future life of an individual settling cypris. Fecundity needs to be investigated over these very different habitats in order to determine whether this has an affect on the barnacles and their number of offspring in order to estimate the fecundity of the whole area.

3.2 Aims of chapter

A method to estimate fecundity of *Semibalanus balanoides* was required, that was non-destructive, in order for future estimates not to alter the ecology of small or even large areas of settlement. It was hypothesised fecundity was related to external shell size.

Rock type varies in the Clyde Sea and it was hypothesised that this would have an effect on the number of barnacles on different rock types and their fecundity.

Adult barnacles are found in exposed and semi-sheltered areas of the shore, as well as over a settlement zone of various heights up the shore. It was hypothesised that both exposure and an individual's position on the shore, would have an influence on its fecundity, due to being covered for longer by the tide (therefore longer to feed), and a greater availability of food and fewer predators on exposed shores.

3.3 Methods and materials

3.3.1 Sample collection

Collections of barnacles were made in December 2004, 2005 and 2006, as the breeding season should have finished by then and fertilized eggs (embryos) would be in the mantle cavity (Barnes & Barnes 1968). For the December 2004 collection of the fecund barnacles, seven sites (Carradale, Cloch Point, Culzean, Inverkip, Lendalfoot, Strachur and Tighnabruaich) were chosen as open and sheltered sites, and settlement was found on all three of the rock types igneous, metamorphic and sedimentary - (refer to Chapter 2, Table 2-7 and Table 3-1 for the sites, grid references, years each site was used, exposure and rock type). All sites were easily accessible from a road or car park. The second years collections (December 2005) were reduced to five sites (Ardrossan, Cloch Point, Culzean, North Lunderston Bay and Strachur – see Table 3-1) due to operational restrictions on time for the accompanying under graduate. Lendalfoot was removed from the investigations because the high level of sea due to forcing of the water into the fjordal system made the site potentially dangerous at low water. These were on the west facing coastal strip of the Clyde Sea and Strachur. The third years collections (December 2006) were made at all the eighteen sites listed in Chapter 2, Table 2-7 and Table 3-1. These sites covered all previously used (except Lendalfoot) and ten new sites, so that there were three sites at each exposure (exposed or sheltered) and each rock type (igneous, metamorphic and sedimentary). Barnacles were collected randomly from all sites to give an overall view of fecundity, taking into account potential differences in fecundity between solitary individuals and those in clumps.

Site	**Year**	**Method**	**Replicates**
Ardrossan	Dec 2005	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	8 / quadrat
		MI	8 / quadrat
		Egg weight	8 / quadrat
		Somatic Tissue	8 / quadrat
		Egg No. & size	10 eggs of 5 barnacles / quadrat
	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Brenfield Bay	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Eggs No. & size	10 eggs of 5 barnacles / quadrat
Carradale	Dec 2004	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	8 / quadrat
		Frustum volume	8 / quadrat
		MI	8 / quadrat
		Egg weight	8 / quadrat
		Somatic Tissue	8 / quadrat
		Egg No. & size	10 eggs of 5 barnacles / quadrat
	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Cloch Point	Dec 2004	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	8 / quadrat
		Frustum volume	8 / quadrat
		MI	8 / quadrat
		Egg weight	8 / quadrat
		Somatic Tissue	8 / quadrat
		Egg No. & size	10 eggs of 5 barnacles / quadrat
	Dec 2005	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	8 / quadrat
		MI	8 / quadrat

		Egg weight	8 / quadrat
		Somatic Tissue	8 / quadrat
		Egg No. & size	10 eggs of 5 barnacles / quadrat
	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Culzean	Dec 2004	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	8 / quadrat
		Frustum volume	8 / quadrat
		MI	8 / quadrat
		Egg weight	8 / quadrat
		Somatic T.	8 / quadrat
		Egg No. & size	10 eggs of 5 barnacles / quadrat
	Dec 2005	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	8 / quadrat
		MI	8 / quadrat
		Egg weight	8 / quadrat
		Somatic T.	8 / quadrat
		Egg No. & size	10 eggs of 5 barnacles / quadrat
	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Cumlodden Cottage	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Dunure	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Inverkip	Dec 2004	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	8 / quadrat
		Frustum volume	8 / quadrat
		MI	8 / quadrat

		Egg weight	8 / quadrat
		Somatic T.	8 / quadrat
		Egg No. & size	10 eggs of 5 barnacles / quadrat
	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Kildonan	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Lendalfoot	Dec 2004	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	8 / quadrat
		Frustum volume	8 / quadrat
		MI	8 / quadrat
		Egg weight	8 / quadrat
		Somatic T.	8 / quadrat
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Maidens	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Meigle Bay	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
North Lunderston Bay	Dec 2005	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	8 / quadrat
		MI	8 / quadrat
		Egg weight	8 / quadrat
		Somatic Tissue	8 / quadrat
		Egg No. & size	10 eggs of 5 barnacles / quadrat
	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat

Peninver M	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Peninver S	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Saddell Bay	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Strachur	Dec 2004	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	8 / quadrat
		Frustum volume	8 / quadrat
		MI	8 / quadrat
		Egg weight	8 / quadrat
		Somatic T.	8 / quadrat
		Egg No. & size	10 eggs of 5 barnacles / quadrat
	Dec 2005	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	8 / quadrat
		MI	8 / quadrat
		Egg weight	8 / quadrat
		Somatic Tissue	8 / quadrat
		Egg No. & size	10 eggs of 5 barnacles / quadrat
	Dec 2006	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	5 / site
		MI	5 / site
		Egg No. & size	10 eggs of 5 barnacles / quadrat
Tighnabruaich	Dec 2004	NDF	9 photos, ~ 20 barnacles / quadrat
		Frustum measured	8 / quadrat
		Frustum volume	8 / quadrat
		MI	8 / quadrat
		Egg weight	8 / quadrat
		Somatic T.	8 / quadrat
		Egg No. & size	10 eggs of 5 barnacles / quadrat

	Dec 2006	NDF Frustum measured MI Egg No. & size	9 photos, ~ 20 barnacles / quadrat 5 / site 5 / site 10 eggs of 5 barnacles / quadrat
Troon	Dec 2006	NDF Frustum measured MI Egg No. & size	9 photos, ~ 20 barnacles / quadrat 5 / site 5 / site 10 ggs of 5 barnacles / quadrat

Table 3-1 Summary table of sampling locations, years, methods used and the number of replicates (NDF = non-destructive estimation of fecundity quadrats and number of barnacles taken for other analysis – see methods, MI = morphological index).

3.3.2 Non-destructive estimation of fecundity

At each site a quadrat (500 mm x 500 mm) was placed over the rock and a photograph taken (Figure 3-1) using either a Canon Powershot G3 (4×10^6 pixels) or a Canon 350d camera (8×10^6 pixels). Nine quadrats were photographed at each site with three quadrats at the top of the *S. balanoides* settlement zone, three in the middle of the settlement zone and three quadrats at the bottom of the settlement zone. Each vertical line of quadrats was 10 m from the next line. Abundance of live, dead barnacles; and areas of rock and fucoid cover were calculated for each quadrat using ImageJ (Rasband 1997-2008) (Figure 3-2). A 100 ml centrifuge tube or a 125 ml Nalgene bottle with a wide neck, was then filled with barnacles carefully removed from each quadrat using a pen-knife, labelled and 100 % ethanol added as a preservative.

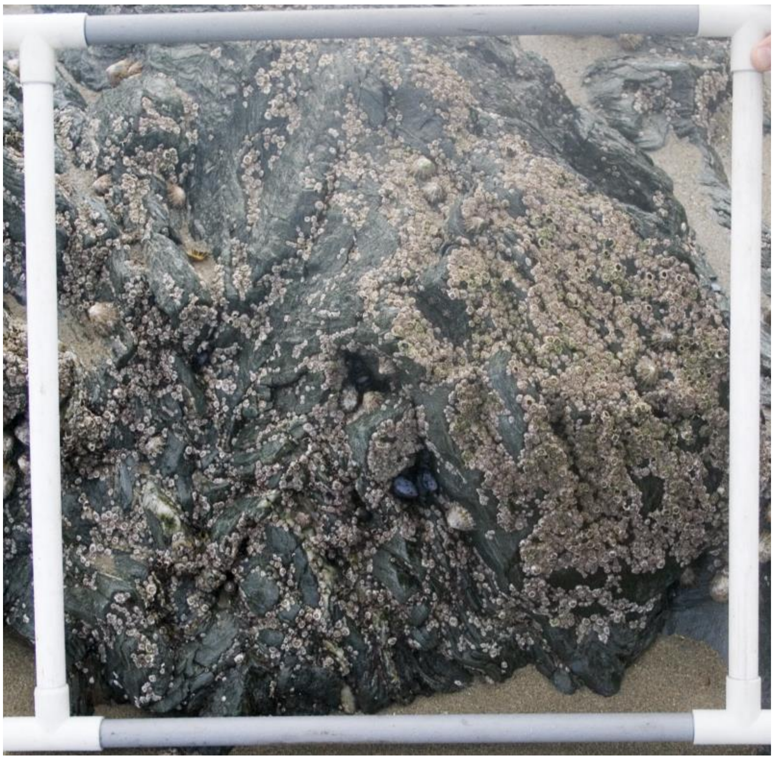
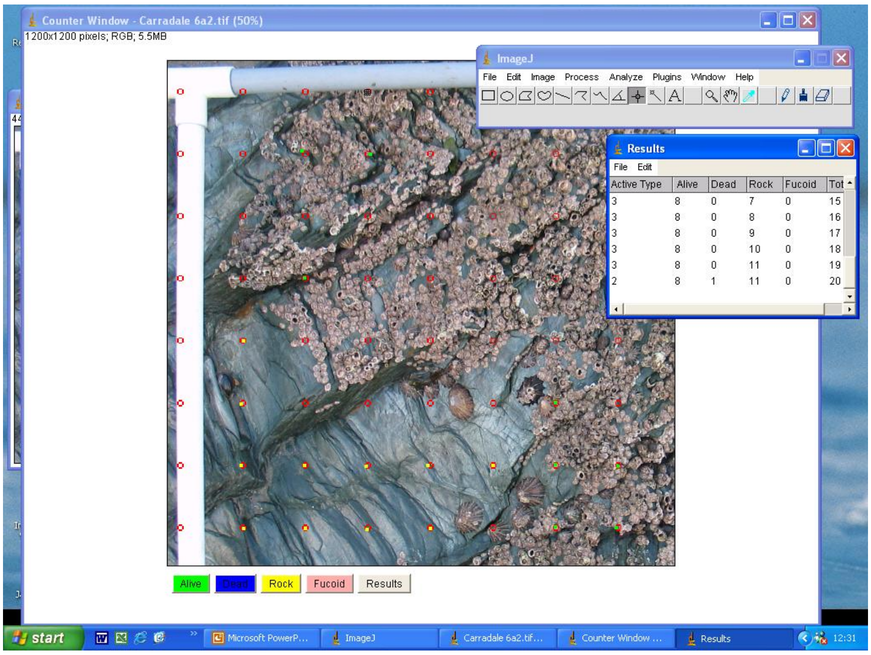


Figure 3-1 Photograph of quadrat taken at Carradale (vertical line A, No. 3 (bottom of settlement zone).



Active Type	Alive	Dead	Rock	Fucoid	Tot
3	8	0	7	0	15
3	8	0	8	0	16
3	8	0	9	0	17
3	8	0	10	0	18
3	8	0	11	0	19
2	8	1	11	0	20

Figure 3-2 Screen shot of ImageJ programme for counting areas of alive and dead barnacles, and area of rock and fucoid cover.

3.3.3 Determination of fecundity

Each individual barnacle collected from 2004 and 2005 was trisected into somatic tissue, egg mass and shell (Hills & Thomason 2003b). The somatic tissue was oven dried at 50^{o}C for 24 hours and weighed (± 0.01 mg) to determine dry weight. The shell was measured for its height, base length and width, and top length and width (opercular diameter) using digital callipers (± 0.1 mm).These measurements were then used to calculate a morphological index (MI) as:

$$M = h/[(o + b)/2]$$

where M is the unit-less morphological index, h is shell height, o is the opercular diameter and b is the basal diameter (Thomason et al. 2000, Hills & Thomason 2003b). Likewise external frustum volume (mm^3) which is the cone volume of shell was calculated as:

$$V = 1/3\ h\ (A_1 + A_2 + \surd\ A_1A_2)$$

where V is the frustum volume, A_1 is the base area and A_2 is the top area and h is the shell height, due to the base and top of barnacles being ellipsoid (Weisstein 2007-2008). Frustum volume can be measured without the destruction of the live barnacle (using external measurements), whereas direct frustum volume would require the destruction of the animal (using dental impression material to gauge the internal volume).

To give an exact measure of volume each shell was filled with dental impression material (DIM) (Kerr Extrude low viscosity polyvinylsiloxane – Kerr Manufacturing Company, Romulus, MI 48174, USA.) (Hills et al. 1998b, Thomason et al. 2002b). This was then carefully removed and weighed (± 0.01 mg) to give an internal volume of the shell by previously calculating weight of dental impression material and how much water it displaced in order to give the volume:

$$V = dI$$

where V = volume (mm^3) and I = weight of impression material in mg and d its density (0.7358).

This was to ascertain whether shell morphology or frustum volume were accurate predictors of fecundity. The morphological index gives an indication of columnar or conical morphology of the shell, the higher the MI ratio the greater the height is to the mean width (the more columnar the barnacle is) although two barnacles with the same MI ratio are not necessarily the same size (Hills & Thomason 2003a, b).

Each barnacle examined had the quadrat noted with its position on the shore, rock type it was on, the exposure and density of live adult barnacles in that quadrat. This allowed analysis of these effects on fecundity which may alter year to year.

To count the eggs a random sample of eight barnacles from each quadrat used in 2004 and 2005 were selected, also eight barnacles (from each of these quadrats for these years) had the egg masses and somatic tissue separated and dried at 85°C for 24 hours to record their mass. For the 2006 sites, five barnacles from each site were dissected, with egg masses removed. Each egg mass was then carefully separated from the somatic tissue, dyed using Rose Bengal (Sigma, 1 mg in 100 ml of 100 % ethanol), and photographed using a Canon 350d camera (8×10^6 pixels) with an 18-55 mm lens at 55 mm supported by a copy stand. All eggs were fertilized and therefore were embryos. Photographs were sharpened in Photoshop CS2 using an unsharp mask filter with a threshold of 0, pixel 1.5, % sharpening 100; converted to grey scale and the background made a uniform colour using the wand dropper and paint bucket. Egg numbers and sizes were then calculated using ImageJ (Rasband 1997-2008). The order of command steps used in the programme were:- the scale was set using the glass Petri dish; circular crop; threshold; analyse particles (size 0.01 – 0.03 exclude on edges). Ten eggs on each photograph were chosen at random, and their area was determined (mm^2). These areas were then used to calculate the mean size of eggs in each sample in case some eggs were larger than others. To estimate the total number of eggs per barnacle, the total area of eggs was divided by the mean area of an egg. This was done for each sample.

3.3.4 Data analysis

Fecundity estimates

In order to ascertain whether fecundity could be predicted from external measurements, multiple and best sub-set regressions were used with the response as the number of eggs per individual and the predictors base length, base width, top length, top width and height.

To establish if calculated frustum volume could be used as a reliable indicator of measured volume, the volume measured for the samples taken in 2004 was used as the response in a regression with frustum volume as the predictor. If volume related to fecundity then frustum volume could be a useful non-destructive indicator of fecundity.

Regression analyses were used to determine the best way to estimate the fecundity of a barnacle from combined external sizes. Predictors were MI and frustum volume, and the response was eggs. A linear regression was used to determine the relationship between the dry weight of eggs per barnacle and its dried somatic tissue.

Relationship between rock type, exposure, density, shore height and barnacle fecundity

This was conducted so that in the future, fecundity could be estimated by evaluating maps of an area, with the rock type, shore height and exposure shown, thereby being able to estimate the density of live adult barnacles and their resulting fecundity.

A univariate GLM ANOVA with Ryan-Einot-Gabriel-Welsch Range *post-hoc* was used to establish whether rock type had an effect on density of adult barnacles for data from all three years; then a regression analysis was performed to determine if there was a relationship between density of adult barnacles and the number of eggs produced. This was followed by a GLM ANOVA with Ryan-Einot-Gabriel-Welsch Range *post-hoc* test, on cube root transformed data for 2006 to ascertain if rock type, position on shore or exposure had an effect on barnacle fecundity. Data for 2006 was used as this year had a full data set, and was considered a ‘normal’ year for the number of eggs produced.

Is there a connection between density of adults and the number and size of eggs produced

An Analyses of CoVariance (ANCOVA) was carried out on one hundred samples, to determine if crowding (increased density of adults) explained the number and size of eggs produced by barnacles on different rock types and exposures. Density was the covariable, egg number and egg size were the respective dependent variables, and the fixed factors were exposure, rock type and position on the shore.

All statistics were undertaken in either Minitab 12 or SPSS 12.

3.4 Results

3.4.1 Fecundity estimates

Researchers have found that basal diameter, aperture length or morphological index are good, non–destructive indicators of fecundity (Hurley 1973, King et al. 1993, McGrath et al. 1994, Hills & Thomason 2003a, b). Therefore external measurements were investigated separately and also combined in order to find the best possible indicator of a barnacle's fecundity. Frustum volume and morphological index were investigated to see if these were good indicators of fecundity, combined data for all years were then split into their individual years to see if any one year had an impact on the results, and possible reasons for this.

Sub-set regression analysis showed the best two external predictors for each variable, to see if any of these external measurements could be used as an indicator of fecundity. As can be seen from Table 3-2, height is the most consistently used predictor. However, height can vary for barnacles depending on the amount of spacing between individual barnacles. This does not allow for tall thin (trumpet shaped) barnacles and wide conical barnacles as these could have vastly different internal volumes.

Predictor or combination of predictors	**r^2**	**adj r^2**	**C-P**
Height	0.375	0.372	**49.2**
Base length	0.372	0.369	**50.6**
Height and base width	0.498	0.493	**2.9**
Height and base length	0.496	0.491	**3.8**
Height, base width and base length	0.505	0.497	**2.4**
Height, base width and top width	0.498	0.491	**4.9**
Height, base width, base length, and top width	0.506	0.496	**4.0**
Height, base width, base length, and top length	0.506	0.495	**4.1**
Height, base width, base length, top width and top length	**0.506**	**0.493**	**6.0**

Table 3-2 Best sub-set regression with predictor as eggs for December 2004, 2005 and 2006

Key to labels: r^2 = measure of the amount of variation accounted for by the regression line (also called the coefficient of determination), adj r^2 = amount of variation in the dependent variable adjusted for the degrees of freedom and an unbiased estimate of the population, C-P = (statistic) best model with p parameters (see Quinn and Keough 2002).

The morphological index and frustum volume were evaluated in order to find out if these calculations were effective in estimating fecundity of barnacles.

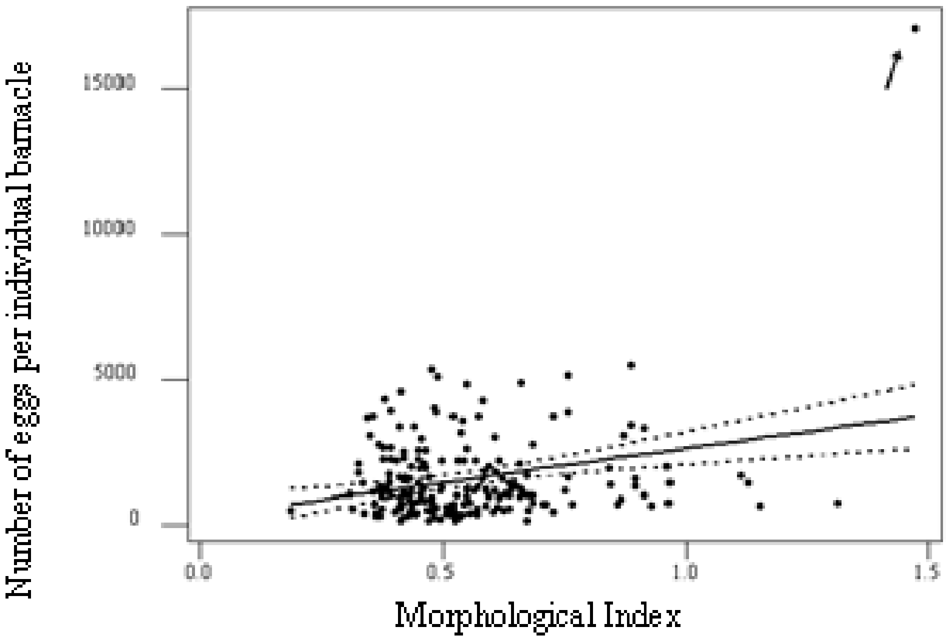


Figure 3-3 The relationship between morphological index (MI) and the number of eggs per barnacle (data from 2004, 2005 and 2006). Regression ——, 95% confidence interval ----, ($r^2 = 0.071$, $p < 0.001$, eggs 286.74 + 2323.73 MI).

The outlier (top right-hand corner indicated by the arrow) was an exceptionally large barnacle collected in 2004.

The morphological index (Figure 3-3), gave a very weak indication for the number of eggs produced by individual barnacles over three years but was significant ($r^2 = 0.071$, $p < 0.001$, number of eggs = 286.74 + 2323.73 MI).

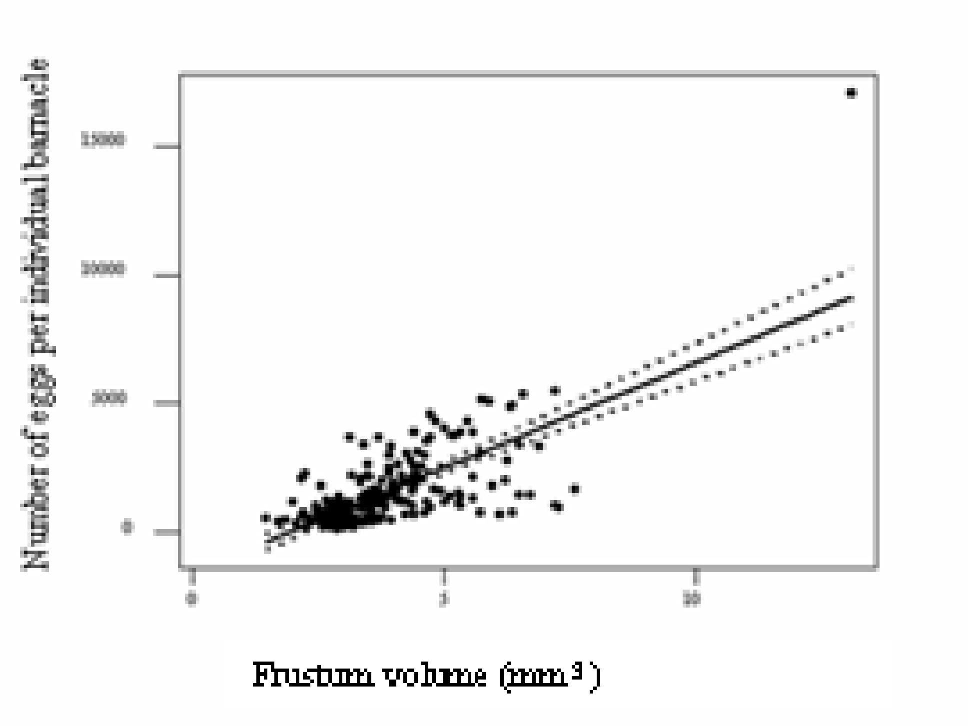


Figure 3-4 The relationship between frustum volume (mm^3) and the number of eggs per barnacle (data from 2004, 2005 and 2006). Regression —, 95% confidence interval ---, ($r^2 = 0.489$, $p < 0.001$, eggs -1612.43 + 819.53 frustum).

The regression for the frustum volume against egg mass for the three years was stronger than the morphological index but it was still a weak relationship $r^2 = 0.489$, $p < 0.001$ number of eggs (see Figure 3-4).

The data of egg numbers were then split into the three years and the regression plot re-run, producing the following graphs (Figures 3-5, 3-6 and 3-7).

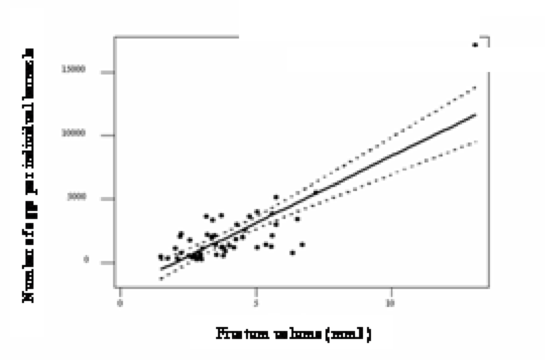


Figure 3-5 Regression fitted line plot to show the relationship between frustum volume and the number of eggs produced for 2004. Regression —, 95% confidence interval ---, $r^2 = 0.631$, $p < 0.001$.

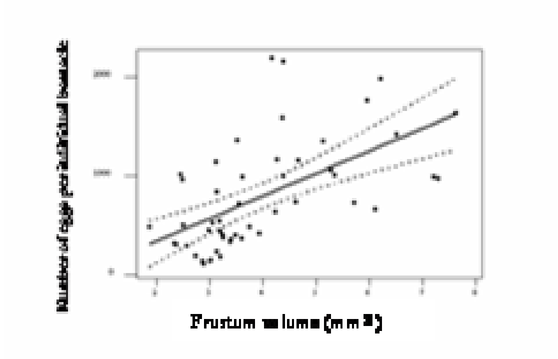


Figure 3-6 Regression fitted line plot to show the relationship between frustum volume and the number of eggs produced for 2005. Regression —, 95% confidence interval ----, $r^2 = 0.34$, $p < 0.001$.

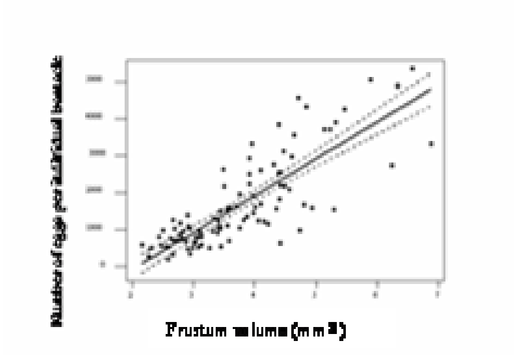


Figure 3-7 Regression fitted line plot to show the relationship between frustum volume and the number of eggs produced for 2006. Regression —, 95% confidence interval ----, $r^2 = 0.682$, $p < 0.001$.

The regression for barnacles collected in December 2004 (Figure 3-5) gave a much stronger relationship of $r^2 = 0.631$, $p < 0.001$, when compared with the first analysis of the combined years dataset ($r^2 = 0.489$). The sample collected in December 2005 (Figure 3-6) gave a much weaker relationship of ($r^2 = 0.34$) whilst the sample from December 2006 (Figure 3-7) gave the strongest relationship ($r^2 = 0.682$).

A regression plot (Figure 3-8) was conducted using the internal measured volume that was directly obtained by using dental impression material (DIM) for the December 2004 barnacles, against frustum volume that was calculated indirectly from external measurements.

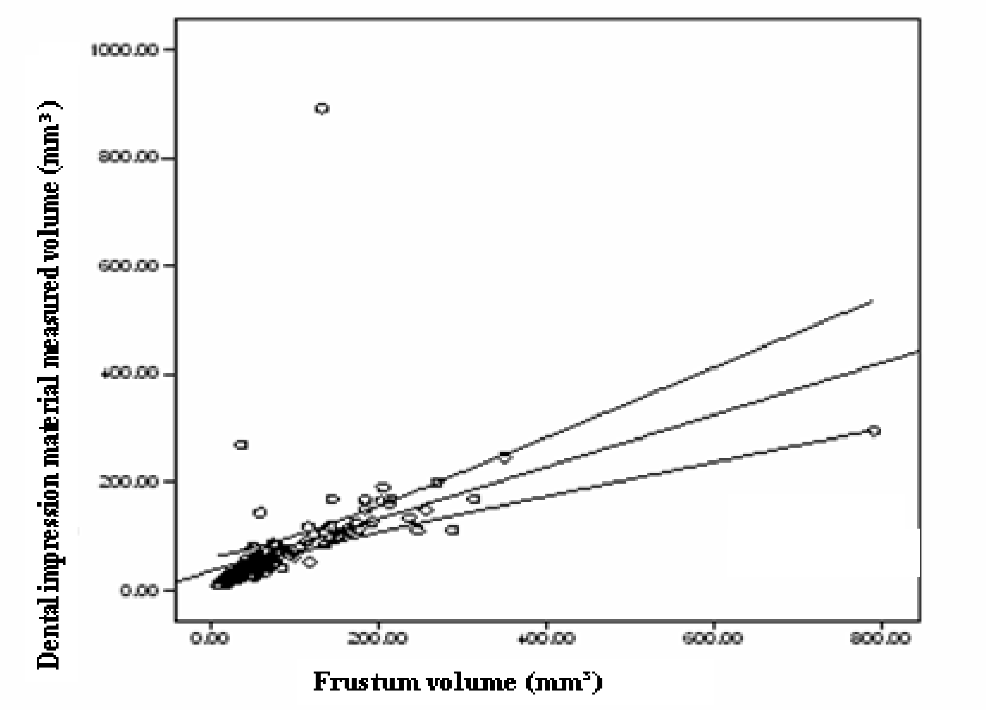


Figure 3-8 Regression fitted line plot of the internal measured volume of the 2004 barnacles (using dental impression material. Regression —, 95% confidence interval ---, $p < 0.001$, $r^2 = 0.24$, frustum = 56.026 + 0.499 measured volume).

The regression for Figure 3-8 was $r^2 = 0.24$, $p < 0.001$, regression line Frustum =

56.026 + 0.499 Internal volume; indicating that frustum volume (an external calculated volume) can be used as an indicator of the internal volume (directly measured volume) of the barnacles even though the relationship is weak.

The results from the regressions (Figures 3-5, 3-6 and 3-7) suggested that something was happening to the soft tissues of the barnacle in 2005, or the lead up to the reproductive cycle of the second year under investigation. The relationship between the frustum volume and the number of eggs produced per barnacle was much lower ($r^2 = 0.34$) for 2005, than for 2004 or 2006. To try and understand this, another linear regression was undertaken on the relationship between somatic tissue and fecundity, measured as dry weight of eggs and somatic tissue (Figure 3-9) using samples taken in December 2004 and 2005.

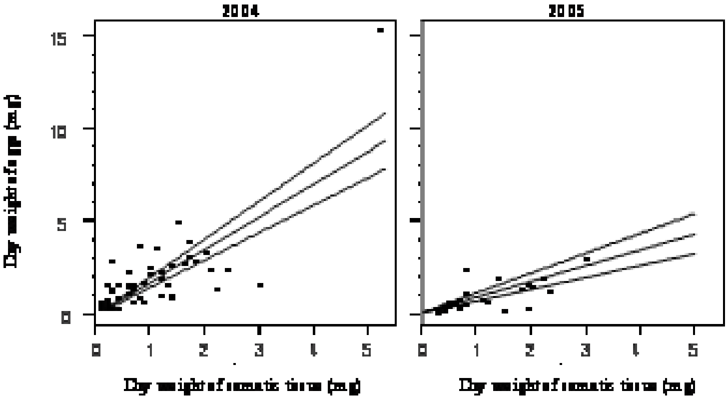


Figure 3-9 Linear regression between the dry weight of somatic tissue (mg^{-1}) and the dry weight of the eggs(mg^{-1}) for the corresponding barnacles for 2004 and 2005, showing 95% confidence intervals.

For 2004 (Figure 3-9) the dry weight of eggs (mg^{-1}) = 1.77 x dry weight of somatic tissue (mg^{-1}), but for 2005 this had dropped to dry weight of eggs (mg^{-1}) = 0.87 x dry weight of somatic tissue (mg^{-1}). This suggests that the barnacles of 2005 were starving when they were producing their reproductive tissues, therefore fewer resources were allocated to reproduction in that year shown by the drop in mean number of eggs per mg of dried somatic tissue from 2114 in 2004 to 613 eggs per mg of dried somatic tissue in 2005.

3.4.2 Relationship between rock type, exposure, density, shore height and barnacle fecundity

The tests of between subjects effects showed that rock type was significant ($p = <0.001$, $r^2 = 0.089$, mean square = 2.495, $F = 9.666$). The Ryan-Einot-Gabriel-Welsch Range *post-hoc* test (Table 3-3) showed that metamorphic rock was in a different subset to igneous and sedimentary rock types. Therefore in the calculations for the mapping of barnacles in the Clyde Sea area, igneous and sedimentary rock types can be grouped together as shown in Table 3-3 and Figure 3-10.

Rock Type	N	Subset	
		1	2
Metamorphic	64	0.700138	
Igneous	59		1.037431
Sedimentary	77		1.039766
p value		1.000	0.980

Table 3-3 Adult barnacle density (adults cm^2) on different rock types for 2004, 2005 and 2006 data combined.

Mean adult density of barnacles per cm^2 was determined in the GLM ANOVA to give an error bar plot of densities on the different rock types (Figure 3-10) using data collected from the three years.

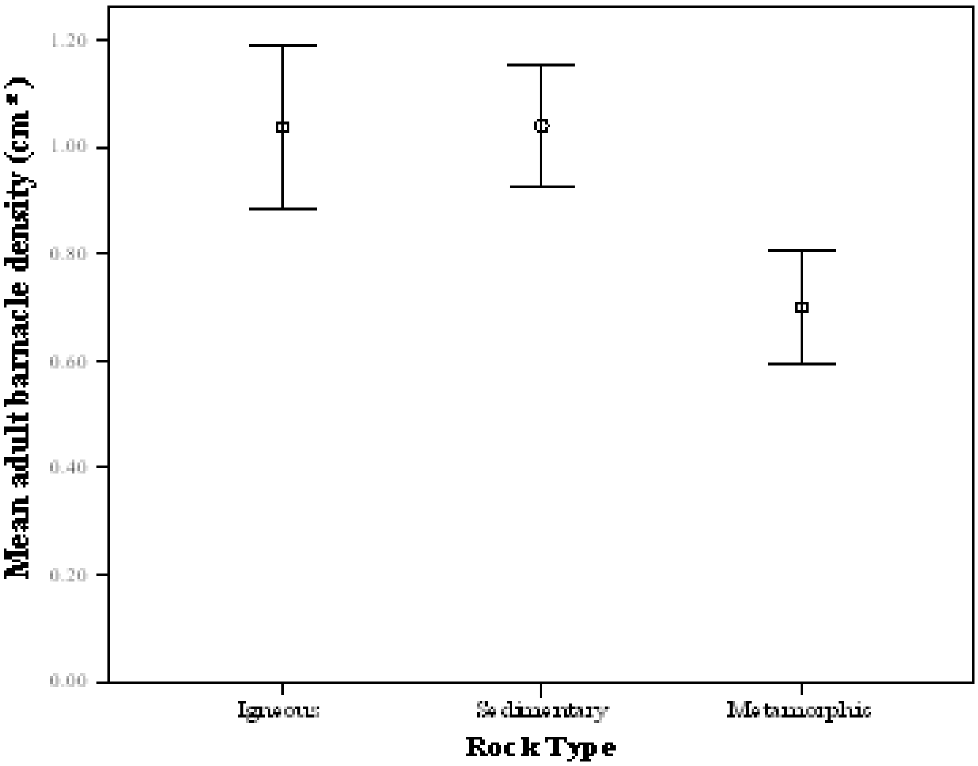


Figure 3-10 Grouped error bar plot (95% confidence interval) for the mean density of adult barnacles (cm^2) on different rock types for 2004, 2005 and 2006; $p < 0.001$, F = 22.302, $r^2 = 0.101$.

A regression analysis was also performed to establish if there was a relationship between density and number of eggs produced by the adult barnacles (Figure 3-11). This analysis was significant ($p = <0.001$, F = 22.302, $r^2 = 0.101$).

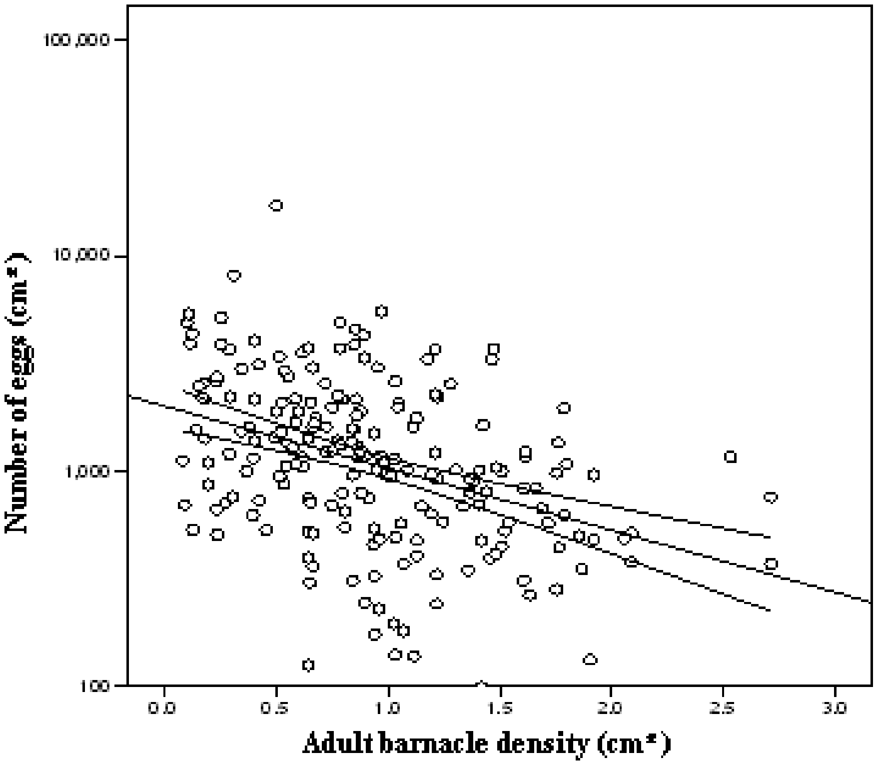


Figure 3-11 Scatterplot for the number of eggs produced (all years, all heights of shore and both exposures) against adult barnacle density (cm^2). Number of eggs = 2510.302 - 1007.74 adult density.

As adult density increases then the number of eggs produced per cm^2 decreases on all rock types (Figure 3-11).

The data was split into years, and the three sites that had been used in all data years were investigated to determine relationships between exposure and fecundity, shore and fecundity, and rock type and fecundity. The 2006 data was used as it was a full data set and assumed to be a ‘normal’ year, as opposed to 2005 where the barnacles appeared to be starving and 2004 the precursor to an abnormal year. As the data was skewed it was transformed by a cube root (0.33) to give a normal distribution.

A GLM ANOVA was run on the data with the Ryan-Einot-Gabriel-Welsch Range *post-hoc* test with the transformed fecundity as the dependent and rock type, position on shore and exposure as the fixed factors for 2006. The only significant factors were the main factors of rock type, position on the shore and exposure, as shown in Table 3-4. Combinations of fixed factors did not significantly affect fecundity over the three year period.

Dependent Variable: Transformed Fecundity

Source	Type III Sum of Squares	df	Mean Square	F	P value
Corrected Model	18.499	17	1.088	4.212	<0.001
Intercept	1781.045	1	1781.045	6893.562	<0.001
RockType	3.905	2	1.953	7.558	0.001
PositionShore	2.492	2	1.246	4.823	0.010
Exposure	11.285	1	11.285	43.680	<0.001
RockType * PositionShore	.097	4	.024	.094	0.984
RockType * Exposure	.683	2	.341	1.321	0.272
PositionShore * Exposure	.359	2	.179	.694	0.502
RockType * PositionShore * Exposure	.251	4	.063	.243	0.913
Error	21.186	82	.258		
Total	1840.440	100			
Corrected Total	39.684	99			

Table 3-4 *Post-hoc* test using transformed fecundity to find significant contributors to fecundity in the Clyde Sea for 2006. The shaded lines show significance for each source (rock type, position on the shore of the barnacles and the exposure).

Error bar plots were performed to perceive whether exposure (Figure 3-12), position on shore (Figure 3-13 and Table 3-5), or rock type (Figure 3-14) were significantly different.

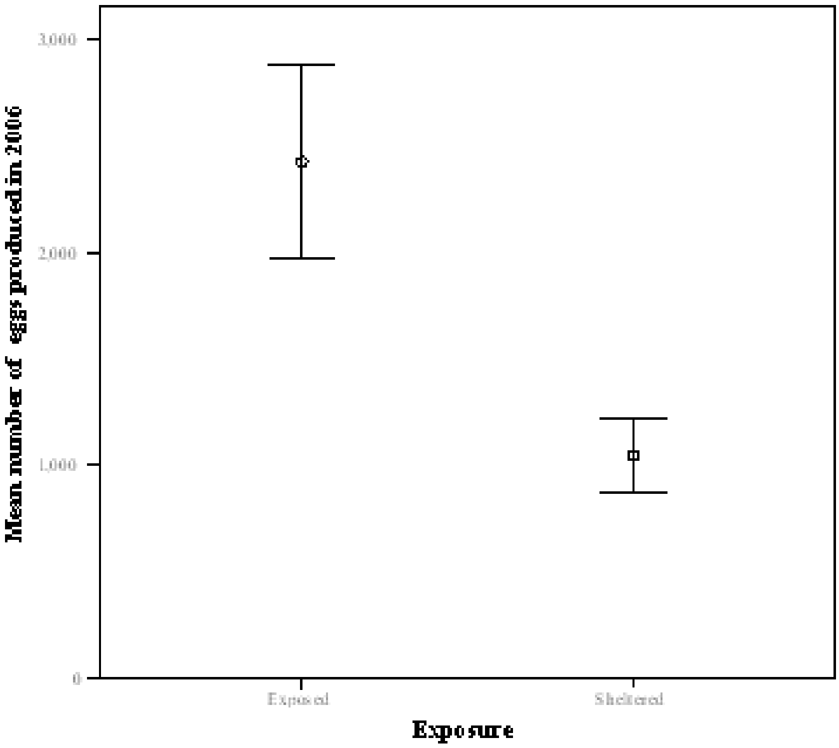


Figure 3-12 Error bar plot (95% confidence interval) showing the mean number of eggs produced in 2006 against exposure, (p < 0.001).

.

A significant difference (Table 3-4) is shown in Figure 3-12 for the effect of exposure on the mean number of eggs produced by *Semibalanus balanoides* for 2006.

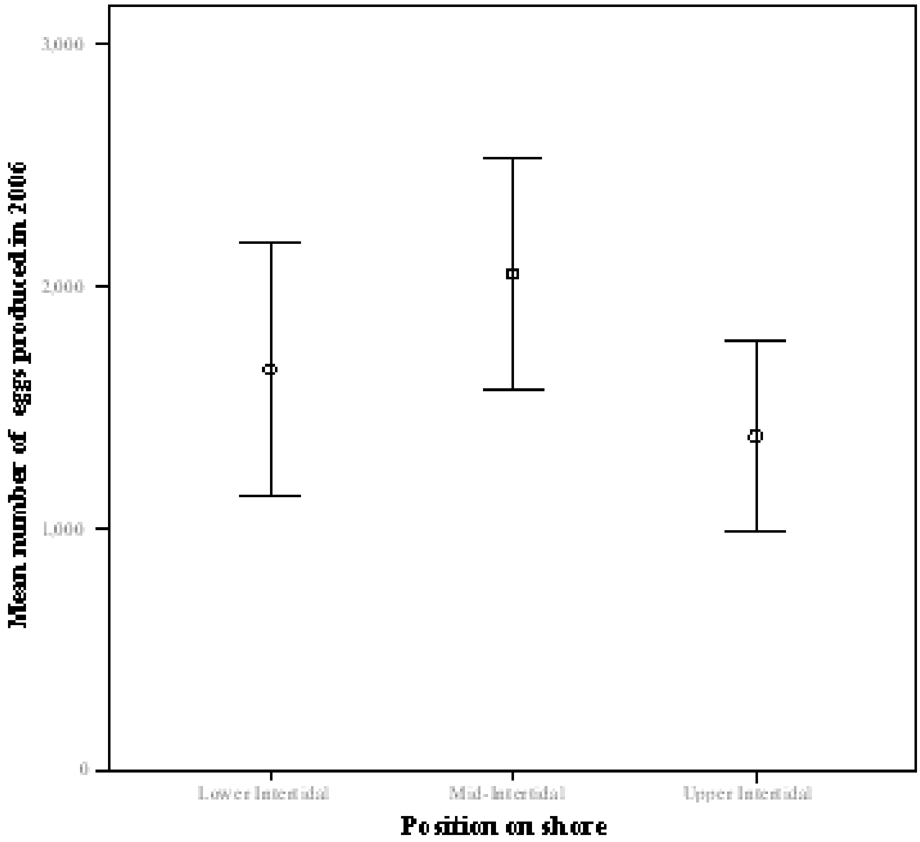


Figure 3-13 Error bar plot (95% confidence interval) showing the mean number of eggs produced in 2006 against position on the shore.

Ryan-Einot-Gabriel-Welsch Range

Position on shore	N	Subset	
		1	2
Upper Intertidal	31	4.0615	
Lower Intertidal	34	4.2176	4.2176
Mid-Intertidal	35		4.4299
p – value		0.230	0.089

Table 3-5 Position on shore sub-sets to find effects of shore height on fecundity for 2006.

Table 3-5 and Figure 3-13 show the lower intertidal appearing in both sub sets of the upper intertidal and the mid intertidal.

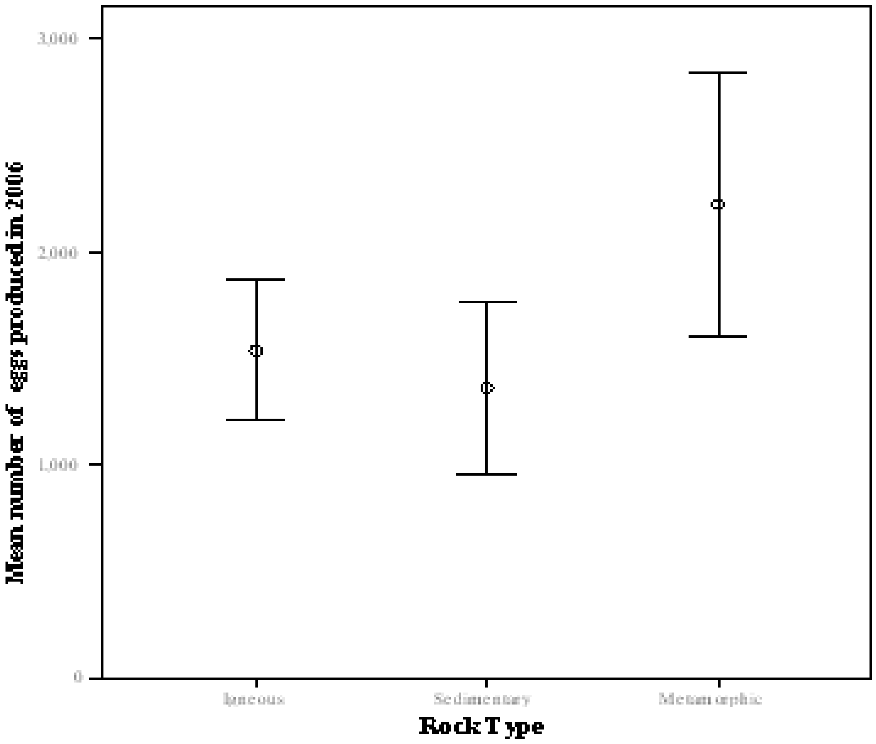


Figure 3-14 Error bar plot (95% confidence interval) showing the mean number of eggs produced in 2006 against rock type.

Figure 3-14 (2006 year data) and Figure 3-8 (combined year's data), both show metamorphic rock as different to sedimentary and igneous rock types, with a lower overall density but a potentially higher fecundity. Thereby confirming the value of dividing rock types for estimation of fecundity using mapping.

		Combined rock type			
		Metamorphic		Sedimentary and Igneous	
		Exposure		Exposure	
		Exposed	Sheltered	Exposed	Sheltered
		Number of Eggs	Number of Eggs	Number of Eggs	Number of Eggs
		Mean & 95 % CI	Mean & 95 % CI	Mean & 95 % CI	Mean & 95 % CI
Position on shore	Lower Intertidal	3597 ± 2242.895	1101 ± 254.724	1748 ± 563.685	1038 ± 427.702
	Mid-Intertidal	3845 ± 1104.550	1565 ± 665.529	2608 ± 647.524	983 ± 227.569
	Upper Intertidal	2760 ± 1127.514	1062 ± 492.156	1487 ± 610.744	771 ± 204.355

Table 3-6 Custom table of mean and 95 % confidence interval values of eggs produced (cm^2) from barnacles on different rock types, different tidal heights on the shore, and different exposure using 2006 data set.

The custom table compiled from the 2006 data (Table 3-6) can be used in combination with the mapping of Chapter 2, to estimate the fecundity of the Clyde Sea without the need for time taking surveys of each shore.

3.4.3 Fecundity, egg size and somatic tissue for 2004 and 2005, fecundity and egg size for 2006

Descriptive statistics were used to estimate the number and size of eggs for each year (Table 3-7).

	Year	**Minimum**	**Maximum**	**Mean**	**95% Confidence interval**
Average size of eggs (mm^2)	2004	0.011981	0.021999	0.016349	± 0.000245
Average size of eggs (mm^2)	2005	0.012115	0.224960	0.016183	± 0.000169
Average size of eggs (mm^2)	2006	0.011094	0.281050	0.018133	± 0.000253
Number of eggs per barnacle	2004	242	17118	2109.34	± 700.77
Number of eggs per barnacle	2005	99	2195	766.96	± 154.92
Number of eggs per barnacle	2006	196	8076	1707.26	± 376.89
Number of eggs per mg dried somatic tissue	2004	189	6336	2113.68	± 362.20
Number of eggs per mg somatic tissue	2005	93	2093	613.24	± 115.11

Table 3-7 Mean egg sizes and the number of eggs per barnacle per year with 95 % confidence intervals.

Table 3-7 shows that the mean number of eggs produced was less in 2005 and although it had increased in 2006 it was still below that of 2004 (the first year under investigation). Ryan-Einot-Gabriel-Welsch Range *post-hoc* test showed that there was a significant difference ($p < 0.001$) between the number of eggs produced in 2005 to those produced in 2004 and 2006 (which were in the same group, see Table 3-8). The maximum number of eggs produced by an individual barnacle was 17118 in 2004, but the lowest number of eggs produced by a single barnacle was in 2005 (99 eggs) for an individual. The number of eggs per mg of dried somatic tissue significantly ($p < 0.001$) dropped in 2005 suggesting that barnacles during this year were using more energy for purposes other than reproduction.

The mean size of the eggs ($n = 2000$ for the 3 years) was not significantly ($p = 0.582$) different for the three years under investigation (see Figure 3-17), according to the Ryan-Einot-Gabriel-Welsch Range *post-hoc* test, even though the descriptive statistics suggest that the mean for 2005 is slightly smaller. The slight difference may indicate fewer resources available for egg production.

An error bar plot (Figure 3-15) was then conducted to find out if the mean number of eggs was the same for each year, produced on all rock types, exposures and positions on the shore.

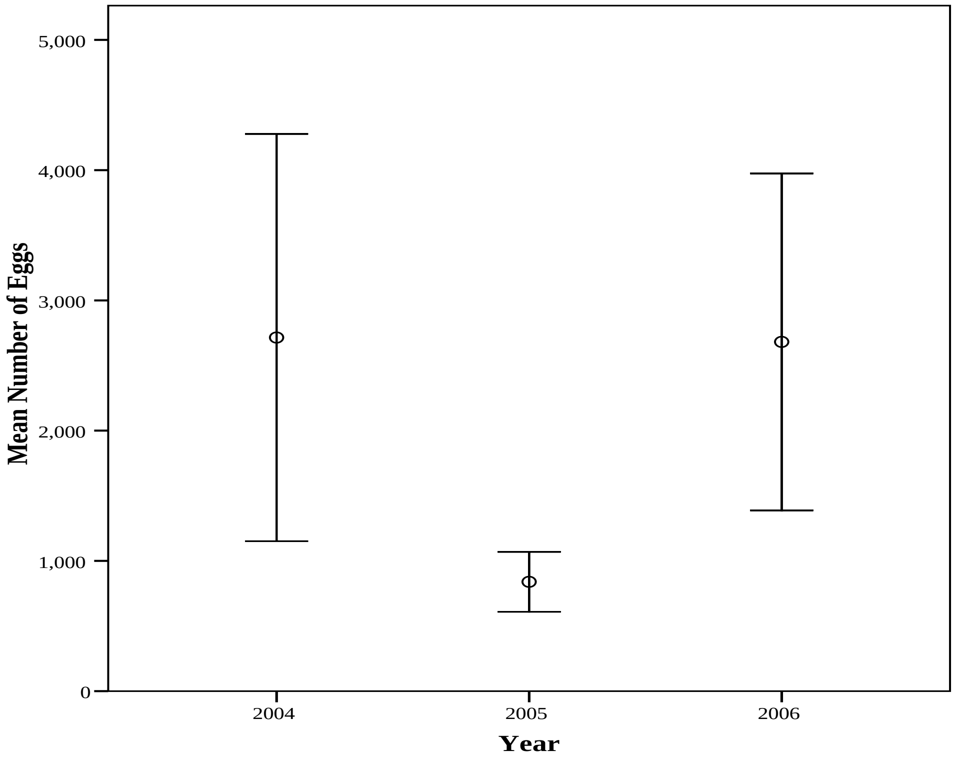


Figure 3-15 Error bar plot for mean number of eggs (95% confidence interval) produced each year. All rock types, exposures and positions on shore ($p < 0.001$, $F = 7.939$, $r^2 = 0.199$).

Ryan-Einot-Gabriel-Welsch Range

Year	N	Subset	
		1	2
2005	30	3.6683	
2004	22		4.4674
2006	15		4.4944
p - value		1.000	0.929

Table 3-8 Sub-sets for the mean number of eggs produced for 2004, 2005 and 2006

The error bar plot for the mean number of eggs produced for the different years shows that in 2005 there was a significant ($p = <0.001$, $F = 7.939$, $r^2 = 0.199$) drop in the number of eggs compared to 2004 and 2006. The Ryan-Einot-Gabriel-Welsch Range *post-hoc* test (Table 3-8) was used to find sub-sets which showed that 2005 was significantly lower than 2004 and 2006 which were grouped together.

The number of eggs per mg of dried somatic tissue was investigated to see if this differed over the two years (2004 and 2005) that there was somatic tissue data for. There was no somatic tissue for 2006, due to time constraints.

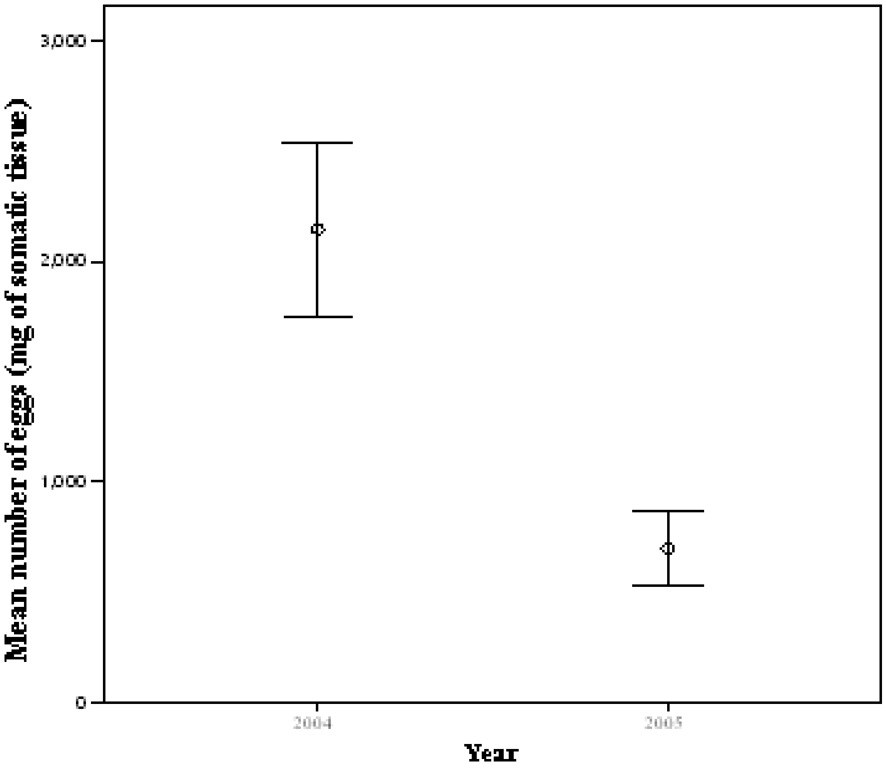


Figure 3-16 Error bar plot for mean number of eggs per mg^{-1} of dried somatic tissue (95% confidence interval, $p < 0.001$, F = 64.451, $r^2 = 0.563$) for 2004 and 2005.

The univariate GLM ANOVA (Figure 3-16) showed that the mean number of eggs per mg of dried somatic tissue was significantly ($p = <0.001$, F = 64.451, $r^2 = 0.563$) different for the two years. There were significantly fewer eggs produced during 2005 than 2004.

The egg size may also alter over the three years under investigation; therefore this was investigated using a univariate GLM ANOVA to produce an error bar plot (see Figure 3-17).

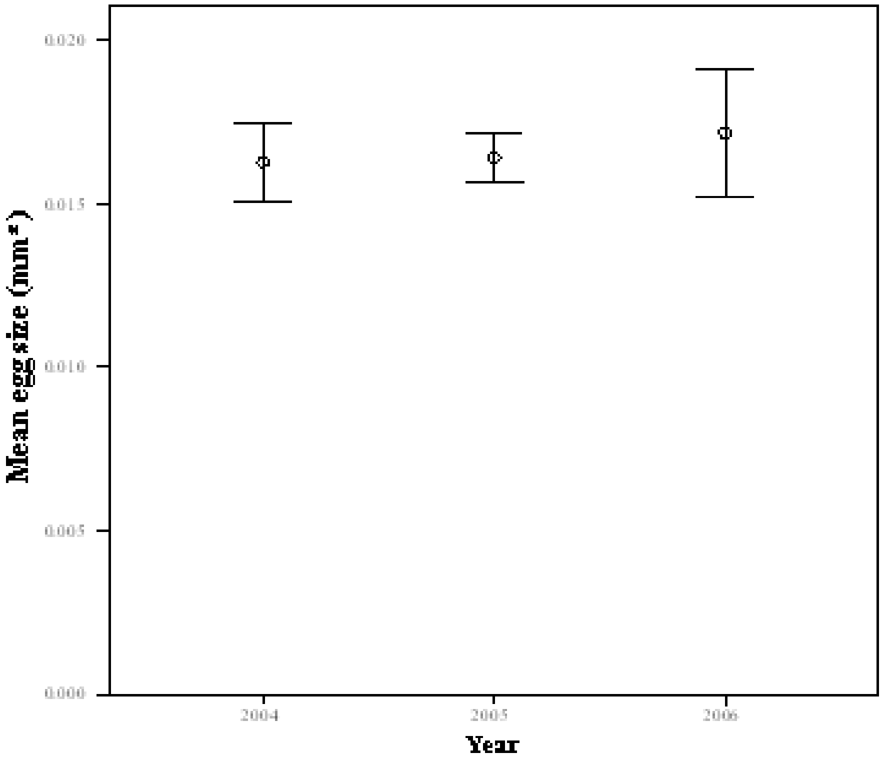


Figure 3-17 Error bar plot for mean egg size (95% confidence interval, p = 0.582, F = 0.545, r^2 = 0.017) for 2004, 2005 and 2006.

The GLM ANOVA showed that year was not significant (p = 0.582, F = 0.545, r^2 = 0.017) for the size of eggs during the three years under investigation. The Ryan-Einot-Gabriel-Welsch Range *post-hoc* test showed that the egg size for each year were in the same set.

3.5 Discussion

The fecundity of *S. balanoides* in the Clyde Sea can be thought of as a closed system because of the long turn-over time for the water over the Great Plateau and very few nauplii will enter from the other side of the Great Plateau, but for individual populations on different shores it is an open system (Jenkins et al. 2000) making the study of fecundity of the area very interesting. This allows the entire Clyde Sea area to be studied at one spatial scale, but at the same time allowing studies at smaller spatial scales of communities on different rock types and exposure.

Using data of the external measurements of *S. balanoides* for the three years under investigation, the best sub-set regression of individual measurements only gave the height of a barnacle as a predictor of egg numbers. However, different shapes of barnacles may have the same height. For example, tall thin ‘trumpet’ shaped columnar shells and the ‘standard’ conical shaped shell may have the same height, but have vastly different volumes internally and externally. King et al (1993) found a significant correlation at one site for the number of embryos and the aperture length (top length), but not at another site. The more measurements used (4 or 5) gave the best r^2 results. The external predictors of height, width, and length gave the highest r^2 values. Hurley (1973) suggested that carino-rostral diameter (base length), was an adequate measure unless the barnacles were crowded. However, for the reason above this suggests that this measurement on its own would be inappropriate to use. For the Clyde Sea, this measurement by itself was the weakest of the predictors (Table 3-2), and it is agreed with Wu (1980) that barnacles when crowded can grow in height. The best-sub set regression did not show any individual or group of measurements (base length, base width, top length, top width, or height) as a particularly good indicator. But it did suggest that all the measurements together may give better results. The measurements were re-evaluated to find a good method of estimating fecundity for the entire Clyde Sea area, which could be used on all sites (exposed or sheltered, different rock types, different heights on the shore and different densities). Morphological index (MI) was a very poor indicator of the numbers of eggs produced by a barnacle (Figure 3-3), probably due to the fact that two individual barnacles could have the same MI, but very different volumes (Hills & Thomason 2003b). The regression of frustum volume with the number of eggs produced over the three years was better than the morphological index, but was weak (Figure 3-4); however this became stronger when the regression was split into the three years, (Figures 3-5, 3-6 and 3-7) with 2005 showing a very weak relationship unlike 2004 and 2006. Ideally it would be possible to determine fecundity from photographs (using photogrammetry) – but unless two photographs were taken of the same group with at least a 40% offset of width and precisely vertical to the colony or individual, with a known magnification, from exactly the same height and analysed using a stereoscopic means, this would be impractical. However, this method has been successfully used by Maleschlijski et al. (2012) in the laboratory to track *S. balanoides* cypris on a glass surface by using two camcorders. Therefore frustum and MI cannot be estimated from photographs due to technical implications and must be obtained using direct measurements of the barnacles.

According to Hills and Thomason (2003a) egg mass and egg number are related in the Clyde Sea, however, in order to obtain these measurements in the future, destruction would be required of individual barnacles. Internal measurements were taken for the barnacles that had been collected in 2004 and 2005 using dental impression material (DIM) to fill the internal cavity of the shell after the somatic tissue and egg sacs had been removed, and the internal volume calculated. The regression showed a significant relationship between indirectly calculated frustum volume and directly measured volume (Figure 3-8), therefore in the future measured volume could be deduced from the frustum calculation and destruction of individual barnacles would not be required for this information.

It was found that dry weight of eggs and dry weight of somatic tissue were linked ($p = < 0.001$), unlike Hills and Thomason (2003b) who did not find that somatic tissue explained egg mass variations while looking at high and low density colonies. But Hills and Thomason (2003b) did state that starvation has been shown to affect fecundity per unit of somatic tissue. This ties in with the finding that dry weight of eggs dropped to give the equation dry weight of eggs = 0.87 * dry weight of somatic tissue in 2005. Where as in 2004 the equation was dry weight of eggs = 1.77 * dry weight of somatic tissue. The dry weighed somatic tissue (from barnacles harvested in December) will indicate the lowest somatic tissue weight for a barnacle for that year, as resources will have been assigned to the production of a penis, sperm and the eggs by the adult for reproduction (Barnes & Barnes 1968).

It is known that *S. balanoides* requires a consistent food supply, and interruption of this can lead to regression of ovarian tissue (Barnes & Barnes 1967, Thomason et al. 2000). It is proposed that at some time over the previous spring, summer or autumn that a phytoplankton bloom may have failed to take place or that fatty acid concentration in the food source may have been lowered. In 2005 diatom cell densities were so low from January to August, that they did not register until September (0.1×10^6 cells l^{-1}) through the winter until March 2006 when levels reached 0.5 x 10^6 cells l^{-1} when 2007 - 2010 they reached at least 2.0 x 10^6 cells l^{-1} by March (taken from Figure 3.8 McIntyre et al. 2012). Similarly in 2005 dinoflagellates did not register until September when their numbers reached about 0.2 x 10^5 cells l^{-1} falling to 0.1 x 10^5 cells l^{-1} until May 2006 when they reached about 0.2 x 10^5 cells l^{-1} (taken from Figure 3.8 McIntyre et al. 2012). The copepod *Calanus helgolandicus* is known to have lower fecundity when food availability is lower (Pond et al. 1996), and in particular particulate fatty acid concentrations, dinoflagellates and ciliates. The zooplankton food environment is taxon specific and it is possible that a particular phytoplankton or microzooplankton may have been in short supply at some time that year (Pond et al. 1996). The possible starvation for the adult barnacles and poor fecundity, as the material investment in eggs, must be linked to the available resources (Jarrett 2003). Reduction of those resources possibly caused the reduction in somatic tissue and the number of eggs (Figure 3-9). The spring following 2005, fecundity measurements were very poor for settlement of cypris along the west facing coastal strip of the Clyde Sea (personal observation).

Much is known about the effect of crowding on fecundity (Wu 1980, Hills & Thomason 2003a, b, Lopez & Gonzalez 2003, Lopez et al. 2007). For example, Hills and Thomason (2003b) found a linear negative relationship between *S. balanoides* fecundity, with increasing population density, and larger egg masses were found in conical barnicles rather than columnar; although Wethey (1984) found in *S. balanoides* and *Chthamalus dalli* that there was an increase in fecundity with crowding. Other barnacles have shown that fecundity was lower in crowded barnacles (Wu 1980, Lopez & Gonzalez 2003, Lopez et al. 2007). Other factors have been found to affect fecundity, such as starvation and sea temperature, and suggested that the density of *S. balanoides* on a shore was not a reliable indicator in which the barnacle has been growing as there is the problem of winter storms (Hills and Thomason 2003a). Determining density requires counts to be made of the number of live adults in a quadrat, but this is time consuming and impractical for an area the size of the Clyde Sea. The geology of the Clyde Sea area is well known, and if this can be used to estimate fecundity, then a technique using rock type would be much quicker. However, nothing is known about the effect of rock type on fecundity in the Clyde Sea from the literature. ANOVA showed that there was a significant different adult barnacle density on different types of rock. Metamorphic rock differed to sedimentary and igneous rock types in the density for *S. balanoides* using both the combined data set for the three years and the split complete set for 2006 (Figures 3-10 and 3-14). The year 2006 was used as a ‘normal year’ as the 2005 barnacles appeared to be starved and 2004 barnacles might have been impacted leading up to this. From this information, the number of eggs produced can be deduced, allowing fecundity to be estimated from the geological maps of a shore. It was found that as live adult density increases on all rock types, the fecundity of the barnacles fall (Figure 3-11), suggesting that barnacles in high density aggregations suffer from increased competition for food, or the need to allocate resources to activities other than reproduction. This reduction in fecundity with increased density of adults has been found by Wu (1980, 1981), working with *Balanus glandula* (Darwin), and Hills and Thomason (2003a) working in the Clyde Sea with *S. balanoides*. But Leslie (2005) found that very low and very high density aggregations of *Balanus glandula* produced fewer eggs than intermediate densities, and in *Austromegabalanus psittacus* there was no difference in fecundity of dense and isolated barnacles (Lopez et al. 2007). However, Wethey (1984a) working on the East coast of North America found that fecundity increased with density in *S. balanoides* and *Chthamalus dalli*, suggesting that in some cases increased density may be beneficial.

Rock type (Figures 3-10 and 3-14), position on shore (Figure 3-13) and exposure (Figure 3-12) were all found to be significant external contributors to a barnacle’s fecundity (Table 3-4). Barnacles on sheltered shores, irrespective of rock type and position had significantly lower fecundity than barnacles on exposed shores. However, barnacles attached to sheltered sedimentary and igneous rock types in the upper intertidal produced the lowest number of eggs for 2006 (Table 3-6), suggesting that the animals on these rock types may have lacked nutrition or have had less access to food sources for longer than other positions, and thereforepartitioning less energy for egg production. The most productive barnacles, for the number of eggs, produced were from exposed, metamorphic, mid-intertidal rocks (Table 3-6). This may indicate higher food abundance in exposed areas (Bertness et al. 1991, Veliz et al. 2004), or the currents stimulating cirral activity (Crisp & Bourget 1985). Jeffery and Underwood (2001) working on *Chamaesipho tasmanica* suggest that larger barnacles are usually found in more exposed sites, due either to more food or because they survive longer due to fewer predators. *Balanus pacificus* is known to have a higher fecundity in older and larger barnacles (Hurley 1973). Although Burrows et al (1992) found with *Chthamalus stellatus* and *Chthamalus montagui* that the larger barnacles and higher proportion of fecundity were found in sheltered locations. Position on the shore had a less obvious effect on fecundity (Table 3-5) with the *post-hoc* test showing an overlap of the number of eggs produced by *S. balanoides* of the lower intertidal position, with both the upper intertidal and the mid-intertidal ranges, as *S. balanoides* is a mid-intertidal species (Stubbings 1975, Southward 2008). The mid-intertidal was the most productive for all rock types and exposures (Table 3-6).

The number of eggs produced in 2005 on all rock types, exposures and positions on the shore was significantly smaller than the number produced in either 2004 or 2006 (Figure 3-15 and Table 3-7). However, egg size did not alter significantly over the three years (Figure 3-17), suggesting that the number of eggs not the size altered when the barnacles were under stress such as starvation. As Barnes and Barnes (1968) point out, from an ecological point of view the more eggs an organism produces the better, although *S. balanoide*s does produce larger eggs to the north of its range to utilize the larger prey available to its nauplii (larger eggs produce larger nauplii) (Stubbings 1975). However, smaller eggs may produce smaller nauplii with fewer resources, which may not be able to disperse as far in the plankton, and may have to metamorphose into cyprids and settle quicker than nauplii from larger eggs.

The number of eggs mg^{-1} of dried somatic tissue in 2004 ranged from 189 to 6336 (Table 3-7), with the number of eggs produced by individual barnacles ranging from 242 to 17118 (Table 3-7) which is the largest number of eggs found in an individual *Semibalanus balanoides* in the Clyde. These results compare with others from the Clyde Sea area: Barnes and Barnes (1968), 3667 – 5333 mg^{-1} dried somatic tissue; Hansson et al (2003), 5333 mg^{-1} dried somatic tissue and 5000 eggs ind^{-1}; Hills and Thomason (2003a), 13333 mg^{-1} dried somatic tissue. In other parts of the world egg numbers per individual *S. balanoides* barnacle were also comparative with King *et al* (1993), 562 – 7120; and Brind'Amour (2002), 2455 – 6532.

This chapter has examined the fecundity of the barnacles in the whole of the Clyde Sea area, and what appears to influence it. The next chapter will investigate cypris and their landing zones.

3.6 Conclusions

- Frustum volume is the best non-destructive way to estimate fecundity of *S. balanoides*
- Fecundity in *S. balanoides* is higher on exposed than sheltered shores in the Clyde Sea
- Fecundity alters with rock type, but igneous and sedimentary rocks in terms of fecundity can be grouped together, with metamorphic in a different group for estimating from geology
- Fecundity alters with a barnacles position on the shore
- Egg size does not significantly alter from year to year

Chapter 4. Cyprid supply and the influence of substratum

4.1 Introduction

In the previous chapter, the fecundity of *Semibalanus balanoides* in the Clyde Sea was assessed in relation to the size of the barnacles, exposure, position on shore and the crowding of individuals; to find out whether fecundity could be gauged from external measurements. The number of eggs and their possible input of stage 1 nauplii into the Clyde Sea system can be quantified (Barnes 1989, Gude 2007).

Many benthic marine organisms have a pelagic larval form for dispersal (Giangrande et al. 1994, Levin & Bridges 1995, Gude 2007), and the link between the population dynamics of the sessile adult population and those of the motile larvae is often obscured by the effects of a suite of abiotic and biotic factors that affect both supply and settlement processes (Miron et al. 1995, Palmer et al. 1996, Todd 1998, Wilson & Harrison 1998, Miron et al. 1999, Olivier et al. 2000, Berntsson & Jonsson 2003, Bellgrove et al. 2004, Watson & Barnes 2004). Quantifying this link and estimating the magnitude of the effects of the impinging factors is central to understanding supply-side ecology (e.g. Grosberg & Levitan 1992, Hughes et al. 2000, Hyder et al. 2001, Delany et al. 2003, Svensson et al. 2004, Gude 2007), and is thus highly important for predictive understanding of marine ecosystems.

Supply – side ecology or supply – side theories relate to the number and delivery of the organism propagules under investigation, to either the substratum or the area (recruitment) where the organism matures into adulthood (Underwood & Fairweather 1989, Todd 1998, Todd et al. 1998, Hughes et al. 2000, Gude 2007). Although marine habitats, especially the rocky shore and fisheries have led the literature in supply – side theories, it is also applicable especially to lentic ecosystems (rivers and streams) and even helminth communities in freshwater fish (Kennedy & Bush 1994, Palmer et al. 1996). The number of larvae at settlement is highly variable both spatially and temporally in marine habitats, which could be due to larval mortality, transport and the larval behaviour prior and during settlement; larval supply is important to settlement rates and sometimes final adult distribution (Gaines et al. 1985, Underwood & Fairweather 1989, Minchinton & Scheibling 1991, Hurlbut 1992, Hughes et al. 2000, Jeffery & Underwood 2000, Bohn et al. 2013). However, adult distribution can also be defined by interactions during and after settlement (Bohn et al. 2013). Todd (1998) suggested that in a rocky intertidal community, larval supply will be very important where recruitment is low, but at high recruitment levels post – settlement processes may be very important. He also suggested that for 'fouling' assemblages, pre and post – settlement processes are very important; and the 'openness' or 'closedness' of a population may have implications for the importance of larval supply over post settlement processes for supply – side theories (Todd 1998).

There are many dispersal techniques used by propagules and methods of dispersal such as: - upwelling's, Eckman transport, upper and lower currents, tidal flow, wind, waves, 'surface slicks', larvae quality (food stores) and behaviour (vertical migrations, responses to cues and salinity), spawning during neap tides and at night; as well as larval retention areas such as bays, therefore it is thought that the offspring are unlikely to return to the parental population (Underwood & Fairweather 1989, Kingsford 1990, Pineda 1991, Grosberg & Levitan 1992, Pineda 1994a, b, Wing et al. 1995, Bertness et al. 1996, Todd 1998, Todd et al. 1998, Hughes et al. 2000, Jonsson et al. 2004, Gude 2007). The lack of offspring returning to the parental population, prevents any local adaptations that are selected for and these selected traits will be useless unless the same adaptations are required in another area (Underwood & Fairweather 1989). If the larvae disperse over large spatial scales, the larvae produced in one area will not necessarily be correlated with adult density or fecundity, but in large areas, such as the Great Barrier Reef, there is a relationship between spawning and recruitment which is also known for fish stocks (Hughes et al. 2000). Todd (1998) found that a planktotrophic species showed genetic homogeneity over 1600 km (showing large – scale dispersal), and a lecithotrophic species showed 'population differentiation' on scales of less than 10 km in a highly dispersive area concluding that they use behaviour to minimise larval transport.

The dispersal phase is fraught with risks for the propagules as they may encounter many dangers not found in the adult's habitat, resulting in differences in survival temporally and spatially for this phase of an organism's life and some potential habitats not receiving any new propagules (Underwood & Fairweather 1989, Grosberg & Levitan 1992, Hughes et al. 2000). The juveniles' habitat may be vastly different from the adults', and this should be understood for an adequate knowledge of the whole organism (Grosberg & Levitan 1992). Local interactions are very important for the number and timing of release of propagules or gametes, but the factors influencing the supply of these organisms to their adult habitat must also be taken into consideration such as oceanographic processes; therefore competition, predation at the shore and at sea must all be taken into account (Underwood & Fairweather 1989, Grosberg & Levitan 1992, Hughes et al. 2000). Wing et al. (1995) found that oceanographic variations were responsible for retaining and returning larvae to inshore waters for urchin and crab settlement, and in some areas oceanographic and local hydrodynamic factors are responsible for recruitment failures over large geographic areas.

Genetic diversity has the potential to be seen in different populations of adults, and may help identify the original populations that new recruits have come from (Grosberg & Levitan 1992). It has been found that some fish larvae that inhabit reefs of the Saint Croix Virgin Islands (US) congregate in offshore areas, moving back to their local area when full grown in pulses of different taxonomic groups, therefore large amounts of larval retention occurs even though larvae are sometimes assumed to behave as passive particles (Warner et al. 2000). As some species of reef fish are endemic to certain islands or reefs, retention of fish larvae must happen, and it has been confirmed by genetic studies in the Caribbean and on the Great Barrier Reef in Australia (Doherty et al. 1995, Warner et al. 2000). This suggests that some species use their swimming and manoeuvrability to stay in an area rather than be advected away (Warner et al. 2000). However, fish from the same species of the Saint Croix Islands, bluehead wrasse (*Thalassoma bifasciatum*) displayed different patterns of movement depending whether they were from the east (had offshore 'blue water syndrome'), or the west that displayed 'near-shore syndrome' during their larval stage (Warner et al. 2000).

Mass spawning by corals can lead to very large numbers of eggs and sperm, that may overwhelm predators, thereby helping to increase recruitment; this spawning occurring during neap tides and at night, might also reduce predation (Hughes et al. 2000). Coral larvae usually aggregate into 'surface slicks' and are moved as a whole before they disperse, and it is hydrodynamic models that predict where larvae will be dispersed around adjacent reefs on the Great Barrier Reef (Hughes et al. 2000). However, recruitment spatially and temporally was linked to variations in fecundity, and as this increased so did recruitment; therefore 'fecundity drives recruitment' in the Great Barrier Reef, but local changes in one part of the reef may affect recruitment on adjacent reefs (Hughes et al. 2000). But some isolated coral reefs such as the Helix reef on the Great Barrier Reef, were found to be reliant on water residence time for the reef to 'self – seed', and the parts of the reef with the highest recruitment had the lowest flushing rates suggesting that these reefs are 'closed' (Todd 1998).

Sometimes the larval supply can be very important if the species is invasive, such as the slipper limpet *Crepidula fornicata* from the North – West Atlantic whose long larval phase and tolerance of wide environmental conditions have allowed its colonisation on Northern European coasts such as Milford Haven (Bohn et al. 2013). The slipper limpet has a larval stage of 2 – 4 weeks; therefore dispersal is thought to be important in its colonisation and regional distribution, with larvae advected away from adults to be transported to new areas (Bohn et al. 2013). Larval supply to two sites was found to be the same, although larval densities in the surrounding water of these sites were different (Bohn et al. 2013). Settlement rates did not differ between the sites, and recruitment was low suggesting that post settlement mortality was important; there was no relationship between adult abundance and settlement rates suggesting that the organism's final distribution was the result of post settlement mortality (Bohn et al. 2013).

Todd (1998) suggested that a new challenge was to evaluate supply – side processes to whole communities. In an open system, a community may be determined by the supply of individuals from a common pool (Caro et al. 2010). Caro et al. (2010) evaluated metacommunities on the rocky intertidal over 800 km, and found that recruitment did not explain the spatial variation of the adult structure; but that post settlement interactions (predation and competition) among species and possibly local environmental factors structure communities, producing relatively convergent community structures.

Cypris respond to biological (biofilms), chemical (settlement inducing protein complex from either other squashed adults and cypris or living animals) and physical cues such as pits and crevices (Eckman 1996, Miron et al. 1996, Minchinton 1997, Hills et al. 1998a, b, Miron et al. 1999, Kato-Yoshinaga et al. 2000, Matsumura et al. 2000, Satumanatpan & Keough 2001, Jeffery 2002, Khandeparker et al. 2003, Czarnoleski et al. 2004, Hung et al. 2008). Settlement – inducing protein complexes (SIPC) can be divided into BSF (barnacle settlement factor), CSF (cyprid settlement factor) and CL (cyprid larvae) which Hills et al. (1998a, b) found encouraged nearly 50 % of settlement within 10 minutes of their experiment, but they had low settlement in untreated pits. Settlement – inducing protein complex is a contact pheromone, a glycoprotein that is, or is equivalent to cypris ‘footprints’ (cypris temporary adhesive); together with a waterborne pheromone (small peptide) released by adults into the water, encourages gregarious settlement (Elbourne & Clare 2010, Khandeparker & Anil 2011, Pagett et al. 2012, Xiaozhen et al. 2013). There have been three types of behaviour described when a cypris encounters the substrata: broad exploration at the scale of approximately 1 m; close exploration at the scale of approximately 1 mm; and inspection which is at the scale < 300 µm (Hills & Thomason 1996, 1998a, b, Hills et al. 1998a, b, Hills et al. 2000, Prendergast 2010). Exploration need not be a long protracted affair, *S. balanoides* cypris have been filmed in the laboratory settling in minutes, and Hills et al. (1998a, b) suggest that inspection and settlement in the field can take minutes even though the cypris are selective (Hills et al. 2000).

Larval supply has been described as the number of larvae transported to the substratum and is different from the ‘planktonic concentration’, i.e. away from the potential settlement site or actual settlement on tiles (Gaines & Bertness 1993, Gude 2007). There are several basic technologies that are used to measure larval supply, i.e. nets, traps, pumps, and adhesive settlement panels. Settlement and recruitment are conveniently measured using standardised artificial panels or existing natural substrata. Settlement panels (some made from ceramic household tiles and panels made from plastic) have also been used to infer supply rates without any demonstrated quantification of the relationship between numbers of planktonic larvae and settled larvae (cf. Hurlbut 1992). Settlement panels (tiles or planks) have included: - Bakelite® (Crisp and Barnes 1954, Crisp 1955), plate glass (Crisp and Barnes 1954), glass beads covered by car finish (Kohler et al. 1999), Pexiglass® (Jarrett & Pechenik 1997, Berntsson et al. 2000, Olivier et al. 2000, Lagos et al. 2008), natural slate (Crisp 1985, Todd 1998), Lego toy building block baseplates (Walters 1992), spruce planks (Miron et al. 1999), ceramic tiles (Pineda & Caswell 1997), polyvinylchloride (PVC) panels (Miron et al. 1996), poly – vinylidene - fluoride (PVDF) panels (Berntsson et al. 2000), polymethyl – methacrylate (PMMA) panels (Berntsson et al. 2004), and filled – polyester resin using polyvinylsiloxane panels (Hills & Thomason 1998b, Thomason et al. 2000, Thomason et al. 2002a, b). Pumps set the standard for quantification of plankton densities as they sample large volumes and larvae have no choice but to be drawn into them. Also the volume they sample can be precisely calibrated (Bertness et al. 1996, Ma & Grassle 2004, Holzman et al. 2005). However, they require a power source and may be relatively expensive for deployment in large numbers. To overcome the difficulties in using pumps in remote locations without a convenient electrical supply, passive traps have been developed (Yund et al. 1991, Castilla & Varas 1998, Todd 2003, Todd et al. 2006, Gude 2007). These entrap larvae below a baffled chamber and have been designed to be cheap and robust enough for deployment on exposed rocky shores. Similarly, passive adhesive panels, using a non-toxic grease to entrap larvae arriving to explore a surface, perhaps provide the most precise measure of supply of competent larvae (Walters 1992, Jonsson et al. 2004). Satuito et al's (1997) original definition of competence is used, i.e. the propensity of larvae to make a positive choice to settle when encountering a suitable substratum: this makes no assumptions about their physiological status (see Miron et al. 1999).

However, panel size, edge effects and hydrodynamic shear, the rugosity of the substrata, gregariousness of the species cypris, patchiness in the water column and phototaxis must be taken into consideration for any investigation of larval settlement behaviour and recruitment (Hills & Thomason 1998a, b, Miron et al. 1999, Hills et al. 2000) Boundary effects of the flow where the area is being filmed, can affect settlement (Hills et al. 2000) as well, and this should be taken into account. It has been suggested by Hills et al (2000), that settlement on the substratum is a combination of day and night-time settlement patterns. In 30 minute filmed trials, 99 % of the cypris explored then left the surface of PVC panels (tiles) with settlement factor on them (Hills et al. 2000).

Crevices and pits are favourable settlement sites for *Semibalanus balanoides* and roughened surfaces on panels have been shown to aid recruitment (Crisp 1961, Hills & Thomason 1998a, b). Panels made of resin have been found to encourage settlement with different roughness in the order of fine texture (< 0.5 mm roughness components) had greater settlement than, medium texture (1 mm - < 2.0 mm ~ 2 mm) than, course texture (2.0 - 4.0 mm) and least on smooth panels with about six times less settling on them than the course texture (Crisp & Ryland 1960, Hills & Thomason 1998a). It has been suggested that larvae settled on surfaces that were similar in size to the larvae themselves, and larger surface - roughness panels may reduce the number of settlement sites (Hills & Thomason 1998a). Other physical and biological factors that have been shown to encourage settlement are conspecific gregariousness at a scale of a few centimetres, availability of space to settle, light, temperature, flow, salinity, biofilms and the age of the cypris involved with a 'desperation' effect of older cypris (Hills & Thomason 1996, Miron et al. 1996, Hills & Thomason 1998b, Keough 1998, Harder et al. 2001, Berntsson et al. 2004, Jenkins 2005). However, settlement has also been argued to be the result of hydrodynamics in the water column (Gaines et al. 1985, Minchinton & Scheibling 1991, Keough 1998, Harder et al. 2001). Experiments with *Balanus amphitrite* on the North American coast showed that larval supply and settlement was not correlated (Olivier et al. 2000).

Semibalanus balanoides settlement has been found to alter with density, at low densities (< 2 cypris cm^{-2}) there is random settlement and these cyprids are to be considered founders, however, at higher densities there is gregarious settlement which interacts with rugosity of the surface (Hills and Thomason1998a, b). In an earlier paper Hills and Thomason (1996) found that *S. balanoides* was aggregated at < 8 cypris cm^{-2} and random at > 8 cypris cm^{-2}, this could possibly be due to each individual of this species requiring finding a mate within a penis length, but then trying to avoid intraspecific competition (Prendergast 2010). However there is a reduced tendency for *S. balanoides* to settle where there are > 10 cypris cm^{2} (Crisp 1961). Although another paper suggests that *S. balanoides* settle amongst conspecifics until 30 % of the free space is taken, and then settlement decreases (Berntsson et al. 2004). Arthropodin (settlement inducer) is a glycoprotein that the adult barnacles produce which encourages settlement now known as settlement – inducing protein complex (SIPC), this encourages the cypris to produce cement and it is slightly different for each species (Crisp 1985, Kato-Yoshinaga et al. 2000, Matsumura et al. 2000, Aldred & Clare 2008). *S. balanoides* cypris have been filmed intertidally at the University Marine Biological Station Kepple Pier, Millport, Isle of Cumbrae KA28 0EG; settling preferentially on conspecific rather than allospecific SIPC (Matsumura et al. 2000). However, it has been noted that slates smeared with oil from a natural seepage attracted more cyprids to metamorphose, than on plain slates used as a control, suggesting that the oil mimicked settlement chemical cues (Pechenik 1999).

The cypris performs an inspection of the substratum that it settles on using its antennules to conduct frequent turns at a speed of up to ~ 2 mm s^{-1}, settled cypris are avoided and the cypris will not touch a conspecific at settlement, allowing space or a territory for growth as an adult (Crisp 1961, Walker & Yule 1984, Crisp 1985, Aldred & Clare 2008, Prendergast 2010). However, it has been suggested that if cyprids don't find a conspecific within 30 mm, they will move off the substratum (Hills & Thomason 1996). Each time a cypris settles on a substratum, it leaves a cypris 'footprint' which is a proteinaceous secretion, but the volume lost each time a cypris lands on the substratum limits the distance it explores, with *S. balanoides* able to explore 638 antennule paces and a distance, with gaps, of 420 mm (Walker & Yule 1984, Aldred & Clare 2008).

On reaching the substratum the cypris can attach temporarily for a whole tidal cycle, then detach and re-enter the water column, but this uses resources and the larvae may become desperate to settle after that and possibly unable to metamorphose (Prendergast 2010). It is suggested that a population on the shore is both a result of Langmuir, surface foam lines, density dependent food competition, predation and other stochastic forces (oceanographic processes, wind driven waves) for the larval dispersal of the blue crab *Callinectes sapidus* (Pile et al. 1996). With the Australian barnacle *Elminius covertus*, vertical settlement was determined by larval behaviour (Satumanatpan & Keough 2001). Experiments in a flume using *Balanus improvisus* by Berntsson et al.

(2004) showed that this species of cypris frequently rejected surfaces in a flow of 10 cm s^{-1} but preferred flows of 5 cm s^{-1}. Svensson et al. (2004) found that recruitment of *S. balanoides* in south west Ireland, Isle of Man and west Sweden was partially dependent on substratum free space; and partially dependent on hydrodynamics, reproductive output of other populations, larval behaviour and survival. Space available to recruits and an area's population is more sensitive to survival and growth of the present population, than to recruitment (Svensson et al. 2004). Osman and Whitlatch (1995) suggest that recruitment pattern isn't substantially different from settlement patterns suggesting settlement may be where the community had most influence (Menge 2000). Recruitment can be reduced on existing populations by overgrowth of older individuals (Osman & Whitlatch 1995). Encrusting algae has been found to have a minor negative effect on barnacles (*Chamaesipho tasmanica*), which they tend not to settle on (Underwood 1999). Minchinton (1997) suggests that gregarious recruitment is dependent on the shape of the free space, for example long narrow patches being preferred to wider shorter spaces. It is also suggested that there is a positive feedback to settlement where the greater the recruitment, the more larvae will be stimulated to settle there (Minchinton 1997).

4.2 Aims of chapter

Ascertaining the number of cypris that will settle from cyprids in the close water column, has met with varying degrees of success in the literature. It was hypothesised that by combining methods to gauge settlement from the water column, a technique to easily forecast settlement would be found.

Artificial rock panels have been used in many experiments in the literature, as they can be easily replicated. It was hypothesised that artificial panels made by the duplication of natural rock from the Clyde Sea (the area where the experiments were undertaken), would be a good alternative to natural substratum and get results statistically similar to natural rock.

Work has been done in flumes examining a cypris investigation of a substratum before settlement - but to my knowledge, cypris have not been filmed in the natural environment exploring a potential settlement site and timed whilst doing so. It was hypothesised that a cypris would be likely to leave after a short period of time, but this needs quantifying.

4.3 Methods and materials

4.3.1 Cyprid supply and settlement frame

A set of pumps, traps, adhesive panels and settlement panels was simultaneously deployed during the spring settlement season of *S. balanoides*, at Fairlie Commercial Pier (O.S. reference NS2065 5630) in the spring settlement season of 2005. It was to compare the usefulness of traps and pumps to quantify planktonic cyprid densities, adhesive panels were used to quantify cypris exploring surfaces, and bare panels used to measure settlement of *Semibalanus balanoides* (Linnaeus) in the Clyde Sea; relating supply to settlement. The design of each component was based on an established protocol and is described in detail below (Figures 4-1 and 4-2).

4.3.2 Pumps

Plankton pumps were designed after Bertness et al. (1996). Each 12 volt Rule® model 24 bilge pump, (nominal throughput of 1360 l hr^{-1}) was attached to a plankton collector via a 19 mm bore reinforced polypropylene pipe. The plankton collector was made from a standard domestic waste water 32mm bore ABS 90^{o} pipe bend (to deflect the vented water away from the other components of the experiment) and 200 µm plankton net (GB Nets, Bodmin, UK). The net was attached reversibly to the bend outflow using a screw ring (Figures 4-1 and 4-2). The intake cover of the pump was attached to a piece of marine plywood (120 mm x 120 mm x 5 mm) to enable the pump and trap to be readily fixed to the deployment frame (Figures 4-1 and 4-2). Flow through each pump was calibrated before use (mean flow = 1322.46 ± 189.69 l hr^{-1}). The pumps were attached to a 12 volt Palstar PS-15® transformer via weather-proof 2 core cable. The transformer was kept in a weather-proof plastic box and connected to an outdoor 240V AC electrical supply via 100 m of outdoor cable and a mini-circuit breaker. It was found that the zooplankton passed through the pumps undamaged on a trial run. Therefore identification of zooplankton collected with the pumps was easily conducted using the book Marine Plankton A Practical Guide (Newell & Newell 2006).

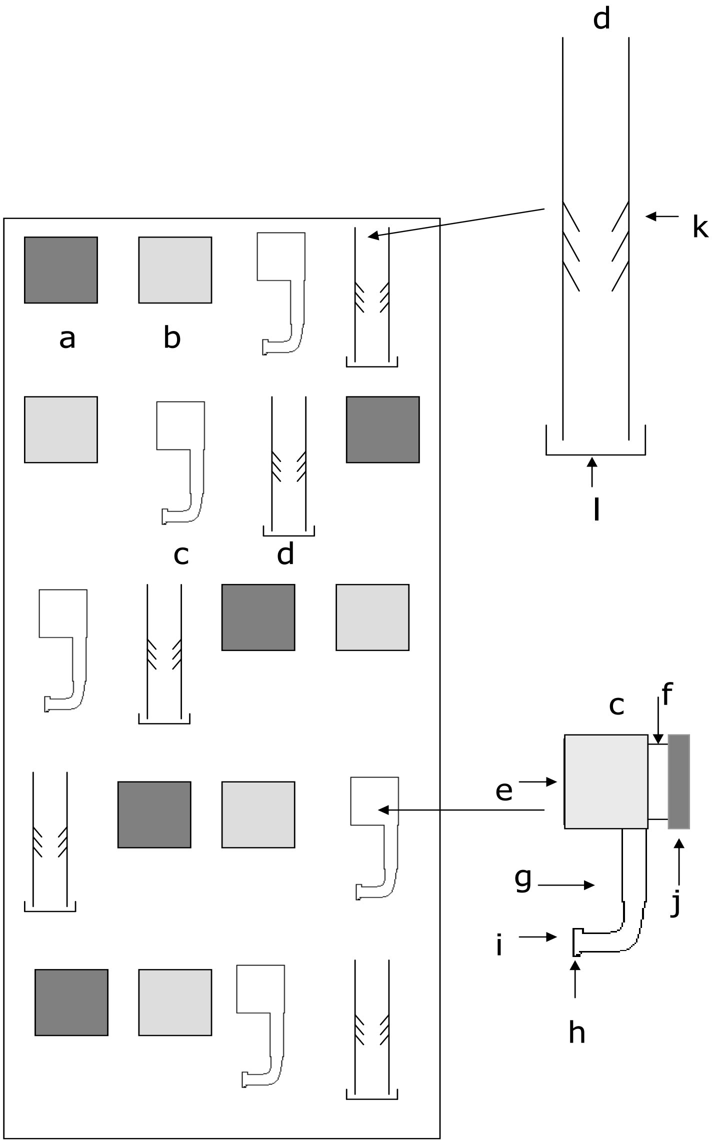


Figure 4-1. Diagram of pumps (c) with the 12v pump housing (e) water intake behind housing (f) exhaust tube (g) plankton net (h) water outflow (i) on mounting plate (j), traps (d) with baffles (k) and inspection end for removal of cypris (l), settlement panels (a) and adhesive panels (b) arranged on frame in Latin square design for deployment.

Figure 4-2 Photograph of Cyprid supply and settlement frame (a = plain settlement panel, b = adhesive settlement panel, c = pump, d = trap) in Latin square design.

4.3.3 Traps

Tubular traps were made with an aspect ratio of 10:4 according to Todd (2003), but were 1.5 times larger (Figures 4-1 and 4-2). They were made from 32 mm bore standard domestic ABS waste pipe and had a total volume of 268 ml. Each trap comprised three sections. The top section was 86 ml. The central section (61 ml) contained baffles made by solvent welding a series of three polypropylene funnels (10 mm, 10 mm, and 6 mm hole diameters; Scientific Laboratory Supplies, UK) into the pipe. Tube baffles of 0.5 or 1.0 cm^2 are optimal for *S. balanoides* (Chen et al. 2013). The baffles prevent advection of larvae back into the water column. To enable ease of fitting of the funnels, they were fixed between two sections of solvent welded pipe connected using external jointing sleeves. The bottom section (121 ml) had a screw cap for removal of trapped larvae and was filled with 4M urea in filtered seawater (Fisher Scientific) to act as a killing solution (Todd 2003).

4.3.4 Plain settlement panels

Settlement panels used were 120 mm x 120 mm x 2 mm roughened (120 grit, orbital sander for 30 seconds) grey PVC. In each corner 4 mm diameter holes were drilled for attachment to the frame, leaving a 100 x 100 mm area in the centre of each panel for counting cypris.

4.3.5 Adhesive settlement panels

Settlement panels (as above) were covered with silicone grease (RS Components Ltd, UK) to a depth of approximately 0.5 mm. Silicone grease does not dissolve, remains tacky underwater and is non-toxic. This was applied to prevent exploring, but non-settling, cyprids leaving the surface (Walters 1992).

4.3.6 Deployment

The pumps, traps, adhesive panels and settlement panels were attached to a frame using 4 mm nylon cable ties (RS Components Ltd, UK). The frame was made out of four vertical 2 m painted steel struts (Dexion Ltd, UK) positioned 0.5 m apart and 5 equally spaced 1.5 m horizontal cross struts. Pumps, traps, adhesive panels and settlement panels were placed in a Latin square design across the frame, with one of each in a row (Figures 4-1 and 4-2). The frame was deployed from a floating pontoon situated adjacent to Fairlie Commercial Pier, Clyde Sea, Scotland (55° 45.6’ N, 4° 51.7’ W). There is intense and predictable monospecific settlement of cypris of *S. balanoides* in the Clyde Sea each spring (Hansson et al. 2003) and this site is less than 3.4 km from Keppel Pier, University Marine Biological Station, Isle of Cumbrae, Scotland (O.S. reference NS1755 5435) where much previous work has been done (e.g. Hills & Thomason 1998a, b, Hills & Thomason 2003a, b, Prendergast 2007, 2010). The frame was positioned 0.1 m below the surface of the water by using two polypropylene ropes, such that each row of a pump, trap, adhesive panel and plain panel were at a depth of 0.1 m, 0.6 m, 1.1 m, 1.6 m and 2.1 m. The pontoon permitted the apparatus to remain at a constant height in the water column to help prevent vertical spatial variability in cypris densities adding further noise to the data. The frame was deployed eight times for 2 to 4.5 hr. during daylight on rising and high tides between 11/04/05 – 15/04/05. Mean volume pumped was 5346.1 l ± 1334.6 l hr^{-1}.

Cypris caught in the pumps and traps were transferred to buffered seawater formalin 6% (v/v) formaldehyde and ~0.01% (w/v) borax in filtered seawater as Prendergast (2007) for subsequent quantification in the laboratory. Photographs (Canon Powershot G3; 4 M pixel; RAW format) were taken of every adhesive and settlement panel after each deployment for later analysis.

4.3.7 Filming of cypris arrival, departure and settlement on artificial rock panels

Rocks of the five rock types of the Clyde Sea (red sandstone, yellow sandstone, igneous, metamorphic from Loch Fyne and metamorphic from the main Clyde Sea basin) and larger than 120 mm x 120 mm were cut - off using a Stihl® saw, scrubbed using bleach, and left in running tap water for 1 week to wash away any chemicals and then left in filtered seawater, filtered using a 200 µm plankton net (GB Nets, Bodmin, UK) for one day. The moulds for the artificial rock panels were made using extrude by Kerr®, Polyvinylsiloxane Impression material (Type 3: low consistency and light bodied in 50 ml packs with a gun attachment to tubes) in order to spread over the rocks.

Resin panels were made by using a wooden mould (with a base and sides) divided into five 120 m x 120 mm compartments (Prendergast 2007). Dried extrude was left for one day, then the impressions were placed in the individual compartments. The resin panels were then constructed by using Filled Casting Resin FC702PA by Trylon (contains styrene), Llewellyn Ryland Polyester with the colour described as Paste. This was mixed with 1 % catalyst (Trylon which contains methyl ethyl ketone peroxide in phthalate plasticizer) in a fume cupboard, avoiding trapping air in order to have an accurate impression of the natural rock (Prendergast 2007). To make the five artificial rock panels 900 ml of the mixture was used. Two holes were made in the panels by placing a nail in before the plastic was poured in (in order to be affixed to a cradle or frame). The artificial panels had a 100 mm x 100 mm centre entirely a replica of the natural stone. The panels were left in the mould for 2 days in order to cure completely. Five panels of each rock type were made in case of losses during the experiments. Panels were left in running tap water for one week to wash away chemicals, then left in filtered seawater for one day to wash away the tap water.

Filming of cypris arrival, departure and settlement was undertaken using the artificial rock panels rather than natural rocks. An initial test film was made and cypris were very hard to distinguish on all natural rock types. An artificial rock panel was attached to a frame using 4 mm nylon cable ties (RS Components Ltd, UK) to a Dexion™ cradle (at a fixed distance of 1 m long) holding an underwater video camera (Kongsberg-Simrad UK Ltd, OE1366). The camera was connected via an armoured umbilical cable (NC-8) of 100 m to a remote control unit (Kongsberg-Simrad, OE1232), which was then connected to a Sony mini digital video VCR/monitor (GV-D1000 – Pal mini DV) following the procedure of Prendergast (2007) and Thomason et al (2002a). The panels were brought into focus manually in order to make sure passing plankton was not in focus and recording began on immersion of the unit from the pontoon. This was deployed in a random sequence between 11/04/05 – 15/04/05 at Fairlie Commercial Pier, Clyde Sea, Scotland (55° 45.6’ N, 4° 51.7’ W) and at a depth of 0.5 m to make sure wave action did not affect the filming. This experiment was conducted at the same time as the cyprid supply and settlement frame was deployed; then copied onto DVD-R discs using a recordable DVD machine (Toshiba RD-XS25SB) for future analysis.

Each immersion of each artificial rock panel was conducted for one hour. The resulting DVD for each immersion was examined. The panel was examined using acetate stuck to a television screen, then using the magnifying control to its highest setting and dividing the screen into six areas (3 horizontal and 2 vertically), in order to assess the arrival, departure and settlement of individual cypris. Each cypris was plotted for a 2 minute time frame at 1 minute (0 up to 2 minutes), 14 minutes (14 up to 16 minutes), 29 minutes (29 up to 31 minutes), 44 minutes (44 up to 46 minutes) and 58 minutes (58 up to 60 minutes after immersion as Measuring Behaviour An Introductory Guide (Martin & Bateson 2005). Due to time constraints of plotting each cypris, plotting could not be conducted for the full hour of recording obtained. Prendergast (personal communication) suggests that a cypris will usually settle if they have stayed on a panel for two minutes. After each immersion the panels were fully scrubbed with bleach that was then washed off with tap water, and finally immersed in filtered seawater as above for 1 hour.

4.3.8 Cypris settlement on natural rock versus artificial rock panels

Five sets of small frames (three replicas of natural rock and two of the artificial rocks); each frame holding one of each rock type (Loch Fyne metamorphic, igneous, Clyde basin metamorphic, red sandstone and sandstone), were constructed out of Dexion™ in order to hold natural rocks in one and the artificial rock panels in another frame. Each frame was 600 mm long and 120 mm high in order to leave an area of 100 mm x 100 mm clear of rock of each type for cypris to land and be counted (Figure 4-3). The rocks and artificial rock panels were the same as for the filming (Section 4.3.7), with the artificial rocks being the spares (not used in filming), but all treated as above; the rugosity was assumed to be similar for each individual type of rock used. See Chapter 6 for photographs of the different rock types used.

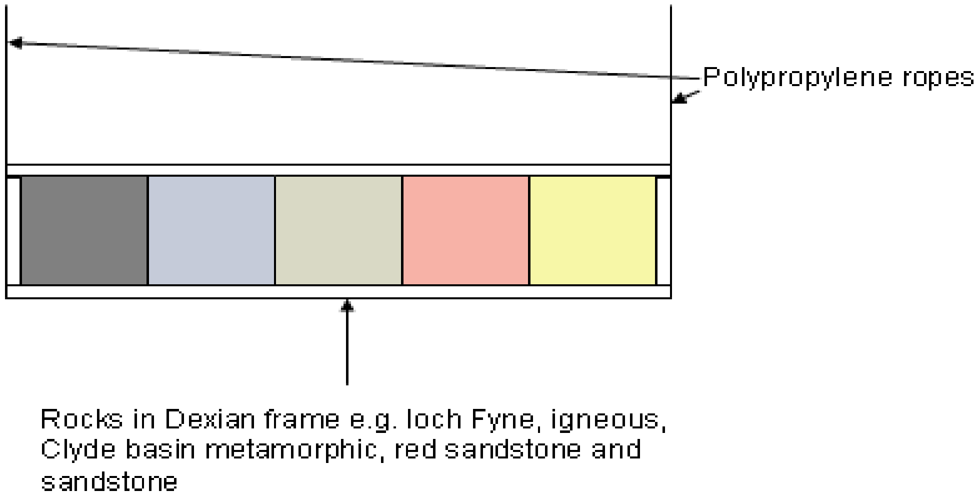


Figure 4-3 Diagram of natural and artificial rock frames

The frames were positioned side by side (although on separate polypropylene ropes in case one set was lost to the sea) at 1 metre depth below mean high water springs on static ropes, at the end of Fairlie Commercial Pier for four daytime tides. This experiment was conducted at the end of the pier, to avoid crowding of experiments on the pontoon.

4.3.9 Data recording and analysis

Image and statistical analyses of cyprid supply and settlement frame

Photographs were converted to tagged image format files (TIF) and automated image analysis of threshold 8-bit greyscale images performed using ImageJ (Rasband 1997-2008) to count cypris. Cypris were counted within the central 100 mm x 100 mm of each panel. Data were recorded as cyprids cm^{-2}.

Cypris from the pumps were decanted into Petri dishes and photographs taken as for the panels. TIFs were spectrally divided into hue, saturation and brightness channels and the saturation channel for each image was threshold and analysed as for the panel TIFs. Pump data were recorded as cyprids l^{-1}. As the urea bleached the cypris caught in the traps, they were dyed using Rose Bengal (~0.5% w/v; Fisher Scientific), before being photographed and analysed as for the pump samples. Data for the traps were recorded as cyprids trap^{-1}.

The relationship between each measure of larval supply, i.e. pump, trap and adhesive panel, and cyprid settlement on the settlement panels was determined using ordinary least squares regression, with model fits checked using fitted line plots.

Cypris arrival, departure and settlement on artificial rock panels

The DVD-R discs were analysed to establish both the time the cypris arrived and how long they explored the artificial rock panels for, in seconds up to 2 minutes (120 seconds). Graphical data is shown for the arrival and exploration of panels, and Chi squared tests for cross tabulation were performed due to the irregular amount of data; filming of rock type igneous (five times), Loch Fyne metamorphic (four times), Clyde basin metamorphic (known as metamorphic - three times), yellow sandstone (known as sandstone – three times) and red sandstone (three times).

Cypris settlement on natural rock versus artificial rock panels

The photographs obtained for both the natural rocks and the artificial rock panels were converted to tagged image format files (TIF), and analysed for cyprid settlement using automated image analysis of threshold 8-bit greyscale images using ImageJ (Rasband 1997-2008). See Chapter 6 – Effect of rock type on settlement for photos of the different rocks (Figures 6-2 to 6-7). Cypris were counted within the central 100 mm x 100 mm of the natural rocks and the artificial rock panels. Data were recorded as cyprids cm^{-2}. Data was log transformed to normalise data and was analysed using GLM ANOVA with transformed settlement as the response, rock and artificial rock panels as fixed factors, and Type III sums of squares with a fully factorial model.

4.4 Results

4.4.1 Cyprid supply and settlement frame

The data were split into surface and sub-surface cohorts and are summarised in Table 4-1. Overall (data for all deployments and depths combined) the adhesive panels, traps and settlement panels recorded 20.8, 2.25 and 0.31%, respectively, of the cypris captured by the pumps (Table 4-1).

	Surface	**Sub-surface**	**Overall % of pumps**
Pumps	627.13 ± 462.10 cypris hr^{-1} (0.11 ± 0.08 cypris l^{-1})	624.79 ± 601.25 cypris hr^{-1} (0.12 ± 0.10 cypris l^{-1})	
Adhesive panels	20.38 ± 23.62 cypris hr^{-1} (0.20 ± 0.24 cypris cm^{-2})	114.56 ± 117.77 cypris hr^{-1} (1.15 ± 1.18 cypris cm^{-2})	20.8
Traps	8.75 ± 7.44 cypris hr^{-1} (2.36 ± 2.01 cypris l^{-1})	10.22 ± 10.51 cypris hr^{-1} (2.66 ± 2.66 cypris l^{-1})	2.25
Settlement panels	6.63 ± 7.11 cypris hr^{-1} (0.07 ± 0.07 cypris cm^{-2}	1.28 ± 2.7 cypris hr^{-1} (0.01 ± 0.03 cypris cm^{-2}	0.31

Table 4-1 The numbers of cypris collected using pumps, adhesive panels, traps and settlement panels at Fairlie Commercial Pier, Clyde Sea. Data are expressed as mean cypris hr^{-1} (and the equivalent per unit volume or area) + Standard Error (SE).

There was no significant linear relationship between cypris settlement and gross or surface larval supply as measured by trap or adhesive panel data, respectively. The single significant relationship between settlement and gross supply measured by the pumps was very weak, with only 18.0% of the variation in settlement attributable to larval supply (Figure 4-4).

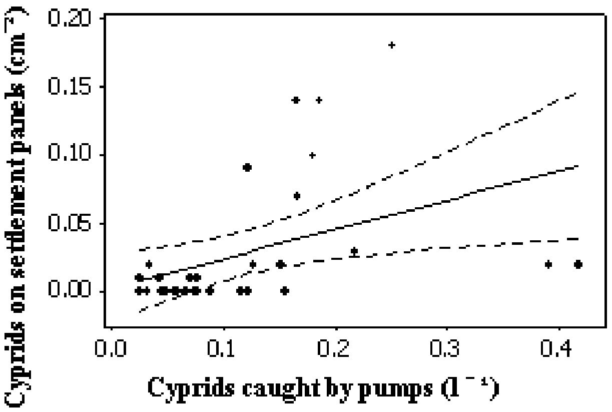


Figure 4-4 ***Semibalanus balanoides*****. Ordinary least squares regression of the number of cyprids (cm^{-2}) on the settlement panels verses the number of cyprids (l^{-1}) caught in the pumps for all depths and deployments ($y = 2.77 \times 10^{-3}\,x + 2.14 \times 10^{-1}$; $r^2 = 0.18$, $p = 0.045$, $n = 40$). Fitted line (—) and 95% confidence bands (- - -) are shown.**

Graphical examination of the data suggested that cypris at the surface were responding differently to those at other depths, therefore regression analysis was repeated after splitting the data into surface (0.1 m) and sub-surface cohorts (0.6 - 2.1 m). Thereafter, strong significant relationships between cypris settlement and both the pump gross supply data and adhesive panel surface supply data were found, but only for the surface cohort (Figure 4-5).

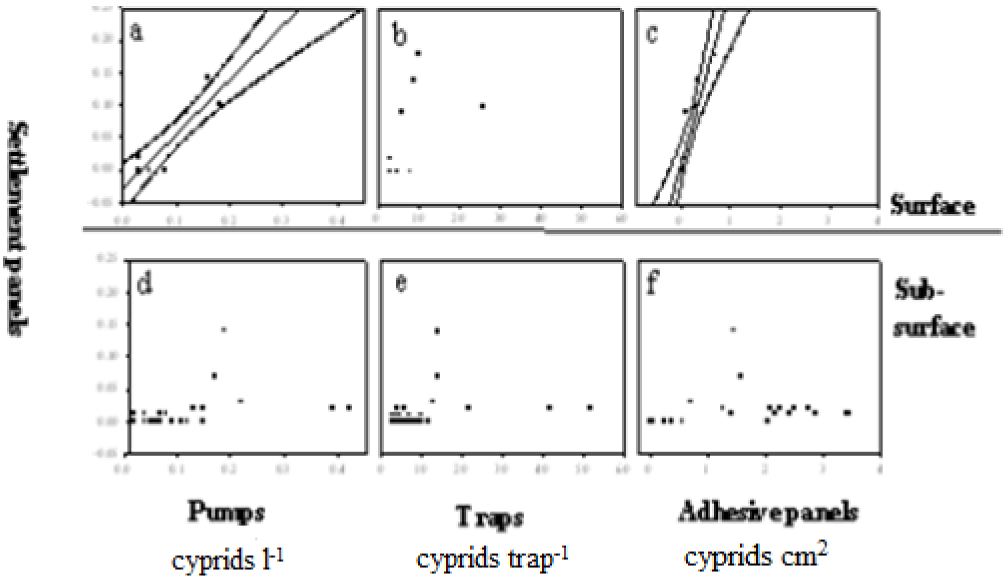


Figure 4-5 *Semibalanus balanoides*. Ordinary least squares linear regression of larval supply (pumps, cyprids l^{-1}; traps, cyprids $trap^{-1}$; adhesive panels, cyprids cm^2) vs. settlement for a) b) and c) surface cohort cyprids and d), e), f) subsurface cohort cyprids. a) $y = -2.900 \times 10^{-2}\, x + 8.450 \times 10^{-1}$, $r^2 = 0.900$, $p = < 0.001$, $n = 8$; b) $r^2 = 0.190$, $p = 0.278$, $n = 8$; c) $y = 1.000 \times 10^{-2}\, x + 2.740 \times 10^{-1}$, $r^2 = 0.830$, $p = 0.002$, $n = 8$; d) $r^2 = 0.120$, $p = 0.097$, $n = 32$; e) $r^2 = 0.060$, $p = 0.186$, $n = 32$; f) $r^2 = 0.070$, $p = 0.138$, $n = 32$. Fitted lines (—) and 95% confidence bands (- - -) are shown.

There was no detectable significant relationship between trap gross supply data and settlement for either surface or sub-surface cohort, or for the sub-surface supply data. To test if these results were consistent over time, the interaction between cohort *vs.* deployment day was analysed using a two-way Repeated Measures GLM ANOVA using cube-root transformed data and type III sums-of-squares. There was no significant interaction (p = 0.11) see Figure 4-6.

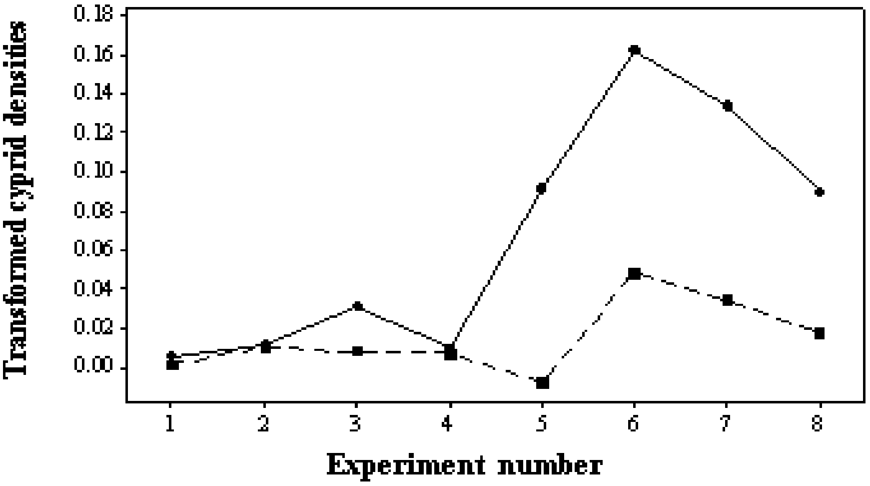


Figure 4-6. *Semibalanus balanoides*. Interaction plot of transformed fitted means from the ANOVA of cyprids densities on plain settlement panels for cohort vs. experiment number. Separate lines are shown for the surface (●) and the subsurface cohorts (■).There was no significant interaction (p = 0.110).

The only reliable measures of supply were found to be pumps and adhesive panels. Regression analysis of the trap and pump data and trap and adhesive panel data found no significant relationships for the surface cohort (traps *vs.* pumps: $r^2 = 0.260$, $p = 0.198$; traps *vs.* adhesive panels: $r^2 = 0.090$, $p = 0.471$) and only found a significant relationship between traps and pumps for the sub-surface cohort (traps *vs.* pumps: $r^2 = 0.840$, $p < 0.001$ $y = 0.114 + 25.840x$; traps vs. adhesive panels: $r^2 = 0.010$, $p = 0.595$). It was found that the density of cypris arriving at the site varied considerably across the different experiments (Figure 4-7).

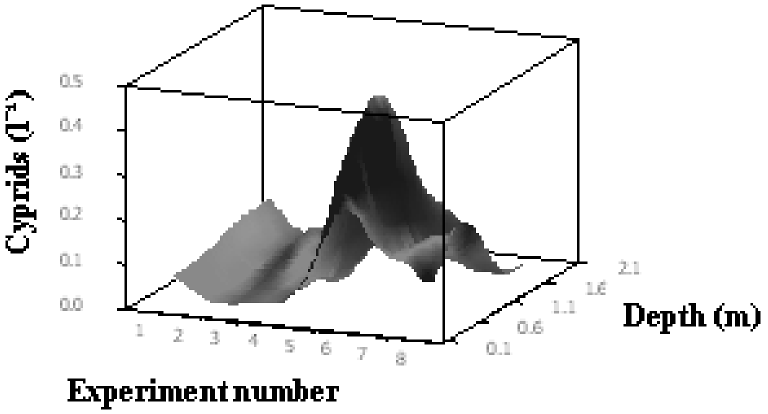


Figure 4-7 ***Semibalanus balanoides*****, 3D surface plot of planktonic cyprid densities (cyprids l^{-1}) by depth and experiment number. Data are from pumped samples.**

4.4.2 Filmed settlement on artificial rock panels

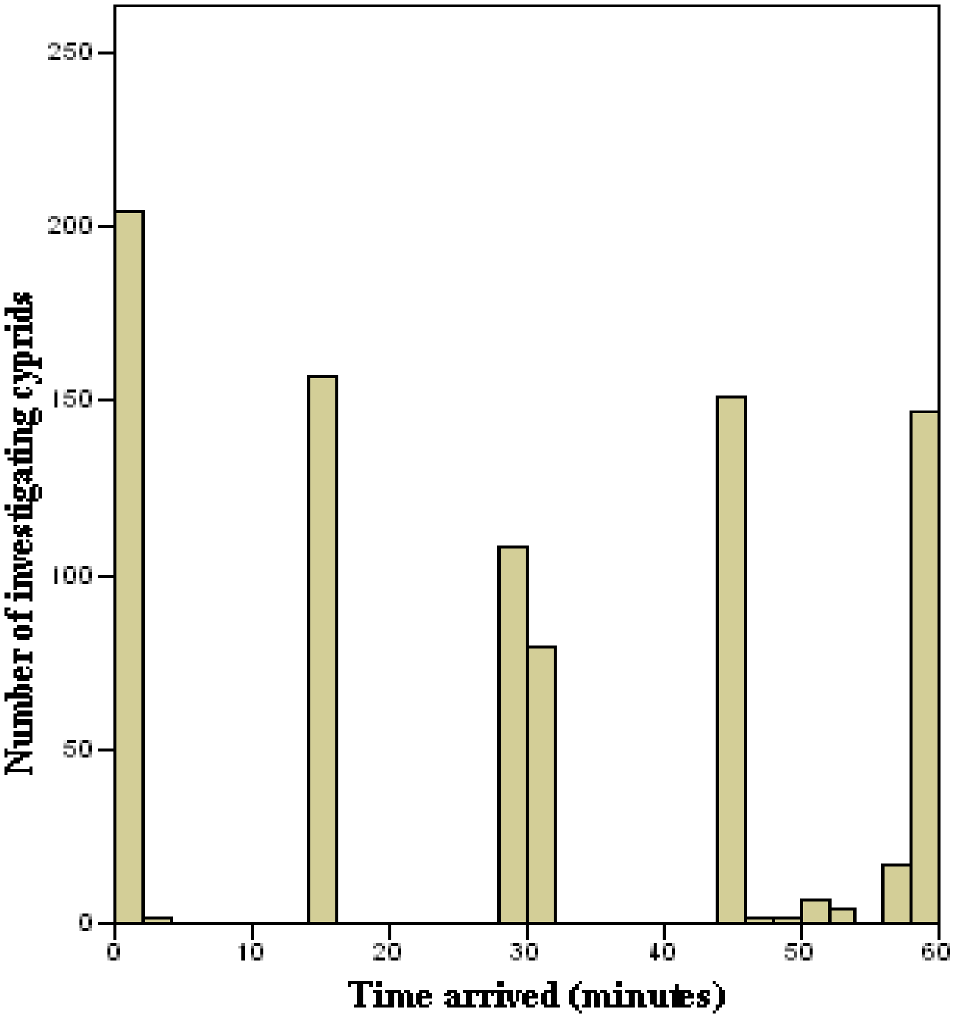


Figure 4-8 Raw data showing the total number of cyprids (880) that arrived on all artificial rock panels against the time in minutes when they arrived.

The individual highest number of cypris arriving during filming of the artificial rock panels was within two minutes at the start of filming (Figure 4-8). Overall arrival appeared to dip at 30 minutes (29 – 31 minutes), although if these two minutes are taken together this time slot under investigation is the second highest arrival time.

	Rock Type					**Total**
	1	**2**	**3**	**4**	**5**	
Left (count)	99	99	125	136	198	657
% within rock type	74.4 %	76.2 %	81.7 %	73.5 %	71.0 %	74.7 %
Stayed (count)	34	31	28	49	81	223
% within rock type	25.6 %	23.8 %	18.3 %	26.5 %	29.0 %	25.3 %
Total cypris	133	130	153	185	279	880

Table 4-2 Number of cyprids that stayed or left from each type of artificial rock panel during the filming period (total of 5 hours filming, 5 separate filming events).

The filmed artificial rock panels are: - 1 = red sandstone, 2 = yellow sandstone,
3 = Clyde Sea main basin metamorphic, 4 = igneous and 5 = Loch Fyne metamorphic

Table 4-2 shows the raw data from all filming of artificial rock panels deployed, indicating that approximately 75 % of the cypris exploring the panels left within two minutes and only 25 % stayed for the full two minutes or longer. Cypris showed a higher propensity to leave rock panels made from Clyde Sea main basin metamorphic rock. This is countered by panels made from Loch Fyne metamorphic rock, cypris showed the highest tendency to stay overall on this rock type.

Chi – Squared Tests for the cross tabulation (stayed * artificial rock panels) for the data in Table 4-2, showed no statistically significant difference in the abundance of cypris staying on each rock type ($p = 0.178$).

No significantly different trends were found from splitting the data into immersions. However for the first immersion there was an overall lower tendency for the cypris to leave, and igneous artificial rock was the most likely of the artificial rock types for the cypris to leave. This was the only occurence when any rock type other than the Clyde Sea metamorphic, had the highest percentage of cypris to leave it. Overall, the first and third immersions each showed that the highest percentage of cypris staying on the artificial rock types was for Loch Fyne metamorphic. The second and fourth immersions, showed the artificial rocks made from red sandstone had the highest percentage of cypris staying, and the fifth immersion showed igneous the highest percentage of cypris for that immersion staying. The individually separate immersions showed that the second and fifth immersion of the Clyde Sea metamorphic had the highest percentages leaving (both were 90.6 %). While the highest percentage of cypris staying, was for the first immersion of the artificial Loch Fyne metamorphic (42 %).

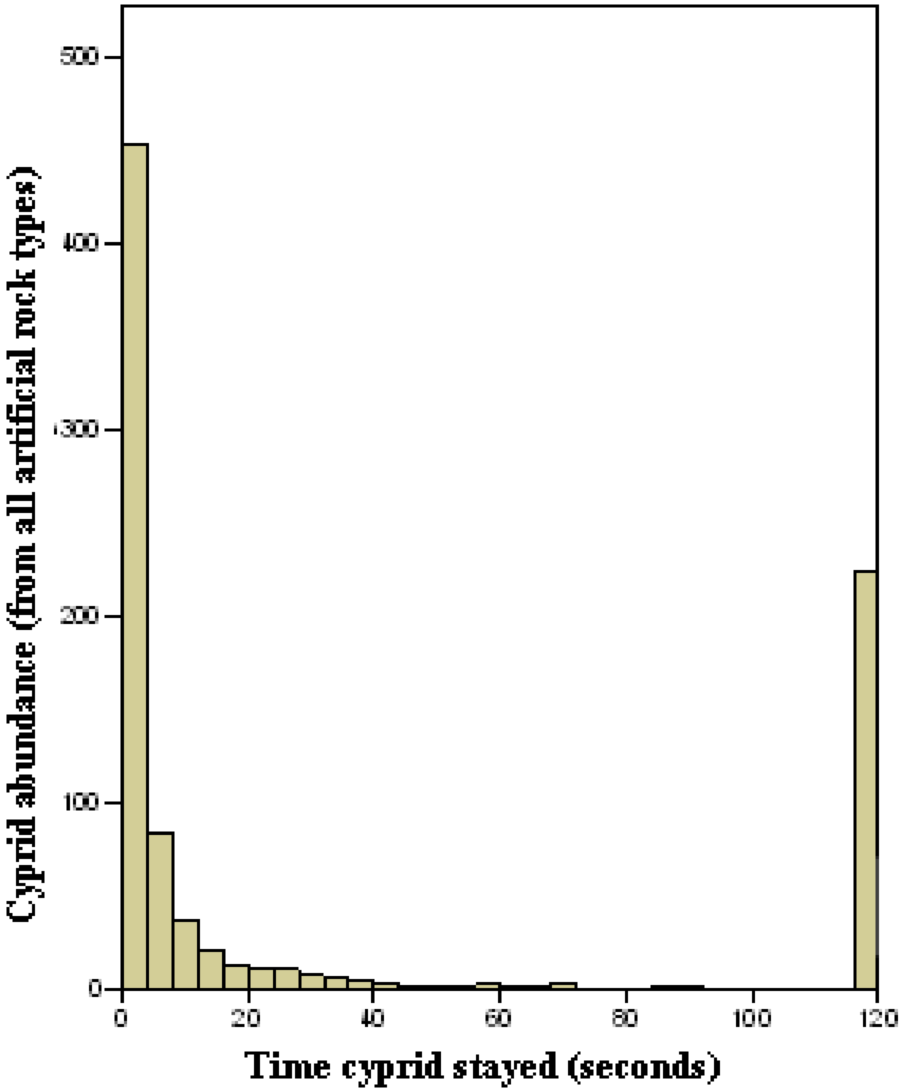


Figure 4-9 Bar graph for the total number of cypris (880) on all artificial rock types against how long the cypris stayed in seconds.

Figure 4-9 graphically shows that the majority of cypris left within the first 4 seconds. The amount of time cypris stayed dropped rapidly until the last four seconds (116 to 120 seconds), when they were considered to have settled (Prendergast personal communication).

4.4.3 *Natural versus artificial rock panels*

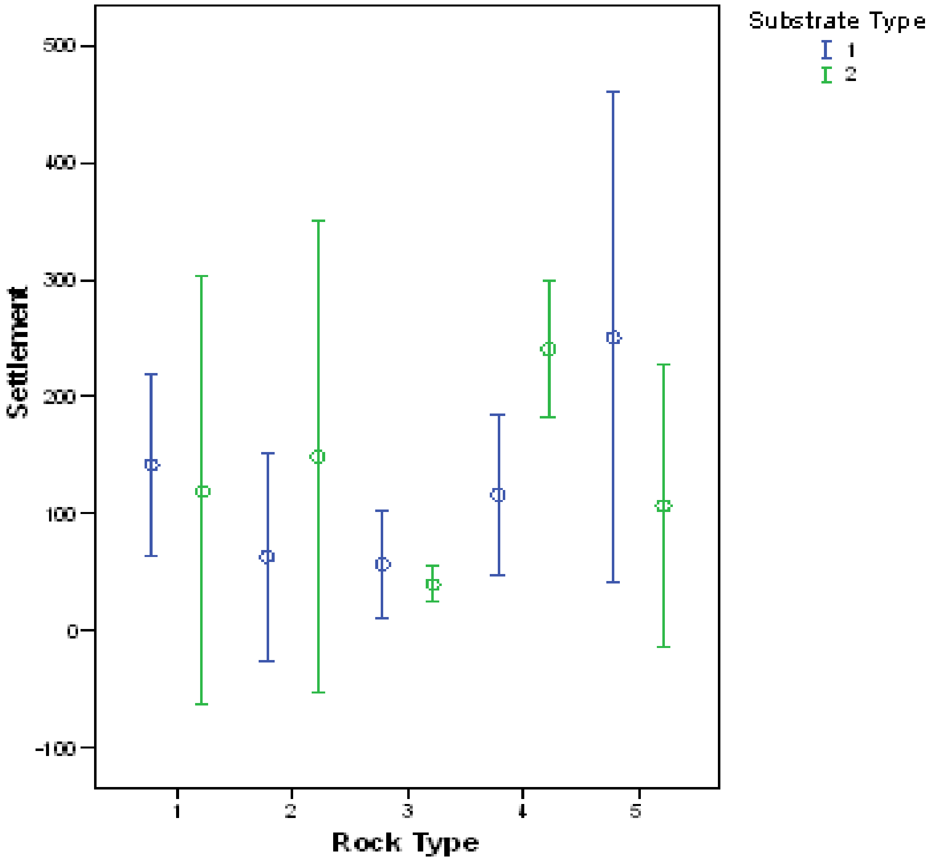


Figure 4-10 Settlement on artificial rock panels (substrate type 1), and natural rock (substrate type 2) showing 95 % Confidence limits.

Rock types for this experiment are: - 1 = igneous, 2 = yellow sandstone, 3 = red sandstone, 4 = Clyde Sea main basin metamorphic and 5 = Loch Fyne metamorphic.

There was a significant interaction between natural rock type and artificial rock panels affecting the settlement of cypris (tests of between subjects tests p = 0.022). In order to find where the significant differences lay all pairwise comparisons for rock type x artificial rock panels were analysed using contrast tests with sequential Bonferroni adjustment. No significant differences between artificial and natural substrata were found for any rock type (in all cases $p > 0.05$) and no significant differences between natural rock types were found (in all cases $p > 0.05$).

Therefore, although the sensitive ANOVA found a significant interaction between substratum type and rock type the blunter contrast tests could not find where the difference lay. The overlapping 95% confidence limits of the un- transformed data (Figure 4-10) indicate the result of the contrast test is the most appropriate one to use.

4.5 Discussion

The aim of this chapter was to determine the best technique to use to quantify the relationship between supply and settlement of *Semibalanus balanoides* barnacle cypris, to find out how long the cypris explored the substratum in the natural environment, and to ascertain if artificial rock panels were a true alternative to natural rock.

The experiments have shown that the strongest linear relationship between gross supply and settlement was quantified using bilge pumps for the surface cohort of *S. balanoides* cypris. A significant, but slightly less strong, relationship was also found between supply measured by adhesive panels (surface supply) and settlement of the same surface cohort. Jonsson et al. (2004) found a similarly robust relationship ($r^2 = 0.75$) between the settlement of *Balanus improvisus* cypris and their supply to surfaces as measured by adhesive panels. However, in their case the relationship was non-linear, though this difference may be due to species specific behaviour or local conditions. The results also suggested that the traps selectively trapped only deep-swimming non-competent larvae, and indicated that cyprids swimming in the top 0.1 m of the water column had a consistently higher propensity to settle than those in deeper water. This was not simply a function of the density of cyprids down the water column as this varied considerably during the course of the experiment (Figure 4-7), and it was not due to cyprids settling within the traps, as when they were dismantled there were no settlers present.

Therefore the choice between the two techniques largely becomes one of practicality. If a 12V DC power supply is available then the small robust bilge pumps are ideal, sampling a large volume of water for little cost, (See Bertness et al. 1996). The large sampling volume may be an advantage where larval densities are low. It should be simple to deploy them autonomously with a self-contained battery supply and timer for control. However, the cost for the pumps is higher (about 18 times) than that of the adhesive panels and care needs to be taken to ensure that the outflow does not affect larval supply to adjacent settlement panels. Chen et al. (2013) suggests that pumps can only be used in wave sheltered areas; however, they worked well at Fairlie Pier that was experiencing intermittent large waves, although not tidal amplitudes of 6 – 10 m as suggested. The cheaper adhesive panels are the same size, shape and material as the plain settlement panels, and they measure a much tightly defined type of supply than the pumps, *i.e.* only those larvae that explore the surface, and therefore they strictly sample the supply of potentially competent larvae. Strong tidal flow and rough seas may transport cyprids to the adhesive panels and entrap them, even though they may not normally have explored that surface. Filming cypris arriving, exploring and departing from the substratum is the ideal technique, although artificial rock panels had to be used for this because observation of the cypris against natural rock was very difficult. Purchasing the filming equipment can be very expensive, and a 240V AC outdoor marine electricity supply is required. However, filming costs have decreased as the technology develops and outdoor waterproof cameras become smaller and cheaper. Analysing the filmed cypris on the substratum is very time consuming due to repeatedly going over the same time frames until all cypris have been accounted for. Adhesive panels may also be used to compare settlement pattern with supply pattern as the entrapped larvae on the adhesive surfaces are not able to explore and thus differences in pattern of larval aggregations between settlement panels and adhesive panels will be simply due to larval exploration (see Walters 1992, Hills & Thomason 1998b, Hills et al. 2000).

The tubular traps caught larvae but there was no detectable relationship between trapped larval numbers and settler numbers. This was rather surprising as prior work has shown that data collected using this type of trap has a strong correlation (r >0.900) with settlement of *Semibalanus balanoides* cypris (Todd 2003, Todd et al. 2006) for the same species on several rocky shores on the east of Scotland. This contradictory result is not due to differences in trap performance as we recorded mean trap capture rates 9.9 cyprids trap^{-1} hr^{-1} and Todd et al found 13.7 cyprids trap^{-1} hr^{-1} (interpolated from Todd et al. 2006 , pg. 12, Figure 8). There were however notable differences between this study Todd (2003) and Todd et al’s (2006) as their traps were deployed intertidally, and that settlement was measured on adjacent rocks or on black acrylic settlement panels that were enhanced by grooves and the application of crude adult extract, both of which alter settlement pattern and behaviour of *S. balanoides* cypris larvae (Hills & Thomason 1998a, Hills et al. 2000). Chen et al. (2013) suggests that the tube traps are probably biased in their estimates of larvae. However, Gude (2007) found that although there wasn’t a significant difference in the raw numbers of cypris caught between resin 1 cm^2 and 2 cm^2 traps, he did find that due to the 1 cm^2 trap having a smaller aperture, there was less sediment, detritus and shell fragments in them. The 1 cm^2 were therefore less prone to blocking, but 0.25 cm^2 traps caught significantly fewer cypris (Gude 2007). The traps used in this experiment did not block or have sediment and shell fragments in them, possibly due to the traps being held at a constant depth and not on the actual substratum, and were deployed for shorter lengths of time rather than a whole tidal cycle. It has been suggested (Gude 2007) that traps are unlikely to saturate with cypris, but it is possible that pumps may clog and thereby lose pumping power, and therefore it may be difficult to accurately count the number of cypris per litre over a whole tidal cycle. Traps (Gude 2007) allow for collections to be made over an entire tide or day, allowing for temporal and spatial variation that may not be obvious over the time the frame was deployed (2 – 4.5 hours on rising and high tides). The frame gave snapshots of time and unlike Gude (2007), not the entire tidal cycle. Pineda et al. (2002) also demonstrated that for one of their two sites there was no relationship between near-shore abundance of *S. balanoides* cypris and settlement, with peaks in settlement occurring at very low pelagic larval concentrations, which may explain some of the variation in the filming experiment. The two sites that Pineda et al. (2002) used were only ~ 1 km apart and the local environmental conditions would appear to have had a strong influence in certain circumstances on the relationship between supply and settlement. Although the settlement during these studies were low by Clyde Sea standards (Hansson et al. 2003), it is comparative to settlement of S. *balanoides* in other parts of Scotland (Todd et al. 2006, Gude 2007). Gude (2007) found higher cypris abundance in his traps in 2006 than 2005, but the potential input into the system was unknown. The frame in the Clyde Sea was deployed in 2005, but the December 2005 egg numbers were low (Fecundity Chapter 3) which would have shown fewer cypris in spring 2006, however 2005 was the poor settlement year for the Clyde Sea. The December 2004 number of eggs were the highest, therefore would have expected April 2005 settlement to be very high.

The pump, trap, adhesive and settlement panels frame was deployed from a pontoon so that each array was held at a constant depth to reduce variation in larval supply to the pumps, traps and settlement panels. Gude (2007) suggests that pump or net sampling is not an accurate measure of larvae delivered to the substratum, and that the concentration would alter over a tidal cycle. However, placing small pumps on the frame in an array near settlement panels was intended to overcome this problem, and the problem of over – saturated silicon greased panels were not found to reach saturation point in this experiment. Although *S. balanoides* is an intertidal species as an adult, its larvae settle out from the water column when the intertidal zone is submerged. This experimental design also enabled the detection of the planktonic stratification of competent larvae. Gude (2007) on the east coast of Fife, Scotland; using depths from 1 m to 3 m also found that cypris were less abundant with increasing depth indicating neustonic behaviour. Having the supply of larvae of an intertidal species in the surface 0.1 m of the water column will ensure that the larvae will settle in the approximately “correct” location (McDougal 1943), with other biological and physical settlement and post-settlement factors determining the subsequent distribution of recruits (Minchinton & Scheibling 1991, Jenkins et al. 1999a, b, Miron et al. 1999). However the filming cradle was required to be deeper in order that the camera was not affected by surface wave action and may have been responsible for the lower number of cypris arriving and settling on the artificial rock panels than expected (Hansson et al. 2003). The static depth of the artificial rock panels versus natural rock panels allowed for the cypris coming into contact with them, but not affecting the other experiments by altering small current flow. The exact depth of stratification will be species specific. For example, Grosberg (1982) found that 94% of the variation in abundance of adults of the intertidal barnacle *Balanus glandula,* was explained by congregation of larvae at the surface. Conversely, larvae of the exclusively subtidal barnacle *B. crenatus* were found to concentrate relatively close to the sea floor. It was speculated that the former may be more buoyant, positively phototactic, or respond to pressure minima, and that *B. crenatus* may have the opposite attributes, allowing both species to settle at the same time without spatial competition. The pump, trap, adhesive and settlement panels of the frame array have shown planktonic stratification not of several species, but of competent/non-competent larvae of a single species. Satuito et al. (1997) showed that as barnacle larvae acquired competence their behavioural responses to settlement cues changed. The results suggest that competency also changes responses in the planktonic phase, priming the larvae for intertidal settlement as the majority stay for up to 4 seconds then leave or stay for at least 116 seconds (Figure 4-9). This may be a change in phototaxis (see Crisp 1974, Hills et al. 1998a, b), buoyancy, or geotaxis (Grosberg 1982). In a study of *Semibalanus balanoides* Miron et al. (1999) using the triacylglycerol/cholesterol ratio (TAG/CHOL) as an indicator of physiological condition, found stratification of planktonic cyprids but not of competent/non-competent larvae. However they did find the TAG / CHOL ratio in newly settled larvae decreased with decreasing intertidal height. Further work combining traps, pumps, adhesive tiles and plain settlement tiles with analysis of larval physiological markers to fully elucidate the interactions between larval supply, competency and settlement and how these determine variation in temporal and spatial variation in cyprid settlement (see Jarrett 1997, Jarrett & Pechenik 1997, Jenkins et al. 2000, Jarrett 2003) is required. However, this experiment has provided calibration data for the passive collectors (traps and adhesive and plain panels). It should now be possible given the capture ratios between pumps and the passive collectors to estimate total planktonic densities of cypris. The natural versus artificial rock panels showed that artificial rock panels could be used as an alternative to natural rock. However, similar work should be carried out using roughened PVC panels against natural rock that both were free of settlement cues. Filming of artificial rock panels showed that initial settlement for all artificial rock types was about 25 %, and there was no significant difference ($p = 0.178$) between artificial rock types. Cypris may have moved off at the next tide but logistical constraints did not permit the study of this.

This work has highlighted the need for more research to be conducted on exactly how passive tubular traps function before the data they provide can be reliably interpreted, and this is continuing by C. D. Todd and his team (cf. Todd et al. 2006, Chen et al. 2013). If they do preferentially trap non-competent larvae then their use in field experiments in combination with panels or pumps may be very beneficial. Likewise, further work is required to test the competence of larvae entrapped on the adhesive panels.

This chapter looked at cyprid supply and prospective settlement substratums - both artificial and natural. The following chapter will examine cannibalism.

4.6 Conclusions

- To measure supply, pumps or adhesive panels are the most appropriate
- Stratification of competent / non-competent larvae should be considered in experiments
- Artificial rock panels can be used as an alternative to natural rock
- The majority of cypris left the artificial rocks within 4 seconds

Chapter 5. Cannibalism

5.1 Introduction

In the previous chapter, larval supply and settlement was assessed by means of a Latin square of panels and pumps to collect the cypris in the plankton adjacent to settlement panels. The filming of cypris on replicate stone panels was also conducted, finding that cypris usually settle within four seconds of exploration or move off to another potential settlement site. In this chapter, cannibalism is investigated as it is a potentially important intraspecific predation act, potentially affecting every cypris that wants to settle with conspecifics, as the cypris have to settle within a penis length of other individuals either adult or cypris.

Cannibalism has been reported in many groups of animals, both aquatic (copepods, rotifers, fish); and terrestrial vertebrates (birds, mammals) and invertebrates (insects, mites and arachnids) (Fox 1975, Rudolf 2008). It is possible that cannibalism can affect trophic levels: by the cannibal reducing the impact its species has on the prey species by reducing the potential number of predators (Rudolf 2008), and thus can be an important regulator of populations (Fox 1975, Amaral et al. 2009) by both intra and intersize cannibalism (Fox 1975, Amaral et al. 2009). The magnitude of predation on earlier stages may be of vital importance in understanding the population dynamics of a species (Tamburri et al. 2007, Porri et al. 2008, Amaral et al. 2009) and recruitment to populations (Sardina et al. 2009). It may also be a type of interference competition, limiting the population or potential population size before other resources become limiting (Fox 1975). Adults within a population may therefore actively control recruitment (Porri et al. 2008). Cannibalism within barnacles may prevent or limit cypris from settling on benthic adults, with subsequent metamorphosis and smothering of older conspecifics. Therefore older individuals in the population are protected (Fox 1975).

Cannibalism

The risk of cannibalism is distinct for larvae that necessitate settlement among relatively dense aggregations of adults if these adults are suspension feeders (Tamburri et al. 2007, Sardina et al. 2009). *S. balanoides* produces planktonic larvae that must settle among conspecifics in order to reproduce (see Fecundity chapter). Other benefits of communal living for this species possibly include, increased feeding currents and reduced predation by being in a large congregation (Navarrete & Wieters 2000, Tamburri et al. 2007, Sardina et al. 2009).

If a species indiscriminately cannibalises conspecifics, there must be over-riding benefits from gregarious settlement (Tamburri et al. 2007, Sardina et al. 2009). At the population level a high larval production would offset cannibalism and other larval losses (Sardina et al. 2009), maintaining the overall population but preventing population explosions (Fox 1975).

Food availability, density or starvation affects cannibalism (Fox 1975, Amaral et al. 2009, Sardina et al. 2009). *S. balanoides* ingests many sizes of food from 2μm to 1mm in length which includes the range of sizes for nauplii and cypris (Southward 1955, Southward & Crisp 1956, Stubbings 1975). Nauplii and cypris of *S. balanoides* in the Clyde Sea area are only available from about February / March (for nauplii) to April / May for the settlement of cypris (Stubbings 1975, Southward 2008). Cannibalism may then be due to the presence of these larvae in the plankton, and be a response to the probability of an adult encountering a larva in its cirral net (Fox 1975, Porri et al. 2008, Camus & Zeng 2009, Plourde et al. 2009). Cannibalism is then variable in time and may account for a small percentage in an adults overall diet, and be a normal response to nauplii and cyprids in the plankton (Fox 1975, Porri et al. 2008). It has been suggested that cannibalism takes place in this species by Todd et al. (2006), Gyory and Pineda (2011), and Gyory et al. (2013).

Advantages to the adult population include the seasonal intake of a nutritious food source and reduction in potential conspecific competitors (Fox 1975, Navarrete & Wieters 2000, Tamburri et al. 2007). Also cannibalism reduces the potential population to the carrying capacity of the immediate area and therefore more food is available to the survivors (Fox 1975, Tamburri et al. 2007, Porri et al. 2008).

The disadvantage to individuals of a species practising cannibalism includes ingesting your own offspring, and reducing potential mating opportunities (Fox 1975). *S. balanoides* lives within the intertidal zone, and the currents and swell are likely to distribute nauplii soon after release, therefore reducing the chances of parents or genetically close relatives consuming their young and filial cannibalism is unlikely (Klug 2009). It has been suggested that the further genetically distant the cannibal is from the victim, the more likely the trait increases in the population for even a small benefit to the cannibal (Fox 1975, Navarrete & Wieters 2000, Banerji & Morin 2009). It is known that the size of the barnacle affects its feeding, which may also affect cannibalism (Thomason et al. 1998). The efficiency of the cirral fan capturing prey, and the swimming capabilities of the cyprids must play an important part of preventing cannibalism, enhancing the evolution of an aggregated life style for this barnacle species (Trager et al. 1994, Tamburri et al. 2007). It has been suggested by Gyory and Pineda (2011) and Gyory et al. (2013), that nauplii abundance coincides with winter storms on the east coast of North America; and that nauplii may be released at approximately the same time as phytoplankton blooms in order to prevent or lower, nauplii being cannibalised because of plenty food. During active feeding, the water disturbances caused by the cirral fan may produce warning signals to potential prey (Trager et al. 1994), but may also create a vortex allowing neighbouring barnacles to catch the prey (Trager et al. 1994). During strong currents (e.g. > 4 cm s^{-1}) *S. balanoides* holds its cirral fan across the current as the currents supply enough food, and potential prey does not have time to avoid capture (Trager et al. 1994).

As oophagy is a relatively common phenomenon in nature providing adults with a source of protein and energy, the ingestion of larvae may also provide similar benefits (Southward 1955, Tamburri et al. 2007). Larviphagy may be higher at different times of the tide for *S. balanoides,* for example Porri et al. (2008) found in mussels that it was higher during ebbing tides, and be a very important source of mortality in the species (Porri et al. 2008) Recruitment may be affected by the number of larvae in the adjacent water column. With *Semibalanus cariosus* (Pallas) (a common barnacle species found on the west coast of San Juan Island, Washington, USA), during low recruitment the adult barnacles remove proportionately more cypris, but during high recruitment the resident adults are overwhelmed by the numbers of cypris (Navarrete & Wieters 2000).

5.2 Aims of chapter

Cannibalism has been suggested to take place in *Semibalanus balanoides* on the east coast of Scotland, but it is known in other populations of animals that this can be a behaviour due to extremes, and not normal. Therefore it was decided to discover whether cannibalism took place in the Clyde Sea (Millport) population. It was hypothesised that cannibalism did take place in the Clyde Sea, with adults consuming nauplii and cypris. If cannibalism took place, to what extent would this be?

If cannibalism took place, were larger barnacles or taller barnacles more cannibalistic? It was hypothesised that larger and taller barnacles would be more cannibalistic, as larger barnacles would have larger cirri, and would be able to entrap and consume more barnacle larvae.

Did the state of the tide affect consumption of larvae? For example were cyprids and nauplii more likely to be caught during one particular state of the tide. It was hypothesised that state of the tide would affect consumption and therefore cannibalism.

5.3 Methods and materials

Two approaches were used, namely analysis of gut contents of adults collected from the shore, and filming of submerged adults when feeding.

5.3.1 Gut content analysis

Semibalanus balanoides were collected from the shore for a period of 28 days from 21/03/2007 up to and including 18/04/2007, which covered the spring settlement season. However, on the 22nd, 24th to 29th, 31st of March; and the 1st of April, there were no collections made due to bad weather and other commitments. Fifteen to twenty barnacles were removed from the substratum, with a range of sizes from small to large, selected randomly from the quadrat areas. The barnacles were taken from the centre of the *S. balanoides* settlement zone (2.6 m above chart datum), and from the same area to reduce anomalies caused by currents or exposure which was at O.S. reference NS177 545 (just north of Keppel Pier on the Isle of Cumbrae). Collected barnacles were placed in centrifuge tubes filled with 100% ethanol and labelled. Five barnacles were then taken from the centrifuge tube for each date, with a range of sizes from small to large, making a total of one hundred barnacles examined. Frustum volume was calculated for each adult. Dissection of *S. balanoides* collected from the shore was undertaken using a dissecting microscope (Nikon 87989), and faecal pellets were viewed under a high contrast bifocal microscope (x100 magnification). The microscope (Olympus GM180M HBG) was connected to an analogue live video camera with CCD chip video output (JVC TKC1431EG) with a 0.5x C-Mount camera adaptor (GXM180M C-M 0.5x), this was then connected to a 28 inch television (Welltech) used as a monitor. Faecal pellet composition was investigated using Marine Plankton A Practical Guide (Newell & Newell 2006), Handbook of the Marine Fauna of North-West Europe (Hayward & Ryland 1990), and Stubbings (1975) for cyprid and naupliar remains which could be identified. Other zooplankton were also observed such as copepods and a dinoflagellate (*Ceratium fusus)*. However, smaller particles were more difficult to discriminate and were not classed as barnacle. Shore barnacles were taken for gut content analysis over a longer period than filming in order to give an overall view of the settlement season.

5.3.2 Filming of cannibalism

This experiment was conducted from 11/04/2007 until 13/04/2007 at University Marine Biological Station, Isle of Cumbrae, Scotland (O.S. reference NS177 544).

Fifteen small rocks (approximately 15 cm x 5 cm x 10 cm), with at least 5 live *S. balanoides* positioned in a single plane of view (sometimes barnacles had to be removed in order to achieve this) were collected. A rock was attached by cable ties (RS Components) to a Dexion™ frame (at a fixed distance of 1 m long) holding an underwater video camera (Kongsberg-Simrad UK Ltd, OE1366). The camera was connected via an armoured umbilical cable (NC-8) of 100 m to a remote control unit (Kongsberg-Simrad, OE1232), which was then connected to a Sony mini digital video VCR/monitor (GV-D1000 – Pal mini DV) following the procedures of Prendergast (2007) and Thomason et al. (2002a). The unit was then suspended from Kepple pier, University Marine Biological Station, Isle of Cumbrae, Scotland (O.S. reference NS177 544), to a depth of 2.6 m above chart datum (this corresponded to the middle of the *S. balanoides* settlement zone). Barnacles on the rocks were brought into focus manually in order to make sure the barnacles, and not the rock or passing plankton was in focus. Recording began on immersion of the unit, to 60 minute JVC or Panasonic mini digital videocassettes. The mini videocassettes were then copied onto DVD-R discs using a recordable DVD machine (Toshiba RD-XS25SB) for future analysis. Recording was conducted for three high, three low, and three ebbing tides. With 5 barnacles examined for each film. After filming for one hour the frame was brought to the surface, the barnacles placed in individual centrifuge tubes labelled and filled with 100% ethanol. The DVD's were analysed by stepping through each frame individually for each barnacle to observe what it was catching. Therefore each DVD of each group of barnacles on each rock was stepped through five times in order to get a record of their feeding. Each individual barnacle's placement in the group was noted, as was if they caught and let go, or caught and ingested a nauplii or cyprid.

5.3.3 Data analysis

Is Semibalanus balanoides cannibalistic?

Using data from the barnacles taken from the shore, descriptive statistics were used to determine the percentage of barnacles with identifiable gut contents and those without. Then the percentages of barnacles containing larvae were calculated. Both nauplii and cypris were grouped together in this analysis, and it is assumed that the nauplii and cypris were *S. balanoides* as this is the only species of barnacle reproducing at this time of year in the Clyde Sea area (Thomason et al. 2002a). A graph showing dates of gut contents and larvae was also produced.

Influence of the size of the adult on cannibalistic tendencies

A regression was conducted to determine if larger adults (i.e. those with a larger frustum volume; see Fecundity chapter for information on frustum volume), were more likely to be cannibalistic, using data from barnacles collected from the shore. Frustum volume was the predictor and larvae found in the faecal pellet the response.

Determination of encounter and capture rates

Descriptive statistics were obtained for the data of the encounter and capture rates. The encounter rate was worked out by adding the number of cypris caught in the cirral fan on film, with the number of cypris that had escaped from the cirral fan on film. The total was then divided by the number of cypris captured. This gave the number of cypris encountered whilst the frame was immersed, so the result was divided by the number of minutes that the barnacle was actively hunting (to give encounters / min^{-1}).

Capture rate was worked out by using the number of cypris caught on film divided by the total number of encounters. This total was then multiplied by one hundred to give the percentage of cypris caught per hour. Capture rates were expressed as percentages of encounter rates.

Influence of barnacle height as an indicator to cannibalism

Regression analysis was conducted using the height of the barnacles used during filming as the predictor and the encounter rate as the response.

Influence of tidal state on cannibalism

Two univariate GLM ANOVAs were conducted to find out if the state of the tide had an effect on cannibalism. The first univariate GLM ANOVA used the state of the tide as the predictor and the mean encounter rate as the response. The second univariate GLM ANOVA used the state of the tide as the predictor and the capture rate as the response. The *post-hoc* tests for both ANOVA's were Ryan-Einot-Gabriel-Welsch.

5.4 Results

5.4.1 Is Semibalanus balanoides cannibalistic?

Barnacle Gut Contents	Frequency / percentage %
Empty	3
Full	97
Total	100

Table 5-1 Gut contents of 100 *Semibalanus balanoides* collected from the Isle of Cumbrae shore in March and April of 2007

Table 5-1 shows that 97% of the barnacles examined from the shore had full guts, suggesting that they had fed during the last high tide when they would have been covered.

Number of larvae	Frequency / percentage %
0	56
1	20
2	13
3	8
4	3
Total	100

Table 5-2 Number of larvae (nauplii and / or cyprids) in gut contents of 100 *Semibalanus balanoides* collected from the Isle of Cumbrae shore in March and April of 2007

Table 5-2 shows that from the adult barnacles taken from the shore with full guts (have a faecal pellet), that 44% of these adults contained either nauplii or cypris remains. From the raw data, the cypris nauplii ratio (cypris / nauplii) gives a result of 1.6 cypris eaten for every nauplii eaten. This is to be expected as cyprids are more likely to come near the substratum that adults are residing on in order to find a suitable settlement place, and the experiment was conducted during the cyprid settlement period.

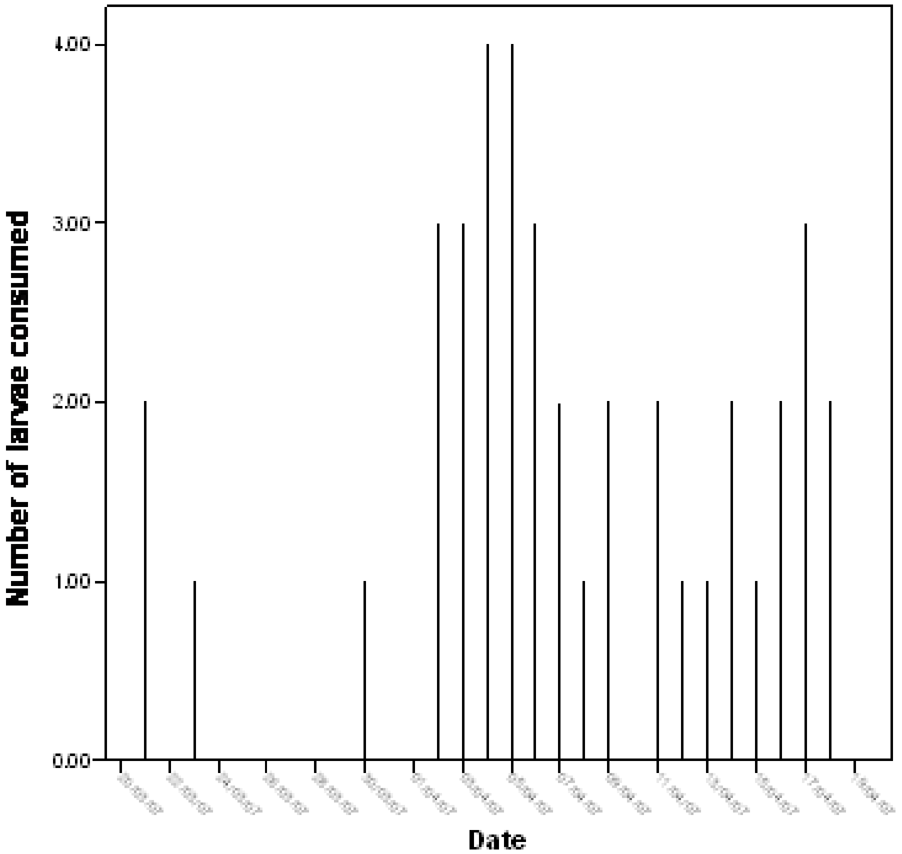


Figure 5-1 Number of larvae eaten by date using remains of gut contents from *Semibalanus balanoides* removed from the shore of the Isle of Cumbrae in March and April of 2007.

The graph (Figure 5-1) produced from the descriptive statistics shows a variable pattern of larvae found in the gut. Larvae were found every day from 02/04/2007 until 18/04/2007 (except 10/4/2007).Cypris may have been approaching the substratum in order to find a suitable settlement site where they would be within a penis length of an adult or another cypris (see Fecundity chapter). It also indicates that the experiment was conducted during the cyprid settlement period, due to consistent cypris remains.

5.4.2 Does the size of the adult affect cannibalistic tendencies?

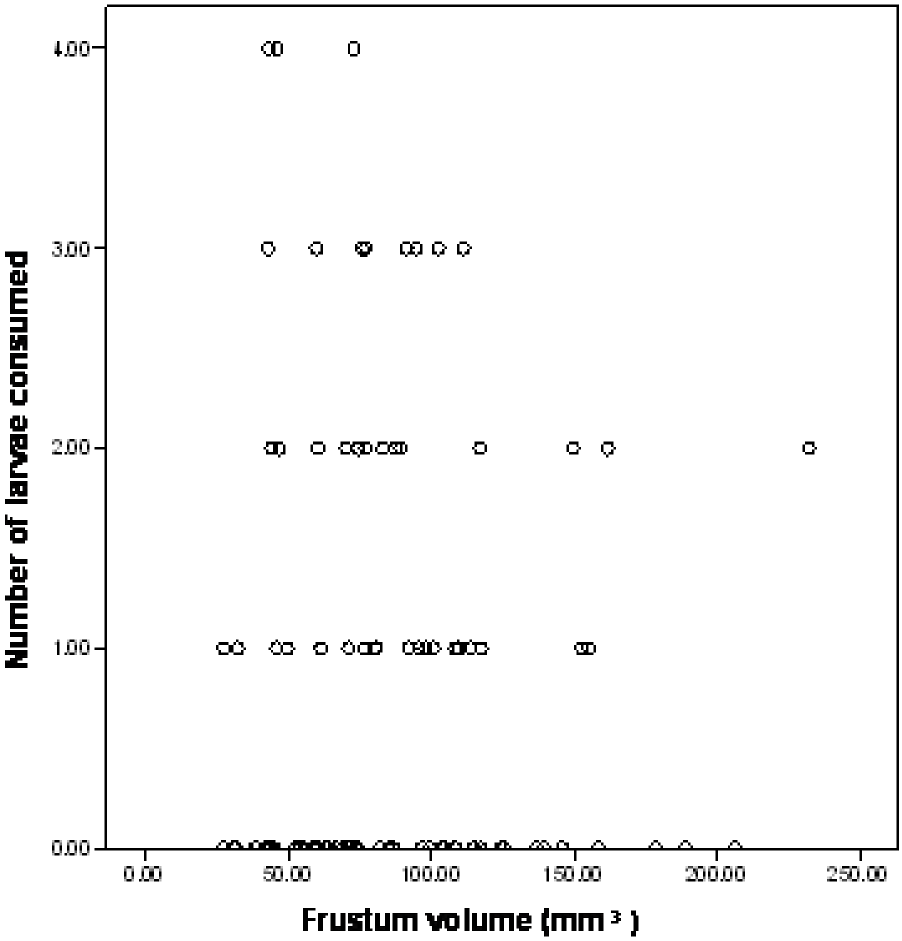


Figure 5-2 Scatterplot of frustum (mm^3) volume against number of larvae consumed by *Semibalanus balanoides* collected from the Isle of Cumbrae shore in March and April of 2007 ($r^2 < 0.001$, F = 0.001, p = 0.974).

The scatterplot (Figure 5-2) shows a broad size range of adults (as the adults were randomly collected) and no relationship between adult size and larvae consumed ($r^2 < 0.001$, F = 0.001, p = 0.974). Therefore size does not affect the propensity of *S. balanoides* to consume nauplii or cypris of their own species. It is assumed that larger volume barnacles have a larger cirral fan (personal observation from the filmed barnacles), but these barnacles were no more likely to consume their own species than their smaller counterparts.

5.4.3 Encounter and capture rates

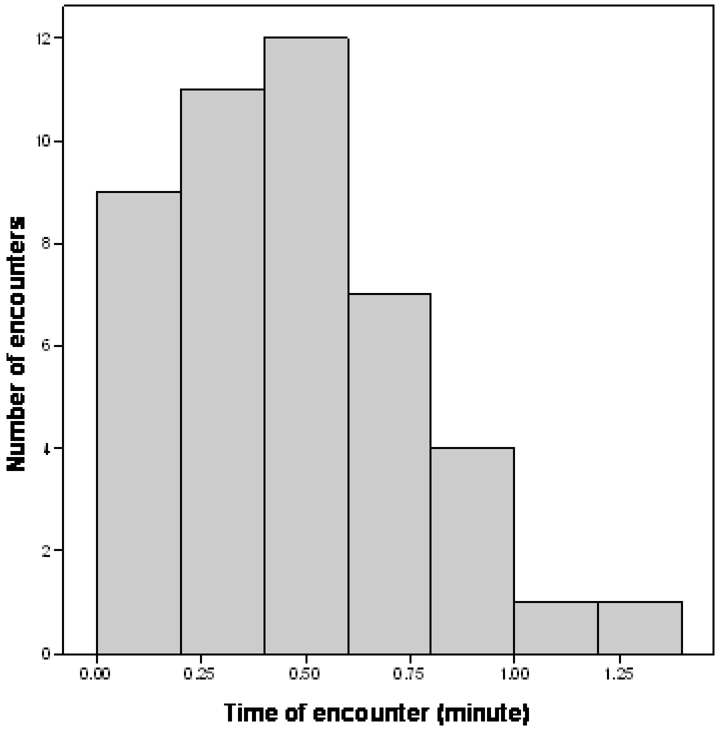


Figure 5-3 Encounter rates per minute of larvae by adults of *Semibalanus balanoides* on the Isle of Cumbrae shore in April of 2007 (n = 45, SD = 0.302, mean = 0.472 min^{-1}).

The encounter rate (Figure 5-3) shows that the cyprids and / or nauplii were bumping into the cirral nets of the adults most frequently every half minute, and the other most frequent encounters were leading up to half a minute during filming. This may suggest that potential food was very abundant in the area where filming occurred (University Marine Biological Station). This encounter rate did not include other plankton hitting the cirral fans. The encounter mean rate of 0.472 larvae per minute encountering the cirral net is equivalent to 28.332 larvae an hour.

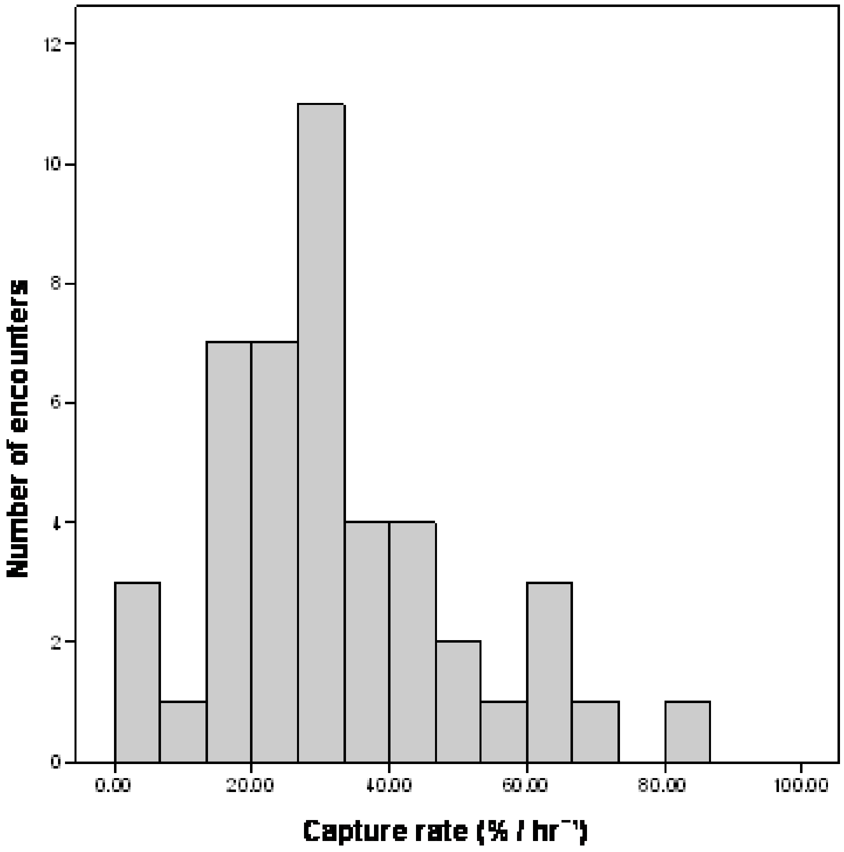


Figure 5-4 Capture rates of larvae by adults of *Semibalanus balanoides* on the Isle of Cumbrae shore in April 2007 for all tides expressed as percentages of encounter rates per hour (n = 45, SD = 18.203, mean = 32.109).

The capture rate (Figure 5-4) was highest around 30 % of the encounter rate (Figure 5-4). There was an increase from about 15 %, a decline after about 35 % through 40 % to about 45 %. The mean capture rate was 32 %, therefore 32 % are ingested with a mean encounter rate of 28.332 per hour. Therefore nearly 1/3 of the 28 cyprids and or nauplii encountered every hour are eaten which is (32.1091 x 28.332) / 100 = 9.0972, this suggests that within the Clyde Sea at cyprid settlement season, 9 cypris are eaten every hour by an adult.

5.4.4 Is increased height of adult an indicator to cannibalism?

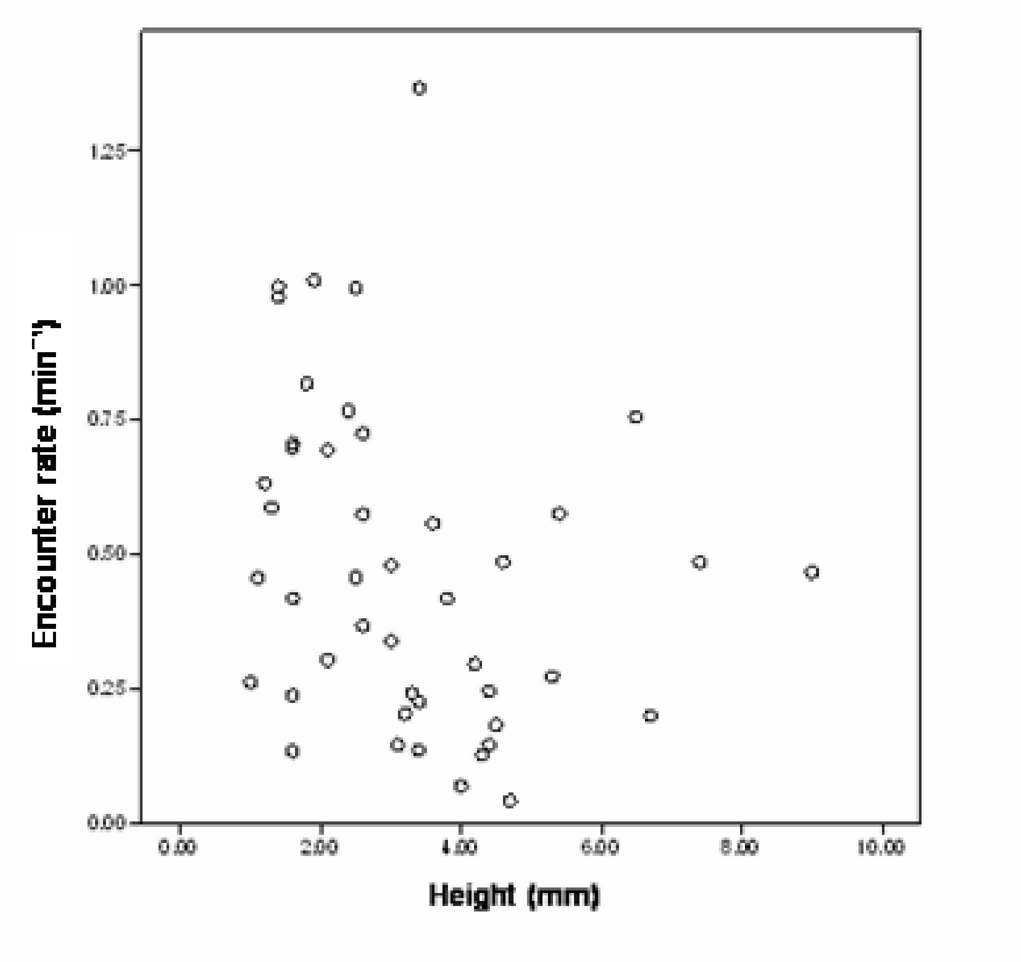


Figure 5-5 Regression heights (mm) of adult barnacles *Semibalanus balanoides* against encounter rate per minute ($p = 0.081$, $F = 3.200$, $r^2 = 0.069$).

Scatterplot analysis (Figure 5-5) of the effect of barnacle height on encounter rates by GLM ANOVA, showed that there was no relationship between barnacle height and encounter rate with larvae ($p = 0.081$, $F = 3.200$, $r^2 = 0.069$). Therefore barnacles of all heights were as likely to encounter cyprids and nauplii in their cirral nets, and the larger barnacles were no more likely to encounter the cypris than their shorter counterparts.

5.4.5 Does state of the tide affect cannibalism?

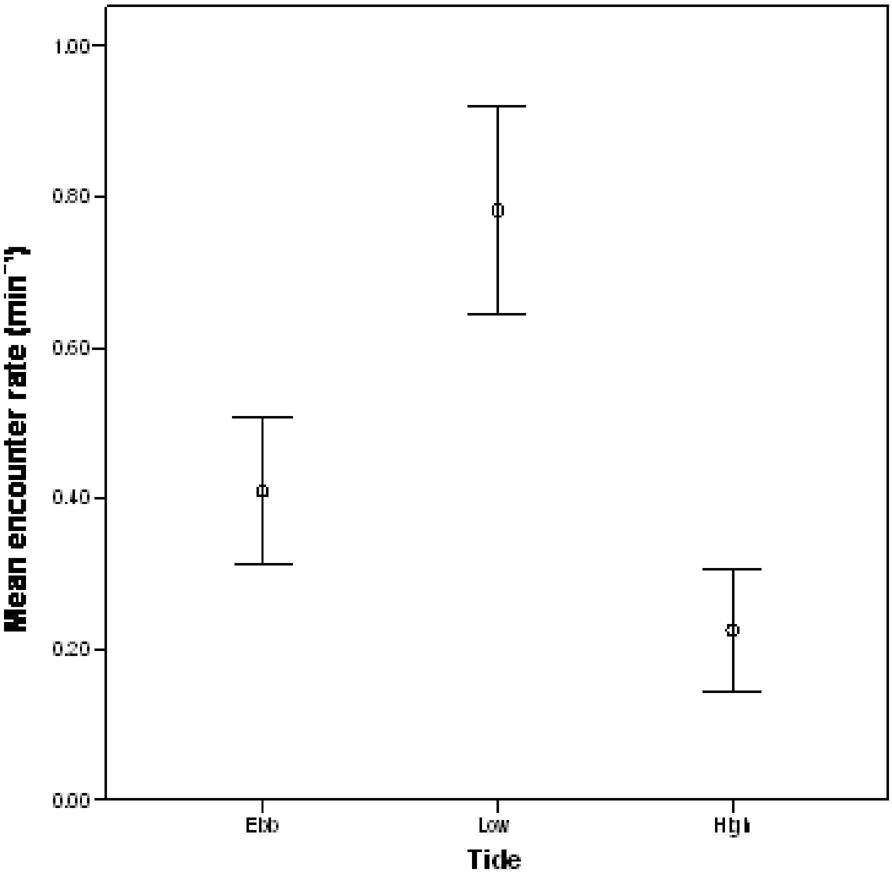


Figure 5-6 Grouped error bar plot (95% confidence interval) for the mean encounter rate (per minute) of cypris and adult *Semibalanus balanoides* and the state of the tide ($p = <0.001$, $F = 31.944$, $r^2 = 0.603$).

Ryan-Einot-Gabriel-Welsch Range

Tide	N	Subset		
		1	2	3
High	15		0.225	
Ebb	15			0.409
Low	15	0.782		
P value		1.000	1.000	1.000

Table 5-3 Sub-sets for the mean number of encounters per minute and the state of the tide

The error bar plot (Figure 5-6) for the mean encounter rate of the different states of the tide shows a significant ($p = <0.001$, $F = 31.944$, $r^2 = 0.603$) difference, with adults during low tide having the most encounters, and during high tide the fewest encounters. The Ryan-Einot-Gabriel-Welsch Range *post-hoc* test to find sub-sets (Table 5-3), showed that all three states of the tide were significantly different from each other.

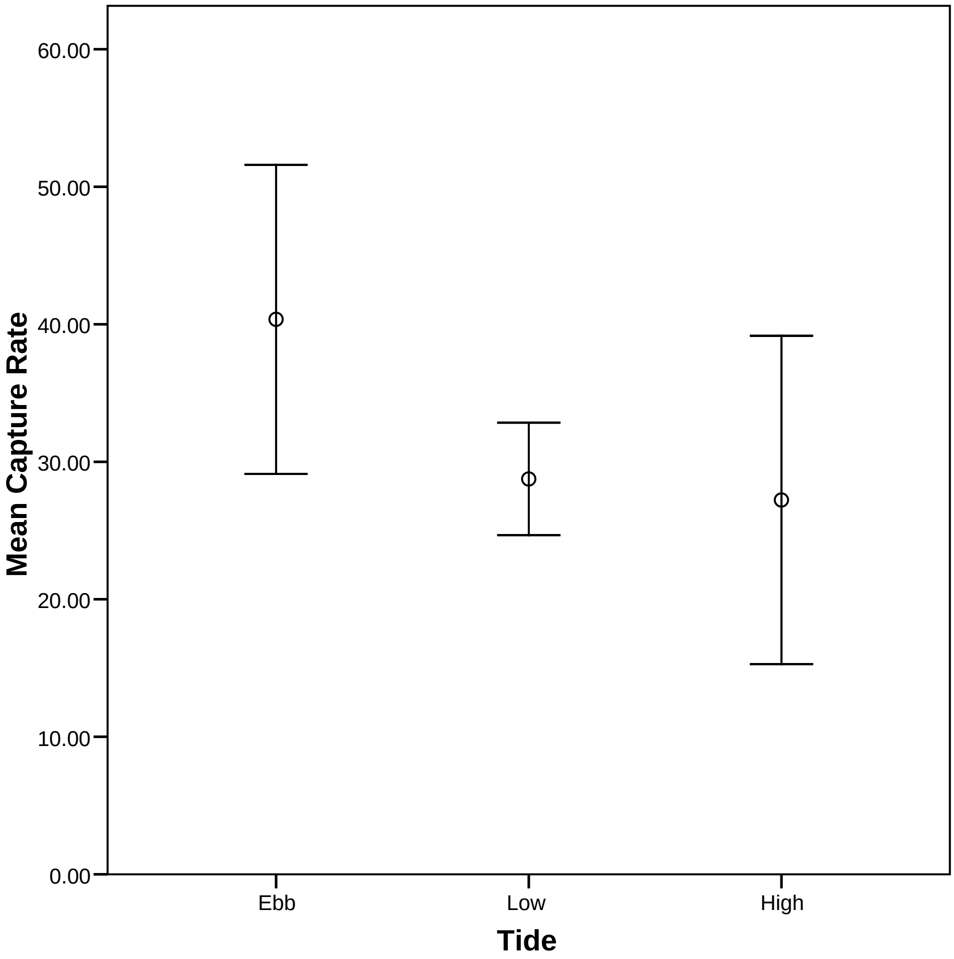


Figure 5-7 Grouped error bar plot (95% confidence interval) of univariate GLM ANOVA for the mean capture rate by adult *Semibalanus balanoides* percentage per hour and the state of the tide ($p = 0.095$, $F = 2.492$, $r^2 = 0.106$).

Ryan-Einot-Gabriel-Welsch Range

Tide	N	Subset
High	15	27.223
Low	15	28.751
Ebb	15	40.354
P value		0.115

Table 5-4 Sub-sets for the mean capture rate percentage per hour and the state of the tide.

The error bar plot for the mean capture rate of the different states of the tide (Figure 5-4) shows that this is not significant ($p = 0.095$, $F = 2.492$, $r^2 = 0.106$). Adults during all states of the tide are as likely to capture larvae. The Ryan-Einot-Gabriel-Welsch Range *post-hoc* test to find sub-sets (Table 5-4), showed that all three states of the tide were in the same sub-set confirming the findings of Figure 5-7.

5.5 Discussion

It has been suggested that a strong selective pressure operating on larval settlement is the risk of predation, including cannibalism (Tamburri et al. 2007). Cannibalism does take place in the Clyde Sea population of *Semibalanus balanoides* with approximately 44 % of the adults that were examined from the shore containing larvae. Navarette and Wieters (2000) had found that conspecific larvae (nauplii) represented 16 % of food found in the guts of adult *Semibalanus cariosus* (Pallas) on the Washington coast of North America, and thought that this was a consequence of filtering. This mid – intertidal barnacle, can interfere and reduce settlement and recruitment of conspecifics and other barnacle species by between 65 – 100 %. However, this disappeared in years of very high recruitment (Berlow & Navarrete 1997, Navarrete & Wieters 2000). It is known that chemical cues (Prendergast 2007, Tamburri et al. 2007) induce *S. balanoides* to settle within a congregation of conspecifics that feed on plankton. It has been suggested by Young and Gotelli (1988) that larval predation may be higher in the rocky intertidal and areas of high barnacle settlement; which may also have a negative effect on oyster recruitment (Osman et al. 1989, Berlow & Navarrete & Wieters 2000). This occurs with other benthic settlers such as oysters, mussels, tubeworms and sand dollars (Fox 1975, Tamburri et al. 2007).

If the adults in an single species congregation are likely to indiscriminately practice cannibalism, a barrier to this is required by the population (Fox 1975, Tamburri et al. 2007), which could be large numbers of larvae which swamp the feeding adults (Navarrete & Wieters 2000, Tamburri et al. 2007). It has also been suggested that cannibalism of larvae by adults (a form of intraspecific competition), reduces a population to its carrying capacity, but the risk of this may be to reduce genetic diversity (Tamburri et al. 2007). Copepods can avoid the cirri of *Nobia grandis* whilst actively feeding (during laboratory experiments); and if caught by the cirri, they can swim away within 40 ms due to their rapid acceleration (Singarajah 1969, Trager et al. 1994). *Calanus finmarchicus* also cannibalise their larval stages (nauplii), and possibly greater numbers of its egg stage to levels independent of the egg production; the eggs will be consumed even though there is alternate algal food supply unless there is a phytoplankton bloom reducing cannibalism (Plourde et al. 2009). In laboratory experiments, it has been found that cannibalism in the copepod *Acartia sinjiensis*, increases with predator density (Camus & Zeng 2009). When larvae of oysters get below the level of the adults ‘gape’, then they can settle in a known habitat conducive to adult life (Tamburri et al. 2007). *Perna perna* and *Mytilus galloprovincialis* mussels cannibalise up to 77 % of potential larval settlers to the adult habitat, and this was highest when settlement was the most intensive (Porri et al. 2008). However, it was suggested that the larviphagy was opportunistic, and highest during the incoming tide, but reliant on adult density and is a proportion of potential settlers in the water column (Porri et al. 2008). Mussels are broadcast spawners and due to larval dispersal it is unlikely that adults ingest their own larvae, and this helps the adults’ fitness and ultimately success of the population (Porri et al. 2008). It was found in *Limnoperna fortunei* that there was a larger density of juveniles where there were dead adults rather than live, possibly due to cannibalism and / or adults reducing food resources (Sardina et al. 2009). Although the larvae that managed to settle, benefited from more food due to more active minor currents caused by the filtering adults, and fewer predators due to the physical barrier of surrounding adults (Sardina et al. 2009). In a mesocosm constructed in a laboratory, *Cancer pagurus* and *Porcellana platycheles* were investigated in connection to their cannibalistic interactions by Amaral et al (2009). Cannibalism was only found in *C. pagurus* juveniles preying on moulting conspecifics that were vulnerable (Amaral et al. 2009). However, intersize cannibalism was common in *C. pagurus* which may be the reason why juveniles and adults are segregated in the natural environment; and why complex habitats (pebbles and *Fucus serratus*) had higher survival, than simple habitats (sandy areas) (Amaral et al. 2009). When alternative prey (*P. platycheles*) were available they were consumed, but no cannibalism was found in either intersize or intrasize cannibalism of *P. platycheles*; however cannibalistic behaviour has been found in other decapod species such as *Carcinus maenas*, *Callinectes sapidus* and *Cancer magister* (Amaral et al. 2009).

In order for male flagfish to increase either their current or future reproduction, they were found to consume some of, or their entire clutch of eggs, when the eggs were spawned by relatively large females to smaller males; and the eggs themselves were very large (with a high energetic content) thereby ensuring the fitness of the adult male (Klug 2009).

During the spring settlement period there are approximately 30 encounters every hour by an adult, of the *S. balanoides* larvae in the water column. During these encounters approximately a third of the larvae are caught and ingested per hour, but two-thirds escape possibly due to drag on the cirral fan (Trager et al. 1994), or are caught then released from an inappropriate part of the cirral net (Southward 2008). Stubbings (1975) suggests that feeding is advocated by potassium ions, L-glutamic acid and L-proline of the foodstuff, thereby enabling the barnacle to recognise food when it is pierced by setae of the cirri. This may be why some barnacles escaped being ingested, but other barnacles i.e. cannibalistic ones (44 % of the shore population examined) ate their own species.

Because *S. balanoides* is a boreo arctic species, the lower the sea temperature that the nauplii and cyprids are in, the less chance they will be caught and ingested by other species that are not reproducing at that time, due to warmer water species not being as active as *S. balanoides*, therefore they may be ‘safer’, except for cannibalism, than if released later in the year (Southward & Crisp 1956).

In the present study the earlier intermittent dates where cypris or nauplii remains were found in the guts of adults could be due to nauplii being released prematurely by damaged adults, then growing into cyprids that have come into contact with cannibalistic adults. Rough seas may also mix the layers, and transport cyprids and nauplii from surface layers to the substratum during the time that the adults are immersed.

Taller barnacles were thought to encounter more cyprids and nauplii due to their cirral nets being higher in the water column. This was not the case, and height did not alter the propensity of an adult to cannibalise larvae. It is possible that vortices created by the adults may carry larvae into the cirral net of neighbouring adults (Shimeta & Jumars 1991, Trager et al. 1994). Trager et al (1994) found from video analysis of *S. balanoides* that when the cirri actively swept, it could create a ‘feeding vortex’ which caused particles to be available on the next sweep. The swimming capabilities of the cyprids play an important part of preventing cannibalism by sensing flow disturbance, and escape by swimming away from it before the cirral fan is able to entrap it (Tamburri et al. 2007). Water movement and current velocity may affect the escape capabilities of prey. The speed of the current flow may also reduce escape action due to the prey being carried rapidly into the net and the prey being unable to mobilise an escape manoeuvre before contact (Trager et al. 1994). At high flow speeds (> 40 mm s^{-1}) it is known that adult *S. balanoides* stop beating their cirral fans and turn it into the flow in order to catch zooplankton (Southward 1955) therefore the feeding mode (active and passive) depends upon conditions of the current (Trager et al. 1994). Zooplankton may escape due to the drag of the current removing them, before the barnacle can withdraw the food. Todd et al (2006) suggests that *S. balanoides* cypris behave as passive particles in the water column when they encounter substratum, then they explore the surface before final settlement.

Although the encounter rate was significantly different for the state of the tide, and formed three sub-sets in the *post-hoc* test with low tide having the most encounters and high tide the least, there was no significant difference for the mean capture rate (adults at any state of the tide were equally likely to capture prey of cyprids and nauplii if they were in the water). The encounter rate may be due to the fact that nauplii and cyprids are unlikely to be found in a regular distribution in the plankton, and distributions can vary over a short time scale and with vertical mixing due to currents (de Wolf 1973). It has been suggested by Shimeta and Jumars (1991) that suspension feeding is a form of hydrosol filtration, with retention converting the encounter of a particle into capture, but this may decrease with an increase of the size of the particle and increased velocity of the current. Local hydrodynamic forces are also known to affect encounter rates, which can alter over small spatial scales (Larsson & Jonsson 2006); for example adults in a strong constant flow may have more opportunities to feed from passing plankton as they are brought into contact with the feeding barnacles cirri, before they are able to avoid it (Trager et al. 1994). Suspending the filming unit in one area and at a constant depth ameliorated the affect of different hydrodynamics as much as possible.

The filming could have been improved by using two video cameras to make a three dimensional recording, and using an electronic flow meter to record the flow speed as Trager et al (1994). The flow could have been controlled by using a flume; however natural cues (chemical and biological) and a wide range of zooplankton and phytoplankton may have been absent. If three or more filming cradles, each with its own flow meter were deployed at the same time in the same area, it would have given a more accurate view of the cannibalism at that precise time, water movement, temperature of the water and height of the sun. Also recording a natural group of barnacles on the substratum would have increased the reliability of the results, but may have increased flow disturbance to other barnacles in the immediate area.

5.6 Conclusions

- Cannibalism does take place in the Clyde Sea area (at Millport)
- Cannibalism was found in 44% of the adults investigated on the shore
- Frustum size of barnacle did not affect propensity to cannibalise
- Height of barnacle did not affect propensity to cannibalism
- State of the tide had a significant effect on encounter but not on capture
- Filming of adults feeding showed a mean 9 cypris and / or nauplii eaten per adult per hour

Chapter 6. Effect of rock type on settlement

6.1 Introduction

In the previous chapter it was found that *Semibalanus balanoides* is cannibalistic to larvae. In this chapter the effect of rock type on settlement for the cypris is examined, providing that they are not consumed before examining the substratum.

The settlement stage for a cypris can be considered the single most important event, as the animal's subsequent survival and reproductive success depends upon this (Kent et al. 2003). From many experiments conducted using different species of barnacles throughout the world, it has been suggested that barnacles react similarly to contact stimulation of the substratum (Crisp & Barnes 1954, Wethey 1983, Minchinton & Scheibling 1993, Bertness et al. 1996, Jeffery 2000, Pineda & Lopez 2002, Herbert & Hawkins 2006) even over time (Aguirre et al. 2008).

In Chapter 2 it was shown that the Clyde Sea has sedimentary, metamorphic and igneous rock types. Adult *Semibalanus balanoides* are found on all these rock types (see Chapter 3: Fecundity), but it was not known if the cypris had a preference for the type of rock found in the Clyde Sea or just settled on the first available rock type that they encountered, and could have been washed off by storms in later life, if the rock type was very friable, for example mudstone which may be as friable as chalk in the English Channel (Herbert & Hawkins 2006).

The restricted swimming ability of cyprids in hydrodynamic flow (Crisp 1955), and relatively small time frame for settlement, with only 3-4 weeks between the release of the nauplii and settlement of the cyprids, suggest that the larvae have only a limited degree of choice of locality for settlement at the large spatial scale e.g. tens of metres to kilometres (Hills & Thomason 1996, Hansson et al. 2003, Head et al. 2004). Settlement sites for cyprids can be dictated by the actions of the sea and wind, either stratification of the water column, currents or onshore winds, as their rate of swimming is less than the rate of motion in the water column (Moore 1975, Hawkins & Hartnoll 1982, Walters 1992, Bertness et al. 1996, Jenkins et al. 2000, Pineda & Lopez 2002). At the smaller spatial scale of less than a metre, settlement is dictated by larval behaviour which is influenced by physical, chemical and biological characteristics of substratum (Crisp 1955, Hills et al. 1998a, Navarrete & Wieters 2000, Jeffery 2002, Aguirre et al. 2008, Prendergast et al. 2008), but this specificity for certain settlement areas may alter as the cyprids age, when they are more likely to settle on any substrata which is called larval desperation (Jarrett 1997, Jarrett & Pechenik 1997, Harder et al. 2001, Tremblay et al. 2007). The settlement cues will indicate whether the site is a suitable substratum, and has conspecifics to mate with and potential prey for an adult (Thomason et al. 2000, Berntsson et al. 2004). Gregarious settlement cues where adults are present, is an indicator to a cyprid that survival is possible in this locality (Toonen & Pawlik 1994, Kent et al. 2003). It has been suggested that chemical cues from dead adults will be short lived, and that other cyprids settling may be more important to an individual cypris as they appear to prefer to settle next to other unmetamorphosed cypris (Wethey 1984b). If inappropriate cues are present the cyprid has the ability to re-enter the water column and search for a new settling site (Kent et al. 2003, Berntsson et al. 2004, Bullard et al. 2004). It is known from laboratory work that *S. balanoides* is able to delay settlement for two weeks after moulting into a cypris, however if it is unable to find a suitable settling site within this time, the cypris fail (Knight-Jones 1953).

Therefore a cypris must find an appropriate substratum with conspecifics to settle near before it uses up its reserves of energy to metamorphose. Caffey (1985) found that rock type had no effect on settlement of *Tesseropora rosea* when rocks were transposed from different sites within a large settlement area. Transplantation of basalt and granite rocks by Raimondi (1988) found that cypris of *Chthamalus anisopoma* settled in larger numbers on the granite, but on the shores where the rock was actually from, recruitment was independent of the rock type. The colour and type of rocks was found to have an effect on recruitment of spirorbid tubeworms (James & Underwood 1994), where dark grey shale was preferred to light yellow sandstone irrespective whether they were painted or not; although dark undersides of rocks were preferred to light irrespective of the surface rock colour, then shale was preferred to sandstone. However, Holmes et al. (1997) working with tiles of milled rock proposed that cyprid settlement was affected by rock type, and that this was not attributed to colour of the rocks or surface rugosity, as his experiments were conducted in the dark and on smooth surfaces with many of the surfaces indistinguishable from other rocks in the survey. Omitting texture may have caused lower settlement rates as Hills and Thomason (1998a, b) found with their experiments at Millport. Research on the south coast of England found that rock type was less important for the settlement of cypris than the profile or angle of the rock, although soft limestones, chalk and sandstones were thought to be unsuitable to barnacle long term survival due to the 'softness' of the rocks accelerating erosion (Herbert & Hawkins 2006).

Information on the orientation of barnacles has a long history, with Moore (1933) observing curvature in the pores of *Balanus improvisus*, due to realignment of the barnacle as it grew to the flow in a pipe. Crisp (1953) also found that *B. improvisus* and *Balanus amphitrite* could rotate during growth, and this was shown by examining the radial cones. This rotation was to allow the barnacle to beat its cirri into the prevailing water current, and it was found that these cypris originally orientated to the direction of the light intensity (Crisp 1953). It was suggested that the rotation after metamorphosis was due to the body twisting in relation to the shell, to the predominant feeding direction; the greater the shift in direction that the cirri had to make, the greater the observed torsion in the radial canals (Crisp 1953) However, Barnes et al. (1951) found no rotation after metamorphosis for *B. balanoides, Balanus crenatus* or *Elminius modestus*, and that their orientation did not have a significant effect on growth rate for these species; although the dominant orientation was posterior (rostrum) to the predominant current on panels, and in the intertidal. Orientation to the vertical (scuto – tergal axis pointing upwards i.e. carina at the top) was found on settlement panels at the intertidal where light was mainly from above (Barnes et al. 1951). Where illumination was from below (due to shading), the carina was at the bottom (Barnes et al. 1951). Barnes et al. (1951) found that *E. modestus* and *B. balanoides* preferentially settle in grooves and cracks, with orientation to very shallow grooves ascribed to 'thigmotrophic' response (rugotrophic behaviour). The adult may also respond to moving objects such as predators, if it is settled in the orientation where the cirri are beating away from the light; although orientation towards the light might result in settlement closer to the surface and away from deep cracks that may silt up resulting in mortality (Barnes et al. 1951, Mauck & Harkless 2001). Crisp and Barnes (1954) found that *E. modestus* and *B. balanoides* primarily orientated to the grooves, and light direction had less influence than grooves where the cypris orientated along the grooves. When pits were introduced, the cypris orientate to the light, as if they were on a plane surface (Crisp & Barnes 1954). Then on work conducted on a flat bottomed barge, virtually every *B. balanoides, B. crenatus* and *E. modestus* had its carinal end facing into the water current, but the authors suggested that especially on the rocky intertidal with changes in currents, orientation is not noticeable (Crisp & Stubbings 1957). It was suggested that water current didn't have a significant effect on cyprids at settlement or metamorphosis, but that the current influenced the metamorphosed barnacle during early growth (Crisp & Stubbings 1957).

The whale barnacle *Coronula diadema* (L.) has its carinal (or anterior) end facing the posterior of the whale on its ventral surface, so that the cirral net faces into the current (Crisp & Stubbings 1957). Work on *Balanus balanus* (a sublittoral species) on the great scallop *Pecten maximus* showed that the youngest barnacles were found on the periphery of the shells, but this declined with the increasing ages of barnacles (Barnes 1953b). Older barnacles were present at the centres of the scallop shells probably due to shell growth (Barnes 1953b). It is thought that the orientation of *B. balanus* tended to be along the lamellae of the scallops due to a rugotrophic response, rather than light due to the depth that they are found at (Barnes 1953b). Orientation was also found to be important in a tropical to sub–tropical barnacle *Balanus trigonus* in the Colombian Caribbean, with the majority of cypris settling 0^{o} – 60^{o} from the horizon, possibly due to either surge movement or light stimulus (Garcia & Moreno 1998). However, there was a greater survival of adults orientated 30^{o} – 90^{o} to the horizon, which was thought to be solely through survival, as older barnacles didn't show torsion of the radial canals; indicating what was a preferential angle for cypris, was not preferential to adult survival (Garcia & Moreno 1998). Crisp and Bourget (1985) suggested that surface grooves were the strongest stimuli for orientation in barnacles, with light the second strongest stimulus.

However, it has been found that increased UV-B radiation due to ozone depletion can cause ocular damage to nauplii and cyprids, impairing their phototactic behaviour and reducing individual cypris settlement which may be important to future barnacle settlement numbers (Chiang et al. 2003). It has been found that *Balanus (=Amphibalanus) amphitrite* cypris can locate adults in the absence of chemical cues, and can discriminate between colours preferring red with adults emitting a red fluorescence (Matsumura & Qian 2014).

Orientation is also very important in other marine epifauna, for example spirorbid tubeworms on Long Reef, Sydney. Spirorbids are negatively phototactic before settlement, and it is thought that for this reason, they prefer to settle on dark coloured substrata, orientating themselves away from the light preferentially living on under surface environments (James & Underwood 1994). In Sydney Harbour, Australia it has been found that subtidal epibiota on the vertical sides and horizontal undersides of fibreglass pontoons, were distinct from each other at all sites (Connell 1999). The effect was prominent on algae, and although the invertebrates were similar at both sites, a bryozoan *Watersipora subtorquata* was similar to the algae and dominant on the vertical sides, with other bryozoans dominating the shade (Connell 1999). Pontoons appear to act differently as habitats to panels on natural substratum, and were found to support different epibiotic assemblages; with this being more important than orientation which was vertical as opposed to the underside of horizontal, however, this also affected general assemblages (Glasby & Connell 2001). Barnacles and tubiculous polychaetes were influenced by orientation (being more numerous on horizontal undersides), with barnacles and bryozoans more abundant on suspended substrata, suggesting work on natural substrata should be conducted on it and not piers or pontoons (Glasby & Connell 2001). Seven major functional groups were investigated using the groupings: - bryozoans, molluscs, polychaetes, ascidians, soft corals, crustose coralline algae and macroalgae in the Red Sea, with different texture of settlement tiles compared with orientation (Thomason et al. 2002b). It was found that for total percentage cover of tiles, functional group richness and dominance of the fouling community; orientation (upper and lower surfaces) were significant for all the measures, with lower surfaces having the highest group richness and percentage cover, but the lowest dominance of fouling community (Thomason et al. 2002b). When the tiles of various rugosities were examined using the total percentage cover on the tiles, the dominant effect for fouling the tiles was texture, whereas for the functional group richness, orientation was the most important factor which shows the importance of orientation (Thomason et al. 2002).

This chapter sets out to ascertain if settlement is different on the rock types of the Clyde Sea with the hypothesis that rock type influences settlement of *S. balanoides.* Rocks found within the Clyde Sea were used for the rock type investigation at one experimental site, where current influences on cyprids and cyprid numbers should be similar on all the rock panels. These rock types all had had, adult settlement where they originated from which were then cleaned off. The rocks were cut into panels as many field experiments use panels (tiles), in order to make the rocks as comparable as possible.

6.2 Aims of chapter

Settlement appeared to be on all major rock types of the Clyde Sea, but was this really the case; and do cypris have a preference of one rock type over others for settlement in the Clyde Sea? The hypotheses were that settlement occurred on all major rock types of the Clyde Sea, and that given an option they would have a preference for one type.

There is much in the literature about orientation and settlement (especially vertical / horizontal), but did orientation of settlement panels north / south affect settlement? The hypothesis was that orientation would affect the settlement of cypris in the Clyde Sea.

6.3 Method and materials

Figure 6-1: Fairlie Commercial Pier where the effect of rock type on settlement experiment was conducted.

This study was conducted at the end of Fairlie Commercial Pier, Scotland (NS2050 5620), (Figure 6.1) during the *Semibalanus balanoides* settlement season between 12/4/2005-16/4/2005.

Rocks collected from sites around the Clyde Sea area (Figures 6-2 to 6-7), were cut using a Stihl Saw® into 12 cm x 12 cm squares, 2 cm thick with both original large faces each measuring 100 cm^2 (1 cm at each edge was ignored as Dexion® or cable ties may cross this area) (Figure 6-2). The rocks selected were igneous (Figure 6-3) from Lendalfoot (the same extrusion as Cloch Point - extrusive, basic lava, igneous), red sandstone (Figure 6-7) from Culzean Bay (Devonian sandstone, sedimentary), sandstone (Figure 6-6) from Inverkip (Devonian sandstone, sedimentary), metamorphic (Figure 6-4) from Strachur within Loch Fyne (Argyll group, dalradian, Easdale subgroup), and metamorphic (Figure 6-5) from Carradale (Southern Highland group, dalradian). See Chapter 2 for geology maps and sites. All sites had settlement on their respective rock types and were felt to be accurate major representations of the rocks found within the study area of the Clyde Sea.

Figure 6-2: Frame 1 front showing igneous, metamorphic from Carradale, red sandstone, Loch Fyne metamorphic and yellow sandstone.

Figure 6-3: Igneous rock.

Igneous rock is formed by the solidification of molten magma and the rock formed at these two sites is an extrusive (volcanic), basic lava (lithological ornament) of the

Permo-carboniferous (Carboniferous period 365 – 290 M year to the Permian period 290 – 245 M year); which is part of the Palaeozoic era (Brown et al. 1992, Timmerman 2004). This cooled quickly forming a smooth crystalline and fine grained rock. Its texture is based on the size and shape of the mineral crystals that form it known as aphanitic (crystals too small to be seen with the naked eye) (Brown et al. 1992, Allaby & Allaby 1999). Most magmas contain oxygen (~ 46.6 % by weight in Earth's crust), silicon (~ 27.7 wt. %), aluminium (~ 8.1 wt. %), iron (~ 5 wt. %), calcium (~ 3.6 wt. %), sodium (~ 2.8 wt. %), potassium (2.6 wt. %), magnesium (~2.1 wt. %); with manganese and titanium in smaller concentrations (Brown et al. 1992). Oxygen and silicon usually combine to make silicate minerals accounting for 90 % of igneous rock types, but the chemistry depending on whether it is a major, minor and trace element is expressed differently (Brown et al. 1992). Major and minor elements are declared as weight percent oxides e.g. 1.50 wt. % TiO_2 titanium oxide or 51 wt. % SiO_2 silicon oxide, trace elements are expressed as parts per million by weight for example 420 ppm Ni nickel (Brown et al. 1992).

The igneous rock used in all of the experiments (Figure 6-3) was basalt due to its fine graining and dark coloured silicates (ferromagnesian) with rocks high in these, about 50 wt. % silica and also have iron, magnesium and pyroxene in them (Brown et al. 1992, Sobolev et al. 2007). Basalt is mafic, which means it has a low amount of silicon (45 – 52 wt. % SiO_2) with < 20 wt. % quartz, < 10 wt. % feldspathoid by volume and ≥ 65 wt. % of the feldspar is plagioclase (series of tectosilicate minerals); the lava has > 4 wt. % of magnesium oxide (MgO), therefore making it basic or alkaline (Timmerman 2004). It is dark grey in colour but can weather to a brown / reddish colour due to the oxidation of its iron rich minerals (Sobolev et al. 2007).

It has been suggested that basaltic rocks, due to the iron (FE (II)) and manganese (Mn(II)) may provide an energy source for bacteria, which have been cultured from these weathered rocks (Edwards et al. 2003). This may encourage biofilms that might encourage the settlement of cyprids. It is known that seawater, water and air will oxidise the iron rich minerals releasing calcium, sodium, and magnesium which may help the seawater in direct contact with the rock to buffer against acidification (Templeton et al. 2005) possibly promoting cypris settlement.

Figure 6-4: Loch Fyne Metamorphic (Dalradian, Argyll group, Easdale subgroup).

Figure 6-5: Metamorphic from Carradale (Dalradian, Southern Highland group).

Both metamorphic rock groups are late Precambrian (early Palaeozoic era ~ 570 – 504 M year) (Plant et al. 1984), although the geology map defines the age as Cambrian (Plant et al. 1984, McAdam et al. 1985). Metamorphic rocks are formed by high temperatures, and pressure from the rocks above, inside the Earth's crust; for example tectonic collisions with horizontal pressure and friction causing heat, or by intrusion of magma (Brown et al. 1992). Metamorphic rocks may have pyroxenes, feldspars, quartz, micas and olivines that are from crystallization of igneous rock, and are stable at high pressures and temperatures therefore remain chemically unchanged (Brown et al. 1992). Metamorphic minerals formed by the process include kyanite, andalusite, staurolite and some garnets (Brown et al. 1992). Where stripes are seen in the rock, the layering is called foliation (and can show the colours of the constituent minerals), for example slate is very fine grained, phyllite medium grained, schist coarse grained and gneiss very coarse grained which allows description of the rock (Brown et al. 1992). The chemical composition of metamorphic rock can be different from its original rock due to metasomation where water may transport chemicals away and to the process (Brown et al. 1992). The texture of the rocks even where foliated are generally smooth to the touch like the igneous, this is possibly due to weathering of their actual faces and due to all the rock types used having natural faces not just mechanically cut. Due to the chemicals in the metamorphic rocks used, they had a slight green / grey tinge to the grey rock in sunlight.

The Argyll group (Figure 6-4) has psammites (from metamorphosed sandstones), pelites (from metamorphosed fine grained sedimentary rock e.g. mudstone), black pelites and some limestones embedded within it; whilst the Southern Highland group (Figure 6-5) is dominated by turbidites (Plant et al. 1984, Anonymous 2014). There is some mineralization in the Easdale subgroup where barium, zinc and lead have passed into Ni-pyrrhotite, possibly due to hydrothermal fluids (Plant et al. 1984). Both groups consist of aluminium oxide (Al_2O_3), boron (B), copper (Cu), iron (Fe), lithium (Li) and zinc (Z) in large amounts (Plant et al. 1984). The Argyll group is ultrabasic to basic in its rock chemistry with nickel, chromium, cobalt, vanadium and magnesium oxide (MgO) in large amounts, whereas the Southern Highland group shows a decrease in beryllium but increased lithium and boron due to the marine clays that have formed it

(Plant et al. 1984). The Dalradian rock group includes the elements with the mean continental crustal values (Clark) of about: - boron 4.5, lead 3.25, nickel 2.75, manganese 2.5, lithium 2.5, zinc 2.1, barium 1.75, rubidium 1.5, uranium 1.5, cobalt 1.25, yttrium 1.25, potassium 1.1, iron 1.1, chromium 1.0, beryllium 0.75, magnesium 0.6, copper 0.5 and strontium 0.5 (taken from Figure 4 of Plant et al. 1984).

Figure 6-6: Yellow sandstone.

Both sandstones are sedimentary rocks probably formed during the Upper Palaeozoic era (~ 570 – 245 M year), comprising of the Devonian (413 – 365 M year), the Carboniferous (365 – 290 M year) and the Permian (290 – 245 M year) (Brown et al. 1992, Boggs 2000). Sedimentary rocks (of which sandstone is one type) are formed by the deposition of particulate matter for example the weathered remains of igneous and metamorphic rocks, which accumulate and are then compacted due to the pressure of overlying deposits. Sandstone is an arenite which implies it has no or a small amount of matrix (< 15 % clay), which is a fine material of silica and / or calcium carbonate that lies between the grains (Pettijohn et al. 1987). The grains in sandstones are 0.0625 mm to 2 mm diameter fragments, primarily of quartz with feldspar grains being the second most abundant minerals that have become rounded over time (Pettijohn et al. 1987). The feldspar grains can be alkaline ranging from $KAlSi_3O_8$ to $NaAlSi_3O_8$, and plagioclase feldspar with the chemical composition $NaAlSi_3O_8$ to $CaAl_2Si_2O_8$ which must be deduced using a photographic microscope (Boggs 2000). The yellow or tan colour (Figure 6-6) is due to the mixture of clear quartz and orange feldspar (Pettijohn et al. 1987).

It is possible that the sandstone or yellow sandstone taken from Inverkip is Lower Old Red Sandstone rock, as there are four major basins of this in the Clyde Sea area :- Hunterston formation, Ardmore Point, Wemyss Bay and Galston (the outcrop known as Wemyss Bay may include Inverkip) (Bluck 1980).

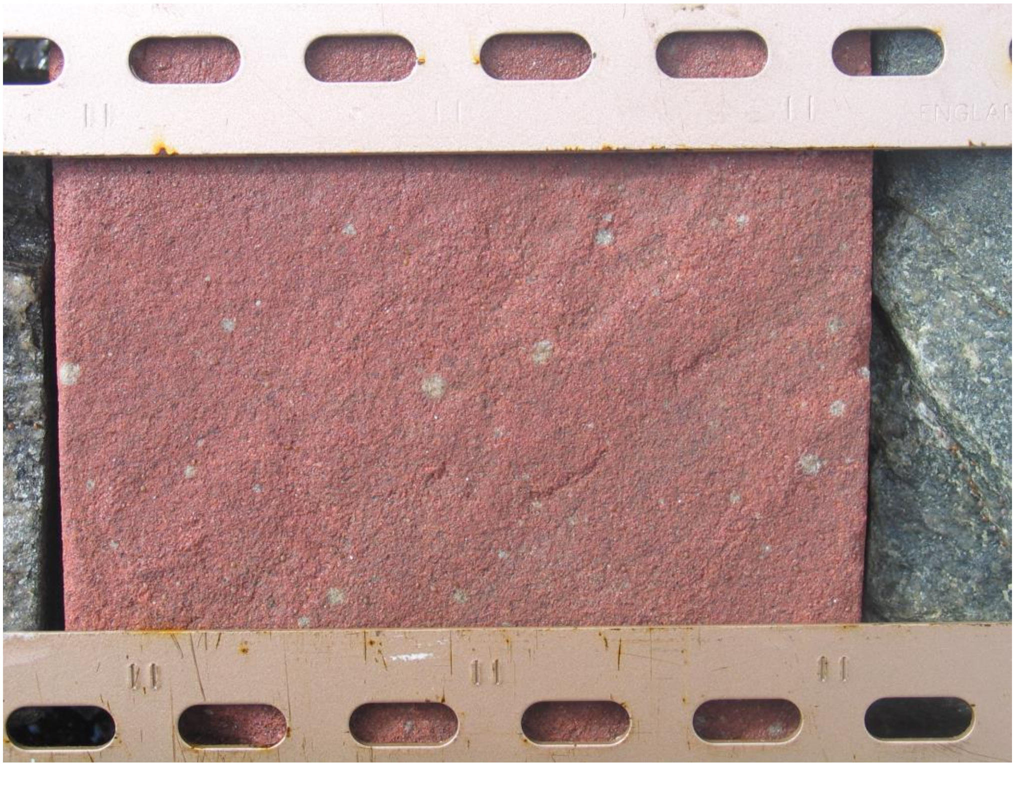


Figure 6-7: Red sandstone.

The red sandstone contains iron oxide and is due to erosion of the Caledonian mountains and is associated with the volcanic rocks due to sub – lithic arenites which also indicate the Dalradian source, forming a calc – alkaline rock and are rich in nickel and chromium (Bluck 1980, Thirlwall 1981). It is constructed from alluvial fans due to movement on the Highland Boundary fault which formed blocks of sandstones due to major fractures and is a response to subduction on the margin of the Lapetus Ocean (Bluck 1980, Thirlwall 1981). Thirlwall (1981) in Table 4 gives the following mean compositions of chemicals to be found in the red sandstone in the South Midland Valley (incorporating Culzean and Inverkip) as: - SiO_2 54.66 wt. %, Al_2O_3 16.86 wt. %, Fe_2O_3T 8.10 wt. %, CaO 8.36 wt. %, MgO 5.09 wt. %, Na_2O 3.72 wt. %, K_2O 1.43 wt. %, TiO_2 1.28 wt. %, P_2O_5 0.27 wt. % and MnO 0.10 wt. % for the oxides; with Sr 515 ppm, Ba 437 ppm, Cr 302 ppm, Zr 197 ppm, V 175 ppm, Ni 143 ppm, Zn 133 ppm, Ce 49 ppm, Cu 34 ppm, Rb 34 ppm, Sc 26 ppm, Nd 25 ppm, Y 25 ppm, La 22 ppm, Nb 9 ppm and Th 7 ppm for the trace elements.

It was impossible to measure the rugosity of the rock types (for example with a machine similar to a Taylor–Hobson Form Talysurf 120 L) (Herbert & Hawkins 2006), due to external naturally weathered faces of the rocks used in the experiments with some deep grooves and chips (> 5mm) formed on them. Both sandstones marked easily forming a scratch that was easily visible but ~ 0.2 – 0.3 mm depth, when a nail was drawn over them with minimal force; however the metamorphic and igneous rocks just formed scratches that were 0.1 mm at most in depth.

The rock panels were scrubbed in 10% sodium hypochlorite solution to remove any residual settlement cues from adult barnacles, and adults were removed (Prendergast 2007). The rocks were then rinsed in tap water for 10 minutes to remove residues of sodium hypochlorite, and then rinsed with filtered seawater to remove any resides left by the tap water. Each panel type was placed in one of three frames in a random configuration. There were four replicates of each of the five rock types for the artificial panels, and three replicates of the five rock types of natural rock panels. The frames were constructed out of Dexion®, with the panels held in place using cable ties. The rocks were orientated so that one face of the panel was always to the south, the other faced north in order to find out if orientation had an effect on settlement. The frames were then lowered off the pier at daylight during low tide, to a height 2.5 m above chart datum (half way up the intertidal). The frames were then left for both a night and day high tide (approximately 26 hours) and a photograph was taken using Canon PowerShot

G3 (4 x 10^6 pixel) of attached cypris the following day at low tide, this continued for four days. Counts were made using photographs of each rock face and using the cell counter in ImageJ (Rasband 1997-2008), recording orientation of rock face.

6.3.1 Data analysis

Raw data was found to have a non – normal distribution and was therefore subject to Box-Cox procedure to give Log (X + 1) transformed data in Minitab 12 to achieve normality. The data was then copied into SPSS 15, where a Repeated Measures GLM ANOVA and the Greenhouse-Geisser test were conducted. The within subject factor was day, and the between subject factors were rock type and orientation, with the response as both together and separate. The variable was day, and the model was fully factorial with a *Post hoc* test (Ryan-Einot-Gabriel-Welsch Range) on rock type.

6.4 Results

6.4.1 Effect of rock type on settlement

Repeated Measures GLM ANOVA tests of within-subjects effects (Figure 6-8), found that day showed a significant ($p<0.001$) effect using Greenhouse-Geisser for settlement on the different rock types. However, the interaction between day and rock type ($p=0.257$), and the interaction of day with rock type and orientation ($p=0.278$) showed no difference. Therefore settlement was dependent on the day, but not dependent on the rock type or orientation.

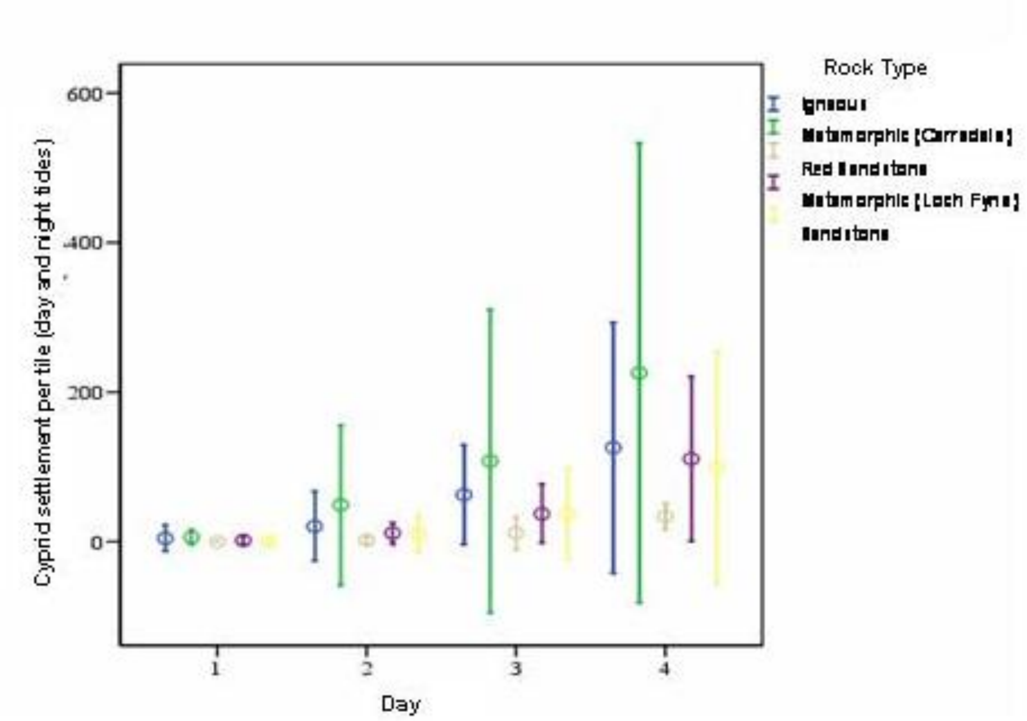


Figure 6-8: Mean cumulative cyprid settlement per tile over a day and night high tide (classed as a day) over the four days (95 % confidence interval shown) using six replicates.

Tests for between-subjects effects, looking at the interaction of different rock types for settlement, found that rock type was significant (p=0.002, F=6.238). The *post-hoc* tests for homogeneous subsets were conducted using Ryan-Einot-Gabriel-Welsch Range found two distinct subsets (see Table 6-1). Red sandstone was significantly different from Loch Fyne metamorphic, igneous and Carradale metamorphic (which had high settlement); and metamorphic was significantly different from red sandstone and sandstone which had the lowest settlements. The two sandstone rock types are grouped together, with the igneous and metamorphic rock types in the other group.

Rock Type	N	Subset		
		1	2	3
Red sandstone	6	1.579		
Sandstone	6	2.415	2.415	
Loch Fyne metamorphic	6		2.788	2.788
Igneous	6		3.086	3.086
Metamorphic	6			3.547
P value		.145	.410	.302

Table 6-1: Ryan-Einot-Gabriel-Welsch Range.

Means for groups in homogeneous subsets are displayed.

Figure 6-8 day one shows little settlement on all rock types with little variance. Day four (cumulative settlement) shows that metamorphic rock had the highest number of settlers but also the highest deviation (225 ± 153.61), followed by igneous rock (125.67 ± 83.84) then Loch Fyne metamorphic rock (110 ± 54.96). Red sandstone had the lowest settlement (33.83 ± 8.95) followed by sandstone (99.33 ± 77.69). Although the sandstone had a large range of cumulative settlement intensification, the red sandstone had consistently low settlement.

6.5 Discussion

The experiments conducted aimed to determine whether rock type influenced settlement of *S. balanoides* cypris. Rock type had an effect on settlement intensification (settlement of cypris, encouraging more cypris to settle in the immediate area – greater the settlement the greater the intensification) but orientation did not, which is important for cyprids finding a place to settle as they are equally likely to settle on north or south facing rock surfaces, rather than taking the risk in only finding one orientation of settlement site. However, cypris were found to have settled on the three major rock types of the Clyde (igneous, sedimentary and metamorphic) this may be due to where the adults are releasing nauplii and the cypris finding a settlement site the same as the releasing parent, due to tides, the desperate larvae hypothesis or another reason that humans have not discovered. Settlement varied significantly for day, using the within subjects effect. This has been found in other studies such as Hawkins and Hartnoll (1982), Jenkins et al. (2000), and Caffey (1985) (who found differences with monthly sampling); all these studies found settlement variation, which could be due to larval supply, this has been linked to wind patterns and hydrodynamics in some instances (Hawkins & Hartnoll 1982, Bertness et al. 1996, Pineda & Lopez 2002).

The tests of between subjects effects showed significance for settlement on rock type which agrees with Holmes et al. (1997) and Raimondi (1988), but disagrees with Caffeys (1985) investigation of *Tesseropora rosea* in Australia, who did not find an effect on settlement densities, when four types of rocks were transplanted on along the coast. Jenkins et al. (2000) working at a European scale on the settlement of *S. balanoides* also found that rock type had no effect.

The five rock types were divided into two groups using the Ryan-Einot-Gabriel-Welsch Range *post-hoc* test. One group was the sedimentary rocks of red sandstone and sandstone, the other group contained the igneous rock and the two types of metamorphic rock (from Loch Fyne and Carradale). The red sandstone had the least combined (north and south) settlement (raw data), with the sandstone the next combined lowest (203 and 596 settlers respectively) after four days. The variance around the mean settlement in Figure 6-8, show that the red sandstone had consistently low settlement on the three settlement frames for both sides. However the sandstone settlement did vary more on days 3 and 4. These findings are in agreement with Holmes et al. (1997), who found that millstone grit sandstone was the least attractive to cypris settlement. The rocks used by Holmes et al. (1997) were milled and had therefore lost rugosity, which Hills and Thomason (1998a, b) found important to cypris settlement. Herbert and Hawkins (2006) thought that sandstones, limestone’s and chalk would be unsuitable settlement substrates for cyprids due to their susceptibility to erosion, and had found that although rocks with greater roughness had highest recruitment, mortality was also the highest on these tiles. However Holmes et al. (1997) had found granitic gneiss (high grade metamorphic rock with the same minerals as granite but subjected to more heat and pressure and has distinctive banding) a less attractive settlement substratum. This is contrary to the findings of this experiment, as igneous rock was the second most densely settled rock type and metamorphic from Carradale was the highest settled rock type. Settlement of igneous and metamorphic rock types had the highest variance around the mean daily settlement, suggesting that cyprids settled differentially on the same rock type.

Berntsson et al. (2004) suggest that if cyprids reject an unsuitable substratum, this will increase the number of larvae that may settle on an adjacent substratum; if this is the case, cyprids that may have settled on sedimentary rock if no other type of substrata was available, may have swum or crawled to an adjacent rock type in the frame. Surface topography is known to influence settlement (Berntsson et al. 2004), and the water flow over the rocks will vary on small spatial scales. Any pit or depression on the rock may offer protection from the local flow or cause turbulence which could cause problem to the cirral fans of young barnacles after metamorphosis, for example *Balanus improvisus* cypris avoid settling where flow speed are higher than 5-10cm/s (Larsson & Jonsson 2006).

There was no significant difference on settlement rates for the north or south of the settlement rocks in the suspended frames. This suggests that the currents in the Fairlie channel were not affecting settlement over the space of a day (two high tides), and that cypris were amenable to different orientations of the rock type they chose to settle on.

From these results ~47% of cypris that settled on the rock panels, settled on metamorphic rock, whilst ~35% settled on igneous rock and ~18% on sedimentary rocks. These results are contrary to the rock type in the Clyde Sea system that is available for settlement where ~31% (~958 Ha) is metamorphic, ~26% (~792 Ha) is igneous, and ~43% (~1300 Ha) sedimentary (see Chapter 2). Therefore the most abundant rock type (sedimentary) has the lowest settlement when the cypris have a choice at relatively small distances. However, if you group the preferences as in Table 6-1 then ~82% inclines towards the metamorphic and igneous rock types, of which ~57% of the rock type available for settlement in the Clyde Sea intertidal is metamorphic and igneous.

A specific rock type may be preferred by settling cypris due to its chemistry. This was not examined in this thesis, but could be suggested by removing the rugosity and topography of the different rocks used as Holmes et al. (1997). Also cyprids are known to prefer artificial settlement panels with a specific rugosity, and they may prefer to attach to rock types with this surface contour (Hills & Thomason 1998a, b). Cypris may be able to distinguish the chemistry of the different rock types and alter their adhesive accordingly (cf. Aldred and Clare 2008, Phang et al. 2008, Aldred et al. 2013a). This may be examined if different rock types were placed in frames in the sea, then remove the frame after enough cypris were available to have their cypris cement investigated (cf. Phang et al. 2006, Aldred and Clare 2008), barnacle adults could be allowed to grow on some frames of different rock types then harvested to find out the adult permanent adhesive (Aldred and Clare 2008).

Settlement for cyprids does not just depend on rock type preference. At smaller spatial scales of less than a metre, larval behaviour can be influenced by chemical and biological cues, with the effect of area for settlement a possible consequence as to whether an individual cyprid may attach and metamorphose. This is investigated in the next chapter 'Effect of available substratum area on settlement intensification'.

6.6 Conclusions

- Settlement occurred on all major rock types of the Clyde tested
- Cypris preferentially settled on metamorphic or igneous rock types
- Red sandstone and sandstone were least preferred by cypris
- Orientation of vertical panels (north or south) did not affect settlement

Chapter 7. Effect of available substratum area on settlement intensification

7.1 Introduction

In the previous chapter (Effect of rock type on settlement), it was found that cyprids preferentially settled on metamorphic or igneous rock types which could be grouped together for the purpose of settlement. The orientation of natural rock types did not have an effect on settlement. In this chapter natural substratum and artificial panel settlement are investigated to discern if the settlement of cypris is the same or different and the effect of the available space is examined.

It has been proposed that barnacle settlement rates increase with decreasing space of suitable substrata for settlement, and it has been suggested that this can cause an intensification effect (Bertness et al. 1992, Pineda 1994b, Pineda & Caswell 1997, Jeffery 2000). However, other studies have shown the opposite indicating that settlement rates increase with enlargement of space availability (Hills & Thomason 1996, Olivier et al. 2000, Petraitis et al. 2003), possibly due to the edge effect of a small settling area which can have consequences for settlement and later competition (Remer & Heard 1998), and larger areas producing stronger gregarious effects. There are also discrepancies in the literature about settlement intensification, regarding artificial substrata such as settlement panels (Pineda & Caswell 1997, Hills & Thomason 1998b) or natural rock (Minchinton & Scheibling 1993, Holmes et al. 1997). However, some investigations have used both settlement panels and natural substrata within the same experiment with the same area (Pineda et al. 2002); finding in one instance higher settlement on artificial plates than actual rock, but at another location artificial verses rock was an unimportant matter for settlement. Others have found that settlement densities were higher on the rock substrata than on settlement panels (Todd et al. 2006). Prendergast et al. (2008) suggest that a roughness (texture) cue of a panel could override even chemical cues from conspecifics in *Semibalanus balanoides.*

Settlement is known to be affected by the area available from previous work by Hills and Thomason (1998a). On artificial panels it was found that cypris were more likely to settle on panels that had a roughness component greater than 0.5 mm to about 1 – 2 mm, than on panels with a roughness outside this range, proposing that cypris settlement in *S. balanoides* was related to a cypris body scale (Hills & Thomason 1998a). Therefore roughness of panels was taken into consideration when using artificial panels in these experiments.

Settlement in areas already colonised by adults and other cypris landing there, is an indication for a cypris that an area is conducive to life as a benthic organism (Bullard et al. 2004). Free space availability and the density of settlers have been suggested as determinants of settlement on the substratum (Minchinton & Scheibling 1993). When there is a high density of settlers this appeared to be a function of available free space. However, when there were low densities of settlers, but an unlimited amount of settlement space, there was no relationship for settlement density and available space. Also areas with more unsuitable settlement sites have been shown to increase the per area settlement on those areas that were suitable for settlement (Pineda & Caswell 1997). High numbers of cypris including non-discriminating pioneer settlers may encourage other gregarious settlers onto a new settlement area (Knight-Jones 1953, Jeffery 2002). Field experiments have shown that settlement intensified after 20 tides on wood, concrete, rusty metal and sandstone; which may have been due to pioneer settlers encouraging gregariousness or due to a build-up of microbial biofilms (Hills & Thomason 1996).

This chapter sets out to ascertain if the area available for settlement influences settlement intensification, as suggested by Pineda and Caswell (1997) under laboratory conditions. Pineda and Caswell (1997) suggest that smaller areas of panel in a flume increase settlement by more cyprids settling (cm^{-2}) on smaller areas than larger areas, therefore it was to be seen if these theories are applicable in the natural environment, by using artificial settlement panels in the natural environment. It was suggested by Prendergast et al. (2008) that laboratory conditions excludes information that is found in the natural environment, therefore these experiments were conducted in the Clyde Sea area. Per unit area settlement has been used by authors of previous experiments to show a relationship between the amount of free space and recruitment of cyprids (Jeffery 2000). Quadrats photographed for Chapter 3, show various free areas of rock between colonies of adult barnacles, and between fucoid cover and barnacle settlement. These images can be used to approximate a total area, or percentage of area available for settlement on various rock types and exposures.

In this study, settlement intensification experiments were first conducted on artificial panels then on natural rock. These experiments were conducted at two different sites and in different years. The artificial panel experiment was conducted first at Fairlie Sands (O.S. reference NS2080 5620) in April 2005 in order to make it similar to Pineda and Caswells (1997) laboratory experiment, by using settlement panels that are in a flow without settlement areas close by for the cypris to settle. As poor settlement was recorded at Fairlie in 2005 for the artificial panels, the experiment was rerun on natural rock, on a rock outcrop during April 2007 at Millport, Isle of Cumbrae (O.S. reference NS1755 5435), where barnacles were present and settlement was known to be high.

7.2 Aims of chapter

There is information in the literature that certain sizes of panels encouraged settlement, some of this information was from flume trials, and others were from information gained in the natural environment. Those experiments in the natural environment to my knowledge did not try different areas, in orders of magnitude. Different areas on artificial panels and the natural environment were investigated, with the hypothesis that certain areas would produce an optimum settlement per area.

From photographs of quadrats taken around the Clyde Sea for Chapter 3, it appeared that some areas (cm^2) were more available than others for settlement on substratum in the natural environment. The hypothesis was that there would be an amount of area more available than others for settlement.

7.3 Methods and materials

Keough (1984) found that in the natural environment (on natural rather than man-made substratum such as breakwaters and piers), the areas available for settlement spanned two or more orders of magnitude. Therefore in these experiments several orders of magnitude were built into the experimental design for both artificial settlement panels and cleared areas on a rock face.

7.3.1 Settlement on artificial panels

The study site was an area of sand, north of Fairlie Commercial Pier, approximately 230 m long (Figure 7-1). The site was chosen because it was naturally unsuitable for settlement by cyprids due to the loose sand, and any small rocks away from the deployment area were covered by fucoids. Therefore there should not be any settlement cues.

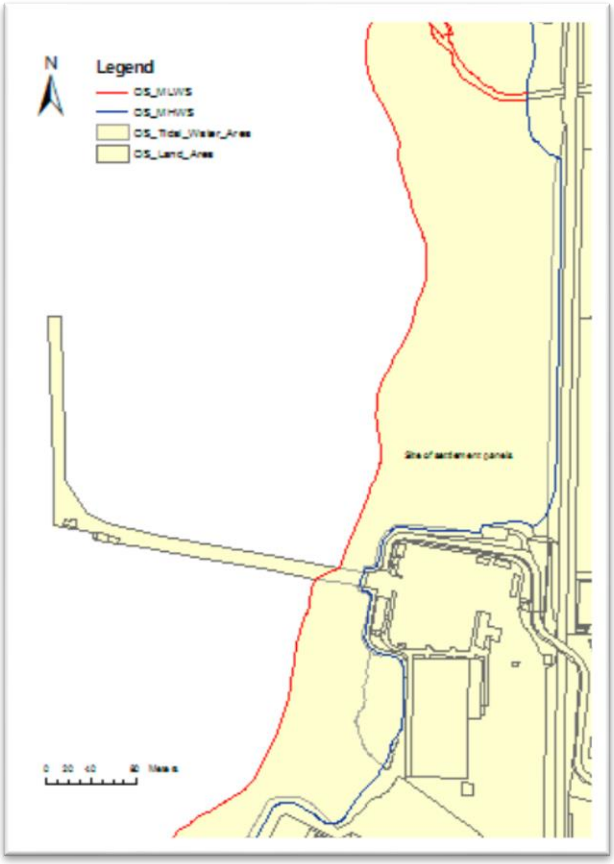


Figure 7-1: Fairlie Sands (OS ref. NS2080 5620), Clyde Sea, showing site of the settlement panels experiment.

The artificial panels that acted as areas for settlement were designed using 1 cm^2, 10 cm^2 and 100 cm^2 grey PVC (Bay Plastics) panels all 2 mm thick to incorporate two orders of magnitude that might be found on natural substratum. The orders of magnitude were used to accommodate the most diverse range of areas possible rather than just a variety of small areas (<10 cm^2). Panels were cut from one sheet of plastic using a mitre saw, which had been sanded using an orbital sander with grit 120, over an area 10 cm x 120 cm for 6 minutes to give a rough texture suitable for settlement see Hills and Thomason (1998a). All panels were free from any biological settlement cues as they were scrubbed using 10 % sodium hypochlorite solution, rinsed in tap water for 10 minutes, then rinsed with filtered seawater to remove residues of tap water (Prendergast 2007). Panels were affixed to 1 mm thick nylon cord 1 m long, by a 2 mm cut cable tie stuck on the reverse of each panel using epoxy resin (Araldite™). The cords were tightly attached to 30 cm long tent pegs with 20 cm above the sand to prevent coverage of the panels by sand via wave action during immersion. The tent pegs were secured to beakers by drilling a hole (8 mm) through the bottom of the beaker that fitted the tent peg, which was then glued securely with the epoxy resin. These were then placed in the sand preventing movement from the deployment area (Figure 7-2).

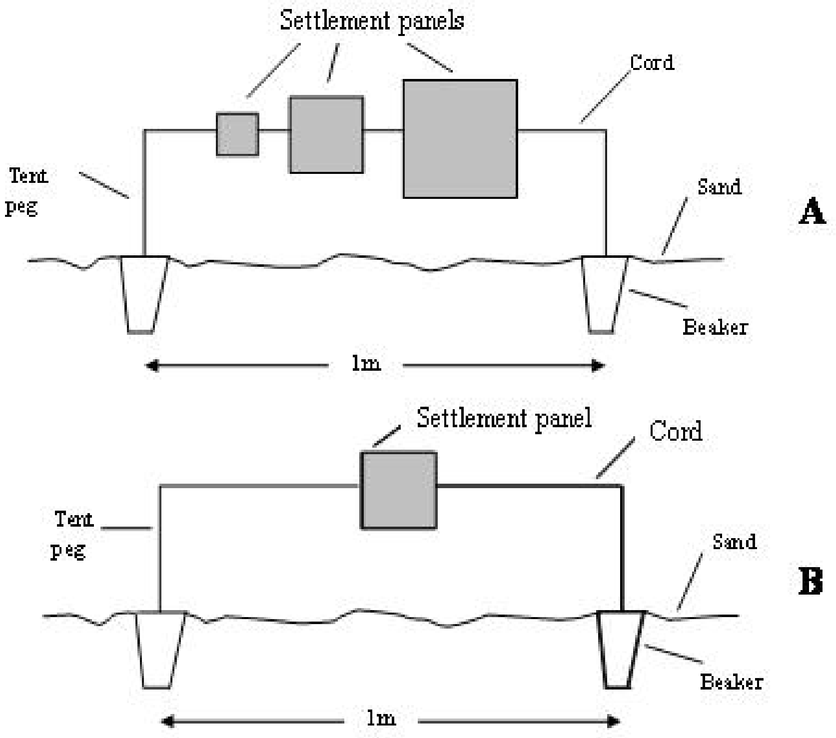


Figure 7-2: Schematic example of arrangement of settling panels for combined A = area settlement and for B = a single area of settlement.

Three replicates of 1 cm^2, 10 cm^2 and 100 cm^2 panels (Figure 7-2 B) were deployed to reproduce separate areas. Three combined areas (Figure 7-2 A) were also deployed which included one of each panel size (a 1 cm^2, 10 cm^2 and 100 cm^2 randomly arranged to give a combined area of 111 cm^2) on 1 m long cord as above. The panels (or settlement areas) were deployed randomly within the study site with 1 m spacing between these artificial areas, at low tide on 13th April 2005. Each area was considered a distinct area for settlement. Settlement was counted by eye for each panel at low tide after two high tides (night and day), tides 2 (14/4/05), 4 (15/4/05) and 6 (16/4/05).

7.3.2 Settlement on natural (rock) substratum

This study site was at a vertical outcrop of intrusive fine grained basic igneous rock (chosen because it was established to be a preferred rock type to settle on in Chapter 6). Located at the University Marine Biological Station, Millport, Isle of Cumbrae, KA28 0EG, Scotland (O.S. reference NS1755 5435). Five replicates of 1 cm^2, 5 cm^2, 10 cm^2, 50 cm^2, 100 cm^2, 500 cm^2, and combined areas that included all these areas to make

666 cm^2 were randomly marked on the rock face on 10/4/2007 (see Figure 7-3). These areas were both the same order of magnitude as settlement on artificial panels, and using areas from 5 cm^2 to a combined area of 666 cm^2 gave a large range of areas.

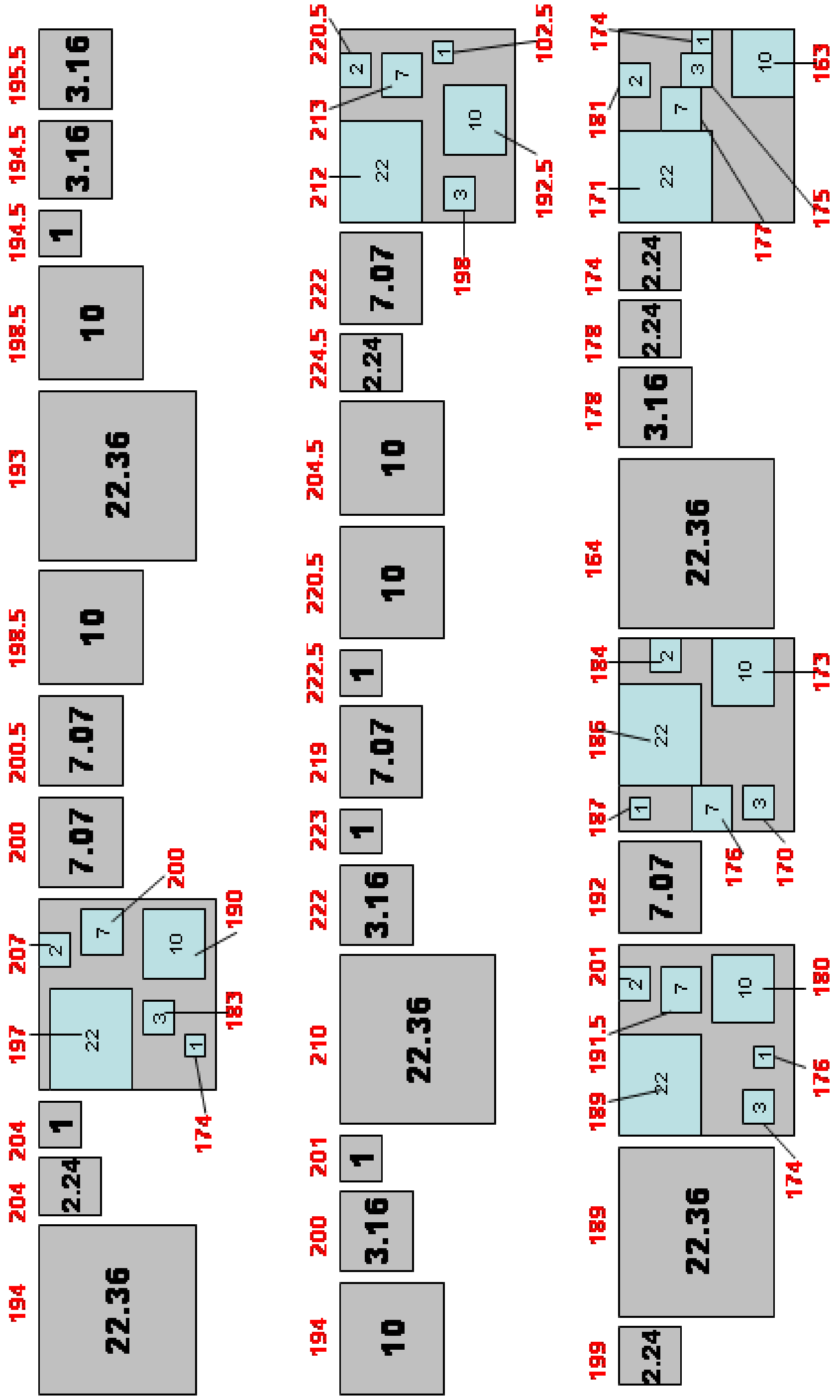


Figure 7-3: Natural rock settlement arrangement to be read as one line (see following paragraph for explanation).

Settlement areas are shown in grey with the length of an edge of each square (in cm) shown inside. Therefore 1 = 1 cm^2, 2.24 (shown as 2 due to lack of space in combined areas) = 5 cm^2, 3.16 (shown as 3 due to lack of space in combined areas) = 10 cm^2, 7.07 (shown as 7 due to lack of space in combined areas) = 50 cm^2, 10 = 100 cm^2 and 22.36 (shown as 22 due to lack of space in combined areas) = 500 cm^2. Combined areas are in grey with arrangement of smaller constituent areas shown in blue = 666 cm^2. Red numbers above or below the areas indicate the height (in cm) above low water on 14/4/07 at 15:53 which was 20 cm above Chart datum (Admiralty's datum shown on Admiralty Charts). At Millport the chart datum is -1.62 m below Ordnance Datum which is shown as Mean Low Water Springs on Ordnance survey maps (see Chapter 2).

The combined areas were within a 50 cm x 50 cm area (areas shown with smaller squares inside) with only 1 – 2 cm between the individual areas within this large area to give both one larger area, and an area that was split up into close small areas as often found in the natural environment. The other individual areas had at least 10 cm between the areas. Each area was scraped clear of adult barnacles, limpets and fucoids using a paint scraper then a wire brush to produce bare rock on 10/4/07 to 1 cm or larger in each direction than the required area. The cleared rock face was then rinsed with fresh water and then filtered seawater (filtered through 200 µm plankton net) to remove any settlement cues left by adult barnacles. The areas were marked onto the rock using Airfix® enamel paint with a colour code to facilitate recording.

The settlement areas were located in the mid intertidal in order that cypris could have a similar residence time near the rock for encountering the substratum and for settlement (Pineda & Caswell 1997). Settlement was recorded using digital photographs taken using a Canon PowerShot G3 (4 x 10^6 pixel), at every low daytime tide following a night time and day time high tide between 11/4/07 – 20/4/07. No data was recorded for 16/4/07 due to technical problems. Flare on night time photography (due to wet rock after the high tide) prevented the cypris being counted at night-time low tides. One final set of photographs was taken on 06/06/2007 giving a total of 57 days.

7.3.3 Space available for settlement

Photographs used for Chapter 3 were taken during the winters of 2004/05, 2005/06 and 2006/07 using a Canon PowerShot G3 and in 2006/07 a Canon 350d (8 x 10^6 pixel).They were re-examined in ImageJ (Rasband 1997-2008) using a grid system to estimate the areas of available space on natural rock types and exposure on shores around the Clyde Sea. The areas (taken from a quadrat 50 cm x 50 cm) were worked out from large bare rock (500 cm^2 = edge lengths of 22.36 cm) to small areas of 1 cm^2 (edge length of 1 cm), using the same areas as the natural rock settlement intensification experiment. Areas less than 1 cm^2 were all grouped together as < 1 cm^2.

7.3.4 Data analysis

Settlement on artificial panels

Data were normalised by log transformation (x + 1) followed by analysis using Repeated Measures GLM ANOVA, where subject factor was day and the between subject factors were area. Mauchlys sphericity test (testing the variances are equal and sphericity is equal) results are shown to authenticate the Repeated Measures GLM ANOVA, and results of the Greenhouse – Geisser test (a correction used on Mauchleys test, if it is significant) are shown.

Settlement on natural (rock) substratum

Data was normalised by log transformation (Log x) followed by analysis using Repeated Measures GLM ANOVA. The within subject factor was day, and the between subject factor was area size. The model was fully factorial with *Post hoc* tests on area (Ryan-Einot-Gabriel-Welsch-Range). The graphs were found to have an S-curve and non-linear regression with the optimum SPSS equation $y = e{**}^{(b}{}_{0}{}^{+\,(b}{}_{1/t}{}^{)}$ where y is the settlement per cm^2, b_0 and b_1 are the slopes, and t is the day. Therefore regression curve estimation was conducted on the raw data up to and including day 10. Curve fit notes were recorded for each area: R^2, F value and P value. A non-linear regression was run using a stack of the number of settlers as the dependent and day as the variable with the non-linear model expression y = a + bx for each area to record parameters a and b estimates and the asymptotic 95% confidence intervals for b (these were recorded in

Table 7-4). This was to see if the rate of settlement for the days was different, as the settlement rate on the smaller cleared areas might have been faster than the larger cleared areas. An ANOVA was completed on the endpoint data (day 57), where in the non-linear regression the dependent was settlement and the fixed factor were the areas. It was not included in the Repeated Measures GLM ANOVA with the data for the first 10 days due to such a large time span between day 10 and day 57.

Space available for settlement

Space was worked out for each quadrat area of 50 cm x 50 cm (2500 cm^2) at the sites photographed around the Clyde Sea. There were 9 quadrats for each site (see Chapter 2, Table 2-7 showing the site, grid reference, exposure, rock type and year that site was used and therefore photographs for); each year producing an area of 22500 cm^2 per site per year. The mean available space for settlement was worked out for each site, each year. The mean was worked out for each site for the three years to get an overall average for each site. The space available for settlement at each site was then converted to a percentage and the exposure for each site, and the rock type noted.

7.4 Results

7.4.1 Settlement on artificial panels

The Repeated Measures GLM ANOVA test of within-subjects effects of settlement were significant for day (p = 0.018). The within-subjects effects for Mauchly’s test showed that the sphericity and the variances of the Repeated Measures GLM ANOVA (p = 0.766) were equal and authenticated, and the Greenhouse-Geisser test (p = 0.009, F = 5.668) did show significance for the GLM ANOVA (not really required as Mauchlys test showed sphericity). The between subjects effects found that area was significant (p = 0.001, F = 7.479). The *Post-hoc* test for homogeneous subsets conducted using Ryan-Einot-Gabriel-Welsch Range (REQWK) test split the areas into two subsets (see Table 7-1). The mean raw data for the settlement panels is shown in Table 7-2 indicating poor numbers of settlers which may have influenced results.

Means for groups in homogeneous subsets are displayed.

Panel	N	Subset	
		1	2
10 cm^2	3	.56	
c10 cm^2	3	.63	
c111 cm^2	3	.64	
c100 cm^2	3	.64	
100 cm^2	3	.71	
1 cm^2	3		0.00
c1 cm^2	3		0.00
P value		.667	<0.001

Table 7-1: Ryan-Einot-Gabriel-Welsch Range.

C before a number refers to it being part of a combined group

The subset 2 contains 1 cm^2 and the combined 1 cm^2; both these areas and their duplicates had no settlement. Only 10 cm^2 and 100 cm^2 contained settlement.

Area	**14/04/2005**	**15/04/2005**	**16/04/2005**
1 cm^2	0.00	0.00	0.00
10 cm^2	0.23	0.47	0.50
100 cm^2	0.18	0.24	0.18
c1 cm^2	0.00	0.00	0.00
c10 cm^2	0.27	0.30	0.43
c100 cm^2	0.18	0.38	0.33
c111 cm^2	0.19	0.37	0.33

Table 7-2: Mean raw data for settlement panels.

7.4.2 Settlement on natural (rock) substratum

Area	**F**	**R^2**	**P**	**a estimate**	**b estimate**	**Asymptotic 95% confidence upper 95%b**	**Asymptotic 95% confidence lower 95%b**
1 cm^2	15.57	0.266	< 0.001	4.148	-1.309	-2.711	0.093
5 cm^2	28.16	0.396	< 0.001	3.179	-1.449	-2.607	-0.291
10 cm^2	62.97	0.594	< 0.001	3.269	-1.584	-2.141	-1.027
50 cm^2	139.22	0.764	< 0.001	2.926	-2.174	-3.204	-1.143
100 cm^2	119.45	0.735	< 0.001	2.510	-1.798	-2.700	-0.896
500 cm^2	211.08	0.831	< 0.001	2.179	-2.857	-3.935	-1.780
combined 1 cm^2	15.91	0.270	< 0.001	4.109	-2.555	-6.514	1.403
combined 5 cm^2	35.55	0.453	< 0.001	3.393	-1.650	-2.641	-0.659
combined 10 cm^2	131.00	0.735	< 0.001	2.817	-1.752	-2.343	-1.161
combined 50 cm^2	105.32	0.710	< 0.001	2.796	-2.047	-2.830	-1.263
combined 100 cm^2	33.53	0.438	< 0.001	2.059	-1.630	-3.143	-0.118
combined 500 cm^2	142.93	0.769	< 0.001	2.159	-3.512	-4.767	-2.257
combined 666 cm^2	220.88	0.837	< 0.001	2.213	-2.701	-3.593	-1.809

Table 7-3: Area results of raw data for settlement intensification.

Table 7-3 shows that the areas were all significant for settlement over the 10 days, using the raw data.

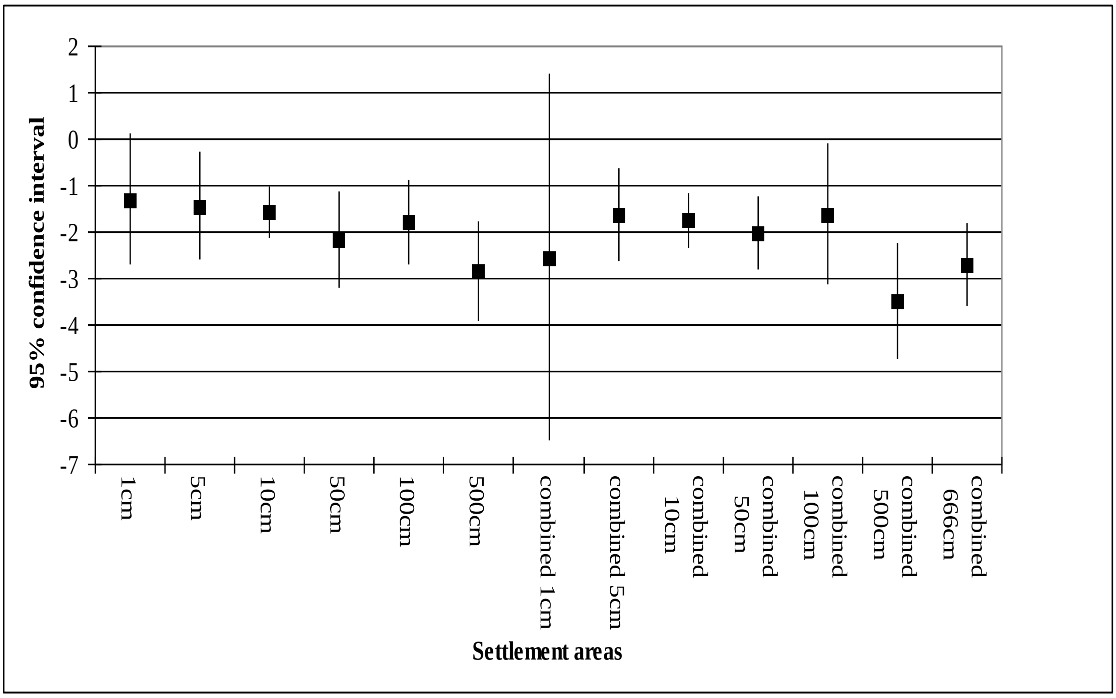


Figure 7-4: Parameter b from Table 7-3 showing 95% confidence interval for settlement intensification (areas).

Figure 7-4 shows the information from the curve fit and the non-linear regression on the raw data per cm^2. Overlap is shown for all settlement areas, from the data of the upper and lower confidence intervals, indicating day had an effect on settlement of each area.

The Repeated Measures GLM ANOVA tests of within-subjects effects, found that day was significant ($p < 0.001$) for settlement on the different rock settlement areas. The test of between subject effect (area size), showed that there was a significant effect of area size ($p < 0.001$, $F = 8.970$). The *Post-hoc* test for homogeneous subsets was conducted using Ryan-Einot-Gabriel-Welsch Range (REQWK) test split the areas into three subsets (see Table 7-4), with two distinct subsets. Areas < 5 cm^2 are grouped in subset three. The combined 10 cm^2 settlement areas are placed with larger areas in subset one.

Area Size	N	Subset		
		1	2	3
c500 cm^2	5	1.19		
c100 cm^2	5	1.21		
500 cm^2	5	1.40		
c666 cm^2	5	1.44		
100 cm^2	5	1.94	1.94	
c50 cm^2	5	2.14	2.14	
50 cm^2	5	2.23	2.23	
c10 cm^2	5	2.25	2.25	
5cm^2	5		2.55	2.55
c1 cm^2	5		2.77	2.77
10 cm^2	5		2.80	2.80
c5 cm^2	5		2.87	2.87
1 cm^2	5			3.50
P value		.087	.215	.135

Table 7-4: Ryan-Einot-Gabriel-Welsch Range

C before a number refers to it being part of a combined group

Means for groups in homogeneous subsets are displayed.

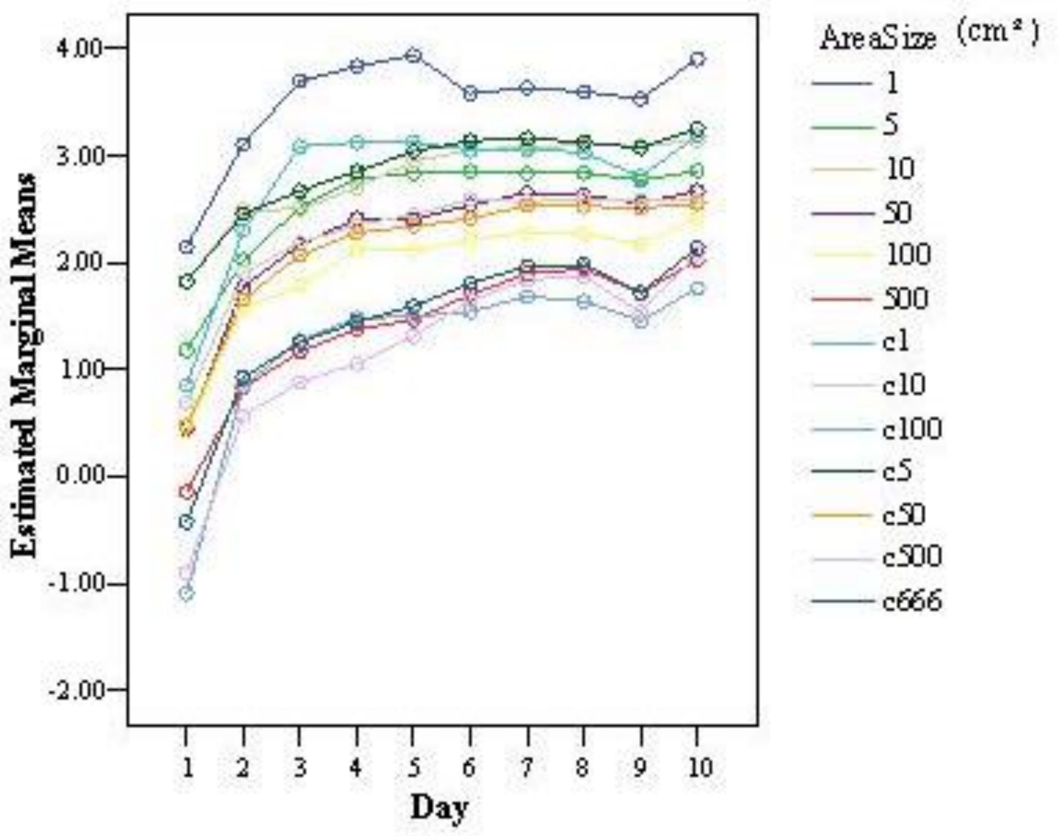


Figure 7-5: Graph showing settlement intensification from the transformed data using Estimate marginal means for settlement (area in cm^2).

Figure 7-5 confirms an increase in density over time, but also shows an intensification of settlement as the area available for settlement decreased. There was high settlement intensification over the first two days on the cleared areas, and then a levelling out of settlement on all areas to a final small increase on the last day. Although final settlement is dependent on area, the days show the same settlement increases each day. After day 2 the settlement increase slows until the day 9 when there is a reduction in settled cypris, this then increases again on day 10. Where there is a drop of settlement (day 9), this shows larvae have left the site either by being washed off if there were rough seas, or by voluntarily releasing to go back into the water column to settle elsewhere. However, if all areas voluntarily released it could not be because of settlement intensification as the larger areas had lower settlement per area than smaller areas such as 1 cm^2. The graph shows 1 cm^2 has the highest settlement per area throughout the settlement period. The combined 500 cm^2 starts off the lowest until day 6 steadily increasing in settlement to have the third lowest settlement overall, with

500 cm^2 the second lowest and 100 cm^2 the lowest settlement after 10 days. The 1 cm^2 and combined 1 cm^2 had drops in settlement on the transformed data after day 5, indicating that cypris left as the ideal settlement intensification had reached an optimal limit. The larger areas with proportionately smaller settlement per cm^2 (combined 100 cm^2, 500 cm^2, combined 500 cm^2 and combined 666 cm^2), increased until day 9 suggesting that the larger areas had not reached an ideal settlement capacity.

7.4.3 Space available for settlement

Free areas of settlement found on natural rock (from Chapter 3) give the following percentages split into rock type and exposure:-

Rock Type	**% Rock available for settlement**	**<1 cm^2**	**1 cm^2**	**5 cm^2**	**10 cm^2**	**50 cm^2**	**100 cm^2**
Sedimentary (exposed)	52.14	47.85	24.80	11.65	11.96	2.42	1.32
Sedimentary (sheltered)	43.18	50.61	24.38	10.39	12.33	2.29	0.00
Metamorphic (exposed)	58.11	39.93	29.98	9.19	17.49	3.22	0.19
Metamorphic (sheltered)	41.56	56.63	21.67	8.97	8.88	3.28	0.57
Igneous (exposed)	37.97	63.14	23.60	7.96	4.26	0.77	0.27
Igneous (sheltered)	50.79	53.81	19.43	10.25	12.56	2.72	1.23

Table 7-5: Raw data of natural rock percentages per area (cm^2), available for settlement (rounded to two decimal places from areas).

Table 7-5 shows the total percentage area available for the spaces indicated on the top bar, for each rock type found in the Clyde Sea, and the percentage of that rock type for settlement found in the Clyde Sea area. For example 52.14 % of exposed sedimentary rock in the Clyde Sea intertidal is available for settlement. Of this area < 1cm^2 spaces occupy 47.85 % this area, 1 cm^2 spaces take up 24.80 % of the free area, 5 cm^2 spaces occupy 11.65 % of 100 % of the free area for this rock type and so on. On photographed natural rock (Table 7-5) there were no natural gaps for settlement of 500 cm^2, however there were a few 100 cm^2 gaps occurring naturally on all rock types except sheltered sedimentary which doesn't have any large spaces. The percentage rock available for settlement is averaged from all the photographs, and the area percentages are of the three years and nine quadrats for each site (3 of low, medium and high settlement heights on the shore for *Semibalanus balanoides*).

As expected the largest percentage across all rock types is for areas < 1 cm^2 a space where a cypris may land and stay, but if food is prolific then there may be competition over growth and crowding may occur. The next largest percentage over all rock types and exposure is 1 cm^2, an area where copulation is easily possible and a cyprid may metamorphose and feed in the adult stage. After these areas available for settlement, there appears to be an overlap of sites where 2 of the 6 possible areas decline in availability, but in the other four areas: - sedimentary (both exposed and sheltered), metamorphic exposed areas and igneous sheltered areas have more 10 cm^2 areas available than 5 cm^2. This may be due to wave action, the effect of flotsam, orientation to prevailing currents, adults being cannibalistic therefore very good at catching cyprids, or another cause not thought of.

7.5 Discussion

This chapter aimed to find out whether the area of substratum available for settlement influenced cypris settlement of *S. balanoides*. The size of the settlement area had an effect on settlement intensification, for both experiments using artificial panels and areas cleared on natural substrata, with areas > 5 cm^2 more likely to be colonised with a higher number of settlers per cm^2.

Although the artificial panel surfaces were conducive to settlement, there was very little settlement on all panels (Table 7-2). This may have been a result of low numbers of cypris in the water column reaching the sandy beach, or a lack of settlement cues in the water column from this area as there were very few rocky areas, as annual variation has been seen in other studies (Caffey 1985). The artificial panels were about 100 m from the pier legs which had settlement on, which may have been too far for settlement cues to have an effect.

Panel colour may have been an issue as other experiments have had panel (tile) colour altered to encourage settlement (Todd et al. 2006). To the human eye the colour was very similar to the metamorphic rock from Carradale and the igneous rock, which had high settlement on during the rock experiment. It is possible that low cypris settlement was due to there being no adults around or that the cypris were not desperate to settle (Knight-Jones 1953, Jarrett 1997, Jarrett & Pechenik 1997, Todd et al. 2006, Tremblay et al. 2007). However, in the past there have been years of very low cypris settlement within the Clyde Sea (Barnes 1956), and low cypris numbers may have been a reason for poor settlement.

The settlement that did take place on the artificial panels (there was no settlement on the 1 cm^2 panels, either in a combined area or individual area) showed that panel size was significant, with there being more settlement on the 10 cm^2 panel sizes (both combined and separate), than the 100 cm^2 panels (combined and separate) or the combined 111 cm^2 areas. These very limited results suggest that edge effect may have been a contributing factor to the 1cm^2 panels, but not the 10 cm^2 panels (Hills & Thomason 1998b). It must be noted that these results were obtained after only three days and not ten days of settlement and intensification as on the natural rock settlement, and that in this case day was not significant for settlement.

Artificial and natural rock areas were not, and are not to be compared directly (or the experiments would have been conducted at the same site and year). However, suggestions for the different results are proposed, because if area had been the only cause of the settlement seen then it is suggested that differing substratum, sites and years would have been irrelevant.

Settlement intensification on the artificial surfaces may have been lower than on the rock at Millport simply because of variation of local settlement areas (Hawkins & Hartnoll 1982) or due to settlers being dislodged by the action of sand particles in the waves, although Pineda and Caswell (1997) propose that sandy areas would receive higher settlement per unit area, due to the lack of settlement sites. The number of cypris may have been smaller due to larval input differing from year to year (Barnes 1956). It is also possible that there may have been few or no 'founders' (Toonen & Pawlik 1994), or that the shoreline was unsuitable due to its flow regime (Larsson & Jonsson 2006). Due to the experiments being conducted in different years, no direct conclusions can be made between artificial panel areas on a sandy beach, and settlement on a natural rock face surrounded by barnacles; and the number of cypris in the water columns may have been different for each year.

From the results of settlement on different natural rock substrata (Chapter 6), it was known that igneous rock was conducive to high cypris settlement; therefore the experiment on natural substrata was conducted on this rock type at the University Marine Biological Station, Millport, Isle of Cumbrae, KA28 0EG. Although each area was cleared with an additional area at least 1 cm outside the markings before a cypris would encounter adults, the entire rock face within the intertidal was covered with adults, and there were only very small areas within the adult cover available for settlement. There should not have been any biofilms left on the rock after it being scrubbed and rinsed with fresh water then filtered water. The position of the settlement areas were in the lee of a small headland, encouraging settlement (Mace & Morgan 2006), unlike the artificial panels at Fairlie that had no shelter. This may have had a concentrating effect on settlement cues from the surrounding adults, encouraging the cypris to settle. Pineda et al. (2002) suggested that settlement on quadrats placed on natural rock might be influenced by juvenile and adults producing an intensification effect due to cypris rejecting adults as a settlement substrate and moving a short distance to open rock to settle; this would have the same effect if the settlement areas were marked on the rock face. Berntsson et al. (2004) working with *Balanus improvisus* also suggests gregariousness of conspecifics as a driver of increased settlement producing an intensification effect. These findings are similar to Knight-Jones (1953), who noted that *Elminius modestus* colonised quicker on shores where there were adults.

Chemical cues have also been suggested for the gregarious settlement of fossilised barnacles (Aguirre et al. 2008). The settlement cues from the adults on the natural rock may well have been an influence, if cypris had 'walked' to adults, and explored the areas before settlement in the painted quadrats, which isn't available to cypris on the artificial panels. Although Prendergast et al. (2008) suggested that rugosity may be more important than a chemical cue for *S. balanoides.* However, older and newly metamorphosed barnacles could indicate that these settlement areas were suitable for a benthic life (Bullard et al. 2004); again this cue would be missing from close to the artificial panels on Fairlie sands.

Settlement did differ with the day on natural rock (as can be seen in Figure 7-5), with a large increase during days 1 and 2, a steadier increase through days 3 to 9, with a final increase on day 10 suggesting a settlement 'S' shaped curve, however the day had the same impact on the settlement of each area (see Figure 7-5). Wethey (1984b) found that cypris settling during the same tide tended towards aggregation, which corresponds with settlement changing day to day on all panel sizes.

The tests of between-subject (area size) showed that there was a significant effect of area upon settlement, with the Ryan-Einot–Gabriel-Welsch Range splitting the areas into three subsets, with two distinct groups. Combined and single area sizes from 666 cm^2 to 50 cm^2 as well as the combined 10 cm^2 were in one group, with combined and single areas 1 cm^2 to 10 cm^2 in the other subgroup. Suggesting that apart from the 10 cm^2 combined group, which combining the areas together in a small settlement area had no effect on the settlement for that size of area, than if the area was by itself further from other settlement areas. There was a larger per area settlement on the small areas than the large areas, these findings agree with Pineda and Caswell (1997) who conducted experiments in a flume on settlement area using artificial panels. This disagrees with Hills and Thomason (1998b), who suggested that density on panels ranked 400 cm^2 > 100 cm^2 > 25 cm^2 > 4 cm^2 > 1cm^2, and who recommended that settlement panels should be at least 10 x 10 cm to avoid edge effects of panels where a cypris whist exploring would come to an edge and then return to the water column. Petraitis et al. (2003) who also worked with *S. balanoides* but in the Gulf of Maine found that on natural rock recruitment appeared to be higher in clearings greater or equal to 4 m diameter.

It is possible that intensification on the smaller areas of natural rock may have been due to physical cues of the substratum (Berntsson et al. 2004), or an intensification effect where disproportionately high settlement rates occur where there is reduced suitable substrata (Pineda & Caswell 1997, Jeffery 2000).

Area available for settlement on natural substratum is predominantly < 1 cm^2 (Table 7-5), and there are few areas of 100 cm^2. This is contrary to Hills and Thomason (1998b) who found that the ideal size for artificial settlement panels would be at least 10 x 10 cm and shows there is a discrepancy between natural settlement areas and artificial settlement panels or tiles.

7.6 Conclusions

- Settlement was higher on natural substratum than artificial panels, but this could be because of natural variation over different years
- There was larger per area settlement on smaller areas
- Natural substratum is the best for estimating recruitment of *S. balanoides*
- Caution should be used when comparing artificial and natural habitats
- Smaller areas (< 1 cm^2 and 1 cm^2) are more common as a potential settlement site in the natural environment and this should be considered when designing experiments

Chapter 8. Final Discussion

Semibalanus balanoides, barnacles and other fouling organisms are generally regarded as an expensive problem and inconvenience, or an insignificant organism on rocks that help prevent you from slipping on wet rocks by the general public (personal communications). Few people except marine biologists realise how beautiful (whilst feeding) and important they are to the ecology of the seas by feeding other zooplankton and fish (especially herring in the Clyde Sea area), however the majority of recent work on barnacles has been in antifouling paints and chemicals to prevent fouling commercial ships and leisure craft, inflows for cooling systems and fish farm cages. Fewer places to colonise, alien species out competing *S. balanoides* and global warming (which fouling organisms may exacerbate due to more fuel being used), may push this species further north and / or prevent it from breeding successfully in its present habitats (Barnes & Barnes 1954), before it and its interactions within its environment are fully realised.

Barnacles are generally of value to the marine economy (fishing) and to the ecosystem as an important part of the zooplankton community (Turner 1984). Throughout the world many species of fish are known to consume barnacle nauplii, for example in New Zealand's rocky reef waters: - *Caesioperca lepidoptera* (Butterfly perch), *Chromis dispilus* (Demoiselle), *Pseudolabrus celidotu* (Spotty), *Pseudolabrus miles* (Scarlet wrasse), *Gilloblennius tripennis* (Spectacled triplefin); and some consume the cirri of adult barnacles such as *Parablennius laticlavius* (Crested blenny) (Russell 1983). In the 50 fish species caught and their stomach contents examined, 5 species included barnacle cyprids and 9 included adult barnacle remains including cirri (Russell 1983). Other zooplanktivorous fish and fish larvae that are known to include barnacle nauplii in their diet are *Brevoortia patrons* (Gulf menhaden), *B. tyrannus* (Atlantic menhaden), *Clupea harengus* (Herring), *Harengula pensacolae* (scaled sardine), *Agonus cataphractu* (armed bullhead), *Callionymus lyra* (common dragonet), *Diplecogoster bimaculatus* (two–spotted clingfish), *Gobiusculus flavescens* (two – spotted goby), *Labrus bergylta* (ballen wrasse), *Lagodon rhomboides*(pinsish), *Leiostomus xanthurus* (spot croaker), *Micropogonias undulates* (Atlantic croaker), *Myoxocephalus aenaeus* (little sculpin or grubby), *Myoxocephalus octodecemspinosus* (longhorn sculpin), *Myoxocephalus* *scorpius* (short–spined sea scorpion), *Pomatoschistus microps* (common goby), *Taurulus bubalis* (sea scorpion), *Triglops murrayi* (mailed sculpin), *Psetla maxima* (turbot), *Solea solea* (sole), *Oncorhynchus gorbuscha* (Pink salmon) and the *Merlangius merlangus* (whiting) (Godin 1981, Batty 1987, Turner 1984). Some of these species are / or were commercially harvested, others play an important part in food webs, feeding higher trophic levels that may be commercially caught such as *Gadus morhua* (cod). Herring, *Sprattus sprattus* (sprat), *Scomber scombrus* (mackerel), cod, haddock, *Trisopterus luscus* (whiting), *Pollachius virens* (saithe or coley), *Merluccius merluccius* (hake), *Scyliorhinus canicula* (dogfish), *Raja batis* (skate), *Lophius piscatorius* (angler fish), turbot, and sole are, or have been landed in varying amounts at Clyde Sea fishing ports; but hopefully with increasing knowledge of overfishing and the ecosystem complexities, landings of these species may increase in the future (Bailey et al. 1986, Hislop 1986, Thurstan 2008, Thurstan and Roberts 2010).

The ecological marine diversity of the Clyde Sea area in the 1880's was vast and included whales; porpoises; skates, rays, dogfish (regarded as a pest during the herring fishing), sharks including *Galeorhinus galeus* (tope), *Prionace glauca* (blue), *Alopias vulpinus* (thresher) and *Cetorhinus maximus* (basking sharks); *Hippocampus spp.* (seahorses); cod; *Aequipecten opercularis* ('queenies'), and *Pecten maximus* (scallops) (Thurstan 2008, Thurstan and Roberts 2010). This diversity may re-emerge in the future, if the fishing and other commercial interests are handled sensitively in regard to the natural ecology and food webs of the area. Commercial fishing could be combined with profitable eco-tourism in the Clyde Sea, especially if there are large marine creatures to be regularly seen by the general public (cf. McIntyre et al. 2012).

Within the Clyde Sea, the herring fishery was of huge economic importance for hundreds of years, including the start of the twentieth century; and the Clyde Sea was known as an important spawning area for many fish species some of which feed on cirripedia larvae or other species that feed on them (Bailey et al. 1986, Thurstan 2008). Whales and porpoise were described feasting on the herring on the Ballantrae banks (a major spawning ground, another is Loch Fyne), as well as *Morus bassanus* (gannets) and other seabirds in 1800's (Thurstan 2008). Even during the 1800's the herring fishery could fluctuate widely, for example 1880 about 5500 tonnes were caught, but in

1882 there was only 1500 tonnes, there was a brief revival during the 1920's, but by then herring fishing was unsustainable in the Clyde Sea although small amounts of herring spawn were to be found on the Ballantrae Bank (Thurstan 2008).

The herring fishery was superseded by the fishing for other species such as cod, *Melanogrammus aeglefinus* (haddock), *Psetta maxima* (turbot), *Platichthys flesus* (flounder), *Pleuronectes platessa* (plaice) then 'queenies', and scallops (Thurstan and Roberts 2010). Since the 1950's to present times the fishing for scallops and *Nephrops norvegicus* (Langoustine, Norway lobster, Dublin Bay prawns) have been the mainstay of the Clyde Sea fishing industry with nearly 3000 tonnes of *Nephrops* landed in 2006 (McIntyre et al. 2012, Thurstan 2008). Unfortunately with the fishing for *Nephrops*, there is a very large discard ratio of approximately 9 kg, for every 1 kg of *Nephrops* caught (resulting in approximately 25000 tonnes of discards); with many of the discards (sometimes juvenile cod, haddock and plaice) dying and eaten by scavengers, such as seabirds and marine animals which might have increased their populations (Thurstan 2008, Thurstan and Roberts 2010). The removal of higher predators such as cod may result in an altered ecosystem with lower trophic levels dominating the environment, and lower diversity that may be susceptible from invasive species and disease (Thurstan and Roberts 2010). Fish are known to selectively feed on various zooplankton and phytoplankton, switching to less favoured species and becoming omnivorous if there is an absence of favoured food (Turner 1984).

Phytoplankton growth and the zooplankton are suggested to be healthy in the Clyde Sea according to the review of the Clyde ecosystem, and although it is warming, it is at a rate similar to other coastal areas on the west of Scotland (McIntyre et al. 2012). It is suggested by McIntyre et al. (2012), that the rocky shore of intertidal habitats are deteriorating due to non-native species, for example *Sargassum muticum* (wireweed), but subtidal habitats are in a better state. Many *Cetorhinus maximus* (basking sharks) are seen off the coasts of Arran during the summer to early autumn due to high plankton numbers, which is a valuable eco-tourism resource (McIntyre et al. 2012). Other large marine species that are found in the Clyde Sea are various seabird species, seals, and in recent years (since 1980) 15 different species of cetaceans have been spotted, which encourages ecotourism to the area and these higher trophic level predators rely on lower levels and ultimately phytoplankton and zooplankton (McIntyre et al. 2012).

This thesis endeavours to go some way to identifying and quantifying the risks that *Semibalanus balanoides* is subjected to in its natural environment, through the interpretation of data collected to quantify the ‘jigsaw’ of this species’ life cycle. The Clyde Sea and its associated fjords combine to make the area the largest fjordal system in Britain. The Clyde Sea has tides, is influenced by winds, fresh water inflows from the rivers, and has a residence time for the sea water of two months that allows the assumption that nauplii released will remain until metamorphosed juveniles. This is in effect a microcosm of the Boreo arctic seas (without ice scouring) inhabited by *S. balanoides* and can be used to contemplate how this organism interacts with rock types, exposures of shores to the fetch, settlement and cannibalism.

I concur with Prendergast (2007) that studies should take years of information into account including all biotic and abiotic influences on the species under investigation. Ecologists try to make models that describe patterns in nature, and explain factors that affect species. Especially in the marine environment this is not always possible as there may be many factors that the humans do not understand or cannot monitor as it is not their natural environment.

The thesis provides an insight over a short time (2004 – 2007), and hopefully others may complete counts at a later date in order to find out what happens in the Clyde Sea over a larger time span. During the study period it was found that possible starvation resulted in barnacles producing fewer eggs, therefore changes in nutrition should be taken into consideration as well as the number of fecund adults. The size of cohorts were also found to differ between rock types and may alter site to site not just if it is an exposed or sheltered shore. Barnes & Barnes (1959) found that high temperature during later stages of development prevents viable eggs, which should be taken into consideration.

Assumptions had to be made (due to the constraints of the experiments) in order to ascertain the number of barnacles on the different rock types, exposures and height of the intertidal foreshore. Man-made structures (piers, sea walls etc.) were not taken into consideration as each site should be investigated separately. The area of rock in the Clyde Sea area was halved for each rock type to:-

Igneous	599.6280 ha
Sedimentary	2368.9095 ha
Metamorphic	1306.5765 ha

This is due to *Chthamalus spp.* also being found in the upper intertidal restricting available space for *Semibalanus balanoides*. It was assumed that due to the prevailing winds (from the west) that half the shores were exposed and half sheltered (see Chapter 2 Mapping, Geology, bathymetric data and exposure ratings).

Year three raw data was used to estimate the mean total number of live barnacles in the Clyde Sea (from photographs of nine quadrats for each site – three highest settlement zones, three mid settlement zone, three mean low water springs), as this was the most complete data with three sites each for exposure and rock type:-

Igneous	1.28749×10^{11}	
Sedimentary	4.11180×10^{11}	
Metamorphic	4.05443×10^{11}	
Total	$\mathbf{9.45372 \times 10^{11}}$	*S. balanoides* adult barnacles in the Clyde Sea

Using the mean number of eggs produced per barnacle for the years 2004 – 2006 (2004 – 2109.34, 2005 – 766.96, 2006 – 1707.26) and the total number of live barnacles the possible number of nauplii released in the Clyde Sea in these years are:-

2004	1.9941×10^{15}
2005	7.2506×10^{14}
2006	1.6140×10^{15}

These numbers do not take into consideration unfertilised eggs.

Using the same year 3 quadrat photographs, the area of bare rock that could be settled by *S. balanoides* cypris were:-

Igneous	276.939331 ha
Sedimentary	992.748617 ha
Metamorphic	684.258947 ha
Total	**1953.946895 ha**

This space is available for the cyprids released in spring 2007 to settle.

The mean barnacle size from the three years is: - 52.76 mm^2.

From this information of the bare rock that was available to be settled, it could accommodate:-

$3.703462652 \times 10^{12}$ grown barnacles (from the mean size)

Therefore there is not enough space for the nauplii that are released in year 3 to settle on the spaces between other *S. balanoides*. However, there has been spare rock between adult barnacle settlement for the three years, and a great deal of nauplii and cypris will be lost between release and juvenile barnacles, as it is known for instance that cyprids may be knocked off and consumed by limpets and removed by fucoid frond movements in the water. Wind direction has also been known to influence failure years (Barnes 1956) when the cyprids may not reach a suitable settlement area. However, filming arrival and departure from artificial panels found that about 25 % of cypris settled, when they came into contact with the panels.

It was found in the cannibalism chapter (Chapter 5) that adults did cannibalise both nauplii and cyprids with 44% of adults in the samples collected from the shore and dissected containing remains in them, therefore contributing to possible future generations by feeding present adults. It was also found that one third of the 28 cypris encountered every hour by an adult were consumed, suggesting that nauplii and cyprids provide an important food source to the adults.

The chapters covering settlement intensification and the effect of rock type highlighted the effect of day as significant, and the effect of previous cypris ‘footprints’ increased settlement, suggesting that data must be collected over the most days possible for the settlement period.

Whilst answering some questions this thesis leaves many more. Due to lack of time and data not collected, this thesis has not covered the importance in the Clyde Sea area of the parasitic isopod *Hemioniscus balani* that makes the barnacle sterile although in years one and two, two parasites were found in the barnacles investigated, and none in year three out of a total of 200 barnacles investigated in the three years; but Stubbing’s (1975) suggested that about 2.5 % of *S. balanoides* in the French Atlantic were predated by this isopod. Other questions include the effects of storms and floatsam hitting adults which then release nauplii prematurely before the phytoplankton bloom (how many are likely to survive initially from this?), desperation effect for settlement, other intraspecific and interspecific competition, increases or decreases of plankton consuming fish or other zooplankton, whether cyprids that have been caught then released from cirral nets are still competent, or how many barnacles in the population are sterile each year. This list is by no means complete, but answers to these questions may add a few more pieces to the ‘jigsaw’ of this species life, and its importance to the food web and ecology of the Clyde Sea.

Various species are known to occupy certain amounts of water in fjords, which can be used to predict values for larger, open ecosystems such as oceans and seas, as they often have the same properties, and can be used as a scale model (Buchholz et al. 2010, Brattegard et al. 2011). For example in previous studies in the Clyde Sea area, naked amoebae number up to 156 x 10^5 l^{-1} in the plankton (Butler and Rogerson 1996). From the frame experiment in Chapter 4 there were 0.11 ± 0.08 cyprids l^{-1} at the surface and 0.12 ± 0.10 cyprids l^{-1} at the sub-surface (11/04/2005 – 15/04/2005).

Barnes (1956) studied nauplii and cyprid numbers, and found that in a normal year (1950), in the Fairlie Channel of the Clyde Sea, counts for the different larval stages of *S. balanoides* in a one and a half mile tow using a Hardy Plankton Indicator. On 7th March 1950 for nauplii stages I, II and III, about 260 individual larvae (estimated from

Figure 1 of Barnes 1956), but no nauplii stages IV, V, VI or cyprids were caught. By 15th March, nauplii stages I, II, and III reached about 850 larvae, and the nauplii stages IV, V, VI and cyprids had reached about 60 larvae in total (estimated from Figure 1 of Barnes 1956). The nauplii stages I, II and III then dropped off by the 30th March to about 550 larvae, but the stages IV, V, VI and cyprids had risen to about 1000 larvae; after that date the stage IV, V, VI and cyprids dropped off to about 400 on the 5th April tow, and about 10 individuals of stages I, II and III (estimated from Figure 1 of Barnes 1956). By the 10th April there were no stage I, II and III nauplii in that dates tow, and about 250 stage IV, V, VI and cyprids; on the 15th April there was a spike of 625 larvae of stages IV, V, VI and cyprids, that fell off after this date until the final tow on 17th April when there were only about 100 larvae (estimated from Figure 1 of Barnes 1956). Unfortunately the exact volume of water trawled was unknown from the trawls in the Clyde Sea, but the numbers of *S. balanoides* larvae seem low compared to more northern fjordal systems (however the cirripedia larvae were not identified to species level in other tows of other fjordal systems). If these tows were repeated, with all nauplii statges identified, this could be compared to other fjordal systems.

In the Arctic, Antarctic and temperate seas, zooplankton population dynamics alter through the season for different taxa. At very high latitudes the ecosystems are characterised by extreme seasonality, but copepods large lipid stores are a benefit due to their winter feeding on ice algae (Kwasniewski et al. 2013). Copepods can therefore start their reproduction earlier when the phytoplankton biomass increases, which in Rivers Inlet, Canada starts when the water temperature reaches 7.5 ^{o}C or above (Tommasi et al. 2013). Sørfjorden in northern Norway, is separated by a shallow sill from Ullsfjorden and has a residence time for the water of 200 days (considerably longer than the two months of the Clyde Sea) is nearly enclosed, the zooplankton is dominated by *Calanus finmarchicus* and euphausiids, as herbivore copepods are dominant in Arctic zooplankton (Edvardsen et al. 2002, Arnkvaern et al. 2005). In Sørfjorden the zooplankton between 10^{-1} and 10^{3} mm^{3} were examined using an Optical Plankton Counter, finding that up to 95 % of them were copepods, and 90 % of those were *Calanus finmarchicus* in the mesozooplankton size range, with euphausiids dominated by *Thysanoessa inermis, T. rachii* and *T. longicaudata* (Edvardsen et al. 2002).

The *C. finmarchicus* different life stages had their cylinder body volumes measured, then their numbers were recorded with the Optical Plankton Counter (Edvardsen et al. 2002). The copepodite life stage areas were estimated from Table 3 in order to determine which stages corresponded with *S. balanoides* nauplii and cyprid stages from Stubbings (1975).

Calanus finmarchicus	***Semibalanus balanoides***	
	UK waters	**Arctic waters 74 ^{o}N**
CI ~ 0.19 mm^2	NI ~ 0.06 mm^2	NI unknown
CII ~ 0.34 mm^2	NII ~ 0.12 mm^2	NII ~ 0.21 mm^2
CIII ~ 0.69 mm^2	NIII ~ 0.18 mm^2	NIII ~ 0.30 mm^2
CIV ~ 1.20 mm^2	NIV ~ 0.24 mm^2	NIV ~ 0.49 mm^2
CV ~ 2.38 mm^2	NV ~ 0.47 mm^2	NV ~ 0.71 mm^2
Adult females ~ 2.19 mm^2	NVI ~ 0.68 mm^2	NVI ~ 0.92 mm^2
	Cyprids ~ 0.50 mm^2	Cyprids ~ 0.92 mm^2

Table 8-1 ***Calanus finmarchicus and Semibalanus balanoides*** **larval stage areas for comparison of size.**

Therefore apart from the fifth copepodite stage and the adult females, *C. finmarchicus* are of similar sizes to *S. balanoides* nauplii and cyprid stages from Arctic waters, and similar to nauplii stage III for UK waters, and may fill the same trophic position in the food webs of the different fjords. Unfortunately the calorific value and chemical make - up of the larval stages for these species is unknown, which may alter how many of each species are consumed by higher trophic levels. However, in the summer of 1998 in

Sørfjorden there was a mean plankton abundance of 5000 individuals per m^{-3}, and therefore a very important food source for planktivorous fish (clupeiformes) and sea birds, linking the phytoplankton to higher trophic levels (Edvardsen et al. 2002, Arnkvaern et al. 2005, Kwasniewski et al. 2013, Tommasi et al. 2013).

In 2002 at Billefjorden (a sill fjord opening onto Isfjorden, Svalbard), *Calanus finmarchicus, Calanus glacialis* and *Calanus hyperboreus* of all copepodite stages were found to number 29000 individuals per m^{-2}, with *C. glacialis* 60 – 80 %, *C. finmarchicus* 20 – 30 % and *C. hyperboreus* 5 – 20 % and an important food source for polar cod (*Boreogadus saida*) in that Arctic environment (Arnkvaern et al. 2005). However, in Godthabsfjord in Greenland, 56 mesozooplankton groups were found over a five year study with *Microsetella norvegica* 408125 ± 161387 m^{-3} the most abundant (87 % of copepods) (Arendt et al. 2013). In the Godthabsfjord study there was a mesozooplankton succession of cirripedia nauplii in March and April, dominating the biomass, then *Calanus* spp. in May and June, then *M. norvegica* dominating from July to September (Arendt et al. 2013). This suggests that in March and April, cirripedia larvae were a very important part of the food web.

The fjord Saanich Inlet on the east of Vancouver Island, Canada is sometimes subjected to hypoxia, due to a large amount of organic matter from phytoplanktonic blooms in the spring and summer (Dinning and Metaxas 2013). One of the most abundant groups of invertebrates taking advantage of this was *Balanus crenatus* (both the nauplii and cyprids), and this was the numerically dominant species in the fjord by the summer 2009 (Dinning and Metaxas 2013). After appearing in February 2009 when there were approximately 30 *B. crenatus* individuals per $m^{-2} d^{-1}$, rising to about 90 individuals per $m^{-2} d^{-1}$ in March / April, peaking at about 9000 individuals per $m^{-2} d^{-1}$ in July, steadily falling to approximately 110 individuals per $m^{-2} d^{-1}$ in September in the sediment traps deployed at 97 m (Dinning and Metaxas 2013). Although high oxygen and low temperatures were recorded in this fjord before February, and the larvae of this barnacle had a strong correlation with these parameters, no nauplii or cyprids were recorded before then, indicating that seasonal reproduction overrode these parameters (Dinning and Metaxas 2013). The oxygen level in this fjord was often < 3.09 ml l^{-1}, but many taxa including *Balanus crenatus* were able to adapt to these levels; which may be important for the future of barnacle species in the increasing number of minimum oxygen zones in coastal waters, due to anthropogenic eutrophication and global warming (Dinning and Metaxas 2013).

Therefore not only is research into various species important (both individual species and taxa) in fjords, repeated examination of the same fjordal systems is vital to try and find out what is happening to them, and possibly the wider marine communities and how they will cope with the changing environment. Mesozooplankton and zooplankton reproduction must be in time to take advantage of the phytoplankton bloom, and at high latitudes such as Arctic regions, sea ice is decreasing due to global warming, with the chance that phytoplankton bloom may happen earlier; therefore the zooplankton will need to adjust their reproduction to maintain synchrony (Kwasniewski et al. 2013). For example, in July 2006, in a study of macrozooplankton ten years after an original one completed in Kongsfjorden, Svalbard; Buchholz et al. (2010) found that there were three additional euphausiid species of typically Atlantic origin found in the fjord. In high latitude seas, different waters contain characteristic species of krill, and their movement of range gives suggestions of global warming, for instance when you get warmer Atlantic water species in previously cooler Arctic coastal waters dominated fjordal water, as Kongsfjorden lies at the northern extent of the Gulf Stream that appears to be influencing the fjordal system (Buchholz et al. 2010).

In a separate study of the upper 50 m of the water column of Kongsfjorden in 2003 and 2004, the dominating taxa were copepods (all stages) along with cirripedia nauplii and polychaeta larvae, accounting for over 85 % of the total zooplankton in all trawls (Kwasniewski et al. 2013). The cirripedia nauplii abundance in mid April 2003 were 752 individuals per m^{-3}, dropping to 16 individuals per m^{-3} in the first week of May, before rising to 5520 individuals per m^{-3} on 18th May (up to 80 % of zooplankton abundance that day), dropping to 890 individuals per m^{-3} on the 1st June, then finally dropping to 7 individuals per m^{-3} on 19th July 2003 when hauls finished using a Multi Plankton Sampler (Kwasniewski et al. 2013). Pre bloom (April to the beginning of May) there was low zooplankton abundance with a total of 788 – 1030 individuals per m^{-3}, during the spring bloom (May to June) total zooplankton abundance ranged from 410 – 7065 individuals per m^{-3} (Kwasniewski et al. 2013). In early summer and post bloom (mid June to mid July), the total number of zooplankton stabilized between 795 – 6016 individuals per m^{-3}, including many large herbivorous copepods, especially *C. glacialis* and *C. finmarchicus* (Kwasniewski et al. 2013). In 2004 on the 8th May, the Cirripedia nauplii reached 10801 individuals per m^{-3} dropping to 3401 individuals per m^{-3} on the 14th May (Kwasniewski et al. 2013). Cirripedia nauplii then increased to their highest number of 15222 individuals per m^{-3} on the 18th May (the same date that recorded the highest number the previous year), dropping to 1456 individuals per m^{-3} on 2nd June, then to 13712 individuals per m^{-3} on the last date trawls were made on 8th June 2004 (Kwasniewski et al. 2013). This compares with *Semibalanus balanoides* at Fairlie Pier between 11/04/2005 to 15/04/2005, where cyprids were found to number between 31 – 191 individuals per m^{-3} at the surface (0.1 m), and 20 – 220 individuals per m^{-3} at the sub – surface (0.6 – 2.1 m). During 2004 cirripedia and copepod nauplii predominated the zooplankton abundance, varying between 2205 – 21647 individuals per m^{-3} and although the cirripedia nauplii were extremely high, numbers were very variable (Kwasniewski et al. 2013). During the peak period of spring blooms, cirripedia nauplii were the most important in terms of abundance and biomass which would be of highest importance to higher taxa feeding on the zooplankton at this time (Kwasniewski et al. 2013). *C. finmarchicus* spawning was after the spring bloom, suggesting that females required additional food from the phytoplankton bloom (Kwasniewski et al. 2013). It is suggested that the ecosystem in Kongsfjorden will change even more due to global warming, with more Atlantic zooplankton and copepods such as *C. finmarchicus* competing with Arctic species such as *C. glacialis*, and this phenological shift may affect the whole food web (Kwasniewski et al. 2013).

The studies in the fjords highlight how important zooplankton are to these ecosystems, and the benefit of using these as microcosms of the marine environment; whether the zooplankton under investigation have been cirripedia, copepods or polychaete larvae.

Obviously the studies shown are over different years and separate geographical places, so there is no way of knowing whether a poor year for *S. balanoides* in the Clyde Sea, is a poor recruitment year in these other fjords for instance Kongsfjorden (if *S. balanoides* is found there), unless the investigations are completed in the same years and preferably the same way. It is possible that different cirripedia that were investigated at other fjords may have been *S. balanoides* as they were not identified to species level, but that cannot be assumed, therefore no direct comparisons can be made. As other zooplankton input was not investigated in the Clyde Sea during April 2005, I am unable to categorically say that between March and April, *S. balanoides* is the most prolific and therefore the most important species contributing to its trophic level of the Clyde Sea food web. However from the data produced in this thesis, *S. balanoides* must have a significant input for the ecology of this sea.

Clupeid fish such as the European sprat (*Sprattus sprattus*) and herring (*Clupea harengus*), both species of fish found in the Clyde Sea feed on copepod, meroplankton and phytoplankton (Falkenhaug and Dalpadado 2014). Sprats are capable of selective feeding, so that if copepods, meroplankton (including cirripedia nauplii and cyprids), cladocerans, appendicularians and eggs are scarce, adults as well as juveniles can feed on phytoplankton (Falkenhaug and Dalpadado 2014). Falkenhaug and Dalpadado (2014) found that in Hardangerfjord, ingestion of phytoplankton accounted for 2 – 89 % of the total stomach contents of the sprat caught. This facultative filter – feeding is known to occur in herring, another fish that can feed in fjords if smaller prey is available in sufficient quantities, and larger prey is scarce (Falkenhaug and Dalpadado 2014). In Hardangerfjord the most important foodstuff for the sprat was found to be the copepod *Microsetella norvegica* (0.5 – 0.7 mm long), which has a similar size to *S. balanoides* nauplii stage V, VI and cyprids in Arctic waters, with *S. balanoides* stage IV nauplii being just smaller than the copepods, and they may be consumed in the Clyde Sea (Falkenhaug and Dalpadado 2014). In Europe in 2012, 408509 tonnes of sprats were caught for the food industry (FAO 2014b); and in 2011, 6919 tonnes of herring were caught in the Clyde Sea and west of Scotland (FAO 2014a). Thus *S. balanoides* may be important in sustaining this fishery.

There are several ways in which the studies reported in this thesis could be continued and improved upon. With the recent advance in technology and subsequent reduction in prices, GPS could be used to exactly position quadrats so that sequential yearly counts could be performed on exactly the same part of the intertidal, with the same barnacles, flora and fauna on the substratum. Filming could be done simultaneously at many sites at the same time (man power dependent) due to long life battery powered waterproof cameras, which could also be used with automatic timers. A three-dimensional tracking system for barnacle cyprids has been developed using stereoscopy, allowing a greater understanding of cyprids behaviour to physical and chemical cues (Maleeschlijski et al. 2012). This could be developed to be used in the natural environment using the two offset cameras on a cradle as in Chapter 4, to gauge swimming direction, velocity and angle. Three-dimensional photogrammetry could be used on or near boulders, giving an accurate surface area for settlement (Bythell et al. 2001).

Semi-remote techniques could be used over a much larger area. For example plankton pumps such as those used in Chapter 4 are cheap enough to use in remote areas with batteries and a timer to trap barnacle larvae, zooplankton and phytoplankton over a given time. The plastic panels could also be deployed at various sites and instead of being deployed for one hour; they could be deployed at various sites around a large area of intertidal (or off many piers) for a whole tidal cycle, before being collected.

Also it is suggested that inshore plankton hauls (as well as hauls in the Fairlie Channel), could be completed throughout the nauplii / cyprid season in order to ascertain how many of each stage were found, and record current data. Barnes (1956) work, groups together the first three then the second three stages of nauplii and cyprids, as he was looking at annual variation of the larval population and proposing reasons for this variation, this could be done for each nauplii stage to find exactly where nauplii are lost to the *S. balanoides* population.

It is important to know the overall and potential numbers of nauplii, cyprids and adult barnacles for the future health and monitoring of the marine environment. In the future we need to know apart from cannibalism (shown in this thesis), where cirripedia larvae are lost and in what numbers to give a value of this species to the ecosystem. Different rock types, their exposure and position on the shore have been shown in this thesis to host different numbers of adult barnacles, and rock type has had an effect on the number of eggs produced. All species are important on this planet, and we as so called intelligent animals, should accept this and need to know the possible numbers of all species, not just a red list for an unfortunate few. All species are of value to ecosystems and / or humans, and it is important to either put numbers or monetary value to species.

It is too late when only a few of a species are left alive. Cirripedia can be an inconvenience to shipping, pleasure craft and cooling systems, but their importance to the other species that inhabit this planet is should also be highlighted, such as the fishing industry and the Clyde Sea ecology now and in the future (if it is allowed to recover). In order to start finding a value, we need to know the numbers involved and I hope that this thesis has in some way attempted this. There are uncertainties with the figures presented in this thesis, but it is hopefully a start to finding potential and overall numbers of *Semibalanus balanoides* in the Clyde Sea area.

Chapter 9. References

Aguirre A., Martin J.M., Braga J.C., Betzler C., Berning B., Buckeridge J.S. (2008). Densely packed concentrations of sessile barnacles (Cirripedia: Sessilia) from the Early Pliocene of SE Spain. *FACIES* 54: 193-206.

Albessard E. and Mayzaud P. (2003). Influence of tropho-climatic environment and reproduction on lipid composition of the euphausiid *Meganyctiphanes norvegica* in the Ligurian Sea, the Clyde Sea and the Kattegat. *Marine Ecology Progress Series* 253: 217-232.

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

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

Aldred N., Gohad N.V., Petrone L., Orihuela B., Liedberg B., Ederth T., Mount A., Rittschof D., Clare A.S. (2013a). Confocal microscopy – based goniometry of barnacle cyprid permanent adhesive. *The Journal of Experimental Biology* 216: 1969-1972.

Aldred N., Hoeg J.T., Maruzzo D., Clare A.S. (2013b). Analysis of the behaviours mediating barnacle cyprid reversible adhesion. *PLoS ONE* 8(7): e68085. doi:10.1371/journal.prone.0068085

Alexander S.E. and Roughgarden J. (1996). Larval transport and population dynamics of intertidal barnacles: a coupled benthic/oceanic model. *Ecological Monographs* 66(3): 259-275.

References

Allaby A. and Allaby M. (1999). A Dictionary of Earth Sciences. Oxford University Press, Oxford.

Amaral V., Paula J., Hawkins S., Jenkins S. (2009). Cannibalistic interactions in two co-occurring decapod species: effects of density, food, alternative prey and habitat. *Journal of Experimental Marine Biology and Ecology* 368: 88-93.

Anonymous (2014). Rock Library. Imperial College, London. Retrieved 14/01/2014 from www2.imperial.ac.uk/earthscienceandengineering/rocklibrary/viewglossrecord.php?gID=00000000132.

Arendt K.E., Juul-Pedersen T., Mortensen J., Blicher M.E., Rysgaard S. (2013). A 5-year study of seasonal patterns in mesozooplankton community structure in a sub-Arctic fjord reveals dominance of *Microsetella norvegica* (Crustacea, Copepoda). *Journal of Plankton Research* 35(1): 105-120.

Arnkvaern G., Daase M., Eiane K. (2005). Dynamics of coexisting *Calanus finmarchicus, Calanus hyperboreus* populations in a high-Arctic fjord. *Polar Biology* 28: 528-538.

Bailey R.S., McKay D.W., Morrison J.A., Walsh M. (1986). Resources status, utilisation and conservation. The biology and management of herring and other pelagic fish stocks in the Firth of Clyde. *Proceedings of the Royal Society of Edinburgh Section B -Biological Sciences* 90: 407-422.

Baker J.M. and Crothers J.H. (2009). Intertidal rock. In: Baker J.M., Wolff W.J. (eds) Biological surveys of estuaries and coasts. Cambridge University Press, Cambridge.

Ballantine W.J. (1961). A biologically defined exposure scale for the comparative description of rocky shores. Field Studies 1: 1-10.

Banerji A. and Morin P.J. (2009). Phenotypic plasticity, intraguild predation and anti-cannibal defences in an enigmatic polymorphic ciliate. *Functional Ecology* 23, 427-434.

Barnes H. (1953a). Size variations in the cyprids of some common barnacles. *Journal of the Marine Biological Association of the United Kingdom* 32(2): 297 - 304.

Barnes H. (1953b). Orientation and aggregation in *Balanus balanus* (L.). Da Costa. *Journal of Animal Ecology* 22: 141-148.

Barnes H. (1956). *Balanus balanoides* (L.) in the Firth of Clyde: the development and annual variation of the larval population, and the causative factors. *Journal of Animal Ecology* 25: 72-84.

Barnes H. (1962). So – called anecdysis in *Balanus balanoides* and the effect of breeding upon the growth of the calcareous shell of some common barnacles. *Limnology and Oceanography* 7: 462-473.

Barnes H. (1963). Light, temperature and the breeding of *Balanus balanoides*. *Journal of the Marine Biological Association of the United Kingdom* 43: 717-727.

Barnes H. and Barnes M. (1954). The general biology of *Balanus balanoides*. *Oikos* 5: 63-76.

Barnes H. and Barnes M. (1959). The effect of temperature on the oxygen uptake and rates of development of the egg masses of two common cirripedes, *Balanus balanoides* (L.) and *Pollicipes polymerus* J.B. Sowerby. *Kieler Meeresforschungen* 15: 242-251.

Barnes H. and Barnes M. (1967). The effect of starvation and feeding on the time of production of egg masses in the Boreo-arctic cirripede *Balanus balanoides* (L.). *Journal of Experimental Marine Biology and Ecology* 1: 1-6.

Barnes H. and Barnes M. (1968). Egg numbers, metabolic efficiency of egg production and fecundity; local and regional variations in a number of common cirripedes. *Journal of Experimental Marine Biology and Ecology* 2: 135-153.

Barnes H., Crisp D.J., Powell H.T. (1951). Observations on the orientation of some species of barnacles. *Journal of Animal Ecology* 20: 227-241.

Barnes M. (1989). Egg production in cirripedes. *Oceanography and Marine Biology an Annual Review* 27: 91-166.

Bartolini F., Barausse A., Portner H., Giomi F. (2013). Climate change reduces offspring fitness in littoral spawners: a study integrating organismic response and long - term time - series. *Global Change Biology* 19: 373-386.

Batty R.S. (1987). Effect of light intensity on activity and food – searching of larval herring, *Clupea harenus*: a laboratory study. *Marine Biology* 94: 323-327.

Begon M., Harper J.L., Townsend C.R. (1999). Ecology. Individuals, populations and communities. Blackwell Scientific Publications, London.

Bell J.J. (2012). High connectivity between sea lough populations of a planktonic larval disperser with the adjacent open coast. *Marine Ecology* 33: 516-521.

Bellgrove A., Clayton M.N., Quinn G.P. (2004). An integrated study of the temporal and spatial variation in the supply of propagules, recruitment and assemblages of intertidal macroalgae on a wave-exposed rocky coast, Victoria, Australia. *Journal of Experimental Marine Biology and Ecology* 310: 207-225.

Berglin M., Hedlund J., Fant C., Elwing H. (2005). Use of surface-sensitive methods for the study of adsorption and cross-linking of marine bioadhesives. *Journal of Adhesion* 81(7-8): 805-822.

References

Berlow E.L. and Navarrete S.A. (1997). Spatial and temporal variation in rocky intertidal community organization: Lessons from repeating field experiments. *Journal of Experimental Marine Biology and Ecology* 214: 195-229.

Bernhardt J.R. and Leslie H.M. (2013). Resilience to Climate Change in Coastal Marine Ecosystems. *Marine Science* 5: 371-392.

Berntsson K.M and Jonsson P.R. (2003). Temporal and spatial patterns in recruitment and succession of a temperate marine fouling assemblage: A comparison of static panels and boat hulls during the boating season. *Biofouling* 19: 187-195.

Berntsson K.M., Jonsson P.R., Lejhall M., Gatenholm P. (2000). Analysis of behavioural rejection of micro – textured surfaces and implications for recruitment by the barnacle *Balanus improvises. Journal of Experimental Marine Biology and Ecology* 251: 59-83.

Berntsson K.M., Jonsson P.R., Larsson A.I., Holdt S. (2004). Rejection of unsuitable substrata as a potential driver of aggregated settlement in the barnacle *Balanus improvisus*. *Marine Ecology Progress Series* 275: 199-210.

Bertness M.D. and Gaines S.D. (1993). Larval dispersal and local adaptation in acorn barnacles. *Evolution* 47(1): 316-320.

Bertness M.D., Gaines S.D., Bermudez D., Sanford E. (1991). Extreme spatial variation in the growth and reproductive output of the acorn barnacle *Semibalanus Balanoides*. *Marine Ecology Progress Series* 75: 91-100.

Bertness, M.D., Gaines S.D., Stephens E.G., Yund P.O. (1992). Components of recruitment in populations of the acorn barnacle *Semibalanus balanoides* (Linnaeus). *Journal of Experimental Marine Biology and Ecology* 156(2): 199-215.

Bertness M.D., Gaines S.D., Wahle R.A. (1996). Wind-driven settlement patterns in the acorn barnacle *Semibalanus balanoides*. *Marine Ecology Progress Series* 137: 103-110.

Bertness M.D., Leonard G.H., Levine J.M., Bruno J.F. (1999). Climate – driven interactions among rocky intertidal organisms caught between a rock and a hot place. *Oecologia* 120: 446-450.

Bluck B.J. (1980). Evolution of a strike – slip fault – controlled basin, Upper Old Red Sandstone, Scotland. In: Balance P.F. (ed) Sedimentation in Oblique – slip mobile zones. Blackwell Scientific Publications, London, p 63-79.

Boggs J.R. (2000). Principles of sedimentology and stratigraphy. Merril Publishing Company, Toronto.

Bohn K., Richardson C.A., Jenkins S.R. (2013). The importance of larval supply, larval habitat selection and post – settlement mortality in determining intertidal adult abundance of the invasive gastropod *Crepidula fornicate. Journal of Experimental Marine Biology and Ecology* 440: 132-140.

Brattegard T., Høisaeter T., Sjøtun K., Fenchel T., Uiblein F. (2011). Norwegian fjords: From natural history to ecosystem ecology and beyond. *Marine Biology Research* 7:421-424.

Brind'Amour, A., Bourget E., Tremblay R. (2002). Fecundity, growth rate and survivorship at the interface between two contiguous genetically distinct groups of *Semibalanus balanoides*. *Marine Ecology Progress Series* 229: 173-184.

Broitman B.R., Blanchette C.A., Menge B.A., Lubchenco J., Krenz C., Foley M., Raimondi P.T., Lohse D., Gaines S.D. (2008). Spatial and temporal patterns of invertebrate recruitment along the west coast of the United States. *Ecological Monographs* 78: 403 - 421.

Brown G.C., Hawkesworth C.J., Wilson R.C.L. (1992). Understanding the Earth. Cambridge University Press, Cambridge.

Buchholz F., Buchholz C., Weslawski J.M. (2010). Ten years after: krill as indicator of changes in the macro-zooplankton communities of two Arctic fjords. *Polar Biology* 33: 101-113.

Bullard S.G., Whitlatch R.B., Osman R.W. (2004). Checking the landing zone: Do invertebrate larvae avoid settling near superior spatial competitors? *Marine Ecology Progress Series* 280: 239-247.

Burrows M.T., Harvey R., Robb L. (2008). Wave exposure indicies from digital coastlines and the prediction of rocky shore community structure. *Marine Ecology Progress Series* 353: 1-12.

Burrows M.T., Hawkins S.J., Southward A.J. (1992). A comparison of reproduction in co-occurring chthamalid barnacles, *Chthamalus stellatus* (Poli) and *Chthamalus montagui* Southward. *Journal of Experimental Marine Biology and Ecology* 160: 229-249.

Burrows M.T., Jenkins S.R., Robb L., Harvey R. (2010). Spatial variation in size and density of adult and post – settlement *Semibalanus balanoides*: effects of oceanographic and local conditions. *Marine Ecology Progress Series* 398: 207-219.

Buschbaum C. (2001). Selective settlement of the barnacle *Semibalanus balanoides* (L.) facilitates its growth and reproduction on mussel beds in the Wadden Sea. Helgoland *Marine Research* 55: 128-134.

Butler H. and Rogerson A. (1996). Growth potential, production efficiency and annual production of marine benthic naked amoebae (gymnamoebae) inhabiting sediments of the Clyde Sea area, Scotland. *Aquatic Microbial Ecology* 10: 123-129.

Bythell J.C., Pan P., Lee J. (2001). Three-dimensional morphometric measurements of reef corals using underwater photogrammetry techniques. *Coral Reefs* 20: 193-199.

Caffey H.M. (1985). Spatial and temporal variation in the settlement and recruitment of intertidal barnacles. *Ecological Monographs* 55: 313-335.

Cahill A.E., Aiello-Lammens M.E., Fisher-Reid M.C., Hua X., Karanewsky C.J., Yeong Ryu H., Sbeglia G.C., Spagnolo F., Waldron J.B., Warsi O., Wiens J.J. (2013). How does climate change cause extinction? *Proceeding of the Royal Society (London) Series B* 280(1750): 20121890.

Camus T. and Zeng C. (2009). The effects of stocking density on egg production and hatching success, cannibalism rate, sex ratio and population growth of the tropical calanoid copepod *Acartia sinjiensis*. *Aquaculture* 287: 145-151.

Caro A.U., Navarrete S.A., Castilla J.C. (2010). Ecological convergence in a rocky intertidal shore metacommunity despite high spatial variability in recruitment regimes. *Proceedings of the National Academy of Science USA* 107: 18528-18532.

Castilla J.C. and Varas M.A. (1998). A plankton trap for exposed rocky intertidal shores. *Marine Ecology Progress Series* 175: 299-305.

Castro P. and Huber M.P. (2008). Marine Biology. McGraw-Hill, New York.

Cervin G., Lindegarth M. Viejo R.M., Aberg P. (2004). Effects of small-scale disturbances of canopy and grazing on intertidal assemblages on the Swedish west coast. *Journal of Experimental Marine Biology and Ecology* 302: 35-49.

Chan B.K.K. and Williams G.A. (2003). The impact of physical stress and molluscan grazing on the settlement and recruitment of *Tetraclita* species (Cirripedia: Balanomorpha) on a tropical shore. *Journal of Experimental Marine Biology and Ecology* 284: 1-23.

Charnov E.L. (1987). Sexuality and hemaphroditism in barnacles: a natural selection approach. In Southward A.J. (ed). Barnacle Biology. A.A. Balkema, Rotterdam.

Chen H.N., Todd C.D., Williams G.A., Chan B.K.K. (2013). A new passive plankton trap to quantify the supply of crustacean and molluscan larvae to rocky shores. *Journal of Experimental Marine Biology and Ecology* 441: 80-89.

Cheung W.W.L., Lam V.W.Y., Sarmiento J.L., Kearney K., Watson R., Pauly D. (2009). Projecting global marine biodiversity impacts under climate change scenarios. *Fish and Fisheries* 10: 235-251.

Chiang W.L., Au D.W.T., Yu P.K.N., Wu R.S.S. (2003). UV-B damages eyes of barnacle larvae and impairs their photoresponses and settlement success. *Environmental Science and Technology* 37: 1089-1092.

Clare A.S. (1998). Towards nontoxic antifouling. *Journal of Marine Biotechnology* 6(1): 3-6.

Clare A.S. (2013). A new open access Journal of Marine Science and Engineering. *Journal of Marine Science and Engineering* 1: 1-2.

Cole S.W.B., Scrosati R.A., Tam J.C., Sussman A.V. (2011). Regional decoupling between NW Atlantic barnacle recruit and adult density is related to changes in pelagic food supply and benthic disturbance. *Journal of Sea Research* 65(1): 33-37.

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

Connell J. (2003). Mechanisms determining the limits of vertical distribution of barnacles at Millport, Isle of Cumbrae. UMBSM Annual Report**:** 5.

Connell J.H. (1961a). The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecology* 42(4): 710-723.

Connell J.H. (1961b). Effects of competition, predation, by *Thais lapillus*, and other factors on natural populations of the barnacle *Balanus balanoides*. *Ecological Monographs* 31: 61-104.

Connell S.D. (1999). Effects of surface orientation on the cover of epibiota. *Biofouling* 14: 219-226.

Crisp D.J. (1953). Changes in the orientation of barnacles of certain species in relation to water currents. *Journal of Animal Ecology* 22: 331-343.

Crisp D.J. (1955). The behaviour of barnacle cyprids in relation to water movement over a surface. *Journal of Experimental Biology* 32: 569-590.

Crisp D.J. (1961). Territorial behaviour in barnacle settlement. *Journal of Experimental Biology* 38: 429-446.

Crisp D.J. (1974). Factors influencing the settlement of marine invertebrate larvae. In: Grant P.T. and Mackie A.M. (eds). Chemoreception in Marine Organisms. Academic Press, New York, p 177-265.

Crisp D.J. (1985). Recruitment of barnacle larvae from the plankton. *Bulletin of Marine Science* 37(2): 478-486.

Crisp D.J. and Barnes H. (1954). The orientation and distribution of barnacle at settlement with particular reference to surface contour. *Journal of Animal Ecology* 23: 142-162.

Crisp D.J. and Bourget E. (1985). Growth in barnacles. *Advances in Marine Biology* 22: 199-244.

Crisp D.J. and Ryland J.S. (1960). Influence of filming and of surface texture on the settlement of marine organisms. *Nature (London)* 185: 119.

Crisp D.J. and Stubbings H.G. (1957). The orientation of barnacles to water currents. *Journal of Animal Ecology* 26: 179-196.

Czarnoleski M., Michalczyk L., Pajdak-Stos A. (2004). Substrate preference in settling zebra mussels *Dreissena polymorpha*. *Archiv Fur Hydrobiologie* 159: 263-270.

Darwin C.R. (1854). A monograph on the sub-Class Cirripedia with Figures of all the Species. The Balanidae, (or sessile cirripedes); the Verrucidae of Great Britain. Ray Society, London.

de Wolf, P. (1973). Ecological observations on the mechanisms of dispersal of barnacle larvae during planktonic life and settling. *Netherlands Journal of Sea Research* 6: 1-29.

Delany J., Myers A.A., McGrath D., O'Riordan R.M., Power A.M. (2003). Role of post-settlement mortality and 'supply-side' ecology in setting patterns of intertidal distribution in the chthamalid barnacles *Chthamalus montagui* and *C. stellatus*. *Marine Ecology Progress Series* 249: 207-214.

Denny M.W. (1994). Extreme drag forces and the survival of wind – and water – swept organisms. *Journal of Experimental Biology* 194: 97-115.

Dinning K.M. and Metaxas A. (2013). Pattern abundance of hyperbenthic zooplankton and colonization of marine benthic invertebrates on the seafloor of Saanich Inlet, a seasonally hypoxic fjord. *Marine Ecology* 34: 2-13.

Doherty P.J., Planes S., Mather P. (1995). Gene flow and larval duration in seven species of fish from the Great Barrier Reef. *Ecology* 76: 2373-2391.

Dreanno C., Kirby R.R., Clare A.S. (2006). Smelly feet are not always a bad thing: the relationship between cyprid footprint protein and the barnacle settlement pheromone. *Biology Letters* 2: 423-425.

Dufresne F., Bourget E., Bernatchez L. (2002). Differential patterns of spatial divergence in microsatellite and allozyme alleles: further evidence for locus – specific selection in the acorn barnacle *Semibalanus balanoides*. *Molecular Ecology* 11(1): 113-123.

Eckman J.E. (1996). Closing the larval loop: Linking larval ecology to the population dynamics of marine benthic invertebrates. *Journal of Experimental Marine Biology and Ecology* 200(1-2): 207-237.

Edvardsen A., Zhou M., Tande K.S., Zhu Y. (2002). Zooplankton population dynamics: measuring *in situ* growth and mortality rates using an Optical Plankton Counter. *Marine Ecology Progress Series* 227: 205-219.

Edwards A., Baxter M.S., Ellett D.J., Martin J.H.A., Meldrum D.T., Griffiths C.R. (1986). Clyde Sea Hydrography. *Proceedings of the Royal Society of Edinburgh Section B -Biological Sciences* 90: 67-83.

Edwards K.J., Bach W., Rogers D.R. (2003). Geomicrobiology of the ocean crust: a role for chemoautotrophic Fe – bacteria. *Biological Bulletin* 203: 180-185.

Eerkes-Medrano D., Menge B.A., Sislak C., Langdon C.J. (2013). Contrasting effects of hypoxic conditions on survivorship of planktonic larvae of rock intertidal invertebrates *Marine Ecology Progress Series* 478: 139-151.

Elbourne P.D. and Clare A.S. (2010). Ecological relevance of a conspecific, waterborne settlement cue in *Balanus amphitrite* (Cirripedia). *Journal of Experimental Marine Biology and Ecology* 392 (1-2): 99-106.

Elliott A.J., Ellis K.M., Lynn N.M. (2003). Baroclinic tidal currents in the Gareloch, Scotland. *Estuarine Coastal and Shelf Science* 58(1): 107-116.

Evans F. (1987). The nauplii of *Balanus balanoides* (Cirripedia: Thoracica) taken in January and February in Northumberland waters. *Journal of the Marine Biological Association of the United Kingdom* 67: 815 - 817.

Falkenhaug T. and Dalpadado P. (2014). Diet composition and food selectivity of sprat (*Sprattus sprattus*) in Hardangerfjord, Norway. *Marine Biology Research* 10(3): 203-215.

Finnie A.A. and Williams D.N. (2010). Paint and Coatings Technology for the Control of Marine Fouling. In: Durr S. and Thomason J.C. (eds) Biofouling. Wiley-Blackwell, Oxford, p185-206.

Food and Agriculture Organisation of the United Nations. (2014a). Report of ICES Advisory Committee. Herring in Divisions. Clyde herring, west of Scotland, landings. Retrieved 10/04/2014 from www.fao.org/forms/resource/ReportofICES/10436/en.

Food and Agriculture Organisation of the United Nations. (2014b). Sprattus sprattus Table 1 Catches 2012. Retrieved 10/04/2014 from www.fao.org/fishery/species/2102/en.

Foster B.A. (1970). Responses and acclimation to salinity in the adults of some balanomorph barnacles. *Philosophical Transactions of the Royal Society of London Series B- Biological Sciences* 256: 377-400.

Foster B.A. (1971). On the determinants of upper limit of intertidal distribution of barnacles (Crustacea: Cirripedia). *Journal of Animal Ecology* 40: 33-48.

Fox L.R. (1975). Cannibalism in natural populations. *Annual Review of Ecology and Systematics* 6: 87-106.

Gaines S. and Bertness M.D. (1992). Dispersal of juveniles and variable recruitment in sessile marine species. *Nature* 360: 579 - 580.

Gaines S.D. and Bertness M. (1993). The dynamics of juvenile dispersal - why field ecologists must integrate. *Ecology* 74: 2430-2435.

Gaines S. and Roughgarden J. (1985). Larval settlement rate: a leading determinant of structure in an ecological community of the marine intertidal zone. *Proceedings of the National Academy of Science USA* 82: 3707-3711.

Gaines S., Brown S., Roughgarden J. (1985). Spatial variation in larval concentrations as a cause of spatial variation in settlement for the barnacle, *Balanus glandula*. *Oecologia* 67: 267-272.

Godin J-G. J. (1981) Daily Patterns of Feeding Behaviour, daily rations, and diets of juvenile Pink Salmon (*Oncorhynchus gorbuscha*) in Two Marine Bays of British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 38(1): 10-15.

Garcia C.B. and Moreno I. (1998). Recruitment, growth, mortality and orientation patterns of *Balanus trigonus* (Crustacea: Cirripedia) during succession on fouling plates. *Scientia Marina* 62: 59-64.

Giangrande A., Geraci S., Belmonte G. (1994). Life-cycle and life-history diversity in marine invertebrates and the implications in community dynamics. *Oceanography and Marine Biology an Annual Review* 32: 305-333.

Glasby T.M. and Connell S.D. (2001). Orientation and position of substrata have large effects on epibiotic assemblages. *Marine Ecology Progress Series* 214: 127-135.

Grosberg R.K. (1982). Intertidal zonation of barnacles: The influence of planktonic zonation of larvae on vertical distribution of adults. *Ecology* 63: 894-899.

Grosberg R.K. and Levitan D.R. (1992). For adults only? Supply-side ecology and the history of larval biology. *Trends in Ecology and Evolution* 7: 130-133.

Gude A.R. (2007). Assessment and Implications of the Supply of *Semibalanus balanoides* (L.) Larvae to shores in Fife, East Scotland. Degree of Doctor of Philosophy, University of St. Andrews.

Gyory J. and Pineda J. (2011). High – frequency observations of early – stage larval abundance: do storms trigger synchronous larval release in *Semibalanus balanoides*? *Marine Biology* 158: 1581-1589.

Gyory J., Pineda J., Solow A. (2013). Turbidity triggers larval release by the intertidal barnacle *Semibalanus balanoides*. *Marine Ecology Progress Series* 476: 141-151.

Hall-Spencer J.M. and Atkinson R.J.A. (1999). *Upogebia deltaura* (Crustacea: Thalassinidea) in Clyde Sea maerl beds, Scotland. *Journal of the Marine Biological Association of the United Kingdom* 79: 871-880.

Hancock K.M. and Petraitis P.S. (2001). Effects of herbivorous snails and macroalgal canopy on recruitment and early survivorship of the barnacle *Semibalanus balanoides* (L.). *Journal of Experimental Marine Biology and Ecology* 257(2): 205-218.

Hansson L.J., Hudson I.R., Seddon R.J., Shaw O., Thomason J.C. (2003). Massive recruitment of the barnacle *Semibalanus balanoides* in the Clyde Sea (Scotland, UK) in the spring of 2000. *Journal of the Marine Biological Association of the United Kingdom* 83: 923-924.

Harder T., Thiyagarajan V., Qian P.Y. (2001). Combined effect of cyprid age and lipid content on larval attachment and metamorphosis of *Balanus amphitrite* (Darwin). *Biofouling* 17: 257-262.

Harley C.D.G. (2011). Climate Change, Keystone Predation, and Biodiversity Loss. *Science* 334: 1124-1127.

Havenhand J.N. and Styan C.A. (2010). Reproduction and Larvae / Spore Types. Biofouling. S. Durr and J.C. Thomason (eds). Wiley – Blackwell, Oxford**:** 1-15.

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

Hawkins S.J., Moore P.J., Burrows M.T., Poloczanska E., Mieszkowska N., Herbert R.J.H., Jenkins S.R., Thompson R.C., Genner M.J., Southward A.J. (2008). Complex interactions in a rapidly changing world: responses of rocky shore communities to recent climate change. *Climate Research* 37: 123-133.

Hawkins S.J., Sugden H.E., Mieszkowska N., Moore P.J., Poloczanska E., Leaper R., Herbert R.J.H., Genner M.J., Moschella P.S., Thompson R.C., Jenkins S.R., Southward A.J., Burrows M.T. (2009). Consequences of climate - driven biodiversity changes for ecosystem functioning of North European rocky shores. *Marine Ecology Progress Series* 396: 245-259.

Hayward P.J. and Ryland J.S. (1990). Marine fauna of the British Isles and North-West Europe. Clarenden Press, Oxford..

Head R.M., Berntsson K.M., Dahlstrom M., Overbeke K., Thomason J.C. (2004). Gregarious settlement in cypris larvae: The effects of cyprid age and assay duration. *Biofouling* 20: 123-128.

Helmuth B., Broitman B.R., Blanchette C.A., Gilman S.E., Halpin P., Harley C.D.G., O'Donnell M.J., Hofmann G.E., Menge B.A., Strickland D. (2006a). Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. *Ecological Monographs* 76: 461-479.

Helmuth B., Mieszkowska N., Moore P., Hawkins S.J. (2006b) Living on the Edge of Two Changing Worlds: Forecasting the Responses of Rocky Intertidal Ecosystems to Climate Change. *Annual Review of Ecology, Evolution and Systematics* 37:373-404

Hentschel B.T. and Emlet R.B. (2000). Metamorphosis of barnacle nauplii: Effects of food variability and a comparison with amphibian models. *Ecology* 81(12): 3495-3508.

Herbert R.J.H. and Hawkins S.J. (2006). Effect of rock type on the recruitment and early mortality of the barnacle *Chthamalus montagui*. *Journal of Experimental Marine Biology and Ecology* 334: 96-108.

Highton A. and Hall I.H.S. (1987). Argyll Sheet 55N - 06W Solid Geology. British Geological Survey. N.E.R. Council, Ordnance Survey, Southampton.

Hills J.M. and Thomason J.C. (1996). A multi-scale analysis of settlement density and pattern dynamics of the barnacle *Semibalanus balanoides*. *Marine Ecology Progress Series* 138: 103-115.

Hills J.M. and Thomason J.C. (1998a). The effect of scales of surface roughness on the settlement of barnacle (*Semibalanus balanoides*) cyprids. *Biofouling* 12: 57-69.

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

Hills J.M. and Thomason J.C. (2003a). Recruitment density can determine adult morphology and fecundity in the barnacle, *Semibalanus balanoides*. *Biofouling* 19(3): 205-213.

Hills J.M. and Thomason J.C. (2003b). The 'ghost of settlement past' determines mortality and fecundity in the barnacle *Semibalanus balanoides*. *Oikos* 101(3): 529-538.

Hills J.M., Thomason J.C., Davis H., Kohler J., Millett E. (2000). Exploratory behaviour of barnacle larvae in field conditions. *Biofouling* 16: 171-179.

Hills J.M., Thomason J.C., Milligan J.L., Richardson T. (1998a). Do barnacle larvae respond to multiple settlement cues over a range of spatial scales? *Hydrobiologia* 376: 101-111.

Hills J.M., Thomason J.C., Muhl J. (1998b). A precise and accurate technique for the manufacture of complex three – dimensional surfaces. *Biofouling* 13: 125-136.

Hislop J.R.G. (1986). Resources status, utilisation and conservation: the demersal fishery in the Clyde Sea Area. *Proceedings of the Royal Society of Edinburgh Section B - Biological Sciences* 90:423-437.

Hoch J.M. (2008). Variation in penis morphology and mating ability in the acorn barnacle, *Semibalanus balanoides*. *Journal of Experimental Marine Biology and Ecology* 359: 126-130.

Hoch J.M. (2010). Effects of crowding and wave exposure on penis morphology of the acorn barnacle, *Semibalanus balanoides*. *Marine Biology* 157: 2783-2789.

Holm E.R. and Bourget E. (1994). Selection and population genetic-structure of the barnacle *Semibalanus balanoides* in the northwest Atlantic and Gulf of St Lawrence. *Marine Ecology Progress Series* 113(3): 247-256.

Holm E.R., McClary M., Rittschof D. (2000). Variation in attachment of the barnacle *Balanus amphitrite*: sensation or something else? *Marine Ecology Progress Series* 202: 153-162.

Holmes S.P., Sturgess C.J., Davies M.S. (1997). The effect of rock-type on the settlement of *Balanus balanoides* (L) cyprids. *Biofouling* 11: 137-147.

Holmes, S.P., Walker G., van der Meer J. (2005). Barnacles, limpets and periwinkles: the effects of direct and indirect interactions on cyprid settlement and success. *Journal of Sea Research* 53(3): 181-204.

Holzman R., Reidenbach M.A., Monismith S.G., Koseff J.R., Genin A. (2005). Near-bottom depletion of zooplankton over a coral reef - II: relationships with zooplankton swimming ability. *Coral Reefs* 24: 87-94.

Hughes T.P. (1990). Recruitment limitation, mortality, and population regulation in open systems: a case study. *Ecology* 71(1): 12 - 20.

Hughes T.P., Baird A.H., Dinsdale E.A., Moltsschaniwskyj N.A., Pratchett M.S., Tanner J.E., Willis B.L. (2000). Supply-side ecology works both ways: the link between benthic adults, fecundity, and larval recruits. *Ecology* 81: 2241-2249.

Hung O.S., Thiyagarajan V., Qian P.Y. (2008). Preferential attachment of barnacle larvae to natural multi-species biofilms: Does surface wettability matter? *Journal of Experimental Marine Biology and Ecology* 361: 36-41.

Hurlbut C.J. (1992). Larval Release and Supply Predict Temporal Variation in Settlement of a Colonial Ascidian. *Marine Ecology Progress Series* 80: 215-219.

Hurley A.C. (1973). Fecundity of the acorn barnacle *Balanus pacificus* Pilsbury: a fugitive species. *Limnology and Oceanography* 18: 386-393.

Huthnance J.M. (1997). The PROFILE project: an overview. *Journal of Marine Systems* 12: 249-261.

Hyder K., Aberg P., Johnson M.P., Hawkins S.J. (2001). Models of open populations with space-limited recruitment: extension of theory and application to the barnacle *Chthamalus montagui*. *Journal of Animal Ecology* 70: 853-863.

Hyder K., Johnson M.P., Hawkins S.J., Gurney W.S.C. (1998). Barnacle demography: evidence for an existing model and spatial scales of variation. *Marine Ecology Progress Series* 174: 89-99.

Iliffe J.C. (2005). Datums and map projections for remote sensing, GIS and surveying. Whittles Publishing, Caithness, Scotland.

Inall M., Gillibrand P.A., Griffiths C., MacDougal N., Blackwell K. (2009). On the oceanographic variability of the North-West European Shelf to the West of Scotland. *Journal of Marine Systems* 77: 210-226.

James R.J. and Underwood A.J. (1994). Influence of colour of substratum on recruitment of spirorbid tubeworms to different types of intertidal boulders. *Journal of Experimental Marine Biology and Ecology* 120: 133-143.

Jarrett J.N. (1997). Temporal variation in substratum specificity of *Semibalanus balanoides* (Linnaeus) cyprids. *Journal of Experimental Marine Biology and Ecology* 211: 103-114.

Jarrett J.N. (2000). Temporal variation in early mortality of an intertidal barnacle. *Marine Ecology Progress Series* 204: 305-308.

Jarrett J.N. (2003). Seasonal variation in larval condition and postsettlement performance of the barnacle *Semibalanus balanoides*. *Ecology* 84(2): 384-390.

Jarrett J.N. and Pechenik J.A. (1997). Temporal variation in cyprid quality and juvenile growth capacity for an intertidal barnacle. *Ecology* 78: 1262-1265.

Jeffery C.J. (2000). Settlement in different-sized patches by the gregarious intertidal barnacle *Chamaesipho tasmanica* Foster and Anderson in New South Wales. *Journal of Experimental Marine Biology and Ecology* 252: 15-26.

Jeffery C.J. (2002). New settlers and recruits do not enhance settlement of a gregarious intertidal barnacle in New South Wales. *Journal of Experimental Marine Biology and Ecology* 275(2): 131-146.

Jeffery C.J. (2003). Determination of abundance and distribution of an intertidal barnacle: settlement or post settlement mortality? *Marine Ecology Progress Series* 246: 291-305.

Jeffery C.J. and Underwood A.J. (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.

Jeffery C.J. and Underwood A.J. (2001). Longevity determines sizes of an adult intertidal barnacle. *Journal of Experimental Marine Biology and Ecology* 256: 85-97.

Jenkins S.R. (2005). Larval habitat selection, not larval supply, determines settlement patterns and adult distribution in two chthamalid barnacles. *Journal of Animal Ecology* 74: 893-904.

Jenkins S.R. and Hawkins S.J. (2003). Barnacle larval supply to sheltered rocky shores: a limiting factor? *Hydrobiologia* 503(1-3): 143-151.

Jenkins G.P., Black K.P., Keough M.J. (1999a). The role of passive transport and the influence of vertical migration on the pre-settlement distribution of a temperate, demersal fish: numerical model predictions compared with field sampling. *Marine Ecology Progress Series* 184: 259-271.

Jenkins S.R., Aberg P., Cervin G., Coleman R.A., Delany J., DellaSantina P., Hawkins S.J., LaCroix E., Myers A.A., Lindegarth M., Power A.M., Roberts M.F., Hartnoll R.G. (2000). Spatial and temporal variation in settlement and recruitment of the intertidal barnacle *Semibalanus balanoides* (L.) (Crustacea: Cirripedia) over a European scale. *Journal of Experimental Marine Biology and Ecology* 243: 209-225.

Jenkins S.R., Norton T.A., Hawkins S.J. (1999b). Settlement and post-settlement interactions between *Semibalanus balanoides* (L.) (Crustacea: Cirripedia) and three species of fucoid canopy algae. *Journal of Experimental Marine Biology and Ecology* 236: 49-67.

Johnston B.R., Molis M., Scrosati R.A. (2012). Predator chemical cues affect prey feeding activity differently in juveniles and adults. *Canadian Journal of Zoology – Revue Canadienne de Zoologie* 90(1): 128-132.

Jones S.J., Southward A.J., Wethey D.S. (2012). Climate change and historical biogeography of the barnacle *Semibalanus balanoides*. *Global Ecology and Biogeography* 21: 716-724.

Jonsson P.R., Berntsson K.M., Larsson A.I. (2004). Linking larval supply to recruitment: Flow-mediated control of initial adhesion of barnacle larvae. *Ecology* 85: 2850-2859.

Kasai A., Rippeth T.P., Simpson J.H. (1999). Density and flow structure in the Clyde Sea front. *Continental Shelf Research* 19(14): 1833-1848.

Kato-Yoshinaga Y., Nagano M., Mori S., Clare A.S., Fusetani N., Matsumura K. (2000). Species specificity of barnacle settlement-inducing proteins. *Comparative Biochemistry and Physiology A - Molecular Integrative Physiology* 125: 511-516.

Kendall M.A. and Bedford M.L. (1987). Reproduction and recruitment of the barnacle *Chthamalus montagui* at Aberystwyth (mid-wales). *Marine Ecology Progress Series* 38: 305-308.

Kennedy C.R. and Bush A.O. (1994). The relationship between pattern and scale in parasite communities: a stranger in a strange land. *Parasitology* 109(2): 187-196.

Kent A., Hawkins S.J., Doncaster C.P. (2003). Population consequences of mutual attraction between settling and adult barnacles. *Journal of Animal Ecology* 72: 941-952.

Keough M.J. (1984). Effects of patch size on the abundance of sessile marine invertebrates. *Ecology* 65: 423-437.

Keough M.J. (1998). Responses of settling invertebrate larvae to the presence of established recruits. *Journal of Experimental Marine Biology and Ecology* 231: 1-19.

Khandeparker L. and Anil A.C. (2011). Role of conspecific cues and sugars in the settlement of cyprids of the barnacle, *Balanus amphitrite. Journal of Zoology* 284: 206-214.

Khandeparker L., Anil A.C., Raghukumar S. (2003). Barnacle larval destination: piloting possibilities by bacteria and lectin interaction. *Journal of Experimental Marine Biology and Ecology* 289: 1-13.

King P.A., McGrath D., Morgan R., Fitzgerald O., Mullins P., Raleigh J. (1993). Reproduction and settlement of the barnacle *Semibalanus balanoides* (L.) in Galway Bay. *Proceedings of the Royal Irish Academy B* 93(1): 5-12.

Kingsford M.J. (1990). Linear oceanographic features: a focus for research on recruitment processes. *Australian Journal of Ecology* 15: 391-401.

Klepal W., Gruber D., Pflugfelder B. (2008). Natural cyclic degeneration by a sequence of programmed cell death modes in *Semibalanus balanoides* (Linnaeus, 1767) (Crustacea, Cirripedia Thoracica). *Zoomorphology* 127(1): 49-58.

Klepal W., Retenberger C., Zheden V., Adam S., Gruber D. (2010). Structual peculiarities of the penis of *Semibalanus balanoides* (Linnaeus, 1767) and *Chthamalus stellatus* (Poli, 1791) (Crustacea: Cirripedia: Thoracica). *Journal of Experimental Marine Biology and Ecology* 392(1-2): 228-233.

Klug H. (2009). Relationship between filial cannibalism, egg energetic content and parental condition in the flagfish. *Animal Behaviour* 77: 1313-1319.

Knight-Jones E.W. (1953). Laboratory experiments on gregariousness during setting in *Balanus balanoides* and other barnacles. *Journal of Experimental Biology* 30: 584-598.

Kohler J., Hansen P.D., Wahl M. (1999). Colonization patterns at the substratum – water interface: how does surface microtopography influence recruitment patterns of sessile organisms? *Biofouling* 14(3): 237-248.

Kordas R.L. and Dudgeon S. (2011). Dynamics of species interaction strength in space, time and with development stage. *Proceedings of the Royal Society B (London) – Biological Sciences* 278(1713): 1804-1830.

Kuklinski P., Berge J., McFadden L., Dmoch K., Zajaczkowski M., Nygurd H., Piwosz K., Tatarek A. (2013). Seasonality of occurrence and recruitment of Arctic marine benthic invertebrate larvae in relation to environmental variables. *Polar Biology* 36: 549-560.

Kwasniewski S., Walkusz W., Cottier F.R., Leu E. (2013). Mesozooplankton dynamics in relation to food availability during spring and early summer in a high latitude glaciated fjord (Kongsfjorden), with focus on *Calanus. Journal of Marine Systems* 111-112: 83-96.

Lagos N.A., Castilla J.C., Broitman B.R. (2008). Spatial environmental correlates of intertidal recruitment: a test using barnacles in northern Chile. *Ecological Monographs* 78(2): 245-261.

Largier J.L. (2003). Considerations in estimating larval dispersal distances from oceanographic data. *Ecological Applications* 13(1): S71 - S89.

Larsson A.I. and Jonsson P.R. (2006). Barnacle larvae actively select flow environments supporting post-settlement growth and survival. *Ecology* 87: 1960-1966.

Lathlean J.A., Ayre D.J., Minchinton T.E. (2013). Temperature variability at the larval scale affects early survival and growth of an intertidal barnacle. *Marine Ecology Progress Series* 475: 155-166.

Lawson J., Davenport J., Whitaker A. (2004). Barnacle distribution in Lough Hyne Marine Nature Reserve: a new baseline and an account of invasion by the introduced Australasian species *Elminius modestus* Darwin. *Estuarine Coastal and Shelf Science* 60(4): 729-735.

Leslie H.M. (2005). Positive intraspecific effects trump negative effects in high-density barnacle aggregations. *Ecology* 86: 2716-2725.

Levin L.A. and Bridges T.S. (1995). Pattern and diversity in reproduction and development. In: McEdward L (ed) Ecology of Marine Invertebrate Larvae. CRC Press, p 1-48.

Lewis J.R. (1955). The mode of occurrence of the universal intertidal zones in Great Britain. *Journal of Ecology* 43: 270-290.

Little C. and Kitching J.A. (2005). The Biology of Rocky Shores. Oxford University Press, Oxford.

Lopez D.A. and Gonzalez M.L. (2003). Density-Dependent Effects in *Jehlius cirratus* (Darwin, 1854) (Cirripedia: Chthamalidae) under Different Growth Conditions. *Marine Ecology Progress Series* 24(4): 289-302.

Lopez D.A., Lopez B.A., Burgos G.C., Arriagada S.E., Gonzalez M.L. (2007). Consequences of base modification in hummocks of the barnacle *Austromegabalanus psittacus* New Zealand. *Journal of Marine and Freshwater Research* 41(3): 291-298.

Ma H.G. and Grassle J.P. (2004). Invertebrate larval availability during summer upwelling and downwelling on the inner continental shelf off New Jersey. *Journal of Marine Research* 62: 837-865.

Mace A.J. and Morgan S.G. (2006). Larval accumulation in the lee of a small headland: implications for the design of marine reserves. *Marine Ecology Progress Series* 318: 19-29.

Macpherson E.A., Scrosati R.A., Chareka P. (2008). Barnacle recruitment on ice - scoured shores in eastern Canada. *Journal of the Marine Biological Association of the United Kingdom* 88: 289-291.

Maleschlijski S., Sendra G.H., Di Fino A., Leal – Taixe L., Thome I., Terfort A., Aldred N., Grunze M., Clare A.S., Rosenhahn B., Rosenhahn A. (2012). Three dimensional Tracking of Exploratory Behaviour of Barnacle Cypris Using Stereoscopy. *Biointerphases* 7(1-4). (online paper).

Marfenin N.N., Bolshakov F., Marclashova M. (2013). Fluctuations in settlement and survival of a barnacle *Semibalanus balanoides* in the White Sea intertidal. *Hydrobiologia* 706(1): 51-68.

Marshall D.J. and Keough M.J. (2003). Variation in the dispersal potential of non - feeding invertebrate larvae: the desperate larva hypothesis and larval size. *Marine Ecology Progress Series* 255: 145 - 153.

Martin P. and Bateson P. (2005). Measuring Behaviour. In: Measuring Behaviour. Cambridge University Press, Cambridge, p 6 – 14.

Matassa C.M. and Trussell G.C. (2011). Landscape of fear influences the relative importance of consumptive and non-consumptive predator effects. *Ecology* 921(2): 2258-2266.

Matsumura K., Hills J.M., Thomason P.O., Thomason J.C., Clare A.S. (2000). Discrimination at settlement in barnacles: laboratory and field experiments on settlement behaviour in response to settlement-inducing protein complexes. *Biofouling* 16: 181-190.

Matsuura K. and Qian P-Y. (2014). Larval vision contributes to gregarious settlement in barnacles: adult re fluorescence as a possible visual signal. *Journal of Experimental Biology* 217: 743-750.

Matthews J.B.L., Buchholz F., Saborowski R., Tarling G.A., Dallot S., Labat J.P. (1999). On the physical oceanography of the Kattegat and Clyde Sea area, 1996 - 98, as background to ecophysiological studies on the planktonic crustacean, *Meganyctiphanes norvegica* (Euphausiacea). *Helgoland Marine Research* 53: 70-84.

Mauck R.A. and. Harkless K.C. (2001). The effect of group membership on hiding behaviour in the northern rock barnacle, *Semibalanus balanoides*. *Animal Behaviour* 62: 743-748.

McAdam A.D., Forsyth I. H., Henderson W.G. (1985). Clyde Sheet 55N 06W Solid Geology. British Geological Survey. N. E. R. Council, Ordnance Survey, Southampton.

McClintock J.B., Angus R.A., McClintock F.E. (2007). Abundance, diversity and fidelity of macroinvertebrates sheltering beneath rocks during tidal emersion in an intertidal cobble field: does the intermediate disturbance hypothesis hold for less exposed shores with smaller rocks? *Journal of Experimental Marine Biology and Ecology* 352(2): 351 - 360.

McDougal K. (1943) Sessile marine invertebrates of Beaufort, North Carolina. A study of settlement, growth and seasonal fluctuations among pile dwelling organisms. *Ecological Monographs* 13: 321-374.

McGrath D., King P.A., Reidy M. (1994). Conditioning of artificial substrata and settlement of the marine mussel *Mytilus edulis* L.: a field experiment. *Proceedings of the Royal Irish Academy B* 94: 53-56.

McIntyre F., Fernandes P.G., Turrell W.R. (2012). Scottish marine and freshwater science report: Clyde ecosystem review Report volume 3 number. Retrieved 12/04/2014 from www.nationalarchives.gov.uk/doc/open-government-licence.

McQuaid C.D., Lindsay J.R., Lindsay T.L. (2000). Interactive effects of wave exposure and tidal height on population structure of the mussel *Perna perna*. *Marine Biology* 137: 925-932.

McQuaid C.D. and Miller K. (2010). Larval Supply and Dispersal. Biofouling. S. Durr and J. C. Thomason (eds.). Wiley-Blackwell, Chichester**:** 16-24.

Menge B.A. (2000). Recruitment vs. postrecruitment processes as determinants of barnacle population abundance. *Ecological Monographs* 70: 265-288.

Menge B.A. and Branch G.M. (2001). Marine Community Ecology. Sinauer Associates Inc., Sunderland, M.A.

Meteorological-office. (2012). Western Scotland: climate. Regional Climate Retrieved 14/02/2012, 2012, from http://www.metoffice.gov.uk/climate/uk/ws.

Michaelidis B., Portner H., Sokolova I., Tomanek L. (2014). Advances in Predicting the Impacts of Global Warming on the Mussels *Mytilus galloprovincialis* in the Mediterranean Sea. *The Mediterranean Sea* 1: 319-339.

Mieszkowska N., Kendall M.A., Hawkins S.J., Leaper R., Williamson P., Hardman-Mountford N.J., Southward A.J. (2006). Changes in the Range of Some Common Rocky Shore Species in Britain - A Response to Climate Change. *Hydrobiologia* 555: 241-251.

Minchinton T.E. (1997). Life on the edge: Conspecific attraction and recruitment of populations to disturbed habitats. *Oecologia* 111: 45-52.

Minchinton T.E. and Scheibling R.E. (1991). The influence of larval supply and settlement on the population-structure of barnacles. *Ecology* 72: 1867-1879.

Minchinton T.E. and Scheibling R.E. (1993). Free-Space Availability and Larval Substratum Selection as Determinants of Barnacle Population-Structure in a Developing Rocky Intertidal Community. *Marine Ecology Progress Series* 95: 233-244.

Miron G., Boudreau B., Bourget E. (1995). Use of larval supply in benthic ecology: testing correlations between larval supply and larval settlement. *Marine Ecology Progress Series* 124: 301-305.

Miron G., Boudreau B., Bourget E. (1999). Intertidal barnacle distribution: a case study using multiple working hypotheses. *Marine Ecology Progress Series* 189: 205-219.

Miron G., Bourget E., Archambault P. (1996). Scale of observation and distribution of adult conspecifics: Their influence in assessing passive and active settlement mechanisms in the barnacle *Balanus crenatus* (Brugiere). Journal of Experimental Marine Biology and Ecology 201: 137-158.

Moore H.M. (1933). Change of orientation of a barnacle after metamorphosis. *Nature (London)* 132: 969-970.

Moore P.G. (1975). The role of habitat selection in determining the local distribution of animals in the sea. *Marine Behavioural Physiology* 3: 97-100.

Muxagata E., Williams J.A., Sheader M. (2004). Composition and temporal distribution of cirripede larvae in Southampton Water, England, with particular reference to the secondary production of *Elminius modestus*. *Ices Journal of Marine Science* 61(4): 585-595.

Navarrete S.A. and Wieters E.A. (2000). Variation in barnacle recruitment over small scales: larval predation by adults and maintenance of community pattern. *Journal of Experimental Marine Biology and Ecology* 253: 131-148.

Newell G.E. and Newell R.C. (2006). Marine Plankton a practical guide, Pisces Conservation Ltd, Lymington.

Nybakkon J.W. and Bertness M.D. (2005). Marine Biology an Ecological Approach. Pearson Education Inc., San Francisco.

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.

Olson R.R. (1985). The consequences of short-distance larval dispersal in a sessile marine invertebrate. *Ecology* 66(1): 30-39.

Osman, R. W. and Whitlatch R.B. (1995). The influence of resident adults on recruitment: a comparison to settlement. *Journal of Experimental Marine Biology and Ecology* 190: 169-198.

Osman R.W., Whitlatch R.B., Zajac R.N. (1989). Effects of resident species on recruitment into a community: larval settlement versus post - settlement mortality in the oyster *Crassostrea virginica*. *Marine Ecology Progress Series* 54: 61-73.

Pagett H.E., Abrahams J.L., Bones J., O'Donoghue N., Marles-Wright J., Lewis R.L., Harris J.R., Caldwell G.S., Rudd P.M., Clare A.S. (2012). Structural characterisation of the *N*-glycan moiety of the barnacle settlement – inducing protein complex (SIPC). *Journal of Experimental Biology* 215: 1192-1198.

Palmer M.A., Allan J.D., Butman C.A. (1996). Dispersal as a regional process affecting the local dynamics of marine and stream benthic invertebrates. *Trends in Ecology and Evolution* 11: 322-326.

Palumbi S.R. (1984). Measuring intertidal wave forces. *Journal of Experimental Marine Biology and Ecology* 81: 171-179.

Pechenik J.A. (1999). On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Marine Ecology Progress Series* 177: 269-297.

People J. (2006). Mussel beds on different types of structures support different macroinvertebrate assemblages. *Austral Ecology* 31(2): 271 - 281.

Petraitis P.S., Rhile E.C., Dudgeon S. (2003). Survivorship of juvenile barnacles and mussels: spatial dependence and the origin of alternative communities. *Journal of Experimental Marine Biology and Ecology* 293: 217-236.

Pettijohn F.J., Potter P.E., Siever R. (1987). Sand and sandstone. Springer, New York.

Phang I.Y., Aldred N., Clare A.S., Callow J.A., Vancso G.J. (2006). An *in situ* study of the nanomechanical properties of barnacle (*Balanus amphitrite*) cyprid cement using atomic force microscopy (AFM). *Biofouling* 22 (4): 245-250.

Phang I.Y., Aldred N., Clare A.S., Vancso G.J. (2008). Towards a nanomechanical basis for temporary adhesion in barnacle cyprids (*Semibalanus balanoides*). *Journal of the Royal Society Interface* 5: 397-401.

Phang I.Y., Aldred N., Ling X.Y., Tomczak N., Huskens J., Clare A.S., Vancso G.J. (2009). Chemistry – specific interfacial forces between barnacle (*Semibalanus balanoides*) cyprid footprint proteins and chemically functionalised AFM tips. *The Journal of Adhesion* 85: 616-630.

Phillips N.E. (2006). Natural variability in the size and condition at settlement of 3 species of marine invertebrates. *Integrative and Comparative Biology* 46(5): 598-604.

Pile A.J., Lipcius R.N., van Montfrans J., Orth R.J. (1996). Density dependent settler recruit juvenile relationships in Blue Crabs. *Ecological Monographs* 66: 277–300.

Pineda J. (1991). Predictable upwellings and the shoreward transport of planktonic larvae by internal tidal bores. *Science* 253: 548-551.

Pineda J. (1994a). Internal tidal bores in the nearshore: warm – water fronts, seaward gravity currents and the onshore transport of neustonic larvae. *Journal of Marine Research* 52: 427-458.

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

Pineda J. and Caswell H. (1997). Dependence of settlement rate on suitable substrate area. *Marine Biology* 129: 541-548.

Pineda J. and Lopez M. (2002). Temperature, stratification and barnacle larval settlement in two Californian sites. *Continental Shelf Research* 22: 1183-1198.

Pineda J., Riebensahm D., Medeiros-Bergen D. (2002). *Semibalanus balanoides* in winter and spring: larval concentration, settlement, and substrate occupancy. *Marine Biology* 140: 789-800.

Plant J.A., Watson J.V., Green P.M. (1984). Moine – Dalradian relationships and their palaeotectonic significance. *Proceedings of the Royal Society (London) Series A* 395: 185-202.

Plourde S., Maps F., Joly P. (2009). Mortality and survival in early stages control recruitment in *Calanus finmarchicus*. *Journal of Plankton Research* 31(4): 371-388.

Pol. (2007). Chart datum / ordnance datum. National Tidal and Sea Level Facility, Retrieved various dates in 2007, from www.pol.ac.uk/ntslf/tides/datum.html.

Polman H., Verhaart F., Bruijs M. (2013). Impact of biofouling in intake pipes on the hydraulics and efficiency of pumping capacity. *Desalination and Water Treatment* 51(4-6): 997-1003.

Pond D., Harris R., Head R., Harbour D. (1996). Environmental and nutritional factors determining seasonal variability in the fecundity and egg viability of *Calanus helgolandicus* in coastal waters off Plymouth, UK. *Marine Ecology Progress Series* 143: 45-63.

Porch C.E. (1998). A theoretical comparison of the contributions of random swimming and turbulence to absolute dispersal in the sea. *Bulletin of Marine Science* 62(1): 31-44.

Porri F., Jordaan T., McQuaid C.D. (2008). Does cannibalism of larvae by adults affect settlement and connectivity of mussel populations? *Estuarine Coastal and Shelf Science* 79: 687-693.

Porri F,, Zardi G.I., McQuaid C.D., Radloff S. (2007). Tidal height, rather than habitat selection for conspecifics, controls settlement in mussels. *Marine Biology* 152(3): 631-637.

Prendergast G.S. (2007). Settlement and succession of benthic marine organisms: interactions between multiple physical and biological factors. In School of Biology and Psychology, Degree of Doctor of Philosophy: Newcastle University.

Prendergast G.S. (2010). Settlement and Behaviour of Marine Fouling Organisms. In: Durr S., Thomason J.C. (eds). Biofouling. Wiley-Blackwell, Oxford, p 30-51.

Prendergast G.S., Zurn C.M., Bers A.V., Head R.M., Hansson L.J., Thomason J.C. (2008). Field-based video observations of wild barnacle cyprid behaviour in response to textural and chemical settlement cues. *Biofouling* 24: 449-459.

Przeslawski R., Davis A.R., Benkendorff K. (2005). Synergistic effects associated with climate change and the development of rocky shore molluscs. *Global Change Biology* 11: 515-522.

Pullen J.S.H. and Rainbow P.S. (1991). The composition of pyrophosphate heavy – metal detoxification granules in barnacles. *Journal of Experimental Marine Biology and Ecology* 150: 249-266.

Quinn G.P. and Keough M.J. (2002). Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge.

Raffaelli D.G. and Hawkins S. (1999). Intertidal Ecology. Kluwer Academic Publishers, Dordrecht.

Raimondi P.T. (1988). Rock type affects settlement, recruitment and zonation of the barnacle *Chthamalus anisopoma* Pilsbury. *Journal of Experimental Marine Biology and Ecology* 123: 253-267.

Rasband W.S. (1997-2008). ImageJ. U.S. National Institutes of Health http://rsb.info.nih.gov/ij.

Remer L.C., Heard S.B. (1998). Local movement and edge effects on competition and coexistence in ephemeral-patch models. *American Naturalist* 152: 896-904.

Rilov G., Dudas S.E., Menge B.A., Grantham B.A., Lubchenco J., Schiel D.R. (2008). The surf zone: a semi - permeable barrier to onshore recruitment of invertebrate larvae ? *Journal of Experimental Marine Biology and Ecology* 361(2): 59-74.

Root T.L., Price J.T., Hall K.R., Schneider S.H., Rosenzweig C., Pounds J.A. (2002). Fingerprints of global warming on wild animals and plants. *Nature* 421: 57-60.

Roughgarden J., Iwasa Y., Baxter C. (1985). Demographic theory for an open marine population with space-limited recruitment. *Ecology* 66(1): 54-67.

Rudolf V.H.W. (2008). The impact of cannibalism in the prey on predator-prey systems. *Ecology* 89(11): 3116-3127.

Russell B.C. (1983). The food and feeding habits of rocky reef fish of north-eastern New Zealand. *New Zealand Journal of Marine and Freshwater Research* 17: 121-145.

Sardina P., Cataldo D.H., Boltovskoy D. (2009). Effects of conspecifics on settling juveniles of the invasive golden mussel, *Limnoperna fortunei*. *Aquatic Sciences* 71: 479-486.

Satuito C.G., Shimizu K., Fusetani N. (1997). Studies on the factors influencing larval settlement in *Balanus amphitrite* and *Mytilus galloprovincialis*. *Hydrobiologia* 358: 275-280.

Satumanatpan S. and Keough M.J. (2001). Roles of larval supply and behaviour in determining settlement of barnacles in a temperate mangrove forest. *Journal of Experimental Marine Biology and Ecology* 260: 133-153.

Schmidt P.S., Bertness M.D., Rand D.M. (2000). Environmental heterogeneity and balancing selection in the acorn barnacle *Semibalanus balanoides*. *Proceedings of the Royal Society of London Series B-Biological Sciences* 267(1441): 379-384.

Schultz M.P., Kavanagh C.J., Swain G.W. (1999). Hydrodynamic forces on barnacles: Implications on detachment from fouling-release surfaces. *Biofouling* 13(4): 323-335.

Shimeta J. and Jumars P.A. (1991). Physical mechanisms and rates of particle capture by suspension-feeders. *Oceanography and Marine Biology an Annual Review* 29: 191-257.

Simpson J.H. and Rippeth T.P. (1993). The Clyde Sea: a Model of the Seasonal Cycle of Stratification and Mixing. *Estuarine Coastal and Shelf Science* 37: 129-144.

Singarajah K.V. (1969). Escape reactions of zooplankton: the avoidance of a pursuing siphon tube. *Journal of Experimental Biology and Ecology* 3: 171-178.

Sobolev A.V., Hofmann A.W., Kuzmin D.V., Yaxley G.M., Arndt N.T., Chung S-L., Danyushevsky L.V., Elliott T., Frey F.A., Garcia M.O., Gurenko A.A., Kamenetsky V.S., Kerr A.C., Krivolutskaya N.A., Matvienkov V.V., Nikogosian I.G., Rocholl A., Sigurdsson I.A., Sushchevskaya N.M., Teklay M. (2007). The amount of recycled crust in sources of mantle-derived melts. *Science* 316: 412-417.

Sousa W.P. (1985). Disturbance and patch dynamics on rocky intertidal shores. In: Pickett S.T.A., White P.S. (eds). The ecology of natural disturbance and patch dynamics. Academic Press, London, p101-124.

Southward A.J. (1955). Feeding of barnacles. *Nature (London)* 175: 1124-1125.

Southward A.J. (2008). Barnacles. The Linnean Society of London, London.

Southward A.J. and Crisp D.J. (1956). Fluctuations in the distribution and abundance of intertidal barnacles. *Journal of the Marine Biological Association of the United Kingdom* 35: 211-229.

Starr M., Himmelman J.H., Therriault J.C. (1991). Coupling of Nauplii Release in Barnacles with Phytoplankton Blooms - a Parallel Strategy to That of Spawning in Urchins and Mussels. *Journal of Plankton Research* 13(3): 561-571.

Stubbings H.G. (1975). *Balanus balanoides*. Liverpool University Press, Liverpool.

Sutherland J.P. (1990). Recruitment regulates demographic variation in a tropical intertidal barnacle. *Ecology* 71(3): 955-972.

Svensson C.J., Jenkins S.R., Hawkins S.J., Myers A.A., Range P., Paula J., O'Riordan R.M., Aberg P. (2004). Models of open populations with space-limited recruitment in stochastic environments: relative importance of recruitment and survival in populations of *Semibalanus balanoides*. *Marine Ecology Progress Series* 275: 185-197.

Svensson C.J., Jenkins S.R., Hawkins S.J., Aberg P. (2005). Population resistance to climate change: modelling the effects of low recruitment in open populations. *Oecologia* 142(1): 117-126.

Tamburri M.N., Zimmer R.K., Zimmer C.A. (2007). Mechanisms reconciling gregarious larval settlement with adult cannibalism. *Ecological Monographs* 77(2): 255-268.

Templeton A.S., Staudigel H., Tebo B.M. (2005). Diverse Mn (II) – oxidizing bacteria isolated from submarine basalts at Loihi Seamount. *Geomicrobiology Journal* 22: 129-137.

Tett P. (1992). The ecology of plankton in Scottish coastal waters. *Proceeding of the Royal Society of Edinburgh Section B - Biological Sciences* 100: 27-54.

Thirlwall M.F. (1981). Implications for Caledonian plate tectonic models of chemical data from volcanic rocks of the British Old Red Sandstone. *Journal of the Geological Society* 138: 123-138.

Thomas M.L.H. (1986). A physically derived exposure index for marine shorelines. *Ophelia* 25(1): 1-13.

Thomason J.C., Hills J.M., Bennett A.M. (1997). Dynamics of a massive freshwater influx in the Clyde Estuary. *Estuarine Coastal and Shelf Science* 44(4): 385-393.

Thomason J.C., Hills J.M., Clare A.S., Neville A., Richardson M. (1998). Hydrodynamic consequences of barnacle colonization. *Hydrobiologia* 376: 191-201.

Thomason J.C., Hills J.M., Mapson P. (2000). The consequences of seasonal reproductive strategies for the interpretation of settlement trials. *Biofouling* 16(2-4): 323-329.

Thomason J.C., Hills J.M., Ocampo Thomason P. (2002a). Field-based behavioural bioassays for testing the efficacy of antifouling coatings. *Biofouling* 18(4): 285-292.

Thomason J.C., Le Tissier M.D'A.A., Ocampo Thomason P., Field S.N. (2002b). Optimising settlement tiles: the effects of surface texture and energy, orientation and deployment duration upon the fouling community. *Biofouling* 18(4): 293-304.

Thurstan R. (2008). Changes in the Firth of Clyde Marine Ecosystems over the past 100 years. York University Report. Retrieved 12/04/2014 from www.arrancoast.co.uk.

Thurstan RH, Roberts CM (2010) Ecological Meltdown in the Firth of Clyde, Scotland: Two Centuries of Change in a Coastal Marine Ecosystem. *PLoS ONE* 5(7): e11767. doi:10.1371/journal.pone.0011767

Timmerman M.J. (2004). Timing, geodynamic setting and character of Permo-Carboniferous magmatism in the foreland of the Variscan Orogen, NW Europe. In: Wilson M., Neumann E.R., Davies G.R., Timmerman M.J., Heeremans M., Larsen B.T. (eds) Permo-Carboniferous Magmatism and Rifting in Europe, Vol 223. Geological Society of London, Bath.

Tivy J. (1986). The Geography of the Estuary and Firth of Clyde. *Proceedings of the Royal Society of Edinburgh Section B - Biological Sciences* 90: 7-23.

Todd C.D. (1998). Larval supply and recruitment of benthic invertebrates: Do larvae always disperse as much as we believe? *Hydrobiologia* 376: 1-21.

Todd C.D. (2003). Assessment of a trap for measuring larval supply of intertidal barnacles on wave-swept, semi-exposed shores. *Journal of Experimental Marine Biology and Ecology* 290: 247-269.

Todd C.D., Lambert W.J., Thorpe J.P. (1998). The genetic structure of intertidal populations of two species of nudibranch molluscs with planktotrophic and pelagic lecithotrophic larval stages: are pelagic larvae "for" dispersal? *Journal of Experimental Marine Biology and Ecology* 228(1): 1-28.

Todd C.D., Phelan P.J.C., Weinmann B.E., Gude A.R., Andrews C., Paterson D.M., Lonergan M.E., Miron G. (2006). Improvements to a passive trap for quantifying barnacle larval supply to semi-exposed rocky shores. *Journal of Experimental Marine Biology and Ecology* 332: 135-150.

Tommasi D., Hunt B.P.V., Pakhomov E.A., Mackas D.L. (2013). Mesozooplankton community seasonal succession and its drivers: Insights from a British Columbia, Canada, fjord. *Journal of Marine Systems* 115-116: 10-32.

Toonen R.J. and Pawlik J.R. (1994). Foundations of gregariousness. *Nature* 370: 511-512.

Trager G., Achituv Y., Genin A. (1994). Effects of prey escape ability, flow speed, and predator feeding mode on zooplankton capture by barnacles. *Marine Biology* 120(2): 251-259.

Tremblay R., Olivier F., Bourget E., Rittschof D. (2007). Physiological condition of *Balanus amphitrite* cyprid larvae determines habitat selection success. *Marine Ecology Progress Series* 340: 1-8.

Turner J.T. (1984). The feeding ecology of some zooplankters that are important prey items of larval fish. National Oceanic and Atmospheric Administration Technical Report National Marine Fisheries Service 7, United States of America Department of Commerce. Retrieved 12/04/2014 from spo.nwr.noaa.gov/tr7opt.pdf

Underwood A.J. (1999). Physical disturbances and their direct effect on an indirect effect: responses of an intertidal assemblage to a severe storm. *Journal of Experimental Marine Biology and Ecology* 232: 125-140.

Underwood A.J. and Fairweather P.J. (1989). Supply-side ecology and benthic marine assemblages. *Trends in Ecology and Evolution* 4: 16-20.

Veliz D., Bourget E., Bernatchez L. (2004). Regional variation in the spatial scale of selection at MPI* and GPI* in the acorn barnacle *Semibalanus balanoides* (Crustacea). *Journal of Evolutionary Biology* 17: 953-966.

Veliz D., Bourget E., Bernatchez L. (2006a). Field evidence for an association between growth and protein polymorphism in the acorn barnacle *Semibalanus balanoides*. *Marine Ecology Progress Series* 308: 197-206.

Veliz D., Duchesne P., Bourget E., Bernatchez L. (2006b). Genetic evidence for kin aggregation in the intertidal acorn barnacle (*Semibalanus balanoides*). *Molecular Ecology* 15: 4193-4202.

von der Meden C.E.O., Porri F., Radloft S., McQuaid C.D. (2012). Settlement intensification and coastline topography: understanding the role of habitat availability in the pelagic – benthic transition. *Marine Ecology Progress Series* 459: 63-71.

Walker G. and Yule A.B. (1984). Temporary adhesion of the barnacle cyprid: the existence of an antennular adhesive secretion. *Journal of the Marine Biological Association of the United Kingdom* 64: 679-686.

Walters L.J. (1992). Field settlement locations on subtidal marine hard substrata: is active larval exploration involved? *Limnology and Oceanography* 37(5): 1101-1107.

Warner R.R., Swearer S.E., Caselle J.E. (2000). Larval accumulation and retention: implications for the design of marine reserves and essential fish habitat. *Bulletin of Marine Science* 66: 821-836.

Watson D.I. and Barnes D.K.A. (2004). Temporal and spatial components of variability in benthic recruitment, a 5-year temperate example. *Marine Biology* 145: 201-214.

Weisstein E.W. (2007-2008). MathWorld – A Wolfram Web Resource. Retrieved 12/06 2008, from http://mathworld.com.

Wentworth C.K. (1922). A scale of grade and class terms for classic sediments. *Journal of Geology* 30: 377-392.

Wethey D.S. (1983). Spatial Pattern in Barnacle Settlement - Daily Changes During the Settlement Season. *American Zoologist* 23: 1021-1021.

Wethey D.S. (1984a). Effects of crowding on fecundity in barnacles – *Semibalanus (Balanus) balanoides, Balanus glandula, Chthamalus dalli. Canadian Journal of Zoology – Revue Canadienne De Zoologie* 62(9): 1788-1795.

Wethey D.S. (1984b). Spatial pattern in barnacle settlement: day to day changes during the settlement season. *Journal of the Marine Biological Association of the United Kingdom* 64: 687-698.

Wethey D.S., Woodin S.A., Hilbish T.J., Jones S.J., Lima F.P., Brannock P.M. (2011). Response of intertidal populations to climate: Effects of extreme events versus long term change. *Journal of Experimental Marine Biology and Ecology* 400: 132-144.

White N. (2008). *Semibalanus balanoides*. An acorn barnacle. Retrieved 05/02/2012, from http://www.marlin.ac.uk/speciesbenchmarks.php?speciesID=4328.

Wilding T.A., Duncan J., Nickell L.A., Hughes D.J., Gontarek S., Black K.D., Sayer M.D.J. (2005). Synthesis of Information on the Benthos of SEA 6 Clyde Sea Area. Report to the Department of Trade and Industry, Scottish Association for Marine Science, Oban, Scotland, PA37 1QA.

Wilson J.R. and Harrison P.L. (1998). Settlement-competency periods of larvae of three species of scleractinian corals. *Marine Biology* 131: 339-345.

Wing S.R., Largier J.L., Botsford L.W., Quinn J.F. (1995). Settlement and transport of benthic invertebrates in an intermittent upwellings region. *Limnology and Oceanography* 40: 316-329.

Wright R.A.D. (1981). Wave Exposure Studies on Rocky Shores in Shetland. Imperial College of Science and Technology, University of London, London. PhD.

Wu R.S.S. (1980). Effects of crowding on the energetics of the barnacle *Balanus glandula* Darwin. *Canadian Journal of Zoology - Revue Canadienne De Zoologie* 58: 559-566.

Wu R.S.S. (1981). The effects of aggregation on breeding in the barnacle *Balanus glandula* Darwin. *Canadian Journal of Zoology - Revue Canadienne De Zoologie* 59: 890-892.

Wu R.S.S. and Levings C.D. (1980). Mortality, Growth and Fecundity of Transplanted Mussel and Barnacle Populations near a Pulp Mill Outfall. *Marine Pollution Bulletin* 11: 11-15.

Xiaozhen R., Gang L., Youqin X. (2013). A review on the mechanism of attachment and metamorphosis in barnacle cyprids. *Acta Ecologica Sinica* 33 (16): 4846-4856.

Young C.M. and Gotelli N.J. (1988). Larval predation by barnacles: effects on patch colonisation in a shallow subtidal community. *Ecology* 69: 624-634.

Young E.F. and Holt J.T. (2007). Prediction and analysis of long-term variability of temperature and salinity in the Irish Sea. *Journal of Geophysical Research* 112: C01008 (1-18).

Yund P.O., Gaines S.D., Bertness M.D. (1991). Cylindrical Tube Traps for Larval Sampling. *Limnology and Oceanography* 36: 1167-1177.