

Characterisation of the supply-settlement  
relationship for *Semibalanus balanoides* (L.) along  
a wave swept coast in Fife, East Scotland.

Patrick J. C. Phelan.

Thesis submitted for the degree of Doctor of Philosophy  
University of St Andrews

September 2006

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## **Acknowledgements**

First I would like to acknowledge my supervisor, Professor Chris Todd, for his endless advice, guidance, and support throughout this project. Chris Andrews provided technical support, particularly in the design and construction of the resin cast traps and the experimental array. Adrian Gude and Birgit Weinmann contributed to discussion and decisions as part of the wider mesoscale project of which the present study was a part. Giles Miron and Walter Lambert also contributed advice to this thesis. All of the above mentioned individuals contributed to the collection and processing of the samples and raw data at some point throughout the 3 years of research.

Rory Beaton, Phil Lovell and Colin Hunter at SMRU here in St Andrews designed and developed the wave sensors and software for the present study. Keith Haynes and Andrew Oliver at the Bute workshop constructed the Perspex settlement panels and mounting boards for the experimental arrays. Mike Lonergan provided patient support and advice on statistical matters, particularly the non-linear regression modelling in Chapter 5. Bob Wilson provided technical support in the field in 2003. Pete Baxter and Ian Johnson provided technical and administrative support at the Gatty Marine Laboratory. Mike Ritchie and Jeff Graves offered direction and advice throughout the project. This thesis was funded by a NERC studentship.

Finally I would like to acknowledge Molly LePley, my family and friends for their support, advice and patience. Especially I must thank my parents, whose advice, encouragement, support and, most importantly, their confidence in me spurred me on.

## Abstract

This thesis describes the results of a three year study which collected larvae of the acorn barnacles *Semibalanus balanoides* on a rocky coastline in Fife, East Scotland. The nauplii larvae of *S. balanoides* are released from their parent in springtime in the United Kingdom and develop in the plankton for approximately one to two months. During this period they are transported some distance from the parent population and eventually return to the intertidal shoreline as a cyprid larval stage. The ‘decision’ to settle is a crucial point in the life history of the organism as most sessile organisms cannot move once this has occurred. The supply of larvae to intertidal shorelines was historically neglected until the 1980s. Patterns of settlement were largely considered irrelevant to distribution patterns of adults relative to post-settlement processes such as predation and competition.

Despite the resurgence in “supply-side ecology” in the past 20 years there has been little development towards the measurement of larval supply. Consequently there has been very little description of fine scale or large spatio-temporal studies involving larval supply. This study demonstrates the first study directly addressing larval supply independently from larval settlement at mesoscales (metres to kilometres; days to years). Improvements were made to the passive larval trap described by Todd (2003). A conical opening was combined with the spiral trap design and a number of inlet areas were trialled (0.25cm<sup>2</sup>, 0.5cm<sup>2</sup>, 1cm<sup>2</sup> and 2cm<sup>2</sup>). These were tested across typical wave regimes measured with a new autonomous pressure sensing wave transducer and the 1cm<sup>2</sup> inlet was selected as the most appropriate trap design due to a balance between an increased per unit inlet larval capture and sufficient absolute larval capture so as to identify daily variation in larval supply to a site.

Larval settlement has been studied extensively and is often used as a direct measure of rates of larval supply. It is widely assumed therefore that rates of settlement are a direct reflection of rates of supply, as long as settlement substrates and adult conspecific responses remain uniform. This thesis provides a means of accurately characterising the supply-settlement relationship for *S. balanoides* independent of substrate based responses and demonstrates that this is not the case. The relationship was found to be asymptotic, even at sites where there was low larval supply. It was concluded that density-dependent larva-larva interactions were present during the settlement of larvae and were relevant at daily temporal scales, limiting the rates of larval settlement proportional to larval density. There was no obvious effect on this relationship due to wave action however differences were observed between sites and years. Saturation of preferential environments within the tiles was observed resulting in a settlement preference cascade, with larvae being forced to settle in increasingly less preferable areas of the tiles with increasing larval density. Larva-larva interactions are demonstrated as having a considerable effect on the rates of settlement of *S. balanoides*.

## **Chapter 1 – Introduction**

### **1.1 Larval ecology**

#### **1.1.1 Community dynamics**

The study of the larval ecology of marine communities has a history that stretches back to the 19<sup>th</sup> century. The dual importance of pre- and post-settlement processes in regulating benthic populations was proposed in the 1800s (Morgan 2001). During the 1950s through to the 1970s, British researchers investigated the importance of larval settlement behaviour in maintaining population and community structure (Knight-Jones 1951; Wilson 1953; Meadows and Campbell 1972; Crisp 1974). The benefits for larvae specifically choosing a suitable settlement substratum were recognised as critical to enhancing the survival and reproduction of the many sessile and sedentary benthic organisms that have little or no ability to adjust their position following metamorphosis.

Connell (1961b) demonstrated that larval settlement was not as important as post-settlement mortality in structuring the vertical distribution of barnacles. This study conducted in Scotland led to the historical assumption by investigators from that period that any variation in larval settlement would be damped by variation in post-settlement processes. Competition for space and resources as well as predation (Paine 1974) were emphasised and given precedence in theories concerning the structuring of intertidal communities.

Larval input was seen as important in maintaining diversity on a shore through the intermediate disturbance hypothesis first proposed by Connell (1978). Dominant competitors were restricted from occupying all the available space through a process of regular mortality releasing patches of space that could be re-colonised by the larvae of inferior competitors (Sousa 1984).

A few decades on from this era, the emphasis on post-settlement processes was to shift. Dayton (1984) noted that a considerable degree of variation in rocky intertidal communities was unexplained and there was a failure amongst studies at the time that explored new methods of explaining this variation. Underwood and Denley (1984) demonstrated that competition and predation were responsible for varying degrees of influence on different rocky intertidal communities. If dominant competitors or predators with planktonic dispersive phases did not arrive as larvae in sufficient numbers they could not exert influence over the community structure.

In some communities studied in Australia, where settlement densities were low, it seemed that variation in the rates of settlement regulated community dynamics. Low settlement densities resulted in less competitive interaction. In addition, more available space meant that pulses in larval supply were more likely to survive to adulthood and contribute to the population structure (Underwood and Denley 1984; Caffey 1985).

Connell (1985) was prompted to re-examine previously published data from a wide variety of temporal and tropical latitudes around the world. Although the studies he reviewed were potentially biased (because many measured recruitment some time after post-settlement mortality may have occurred), he found that both larval settlement (= early post-recruitment) and post-settlement mortality were important in the regulation of rocky intertidal communities. The degree to which either pre- or post-settlement processes contributed to community structure generally, however, was still unclear.

To explore and emphasise this newly revised paradigm of rocky intertidal community ecology, a series of studies followed on the coast of California (Gaines and Roughgarden 1985; 1987; Roughgarden *et al.* 1988; Minchinton and Scheibling

1991). These studies provided further empirical evidence that, at low settlement densities, community dynamics were determined by the considerable variation in larval supply and settlement. This was taken a step further by Raimondi (1990) who suggested that even at high densities, patterns of settlement could regulate adult populations. The studies that followed this on rocky intertidal and reef fish communities were reviewed by Booth and Brosnan (1995). They concluded that recruitment limitation regulates a number of species populations when community interactions are low, particularly, for example, where space is not limiting. Recruitment limitation models may be inadequate when community interactions are strong and/or competition for space is high. The recruitment limitation of communities can vary temporally, and therefore over long periods the degree to which recruitment limits populations will depend on the relative temporal frequency of high and low recruitment rates.

### **1.1.2 Consequences of larval recruitment.**

Populations can show distinctive differences in size classes within a shore. This may be as a result of extreme mortality affecting one year class of recruits, and thereby leaving a gap in the population age structure. However, the same result could occur due to variations in settlement between year classes. In addition, high levels of settlement may allow for relatively low per capita rates of predation due to there not being a sufficiently large predator population to affect that year class (Underwood and Keough 2001). Predator outbreaks such as the crown of thorns starfish (*Acanthaster planci*) have been attributed to increased recruitment of individuals producing recruitment-driven top-down regulation in marine communities (Pratchett 2005).

Competition will occur only when there are high enough densities of individuals to limit resources. Then interaction of these individuals through competition for the resource will occur (Underwood and Denley 1984; Connell 1985; Roughgarden *et al.* 1985; Roughgarden *et al.* 1988). The per capita amount of a resource such as food may reduce due to a decrease in the resource, an increase in the number of individuals requiring resource, or interactions between the two (Underwood 1992). Competition is a density-dependent interaction and one of the ways that density can be affected is through differential recruitment. Therefore competition can be 'switched on and off' in communities through variation in supply-side process such as larval input, settlement and ultimately recruitment.

Understanding the role of recruitment in community dynamics requires the development of models combined with empirical work. Roughgarden and colleagues produced a number of detailed models of populations from the middle of the 1980s. In these models settlement is treated as essentially a random factor and the number of settlers per unit area was determined directly by the availability of free space. One of the most important findings was that low recruitment populations can be stable and have diverse size class distributions. Furthermore, populations could fluctuate in stability where recruitment was high. They proposed the destabilising factor was growth. Because sessile benthic organisms such as barnacles grew into one another, interspecific competition would begin affecting mortality, thereby destabilising the population through post-settlement processes as identified by early workers (Barnes and Powell 1955; Connell 1961b; Paine 1974). Primary space would reopen and recruitment and growth would begin again until competition increased, thereby completing the cycle. For low recruitment populations, competition was insufficient

to affect the structure of the community (Gaines and Roughgarden 1985; Roughgarden *et al.* 1985).

### **1.1.3 Larval dynamics: Dispersal and mortality**

Many modern investigators have examined the role of benthic processes in regulating marine populations and communities. The return to the adult population of planktonic larvae was perceived as random and unpredictable. Largely this is due to the difficulty in studying microscopic, widely-dispersed larvae in the ocean (Morgan 2001).

Production of larvae initially requires fertilisation of eggs by sperm. In some species this is achieved through spawning events whereby large numbers of gametes are released into the plankton. Levitan (1995) reviewed studies on the rates of fertilisation success and found that only ~ 20% of the spawned eggs produce larvae across a wide range of organisms and habitats. In high energy habitats, such as the wave-swept intertidal zone or strong tidal flows, propagules may be dispersed rapidly from their source limiting the concentration and proximity of gametes. Sophisticated spawning behaviour exhibited in fish can, however, significantly improve rates of successful fertilisation (Sale 1991). Synchronised spawning of positively buoyant gametes is also thought to improve fertilisation success of many coral species because they concentrate at the surface (Underwood and Keough 2001). Some species such as barnacles utilise internal fertilisation. Aside from the obvious limitations in searching for sessile conspecifics within sparse populations, fertilisation is unlikely to limit these species populations in well populated, aggregated patches.

Following fertilisation, the number of larvae arriving at a shore from the larval pool will be as a result of the larval transport due to water currents, the length of time over which they disperse and the mortality that they suffer during dispersal.

### **1.1.3.1 Larval transport**

All invertebrate larvae can swim and many dispersive stages of algae can swim actively using cilia, flagella or setae. The large majority of larvae, excluding fish and some crustaceans, are unable to attain velocities greater than  $1\text{mm}\cdot\text{s}^{-1}$  (Chia *et al.* 1984). Larvae that disperse over large distances do so passively as a result of tidal and/or ocean currents, rather than active transportation. Larval supply may be determined to a large extent by physical transport processes and therefore understanding these processes in the light of larval transport is an essential pre-requisite to understanding the dynamics of the adult population and community structure.

Pineda (1991; 1994; 1999; 2000) demonstrated larval transport via internal tidal bore warm fronts. These fronts form when warm surface waters recede offshore and cold water surges up from depth. These fronts form up to 2km offshore. As cold water recedes again towards the deep under the influence of gravity the warm water travels back onshore at the surface. 'Slicks' form, indicative of these warm fronts. When the warm fronts enter shallower water they break, much like wind waves and produce an internal bore wave. These bores drag offshore water onshore. This process is common along well-stratified, deep water coastlines such as California. The bores bring with them larvae from the offshore larval pool and provide a mechanism for the coastal return of larvae to the adult populations.

Hawkins and Hartnoll (1982) demonstrated a good correlation with onshore winds and daily patterns of settlement of *S. balanoides* at two sites on the Isle of Man. Daily larval settlement of *S. balanoides* also was correlated with wind patterns in Narragansett Bay, Rhode Island U.S.A., both within and between years. These patterns were reflected in settlement and recruitment on different sides of the bay between years with predominantly northerly or southerly winds (Bertness *et al.* 1996). Todd (2003) quantitatively demonstrated variation in supply and settlement along the Scottish east coast could be driven by onshore and offshore winds.

Jenkins *et al.* (1999) studied fish recruitment in relation to ocean transport models. Fish larvae, like many invertebrate larvae, are capable of vertical migratory behaviour and so passive models of larval transport may not be appropriate. Despite this, they demonstrated a high ability to predict the distribution of larvae based on a hydrodynamic model incorporating prevailing wind conditions and circulation patterns. The addition of behavioural characteristics of the species to the model offered no significant improvement.

Annual patterns in larval supply, settlement and recruitment were influenced by flushing times in Narragansett Bay, Rhode Island, U.S.A. (Gaines and Bertness 1992). In years where there was high river input into the bay, settlement of distinctively large “bay” cyprids was not observed within the bay area. A bimodal size distribution of recruits was observed for the coastal shore population in the same years, and large cyprids were observed settling on the coast and in larval traps deployed close to the coastal shore. Years when flushing times for the bay were long saw retention and recruitment of the “bay” cyprids within the bay area. They were not observed in significant numbers along the open coast.

### 1.1.3.2 Larval delay

The length of larval life is highly variable in marine invertebrate larvae. Additionally, the point at which competence to metamorphose is attained by larvae does not necessarily determine the point at which the larvae will actually metamorphose. This facultative period of delay following competency can vary from hours to many months between species (Pechenik 1990). During the competent/delay phase larvae respond negatively or positively to a suite of cues before selecting a site for settlement and metamorphosis (Crisp 1974). This has been demonstrated extensively in the laboratory but demonstrating larval delay in the field is much more challenging.

Variability in settlement behaviour and the degree to which larvae will delay settlement can be affected by the nutritional state of the larvae. In particular, non-feeding larvae may become increasingly less discriminatory of their settlement habitat with age in the plankton. This is known as the “desperate larva” hypothesis and was first described by Wilson (1953) and Knight-Jones (1951; 1953). Variation in larval size is a good indicator of larval energy reserves for three species of colonial marine invertebrate larvae, the bryozoans *Bugula neritina* and *Watersipora subtorquata* and the ascidian *Diplosoma listerianum*. Marshall and Keough (2003) demonstrated that for all three species larger larvae delayed longer when settlement cues were absent. Large *W. subtorquata* larvae were more discriminatory in their settlement choice than were smaller larvae. They suggested that variation in size of offspring from a given adult may provide a means of varying the dispersal potential of larvae by manipulating the ‘desperation’ of the larvae.

Variation in dispersal also is possible by poecilogony, though its incidence is rare. This is when a species produces two distinctive types of larvae intended for

different periods of dispersal. For example the polychaete *Pygospio elegans* produces two larval forms. Lecithotrophic larvae either remain within the parental tube or hatch early and feed in the plankton before settling (Morgan *et al.* 1999).

Toonen and Pawlik (2001) cultured larvae of the fouling tube worm *Hydroides dianthus* and then allowed them to settle on slides that were biofilmed or slides that were biofilmed and had conspecific adults glued to them. Their results demonstrated the existence of two distinct types of larvae in the population produced from a single parent; larvae that showed a gregarious response and larvae that would settle where there were no conspecifics, just the biofilm cue. The latter were termed ‘founders’ and it was proposed that these individuals allowed for the colonisation of new sites.

### **1.1.3.3 Larval mortality**

A single individual may produce millions of eggs in its lifetime (Barnes 1989). Assuming a population remains stable there must be extremely high mortality between egg production and recruitment to the population.

Estimating larval mortality rates is made difficult by their small size and the large spatial scales over which they potentially disperse. Some studies have identified rates of larval mortality by following larvae from release, avoiding the need for marking larvae (Stoner 1992). However most studies that have successfully followed larvae from release are biased towards species with large larvae that disperse short distances (Underwood and Keough 2001).

Morgan (1995) reviewed the potential causes of planktonic larval mortality. Physiological stress can be effected in six ways:

1. Temperature
2. Salinity

3. Low dissolved oxygen
4. Pollution
5. UV radiation
6. Poor nutrition

Predation is generally considered to be the main source of larval mortality in the plankton. Predation can be attributed to pelagic invertebrates, benthic invertebrates and fishes. There are a number of anti-predatory adaptations exhibited by larvae, which suggest that planktonic predation has a major influence on larvae. Morphological defences include nematocysts, spines and shells amongst others. Chemical defences are found in echinoderm and ascidian larvae. Many behavioural mechanisms to avoid mortality have been observed in larvae. These include shadow avoidance, diel vertical migration and akinesis (playing dead). Additional factors affecting planktonic larval mortality include sinking and advection (Morgan 1995).

Variability in recruitment of species that do not demonstrate an extensive larval phase can still be related to pre-adult mortality. Spight (1974; 1975) demonstrated that the numbers of dog whelks (*Thais/Nucella* spp.) varied because of predation and chance disturbances (e.g. fresh water) of their egg capsules affected hatching and recruitment success. Generally, however the degree of larval mortality can be expected to be positively correlated with the length of the planktonic dispersal period.

Larval mortality and per capita recruitment failure is thought to be large for dispersive larvae. However, widespread dispersal of larvae and the potential for extensive delay of metamorphosis has the advantage that offspring have a higher probability of encountering sparsely available suitable habitat. Animals that are produced and dispersed locally risk the local habitat becoming unsuitable and this

extinguishing the population. Over a long period of time it would be expected that a habitat will eventually experience some disturbance. Understanding the processes affecting larval mortality will enable ecologists to gain an understanding of the regulation of larval supply to adult populations.

## **1.2 Measuring larval supply**

### **1.2.1 Instantaneous methods**

Recently there has been a shift away from a purely post-settlement approach to community ecology, towards a renewed emphasis on the pairing between supply-side processes and recruitment. Patterns of distribution and abundance in the arrival of larvae have received increased attention as a result. Typically larval supply has been measured by way of either net tows with flow meters or pump sampling of known volumes to give concentrations of larvae.

Grosberg (1982) observed larval abundance patterns each hour from a floating dock. He examined three 24hr periods, obtaining three replicate samples of 20L taken at four depths relative to the surface. The samples were obtained using an air lift – a water column sampling tube powered by a SCUBA unit. From these samples Grosberg determined that the vertical distribution of two *Balanus* species in the water column was qualitatively similar to their distribution on the shore.

Gaines *et al.* (1985) were able to sample larval abundances from triplicate plankton net tows taken for 5 minutes, at each of three sites in Monterey Bay, California at irregular frequencies separated by a few days to once every three weeks. The net samples were taken by boat kept at idle speed to maintain location, as close as possible to the rocky shore. The following year they obtained plankton samples using a pump sampler. A hose was run down the shore to within close proximity to the

settlement sites. This enabled more consistent and frequent measures of larval abundance on the shore every 7-10 days. Between years, settlement rates were observed to differ by nearly two orders of magnitude and this was reflected in the pump and net tows. Within years, there was a high correlation between concentration of larvae measured by either pumps or net tows and settlement.

Ross (2001) sampled cyprid abundance of two species through the winter months in the relatively calm sea water conditions of a mangrove swamp in Sydney, Australia. She used plankton net tows taken either by hand or from a boat. Two samples lasting 5 minutes were taken five times over a period of 15 mo. at each of two sites. The concentrations of cyprids at different distances from the seaward edge and different heights in the water column were correlated with patterns of settlement in the mangrove swamp.

Vertical zonation of planktonic larvae of *Semibalanus balanoides* was identified by pump samples taken by Miron *et al.* (1995). Five different depths were sampled daily for 5 min over 10 days. A great deal of the variation in larval settlement at the lower levels was explained by variation in larval concentrations at those depths. However no other locations demonstrated significant correlation between daily larval concentrations and settlement.

All these studies demonstrate that larval availability is crucial to settlement and both supply and settlement can influence population dynamics across spatial or temporal scales. However all these studies imply that concentration alone is sufficient to explain much of the variation in settlement. Variation in rates of flux can interact with concentration to vary rates of larval availability.

### 1.2.2 Integrated methods

Plankton populations are not necessarily evenly distributed throughout the water column. Their distributions are often aggregated in space and time (Roughgarden *et al.* 1988). Larval aggregations in the water column can be due to behavioural characteristics of the larvae such as responses to light and pressure (Stubbings 1975) or hydrodynamic factors concentrating plankton (Eckman 1996; Pineda 2000).

As a result, from the perspective of assessing larval input to the benthos it is important to integrate measures of larval abundance over time. Counts of larval settlement are integrated over time and yet typical measures of larval supply are generally insufficiently integrated to be comparable (Ross 2001). Attempts to integrate over temporal scales with instantaneous measures of supply restrict spatial scales because the methods often require some extent of manual operation by the investigator. Automated devices can be bulky, expensive and impractical for replication over appropriate and ecologically informative spatial scales (Whitlatch and Osman 1998). Similarly, the integration of instantaneous methods over large spatial scales will limit temporal replication (Gaines *et al.* 1985).

A number of devices have been developed in attempts to integrate larval supply over time. Setran (1992) developed a horizontal trap that could sample larvae in the intertidal under mild wave crash conditions. The device comprised a flow-through design with a one-way 'trap door' inlet at one end to allow water to surge through the trap. Netting at the outflow end allowed the trap to filter and retain larvae. Yan *et al.* (2004) developed a vertically oriented flow-through trap design, with a one-way valve system and which also utilised net filtration to capture larvae.

Modified sediment traps comprising a high aspect ratio (= length of cylinder divided by width of inlet  $> 12$ ) and including a killing solution to provide a means of trapping larvae passively encountering the substrate. This is a potentially ideal means for measuring larval supply because the traps continuously integrate over time and do not suffer from fouling to the same extent as net designs (Hannan 1984; Yund *et al.* 1991; Gaines and Bertness 1993). In addition, such devices are inexpensive and easy to build, require limited attention and due to their autonomous nature can be deployed in environments that are difficult to access repeatably (Comtet *et al.* 2000; Metaxas 2004).

Despite their advantages, tube traps have been seldom used in studies of larval supply because their deployment in a wave swept-environment has been untested. The only previous studies used traps whose size required them to be deployed in deep water away from settlement substrate.

### **1.3 Larval settlement**

The dispersive planktonic larvae of most marine organisms undergo metamorphosis upon encounter and/or detection of suitable, site-specific cues. The number of interactions amongst settlement cues can vary but these frequently are complex. Metamorphosis often involves a marked transformation in body structure. Both sedentary and especially sessile, benthic marine organisms usually incur the loss of swimming appendages at metamorphosis and the development of pronounced body structures (and cement secretions) result in the organism being unable to return to the plankton. For truly sessile organisms such as barnacles the point of settlement determines the location of the adult, largely determining the organism's potential for survival and reproduction from that point forward.

### 1.3.1 Settlement in flow

Larvae are able to respond to settlement cues even in the most turbulent regimes by taking advantage of characteristics of the fluid environment. There are areas of low flow in the lee area of rocks and shore structures and these can result in concentrations of larvae able to exhibit responses to cues despite the high mean energy of the fluid environment in which they are located. For example, larvae of *Gobiosoma boscii*, a benthic oyster reef fish, aggregate in low-flow areas, down current of rocks. Breitburg *et al.* (1995) demonstrated that settlement of this species occurred in aggregated patterns that reflected those of the larval distributions at fine scales. Spatial distributions of prey did not explain these patterns.

The low swimming speeds of most planktonic larvae ensure that hydrodynamic processes play a major role in determining the rate of contact of larvae with the substrate (Chia *et al.* 1984; Butman 1987). Because of the turbulent nature of intertidal environments larvae are able to contact the substratum frequently and therefore ‘sample’ a considerable number of substrate locations and select a primary location for settlement (Denny 1988; Denny and Shibata 1989). Behavioural components such as intermittent swimming or sinking may further influence the rate of contact of larvae (Fuchs *et al.* 2004). For most species contact does not necessitate settlement and metamorphosis. Exploration and evaluation of the substrate is common and settlement follows the response to a cascade of positive and negative cues (Crisp 1974; Rittschof *et al.* 1998).

Larvae are able to explore the substrate due to the fundamental properties of fluids. At a fluid-solid interface fluid molecules bind to the surface of the solid creating a phenomenon known as the non-slip condition. The viscosity of the fluid creates a velocity gradient that increases with distance from the fluid-solid interface.

Within this boundary layer organisms experience far less drag than they would in the mid-stream flow. This enables them to explore the substrate more fully even under turbulent or high flow conditions (Denny 1988). Turbulence can penetrate the boundary layer and in high wave crash conditions water velocities can extend right down to the substrate. Upward limits of turbulent flow for attachment of larvae have been identified in some species (Crimaldi *et al.* 2002).

### **1.3.2 Cue responses**

Settling marine invertebrate larvae come into contact with a large number of potential cues as they approach the substrate. The cues they encounter can either encourage or discourage settlement. They can be physical or chemical, soluble or surface bound, and biotic or abiotic in origin. Differential settlement is a large factor influencing recruitment on hard substrata and recruitment is increasingly recognised as playing an important role in community dynamics.

Settling larvae of the barnacle *Semibalanus balanoides* demonstrate a propensity to settle on textured and structured surfaces (Crisp and Barnes 1954). This response has been termed “rugophilia” and encompasses the settlement response to grooves and cracks, pits and edges. These surfaces are likely to provide a better purchase during settlement and protection from hydrodynamic influences. Rough texture also increases the rate of larval delivery and settlement to a substrate (Wisely 1959).

A strong preference is shown for gregarious settlement. The presence of cohorts stimulates settlement as a result of contact with a surface-bound compound termed “arthropodin” by early authors (Knight-Jones 1953; Crisp and Meadows 1962; Crisp and Meadows 1963; Crisp 1974). The examination and characterisation of this

compound has been expanded on extensively since this early work (Clare and Matsumura 2000).

Additional cues to larval settlement of barnacles include flow (Crisp 1955), rock type (Caffey 1982), biofilms (Todd and Keough 1994; Keough and Raimondi 1995; Keough and Raimondi 1996) surface chemistry (Rittschof *et al.* 1998) and colour (James and Underwood 1994).

#### **1.4 *Semibalanus balanoides***

The species chosen for study in this thesis is the acorn barnacle *Semibalanus balanoides* L. (Arthropoda: Crustacea: Cirripedia: Thoracica: Balanomorpha). *S. balanoides* is a boreo-arctic species found as an adult attached to solid substrate in the eulittoral zone of intertidal shores. It is distributed on both sides of the Atlantic and Pacific oceans in the northern hemisphere (Stubbings 1975). In Europe its range extends from NW Spain to Spitzbergen (Jenkins *et al.* 2000). Due to the abundance and ecological importance of *S. balanoides* throughout Western Europe and Atlantic North America, the species is of considerable interest to studies of physiological, behavioural and ecological realms in science. The economic importance of sessile barnacles lies largely in the field of biofouling. The organisms attach to ship hulls and generate drag, slowing down vessels such as naval ships and commercial transporters. The consequences to this are decreased tactical facility of warships and increased fuel costs. Fouling organisms can also affect offshore and coastal marine structures such as oil rigs (Yan and Yan 2003) and power cooling stations (Taylor 2006).

*S. balanoides* is an obligate cross-fertilising hermaphrodite. All individuals have fully functioning male and female reproductive organs. *S. balanoides* has a single breeding season restricted in the British Isles to late autumn and winter.

Fertilisation occurs essentially simultaneously (over a few days) amongst all individuals within a locality. The dates of fertilisation vary from north to south, with more northerly sites generally preceding southerly sites in timing of fertilisation. Fertilisation requires the individual to locate a mate by extending the penis from inside the shell. The penis is inserted into the mantle cavity and a mass of sperm is deposited. Individuals may be fertilised by more than a single male. Oviposition is stimulated through this process ensuring obligatory cross-fertilisation.

Eggs are incubated throughout winter and nauplii larvae are released in springtime. The larvae are released as a result of a stimulating factor produced by the adult when feeding on spring phytoplankton blooms commences following the dark winter months. This ensures the planktotrophic larvae time their release to coincide with a planktonic sufficient food source (Morgan 1995).

*S. balanoides* nauplius larvae progress through six developmental stages in the plankton over a period of six to eight weeks. Advantages to a dispersive phase in an organism's life history are wide distribution of offspring, gene flow and coexistence with disturbance (Abelson and Denny 1997). By dispersing larvae to distant areas over time, species can avoid extinction due to the patchy spatio-temporal nature of suitable habitats (Underwood and Keough 2001).

Following this period they metamorphose into lecithotrophic cyprid larvae. *S. balanoides* can delay metamorphosis up to 28 days (Pechenik 1990) during which time a suitable habitat on which to settle is selected. The cyprid body plan consists of swimming appendages, food reserves upon which it is wholly dependent up to four days following metamorphosis into the adult, and the attachment organs. The attachment organs are antennules that protrude ventro-laterally from the anterior part

of the body. A number of chemo- and mechano-sensory roles have been demonstrated for these organs.

In Fife, Scotland settlement takes place from late March through to early June. When competent to settle, cyprid larvae become photonegative and swim down towards the substrate (Barnes and Powell 1955). Once in contact with the surface cyprids become indifferent to light and exploration of the surface commences (Crisp 1961).

When a sufficient settlement response is initiated the cyprid commences settlement. The larvae will orientate to light and water flow along fine cracks, grooves and depressions. Attachment is by cement secreted from the cement glands, which open at the penultimate, sucker-like segment of the antennules. This cement hardens and from that point the barnacle is unable to move from its position. Metamorphosis into the adult form follows and is completed within 24 hours. This stage is described as the 'spat' and the development of a number of body features such as the shell plates and gut continue from this point (Stubbings 1975).

### **1.5 Aims of study and wider implications**

This thesis will attempt to describe the larval supply of barnacles to the rocky shore and relate that process to the process of larval settlement. Barnacles, like many sessile marine animals, produce dispersive larvae. However unlike many bryozoans, furoid algae and spirobid polychaetes, barnacles produce larvae with extensive dispersal potential, moving them far from the original location of their parent. For species with large dispersive stages, the task of returning to the shore and identifying a new and suitable place to settle is arguably more complex. The supply of larvae to a location and the relative settlement of those larvae is crucial in understanding the

ecology of sessile benthic communities and yet it has not been described in any detail due to restrictions on the measurement of settlement and, particularly, larval supply. This thesis will describe a means of quantifying the supply and settlement of larvae to a shore simultaneously and at fine scales. Understanding this relationship is crucial to distinguishing constraints in settlement driven by larval supply alone from that of physical and biological constraints on settlement rates as well as providing a means for understanding settlement responses beyond that of substrate determination, as previously described in laboratory and field studies, such as wave action.

The rate of observed recruitment of organisms into an environment depends on three things; (1) the largely passive supply of larvae, (2) the rates of settlement, of which the latter are driven by larval behaviour, and (3) post-settlement mortality up to the point of census by the investigator (Keough and Downes 1982). If larvae respond strongly to cues then variation in settlement may be primarily a result of larval behaviour. If, however, variations in larval supply outweigh variation in larval substrate selection then understanding cue responses of larvae alone is of limited predictive value in quantifying recruitment.

Understanding how selective factors influence critical events in the life cycle of species that make up the constituents of intertidal communities is important to developing models and understanding stock-recruitment relationships. Studies of larval mortality, behaviour and post-settlement mortality have been of great interest in marine population biology throughout the 20<sup>th</sup> century, particularly in order to elucidate the stock-recruitment relationship of commercially and ecologically important species (Morgan 2001).

Despite this, the predictive powers of stock-recruitment models are notoriously weak. This is largely because the processes determining variation

throughout the many and complex life history stages of marine organisms are not necessarily examined or quantifiable. Yoshioka (1986) documented discrete events throughout the life history of *Membranipora membranacea*, a species of bryozoan. He demonstrated that stock size predicted recruit density only when larval abundance was incorporated into a model with biotic and abiotic factors (predation, upwelling and temperature). Larvae of this species are particularly large and do not disperse far; hence the tight correlation between stock size and larval abundance. More dispersive species exhibit a much more complex planktonic dispersal period (Eckman 1996). The variation in benthic zooid densities was seemingly unrelated four to six weeks following recruitment in Yoshioka's study, suggesting post-recruitment processes had a large effect on stock densities.

The work in this thesis is focused on the transitional point linking variation in abundance of larvae at the benthic (settlement and beyond) and dispersive stages (planktonic period prior to settlement) by demonstrating variation in larval supply and settlement. Integrating aspects of the biology and ecology of larval and post-larval stages are vital if significant advancement of community ecology is to proceed (Eckman 1996; Morgan 2001).

Due to the considerable spatial and temporal variation in community characteristics and recruitment, describing coastal community dynamics requires studies that cover broad temporal and spatial scales. In the present study, passive larval traps similar to those of Yund *et al.* (1991) were used to quantify rates of larval supply. Further modification were made to traps which had previously been developed by Todd (2003) to increase the per area inlet capture rates so as to provide better resolution and consistency in measuring larval input. The traps were replicated within sites and combined with replicated artificial settlement substrate. This substrate

was developed to provide high rates of settlement per unit area in order to elucidate the true nature of the supply-settlement relationship. Previous studies (Bertness *et al.* 1992) demonstrated a positive relationship between rates of larval supply and settlement that varied temporally and spatially. Despite this the relationship was quantitatively weak and poorly described. This was because the larval traps were deployed sublittorally and 10 – 45 m from the settlement substratum in the sublittoral and collected fortnightly. The array used in the present study utilises a means of accurately and concisely measuring supply and adjacent settlement of larvae. The shores were surveyed daily and provided a profile of within- and between-year settlement across multiple sites. These data were studied in light of abiotic and biotic factors to understand their relative importance to this stage in the life history of a typical benthic marine organism. Variation in the supply-settlement relationship due to variation in turbulent wave action typical of a rocky intertidal shore was examined to demonstrate the applicability of the experimental array in understanding the relative importance of cue responses in larvae compared to rates of larval input in a natural environment.

The short field work season typical of species that exhibit seasonal life cycles restricts the logistical framework of empirical studies in natural environments. The development of autonomous devices that are able to integrate measures of relevant factors over time, whilst minimising cost, are crucial to field ecologists. Demonstrated here is a method for obtaining supply, settlement and hydrodynamic data on a wave swept intertidal shore that satisfies these requirements.

## Chapter 2 - Wave Sensors

### 2.1 INTRODUCTION

#### 2.1.1 Wave action and ecology

The movement of fluids around an organism in the intertidal environment exerts a structuring force of potentially paramount importance on marine organisms, populations and communities. In particular, flow driven by wave action has a considerable effect on these factors. Wave action affects biological organisms in intertidal environments through several factors.

##### 2.1.1.1 External fertilisation

In order to understand the rates of fertilisation an understanding of how water movement affects the mixing and dispersal of gametes is necessary. Predicting fertilisation success in the laboratory is simple; however, measuring the rates of fertilisation in a wave-swept rocky shore environment is far more complex because of the highly variable and inaccessible nature of the environment (Levitan 1995). Organisms that release gametes into the water column potentially suffer from very high rates of sperm and egg dilution in turbulent environments (Denny and Shibata 1989).

Some organisms release gametes only during periods of low turbulence and this sensitivity to wave action can lead to an increase in successful external fertilisation. The macroalga *Fucus vesiculosus* (Baltic Sea) released high numbers of gametes only when maximal water velocities were below  $\sim 0.2\text{m}\cdot\text{s}^{-1}$ . In these instances natural fertilisation success measured in the field was close to 100% (Serrao *et al.* 1996).

### **2.1.1.2 Delivery of food**

Most benthic suspension feeders are sessile and rely on water to bring them food. A regular rate of flow within the environment is therefore important for supplying nutrients to these organisms, however flow rates can also be limiting to feeding at high levels. Moderate levels increase feeding but high levels inhibit them (Ackerman 1999).

Dominant benthic organisms such as *Mytilus* spp. can be influenced in their distributions according to wave exposure. *M. californianus* is a dominant species on wave-exposed coasts of the U.S.A. *M. trossulus* is dominant on wave sheltered environments. Differences in their growth rates across different turbulent flow regimes in the laboratory led Ackerman *et al.* (2004) to suggest flow may be significant in determining the post-settlement ecology of this group of organisms in the field.

Pelagic predators have been shown to increase their feeding rates with turbulence. This was predicted by models of turbulent systems demonstrating increased encounter rates of predators with prey during periods of high turbulence. Adamik *et al.* (2006) demonstrated that the number of prey consumed by *Clytia gracilis*, a passive, pelagic foraging hydroid in laboratory conditions demonstrated a bell shaped pattern with increasing turbulence. This suggested that feeding increased with turbulence initially, then decreased at high levels of turbulence.

### **2.1.1.3 Distribution and settlement of larvae**

Passive delivery and deposition of larvae, combined with settlement behaviour of the organisms, affects the abundance patterns of settlers and recruits. Despite historical perspectives suggesting the two processes have variable relevance to

community structure and distribution patterns, both factors are important in determining the ecology of marine systems (Butman 1987).

In an intertidal environment the instantaneous directional force of a breaking wave and the viscosity of the fluid produce turbulence. Turbulence is the forceful shearing of a fluid producing eddies. Turbulence can influence the distribution patterns of larvae. Sinking or swimming speeds of larvae or spores are small relative to the large speeds and accelerations induced by breaking waves (Chia *et al.* 1984; Denny 1988). This suggests that larvae and spores will often have very limited abilities to affect their delivery rates to the substrate in highly turbulent environments. Denny and Shibata (1989) demonstrated this theoretically. They modelled the rate of contact expected from variation in turbulence and height of larvae in the water column. The predicted rapid mixing of water in the surf zone ensured that larvae were rapidly transported to the substratum from the water column. The models demonstrated that turbulence in the wave-swept environment could provide an important mechanism to facilitate the exploratory behaviour of larvae.

In two experiments Eckman and Duggins (1998) studied the settlement of five organisms (*Balanus* sp., *Mytilus trossulus*, *Psuedochitinopoma occidentalis*, *Tubilipora* spp. and *Eupolymnia heterobranchia*). Larval supply predicted from variable flow explained the majority of variability in settlement on pipe walls. However, orientation of the settlement of larvae varied in a manner not predicted by larval supply suggesting the larvae responded to flow when they settled.

Jenkins (2005) suggests that horizontal adult abundance patterns of two chthamalid barnacle species were determined by patterns of larval settlement. This was not due to heterogeneity of supply but as a result of differential larval settlement behaviour because the larval pool was demonstrated to be well mixed. The sites

between which differences in settlement were observed were distinguished as wave sheltered and exposed (Jenkins 2005).

#### **2.1.1.4 Post-settlement mortality**

Post-settlement processes have long been recognised as having a significant effect on structuring densely populated rocky intertidal shores (Connell 1961b; Paine 1974; Connell 1985) and the effects of wave exposure on marine intertidal communities is long established (Moore 1958; Lewis 1964).

In Monterey Bay, California *Mytilus californianus* dominates shores with high exposure to waves. On wave sheltered shores, *M. galloprovincialis* and *M. trossulus* are prevalent. Johnson and Geller (2006) demonstrated rates of settlement for both species were high at the wave-exposed site. Despite this, adults were dominated by *M. californianus*. This suggests post-settlement mortality affected the adult distribution of *M. galloprovincialis* and *M. trossulus*. *M. californianus* larvae did not settle in high densities on the sheltered shore and, despite not having a measure of larval supply, it was suggested that this pattern of larval abundance on the shore demonstrates a behavioural response to flow (Johnson and Geller 2006).

Studies have demonstrated the effects of sedimentation rates at exposed and sheltered sites on rocky intertidal reefs. Coarse sediment, such as sand and gravel, can scour surfaces and damage organisms or even remove them entirely. Filter feeders can suffer from clogging due to sedimentation. Some species have adapted resistance to sand abrasion. The rates of sedimentation depend to a large extent on the flow and turbulence of water in an environment. This process affects the movement and suspension of particles. The rates of cover, depth and flux of sediments vary with rates of turbulence (Schiel *et al.* 2006).

### **2.1.2 Design specifications for pressure sensing data logger**

Standardising methods within ecology is important if the development of generalised models and the testing of these models is to be accomplished. Lindegarth and Gamfeldt (2005) state that empirical tests of theories require a method incorporating appropriate statistical inferences that account for the natural variability and the sampling error typical of ecological systems and data. Wave exposure has been unequivocally identified as a structuring agent in rocky intertidal marine communities; however due to the physical complexity of wave action, quantitative, continuous data have rarely been obtained in ecological studies (Lindegarth and Gamfeldt 2005). As a result it may be possible that different studies may classify areas of similar levels of wave exposure differently. Quantitative measures of wave action allow for continuous, standardised, categorisation of wave exposed shores.

Also, the role of scale is critical to ecological theory. In order to generalise models the principles must scale accordingly. Denny *et al.* (2004) discuss the importance of measuring variables at relevant scales. Wave action, like many variables, varies considerably within a shore. The ability to accurately relate factors such as settlement and wave action within a highly variable environment such as the intertidal zone requires that these factors be measured within close proximity to one another.

This chapter is concerned with the development and immediate application of an autonomous data logging wave sensing pressure transducer to the measurement of wave crash on the intertidal shores of Fife in Scotland. The sensor was developed for deployment alongside trap and panel arrays as described by Todd (Todd 2003; Todd *et al.* 2006), to quantify tidal immersion and wave action contemporaneous with measures of larval supply and settlement. An important factor in the design of a wave

sensing pressure transducer for use in such ecological studies was that it be easy to deploy intertidally and within close proximity to the experimental array. It should also be resistant so as to withstand impacts and debris moved by wave action common to the intertidal. The sites were often remote and accessible only by walking in from a distance. Therefore the device could not have an independent power supply and data logging system that required cables run up the shore to a point above the high tide. The device would be required to record data and store these for an appropriate period of time so as to limit the amount of time dedicated to the retrieval, downloading and redeployment of the instrument. This arose from daily logistic restrictions due to fieldwork being undertaken at multiple field sites. An autonomous data logging design was necessary if digital data were to be collected. Finally, more than one device would be required and these must show repeatability within the accuracy levels required to allow comparisons within and between shores in terms of variation in wave action and immersion time. This was made possible by adapting seal tagging technology developed by the Sea Mammal Research Unit at the University of St Andrews.

### **2.1.3 Wave Generation**

Energy from the wind is transferred to the surface of the water generating wave patterns. This can occur through variation in pressure of the air caused by the turbulence of the wind pushing and pulling on the surface thereby generating waves. Once a wave begins to form, the wind will accelerate over the crest and in front of the wave. Over a large distance this will add to the wave's energy. This distance is known as the wave fetch. The larger the fetch, the longer the wind has a chance to impart its energy into the wave, and hence the larger a wave can become. For a given wind

speed, the wave height can be limited by the length of time the wind blows, the fetch or both. A fully-developed sea occurs when the waves reach a height unlimited by a short fetch or gusting winds, but have been produced by constant wind of a given speed.

## **2.1.4 Oceanic waves**

### **2.1.4.1 Particle Motion.**

The motion of an ocean wave is easy to distinguish. The crest of the wave can be watched as it moves in a linear direction, parallel to the preceding wave. This motion must be distinguished from the movement of a particle within or on the surface of an ocean wave. In fact there is negligible net movement of fluid at a given point as a waveform passes. At the crest of a wave a particle moves in the direction of wave propagation, but by the time the particle has dropped half the height of the wave (at the still water level) its motion is vertically down. At the trough of the wave the particle is moving in the opposite direction of wave propagation. As it rises back to the still water level its motion moves to directly vertical. As a result, the motion of a particle suspended in a wave is a circular orbit like that of a Ferris wheel turning in the direction of wave propagation.

The size of a particle's circular orbit within a waveform decreases the further it is from the surface of the fluid. Accordingly, the circular orbit of a particle is maximal at the surface. At a depth beneath the wave of around half its wavelength the particle is virtually static. Therefore if the depth of water over which a waveform passes is deeper than half its wavelength, there will be no noticeable effect from the sea floor on the wave. The parts of an oceanic environment that match these criteria are defined as deep water or the offshore zone. The boundary of an offshore or deep water zone is not permanently fixed because it is defined by the size of the waves at

any given time. Larger waves require a greater depth before they begin to become influenced by the ocean floor.

#### **2.1.4.2 Wave Form.**

A perfect waveform can be described mathematically by a sine wave. A sine wave is generated from a circle with a radius beginning at 9 o'clock and moving clockwise in a circular motion. This creates an increasing angle between the original radius of the circle and the new radius. This range (from 0 to 360°) contributes the horizontal or  $x$  co-ordinates of a sine wave graph. The vertical distance between the original horizontal radius and the point at which the radius intercepts the circumference of the circle creates the vertical or  $y$  co-ordinates of the sine wave.

The mathematical derivations of the principles of wave action described from here forth can be found in Denny (1988) and references therein. These have been summarised so as to describe the relevant forces and how they may be theoretically described quantitatively and empirically measured.

When describing a waveform mathematically a number of terms are important. The horizontal distance between two successive crests is the wavelength and is often written as  $\lambda$  or  $L$ . For ocean waves  $\lambda$  is most commonly measured in metres. The time it takes for two successive wave crests to pass a fixed point on the  $x$ -axis is known as the period of the wave and is usually referred to as  $\tau$ . The period depends on the length of the wave and the speed at which the wave is travelling. A wave's velocity is known as its celerity, or phase speed, and is frequently written as  $C$  or  $U$ .

$$\tau = \lambda / C$$

Bernoulli's principle demonstrates that for an ocean wave that conforms to a sine wave form any one of the length, speed or period of the wave can be used to derive the other two functions. For a boat sitting on a theoretical ocean swell with a 10 second period between two consecutive wave crests it is possible to calculate that the wave has a length of 156 metres and a celerity of about  $15.6 \text{ m.s}^{-1}$ .

#### **2.1.4.3 Wave energy**

Wave energy takes two different forms, kinetic and potential.

The kinetic energy (K.E.) in an oceanic waveform that conforms to a sine waveform is due to the velocity of the water's mass. Denny (1988) provides a derivation of the relationship of kinetic energy and wave height.

$$\text{K.E. per unit area} = (1/16) \rho g H^2$$

$\rho$  is the density of the fluid (seawater),  $g$  is the acceleration due to gravity ( $9.8 \text{ m.s}^{-2}$ ) and  $H$  is the height of the column wave. Gravitational potential energy (P.E.) occurs because the mass of water is displaced from the still water level towards the crest or the trough. The higher it is displaced the greater the potential energy held in the mass. The potential energy of a mass is maximal at the crest and minimal at the trough. At the still water mark P.E. is 0. The vertical displacement of water decreases with increasing depth due to the nature of wave motion. By following a similar derivation as that for K.E., P.E. for all particles under a wave can be calculated as

$$\text{Average P.E. per unit area} = (1/16) \rho g H^2$$

Therefore, the total energy in a true sine wave is split evenly between kinetic and potential energy. This can be thought of in terms of the circular shape to the motion in a wave; the vertical fluctuation provides potential energy, the horizontal

direction provides kinetic. Consequently, the area under the wave relates directly to the energy within the wave.

### **2.1.5 Nearshore waves**

#### **2.1.5.1 Shoaling.**

The power of a wave is given by the following relationship

$$Q = nEC$$

where

$$n = 1/2\{1+(2kd / \sinh (2kd))\}$$

$k$  is the wave number or the number of peaks over a given distance. It can be written as  $k = (2\pi / \lambda)$ .  $d$  represents the depth over which the wave is travelling.  $E$  is the energy of the wave and  $C$  is the celerity, or velocity of the wave.

When the depth beneath a wave is deep relative to its length ( $d > \lambda / 2$ )  $\{1+(2kd / \sinh (2kd))\}$  approaches 1, so  $n$  approaches  $1/2$ . However when the wave reaches the shore and begins to shoal,  $n$  approaches 1 and power ( $Q$ ) approximates  $EC$ . The wave no longer acts as a deep ocean wave. The process of moving from deep water to shallow water is referred to as shoaling and changes the shape of an oceanic wave. The new wave form is known as a solitary wave.

The power of the wave is lost when it begins to break and viscosity dissipates power through heat (that is why one hears the sound of a breaking wave). As a wave reaches shallow water the front of the wave slows. This decreases the wavelength and simultaneously increases wave height. Essentially the orbital motion viewed in a deep water wave that decays in magnitude with depth from the surface runs out of depth and becomes elliptical. For this reason nearshore waves are seen to steepen as they approach shallow water.

Particle motion of a nearshore wave is no longer orbital due to the limitation of depth. If particles under a nearshore wave are watched they rotate in increasingly elliptical orbits with depth, decreasing in magnitude with depth, until at the boundary with the sea bottom they can be observed moving in a near horizontal plane.

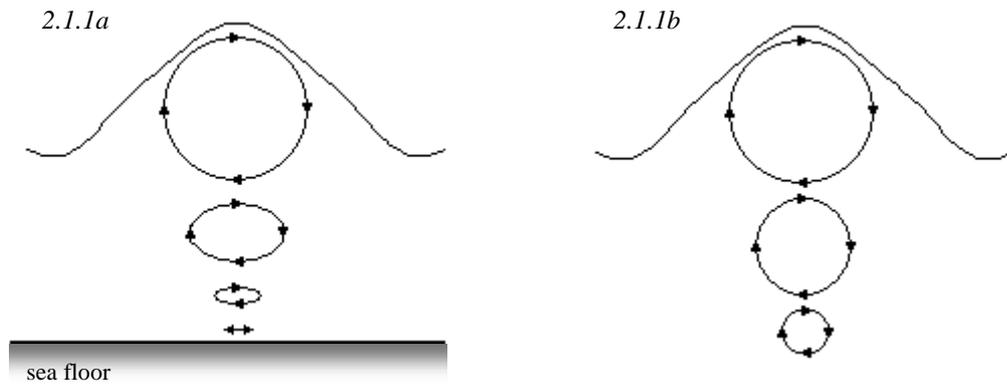


Figure 2.1.1a and b. Schematic representation of particle motion within a shallow and oceanic wave (2.1.1a and b respectively).

A second distinguishing point should be made with regard to nearshore and offshore wave motion. Because of the lack of a significant trough limited by depth in nearshore waves, the negative motion of the trough does not cancel the initial forward motion of an object driven by the positive deviation of the waveform - the motion of the crest. Unlike offshore waves, there is a significant net transportation of particles by nearshore waves.

### 2.1.5.2 Breaking.

At a critical point the velocity of the water at the crest of a wave equals that of the wave celerity. At this point a waveform becomes unstable. McGowan (1894, cited in Denny, 1988) calculated this point of instability as the height divided by the depth having a ratio of 0.78.

In fact this relationship is accurate only for gently sloping shores. On a steep shore the ratio can be considerably larger due to the time it takes for the wave to

break. This delay will take it into a shallower depth before the process begins. The ratio also depends on how steep the wave was offshore ( $H_o / \lambda_o$ ). A steeper wave will break in deeper water.

It is reasonable to state that a wave breaks when its height is equal to the water depth below the trough. This rule will tend to overestimate on shallow sloped shores and underestimate on steep sloped shores but as a general measure it is sufficient.

The manner in which a wave breaks depends on three factors;

- 1 the height of the wave at breaking (H)
- 2 the period of the wave ( $\tau$ )
- 3 the slope of the bottom expressed as  $\tan(\theta)$

These factors can be combined to form a breaking parameter

$$B_b = H_b / g \tau^2 \tan(\theta)$$

Above a value of  $B_b \approx 0.068$  the breaking wave is termed a spilling wave. The wave is nearly symmetrical at breaking except nearest the crest. Initially a small amount of water at the crest spills down the front of the wave. These waves have relatively short periods and are common on beaches with shallow slopes.

Waves with  $B_b$  between 0.003 and 0.068 are termed plunging waves. Breaking is initiated when water at the crest arches over the front of the wave. The classic plunging wave creates a barrel parallel to the crest. The period of these waves is longer than that of a spilling wave and the beach slopes far more quickly. Additionally the deepwater steepness of the wave would be expected to be higher.

A breaker near to  $B_b = 0.003$  is known as a collapsing wave. The water breaks not from the crest as in the previous two types but from the majority of the vertical face of the wave. Through this process the wave is seen to collapse from under the crest of the wave

At a  $B_b < 0.003$  the wave does not break in the conventional sense. The wave simply flattens out and surges up the beach. As a result these waves are termed surging breakers. These breaking wave types are typical of very long period waves and steep shores.

Of course the values demonstrated here are subjectively observed values and the pattern of wave break is a continuous spectrum of waveforms.

### **2.1.5.3 Turbulent bores.**

After a wave has broken part of its energy has been lost to viscous forces, but it still retains some of the energy from its offshore waveform and continues to move inshore. The new wave form, independent of the manner in which it broke, is recognisable as a turbulent bore. The front face of the wave is steep and foaming and there may be a distinctive 'toe' where the foam encounters the water in front of the wave. Turbulent bores are best observed on gradually sloping shores.

The easiest way to envisage a bore is to imagine two unequal masses (depths) of water meeting head on. The larger mass of water will propagate over the smaller mass of water much like a solitary waveform. In a turbulent bore the motion of a particle is similar to that of a solitary wave. As the bore passes, particle motion is in the direction of the bore. Although this motion dissipates to some extent with depth within the bore it is not as marked as that of a solitary wave. As a result there is a greater net motion of water. Usually a counter current from the previous bore runs seaward beneath the shoreward propagating bore.

As a plunging wave begins to break the crest of the wave shoots out as a jet and falls under the force of gravity. This wave will crash into the front of the wave at some point on its face. The water from the jet will be moving in the opposite direction

from the water of the face and the resulting relative velocity at contact will be quite large. The point of contact creates a mixing layer or shear layer. Due to the high velocity of the shear layer substantial viscous forces are created. Essentially however the resulting combination of the opposing fluid motion results in a rotational motion at the point of contact. This takes the form of a series of vortices and eddies within the combined mass of water. Each vortex forms a velocity gradient with its surrounding fluid and this causes secondary vortices to form. These produce tertiary vortices and the turbulent motion propagates through the wave. As the bore propagates, the turbulence streams out behind the crest in a plume that is visible as a frothy mass.

Turbulent flow quickly consumes energy in a waveform through the viscous forces at the shear layers. As the energy decreases the wave height decreases proportionally. Once a wave has broken it becomes smaller as it moves onshore, unlike a shoaling wave.

The nature of the turbulence experienced by a broken wave depends greatly on the type of breaking wave that formed it. Spilling waves produce turbulence that is often confined to the surface. In a plunging or collapsing wave the turbulence begins further down the wave face and propagates further through the depth of the broken wave. The steeper the shore, the deeper the turbulent forces are felt.

### **2.1.6 Refraction and diffraction**

Refraction occurs when a wave moves over a sloped seabed at an angle. Consequently the gradient of depth along the length of the wave the celerity of the wave decreases from offshore to near shore. As the wave advances over the sloped shore the slower end nearer the shore will effectively turn in, to become more parallel with the shoreline.

Wave diffraction can occur when a wave hits an object in its path. Although the depth of the water remains constant, the object can exert a drag force on the wave, turning it in towards the object in question. This is commonly seen on breakwaters, islands or solid piers.

### **2.1.7 The random sea**

Measuring the action of a wave on a rocky shore is a complex and challenging task. Waves in a sea are never really alike which makes a model of wave action unlikely to demonstrate much of the reality of a wave regime. Both height and period at any one place or time can vary from one wave to the next, and often it is very hard to see where one wave ends and another begins.

Waves produced by storms often will outlive the forces that produced them. These waves will travel away from the source and eventually break on a shore. Large propagated waves are known as swell and they can mix with locally propagated wind waves to create a complex wave regime or signature. Waves with different wavelengths travel with different celerities and so will pass through each other. Crests and troughs combine as they are mixed together in a process known as interference creating larger waves or cancelling each other out. The way in which waves are measured therefore depends on the application of the data by the experimenter.

### **2.1.8 Measuring wave forces**

#### **2.1.8.1 Exposure**

An ecologist may wish to know the degree of wave action on a shoreline for many reasons, not least because it has a large effect on the structure and dynamics of the shore community. A general measure of exposure of a shoreline can be obtained

by simply surveying the dominant organisms present on a particular shore, much as one may examine the species of a freshwater stream in order to indicate its levels of anoxia. These dominant species are known as indicator species (Lewis, 1964).

Exposure depends largely on the geographic location of a site. A site such as a headland is exposed to oceanic swells. The level of wave exposure that such a site experiences will be high. The landward side of an island or the head of an enclosed bay, for example, will be relatively sheltered from oceanic swells and as result experience far less wave exposure. Larger waves are generated by prolonged wind stress across a large uninterrupted distance or fetch. It may seem sensible to predict exposure on a shore with an index combining wind strength, direction and fetch. However, waves reaching a shore may have arisen far from local wind conditions and can show little resemblance to wave patterns expected by an index that considers only those wind conditions. Waves arriving from a distance will interact with the local wind conditions further complicating the model. In addition, waves reaching a shore often will have been refracted or turned from their original direction due to the angle at which they approach the shore.

A further, particularly important, factor that complicates the determination of wave exposure at a shore is variation in bottom topography. A gentle sloping shore will have a long distance over which the waves will dissipate their energy. Broken topography will dampen the effects of waves further as they break along the shore. Sloping, broken shorelines therefore tend to show less exposure than steep shores regardless of their geographic exposure to oceanic swells. *In situ* measures of wave crash are useful when an experimenter wishes to record wave crash and relate it to biological factors such as abundance and distribution or physiological response.

### 2.1.8.2 Dissolution

The nature of turbulence is complex and random. Modelling this process based on measures of fluid velocity is difficult and models often are crude approximations. A better method is to directly measure the effects of turbulence, particularly with respect to the consequences of turbulence. The principle action of turbulence is the exchange of fluid or particles within the fluid through the diffusion of eddies, and is important to diffusive mechanisms in a fluid environment. Therefore a novel approach to the quantification of turbulence is to directly measure the rate of dissolution of particles.

Net flux at a surface can be quantified by the rate of dissolution of plaster of Paris balls. Plaster of Paris is soluble in water and it is possible to measure the rate of dissolution in a laboratory flume. These values of the mass loss to the plaster of Paris balls under known flow conditions can then be used to quantify the rate of flux in the field (Muus 1968).

A problem with this method is the relationship between dissolution rate and shape. An angular block will dissolve much faster than a sphere because of its uneven shape. Unfortunately, casting in a uniform, spherical mould is quite difficult. Pre-cast commercially available options are available. Carpenter's chalk or gypsum disks are both useful alternatives to plaster of Paris (Santschi *et al.* 1983). The dissolution rate of soluble substances such as plaster of Paris is also dependent on temperature. This can be calibrated in the laboratory but will require an accurate time series field measurement of average temperature unless the fluid medium is suitably stable for temperature fluctuations.

For a broad measure of turbulence, dissolution materials are appropriate and their affordability is a clear advantage. Also, the ability of the observer to measure a

very local turbulent effect by positioning the block close to a site is a distinct advantage (Cusson and Bourget 1997). This method is, however, generally impractical when attempting to measure variances within 10-20% (Denny 1988). Technological advances have led to the development of a number of instruments that can be used to quantify wave forces and provide a great degree of accuracy if so required.

### **2.1.8.3 Wave staffs**

Resistance staffs contain two fine wires held parallel and vertical from the surface, through the water column. When entirely out of the water the resistance along the wires is nearly infinite due to the conducting properties of air. Sea water is a much better conductor than air so as the staff is increasingly submerged the resistance becomes less. Capacitance staffs work in a similar way but rely on a measure of capacitance (Denny, 1988).

Both these instruments require a mechanism at the surface such as a pole to which they can be firmly attached. This is often impractical away from man-made structures such as piers and therefore these instruments are of limited use in remote locations. They are easily fouled by drift algae, plastic bags, nets etc. and can be damaged as a result. A second problem is they are sensitive to the occurrence of froth, which can be common in the surf zone.

### **2.1.8.4 Electromagnetic flow meters**

When an electrical current is passed through a wire a magnetic field is induced. Coiling a wire around a conductor amplifies the magnetic field. A magnetic field is perpendicularly circular around a wire conducting a current and deteriorates in

intensity with distance from the source. If a magnetic field is generated and a fluid with sufficient conductive properties flows through this field the voltage potential of the field will be increased proportional to the flow. This is the principle on which an electromagnetic flow meter works. This device is known as an inductor and is measured as capacitance in Henrys.

In practice, the voltage produced by the flow of seawater generally is very small and the detection of the voltage difference caused by change in flow relative to other voltage changes is problematic. Devices on the market circumvent this problem by pulsing the magnetic field; the response time of the device therefore is limited by the pulse rate of the magnetic field. A typical pulse rate is  $\sim 30$  Hz but the output from the device is usually “filtered” further to a frequency of  $\sim 1$  Hz.

Inductors measure the flow rate across a 12 cm torus, which means the fine-scale structure characteristic of turbulent flow rate will not be measured. A more serious implication when using the device in the intertidal wash zone is, however, the transient voltage surge that occurs when the sensor is immersed and exposed due to wave action. The device must remain fully immersed during the relevant recording period as a result and will not record periods of early immersion and partial exposure. Inductors suffer from similar physical complications to a wave staff. They are rigidly mounted onto a pole and can be damaged by algae and other flotsam wrapping around or impacting the device.

It should also be noted that these devices are increasingly sophisticated because they are normally used for collecting data on temporally and spatially large-scale oceanographic projects. For this reason they are relatively expensive to obtain on a smaller budget common to ecological studies.

### 2.1.8.5 Pressure

A pressure sensor can be used to determine a wave's height. As a wave passes over a point on the sea floor the height of the water column increases and decreases due to the height of the wave. A water column has a mass proportional to the density of the medium ( $\rho$ ) and the height of the fluid ( $z$ ). Force is mass times acceleration and in this instance the acceleration is that due to gravity ( $g$ ). The force in question is pressure and is usually described by oceanographers as

$$\text{Hydrostatic pressure} = -\rho gz + \text{atmospheric pressure}$$

The negative sign is due to  $z$  being a measure of vertical displacement from the still water level, denoted by oceanographers as  $z = 0$ . Atmospheric pressure is a consequence of the mass of air above the point of reference and is measured relative to a vacuum. Compared to the pressure fluctuations as a wave passes, air pressure remains relatively constant at  $\sim 1$  bar ( $\equiv 1$  atmosphere).

A wave is a temporal fluctuation in height relative to a fixed point and as a consequence will be registered as a temporal fluctuation in pressure at a point on the subsurface. This function can be added to the above equation as

$$\text{Hydrostatic pressure} = -\rho gz + \text{atmospheric pressure} + \{\rho gH / 2\}$$

$H$  represents the height of a wave from trough to peak. The height of a wave in a nearshore environment is related to the depth of the water, the celerity of the wave and the original height of the wave in deep water.

In reality the pressure imparted by a wave will deteriorate with depth. The mathematical function of this relationship originates in a concept of the velocity potential of a wave. It is derived by Denny (1988) and will not be demonstrated here. The deterioration function is represented by an addition to the formula

$$\text{Hydrostatic pressure} = -\rho g z + \text{atmospheric pressure} + \{\rho g H / 2\} \{\cosh(ks) / \cosh(kd)\}$$

$d$  represents the depth below still water, and  $s$  represents the depth from the sensor to the sea floor beneath the wave.  $k$  is the wave number, given by the formula  $k = 2\pi / \lambda$ .  $\cosh$  is the hyperbolic cosine.

A wave in 10 m of water with a height of ~ 2 m has a period of ~ 10 sec and a wavelength of ~ 150 m. The expected pressure from a wave of this type exclusive of atmospheric pressure variation is 100.55 Mb (1 cm water ~ 1 Mb of pressure). However, due to the attenuation effect a pressure reading at 10 m under this wave will in reality give 92.33 Mb. This is an attenuation of < 9% for a wave of 2 m height at deployment depth of 10 m.

Measuring the elevation of water directly can circumvent the problem of depth-related pressure attenuation. Resistance or capacitance wave staffs provide an instrument most capable of achieving this; however, their susceptibility to damage and high cost severely limits their usefulness to rocky shore ecologists. Modern pressure transducer technology is well developed, because there are many modern applications for these devices. It is now possible to buy pressure sensors designed specifically to measure wave height and these are commonly used in nearshore waters. Microchip circuitry, combined with powerful battery technology, has seen the

addition to these sensors of digital data logging which removes the requirement for cables run to power sources, external logging systems or transmitters at remote sites.

### **2.1.9 Quantifying wave action**

An experimenter wishing to measure wave action must convert the data into a relevant format before analysis and tests of hypotheses. Quantifying a wave regime as a usable index of wave action is frequently the approach. In the absence of a suitable instrument an early simple, visual measure was that developed by W. H. Munk (Denny 1988). When left to estimate wave height by eye, it seemed the brain tended to calculate the mean of the one-third largest waves. This is known as the significant wave height ( $H_s$  or  $H_{1/3}$ ).

A more mathematical and commonly used index is the root mean square amplitude of the wave regime. The amplitude of a wave is the vertical deviation of the water surface from the sea water level. Each wave has a positive amplitude (from SWL to the crest) and a negative amplitude (from the sea's water level to the trough). In a basic linear wave the amplitude is half the height of the wave. A time series of wave height (a wave signature) is required for the analysis. The SWL is taken as the average of all the points in the time series. This depth is subtracted from all the individually measured points in the data set. The results will contain positive and negative values so all these values are squared to render them positive. The average of these values is obtained and the square root of this value is taken to give the root mean square amplitude ( $A_{rms}$ ).

In addition to the description and assessment of a pressure sensing wave transducer various, differing indices of wave action are examined here for their suitability for inclusion in ecological experimentation. The specifications for an index

are that it is simple to apply to large amounts of digital data, can be performed in Microsoft Excel or another common statistics package and give an intuitive measure of wave action across temporal and spatial scales relevant to a rocky intertidal ecologist.

## **2.2 METHOD**

### **2.2.1 Transducer Design and Use**

The wave transducer recorded the pressure surrounding the sensor at high frequency (10 Hz). During immersion this pressure is determined by the atmospheric pressure (~ 1 atmosphere and assumed constant over temporal scales relevant to wave frequencies) and the weight of water overlying the sensor. The weight of water is directly proportional to its height so the time-related pressure profile recorded by the sensors could be converted into a time signature representing the wave height. This gives a profile of the surface shape over time known as the wave signature. Data initially stored by the transducer were easily imported into Microsoft Excel.

#### **2.2.1.1 Encapsulation**

Once assembled, the circuitry and components, were set in an Araldite CY1301 low-viscosity epoxy resin (manufactured by Robnor Resins). This could be moulded to any desired dimensions. To minimise its size, and maximise ergonomic design in terms of ease of deployment and handling, a standard SMRU satellite tag mould was used to encase the circuitry and battery in a box form. The transducer measured 110mm by 85mm by 40mm.

#### **2.2.1.2 Sensing device**

The pressure sensor used in the transducer was a piezoresistive absolute pressure sensor capable of sampling pressure over a 5 bar range (Keller AG; [www.keller-druck.com](http://www.keller-druck.com) 5 bar PAA-7 model). This device is built from a tough stainless steel 316L housing. The inside of the sensor contains a conductive diaphragm mounted in silicon oil. This flexes under pressure and its resistance is

altered. The change in resistance effects a change in voltage across the diaphragm that can be read by the circuit.

When the transducer was assembled and set in the epoxy resin the exposed sensor was encased in a plastic housing to protect it from direct impact due to stones or fouling. The housing was perforated so it can flood when immersed. Conductivity sensors positioned outside the epoxy resin allowed the device to detect when it was immersed in salt water.

### **2.2.1.3 Circuitry**

The circuit design allowed for a small voltage differential across the pressure sensor to be amplified and fed into an 8-channel, 16-bit Burr-Brown ADS8344N A to D converter. This is then fed into a flash memory store from which it can be extracted at a later date via an RS232 cable. As a result, the device could be deployed for ~ 3 weeks before requiring downloading to a computer to retrieve data and allow resetting of the flash memory. The sampling frequency of the sensor is variable between 1 and 100 Hz, but for recording a water wave, a frequency of 10Hz was considered to offer reliable and precise measures of individual waves whilst not compromising memory capacity and hence maximising deployment time.

The circuitry was designed to run off a single D cell battery (Saft LSH20 Lithium Thionyl Chloride 3.6v) that lasts for approximately 2 years of continuous recording.

### **2.2.1.4 Software**

When attached to a desktop computer by a RS232 cable the sensor could be programmed using a simple software package called Tag Config v9. Time stamps

including date and time can be placed within the data file at any increment of 1 sec. The sensor can also be programmed to cycle through an on/off sequence. This allows for a sample of the wave regime, which is unlikely to change markedly within short period of time, whilst saving space on the flash memory and battery power.

Once the text file has been downloaded using the Tag Config v9 software the sensor can be reset. A full memory transfer contains 8.5 Mb, which equates to approximately 2241 minutes (37.5hrs) of data. A full download takes < 2 hours. This allows data to be dumped and the device reset within the limits of emersion between tides at sites used in this study. The transducer will remain in a dormant mode following reset of the flash. It will activate when the wet/dry sensors that protrude from the epoxy jacket are immersed in saltwater. As a result, the sensors do not record during the transfer from the laboratory to the shore and prior to immersion by the first low tide, again saving space on the flash memory.

### **2.2.2 Field testing**

Three sensors were deployed alongside each other in the field to ascertain the similarity amongst the sensors when recording a natural wave environment. They were set to record continuously over a single tide. A continuous recording was necessary, because the sensors will switch on only when immersed in salt water. A slight difference in deployment height or excessive splash from a large wave could immerse one sensor enough to start recording earlier than the others. If the sensors were programmed to cycle on and off they may be out of synchronisation with each other and there would be no comparable data.

When deployed individually on separate shores and during an extended experimental period they are set to cycle for 2 min (on) and 13 min (off) so as to save

memory space. For this reason 2 min samples separated by 13 min were isolated and used from each sensor's data set for analysis of the indices. The sensor clocks had been synchronised prior to deployment to ensure the data were contemporaneous.

In order to assess the ability of the sensors to record similarly in the field, two specific aspects of the data returned were examined. The tidal period was examined to ensure similarities between the three sensors. Pressure signatures across the period were also compared to ensure they were recording similar patterns of wave crash.

### **2.2.2.1 Tidal immersion**

Initially the average value for each 2 min section was plotted to smooth the small frequency variation caused by wave crash (sec) and visualise the large frequency variation driven by a tidal pattern (hr). Profiles of pressure fluctuations were examined visually at increasingly smaller scales to ensure they were recording similarly amongst the sensors in terms of wave crash and the end of the period of tidal immersion.

Identification of a sufficient model that would produce an estimate of sea water level and hence tidal variation was undertaken. The period of immersion was defined as having ended when the pressure signature no longer demonstrated a set of complete waveforms with unflattened bottoms indicative of emersion.

Two methods were used to estimate the tidal curve. First, pressure was regressed against time using a quadratic model (SPSS). Second, the average pressure for each 2 min period was obtained over the immersion period. The two methods were plotted in order to compare their accuracy in estimating tidal height over time and immersion/emersion transition.

### 2.2.2.2 Wave action

The data returned from the transducers required a sufficient analysis tool that could quantify a time series analysis of pressure in terms of wave action. Excel provides a simple average deviation function (`=AVEDEV()`) which calculates the average absolute residual using the mean as a model of sea water level. The value for average deviation was obtained for each 2 minute section throughout the tidal period.

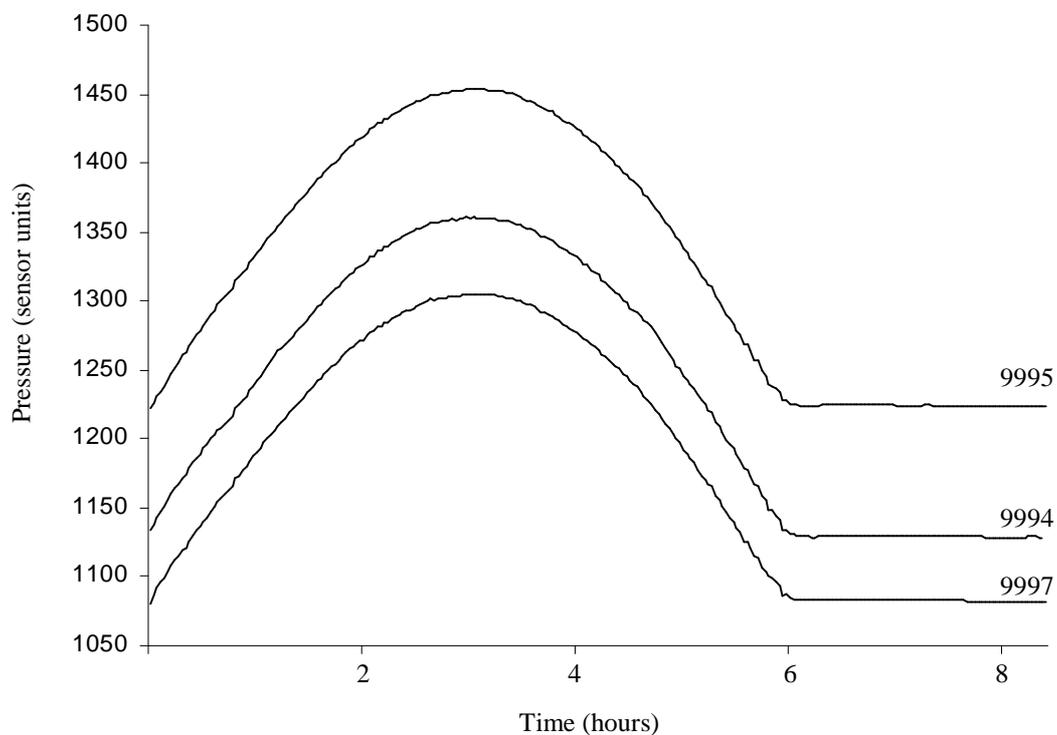
A second method is to use average absolute residual values from the quadratic model. The model was determined from the data following the method described in section 2.2.2.1. The absolute residual values were calculated using the model and the average of these for each 2 minute period was taken.

Both indices were compared with  $\frac{1}{2}$  the maximum wave height and the root mean square amplitude ( $A_{\text{rms}}$ ) for two minute periods throughout the tidal cycle. Periods during the lowest, highest and middle parts of the flood and ebb tides were compared. There were 11 data records either side of the highest tidal period (taken as the largest 2 min average). The 12<sup>th</sup> (high), 6<sup>th</sup> (middle) and 1<sup>st</sup> (low) periods relate to the flood tide and the 14<sup>th</sup> (high), 19<sup>th</sup> (middle) and 24<sup>th</sup> (low) periods to the ebb tide. The smallest values for the three residual indices (AveDev,  $A_{\text{rms}}$ , and the quadratic index) will demonstrate the best estimate of wave action; however the variance between the sensors must also be considered. The index must be sufficiently accurate as to maximise the ability to assess wave action between sites, whilst not being affected by discrepancies caused by sensor error.

## 2.3 RESULTS

### 2.3.1 Tidal immersion

Averaging each 2 min section separated by 13 min breaks gave a smooth tidal curve for each of the three sensors. The tidal curves conformed for all three sensors deployed simultaneously. These are demonstrated in *Figure 2.2*. All three sensors were deployed at the same tidal height however they each have a slightly different offset value (constant starting point). This has not been adjusted for in *Figure 2.2*.



*Figure 2.2.* Average pressure for 2 minutes separated by 13 minutes in sensor units for three sensors deployed simultaneously over a complete tide. The offset values for each sensor has not been removed so as to better demonstrate visually the similarities of the curves. Tag id numbers are included on the plot.

The period of onset of tidal immersion was not demonstrated here because the sensors begin recording after becoming immersed. The end of the tidal period and beginning of emersion is evident, however, because the sensors were left recording for a period prior to retrieval. This period is demonstrated in more detail in *Figure 2.3*.

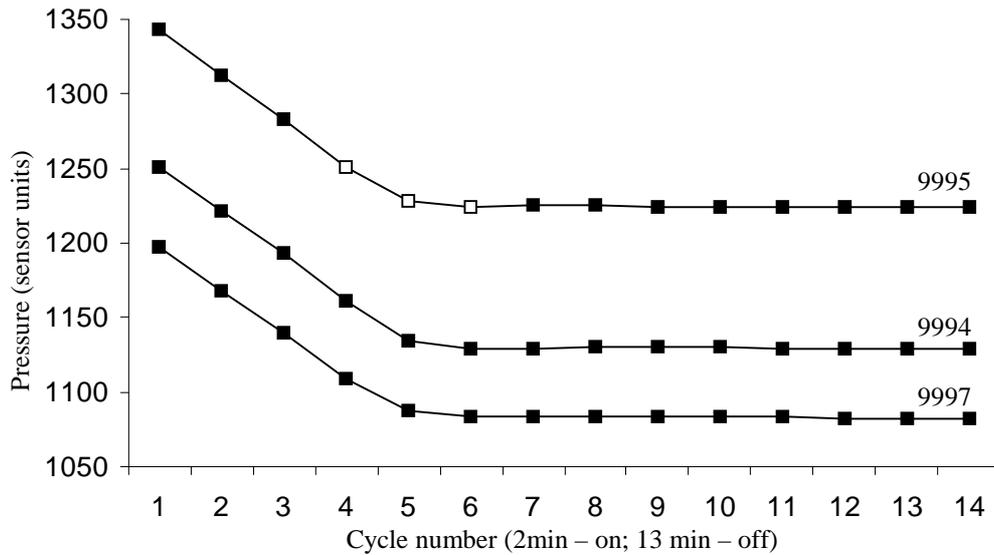


Figure 2.3 Average pressure per two minute section separated by 13 min plotted against time after immersion. Tag id numbers are included on the plot. Pressure signatures are plotted for the open boxes in Figure 2.4.

The point of emersion can be estimated to within 15 min (between 5h 30min and 5h 45min after immersion) by visual inspection of Figure 2.3. This was the same for all three sensors.

The final 30 sec segment of three 2 min periods for one of the sensors shown in open boxes in Figure 2.3 is plotted in Figure 2.4. These data demonstrate a typical wave signature for a fully immersed, partially immersed and emersed period.

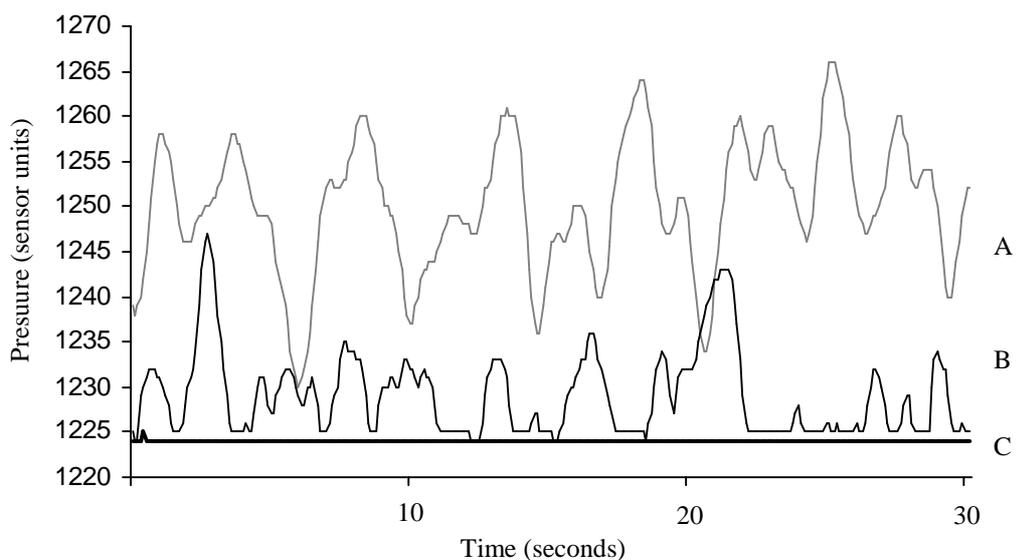
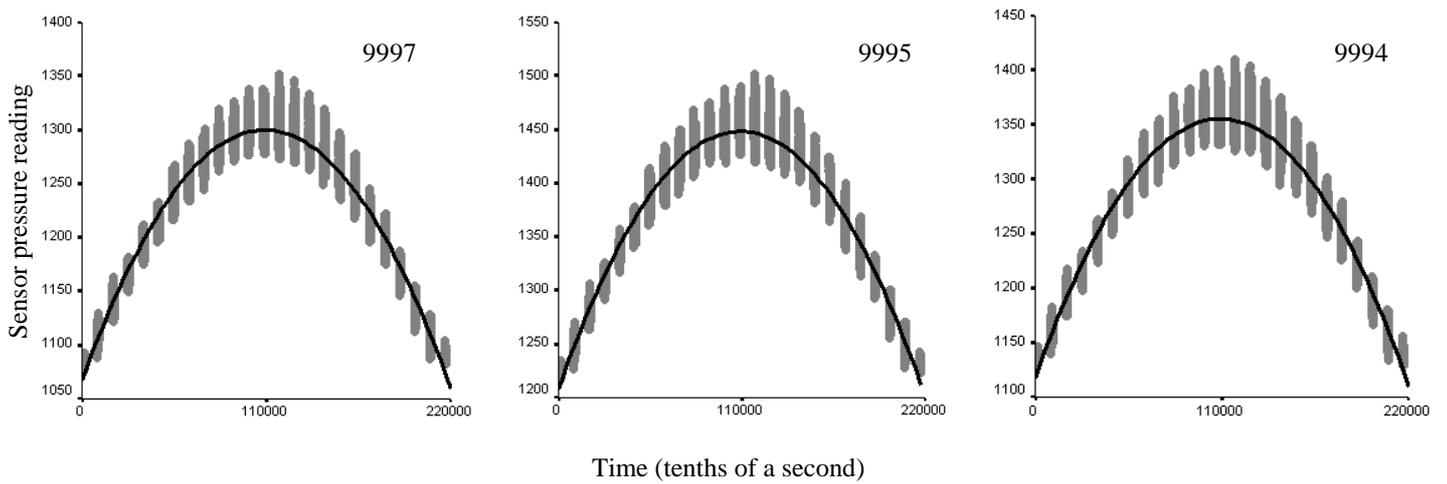


Figure 2.4 Pressure signatures for three consecutive points at the end of a tidal period. Plots are offset due to their tidal height. All three plots are from the same sensor measuring the same tide. A. (grey) = fully immersed. B. (thin black) = partially immersed. C. (thick black) = emersed.



*Figure 2.5* Quadratic regression of one complete tide for three sensors deployed simultaneously on the shore at Fife Ness (their corresponding id tags are included in the top right corner of each plot). The grey points are pressure data from two minute sections separated by thirteen minutes. Each data point represents pressure every one tenth of a second. The dark line shows the quadratic regression model – an estimate of the tidal curve.

*Figure 2.5* shows the results from quadratic regression overlaid onto pressure data from each of three sensors to demonstrate the fit of the quadratic for each 2 min section. The quadratic appears to underestimate the sea water level at the high and low periods of the tide. The quadratic appears to over estimate through the flood and ebb periods of the tide. *Figure 2.6* shows a plot of the pressure data against the average pressure for each 2 min period across the whole tide to demonstrate the fit of the mean at for each 2 min section. The average model appears to underestimate at the low tide but fits the rest of the data well.

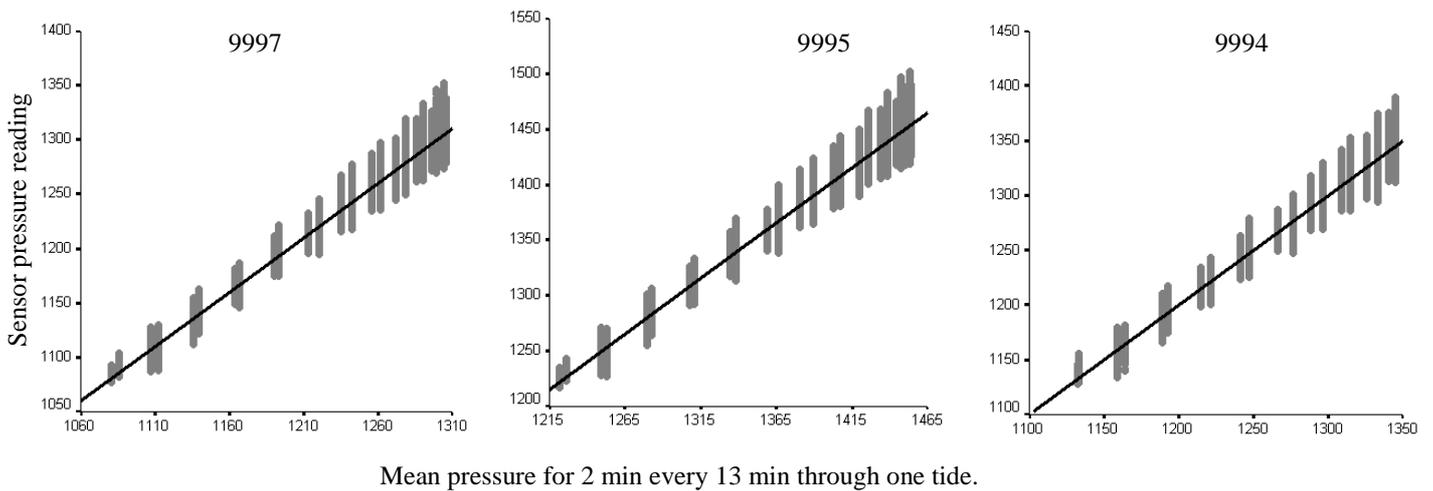


Figure 2.6 Plot of pressure sensor units against the average pressure for two minute periods separated by thirteen minutes for three sensors deployed simultaneously on the shore at Fife Ness. The thick line is a plot of  $y = x$ . The data from each sensor has not been adjusted for the offset, hence the differences in absolute values but not sensor units for the axis.

### 2.3.2 Wave action.

Initial visualisations of overlaid wave signatures from the three sensors deployed simultaneously on the shore, demonstrated they were recording similar pressure fluctuations within 2 min periods. Further analysis of similarities of indices of wave action between the sensors, throughout the tidal period, are demonstrated in Figure 2.7.

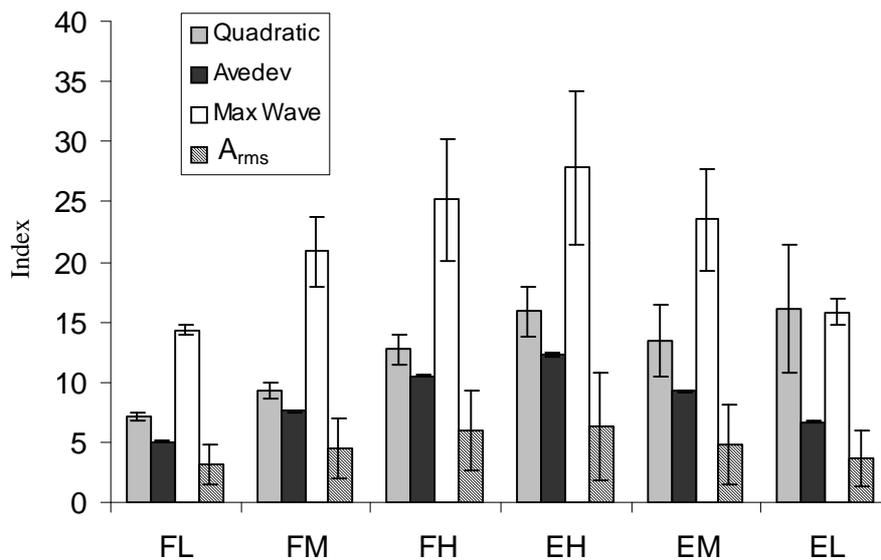
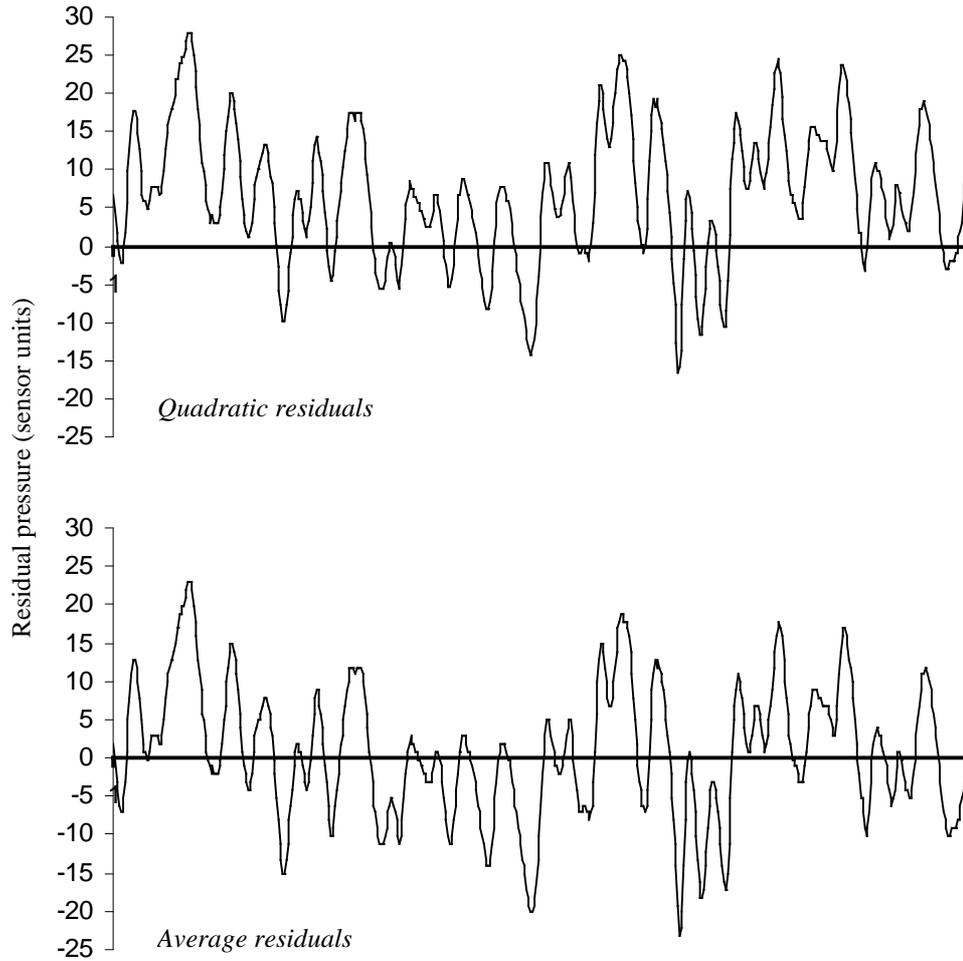


Figure 2.7 Mean  $\pm$  1 s.e. of three sensors for four indices of wave action. Average absolute residuals from a quadratic (Quadratic index); average absolute residuals from the average (Avedev index);  $\frac{1}{2}$  maximum wave height (Max Wave index); root mean square amplitude ( $A_{rms}$  index). A 2 min period of wave crash at 6 points through a tide were examined. FL = low flooding tide; FM = mid flooding tide; FH = high flooding tide; EH = high ebbing tide; EM = mid ebbing tide; EL = low ebbing tide.

The  $A_{\text{rms}}$  has the lowest value for the three residual indices suggesting it estimates the sea water level most accurately. The variance amongst sensors is relatively large compared to the AveDev index.

The maximum wave index increases towards the high tide. This is also seen for the AveDev index and the  $A_{\text{rms}}$ . This pattern is seen on the flooding tide for the quadratic index and not on the ebbing tide. The variance amongst the sensors is seen to increase over time for the quadratic index. The variance between sensors is smallest for the AveDev index (*Figure 2.7*).

The residuals from the quadratic regression and the average of a 2 min period at the end of the tide are plotted in *Figure 2.8*. At  $y = 0$  the residual values for the quadratic model appear to be much lower on the signature than the average residuals. The plot of  $y = 0$  intersects the average residual plot nearly twice as many times as the quadratic residual plot and the average residuals appear far more evenly spread above and below  $y = 0$ .



*Figure 2.8* Plots of the residuals from two models for tidal data from a low ebbing tide. The data are for 30 sec recorded from the start of the 2 min period described in *Figure 3.9* as EL. The quadratic residuals are produced by subtracting a quadratic regression produced from the complete tidal period. The average residuals are produced by subtracting the average of the two minute period.

## 2.4 DISCUSSION

### 2.4.1 Tidal immersion

Initial observation of a plot of the mean of 2 min sections for the whole of the data demonstrates the sensors are measuring broadly similar tidal immersion periods. This period is followed by a period of stable emersion and measurement of atmospheric pressure during low tide that fluctuates at a very low frequency and is barely detectable across tidal scales (*Figure 2.2* and *2.3*). On closer examination it is clear that the exact point of emersion is not easily defined. As the tide drops below the height of the sensors, wave splash can be observed surging over the exposed sensors generating distinctive flat-bottomed wave forms in the signature (*Figure 2.4*). Derivations of site specific immersion times are possible to within minutes and seconds as a result of measuring the time of immersion from these surging waves. The implications for this resolution of immersion time can be important to ecologists. The length of immersion of a site throughout the tidal cycle is an important factor determining survivorship, distribution, abundance and potentially larval supply of organisms on a rocky shore. Tidal charts providing an estimate of 'expected immersion time' are typically used to calculate immersion times. This is based on the fact that tides are predictable as a result of the gravitational pull of the sun and moon and the rotation of the earth. Changes in topography, barometric pressure, wind or wave conditions can markedly affect tides on a more local scale. Immersion time can vary completely independent of tidal surge on small scales. Castilla (pers. obs. 1982 cited in Venegas *et al.* 2002) has observed chthamalid barnacles several centimetres above the highest high tide mark along the coast of Chile. Lewis (1964) observed that the upper limit of *Alaria esculenta* in Co. Kerry, Ireland, lies above the predicted

HWS tide mark as a result of spray and splash from wave action. This is possible as a result of immersion by waves surging up the rocks.

In order to describe the tidal period a number of methods can be used to fit the tidal curve. If a complete model of a tidal curve was required then a quadratic regression provides a simple but imprecise method of estimating a curve across the tidal period (*Figure 2.5*). A polynomial function or a sine curve would possibly have provided a more accurate description of the tidal curve here. The bias seen in *Figure 3.4* may be due to the turning of the tide, which is not predicted by a quadratic. The 2 min mean appears to have provided a very good fit to the tidal data. The under estimation at the periods of low wave crash are likely to be as a result of the biased distribution of large waves that would occur at these shallow periods.

#### **2.4.2 Wave action**

Several indices were compared with the data for their ability to quantify wave action measured by the pressure transducers in relation to deployment of the devices across a range of sites. They would be required to quantify variation in relevant wave forces between sites and tides and possibly within tides.

Wave height is a function of energy in unbroken waves. The greater the energy, the higher the wave form. Once a wave enters shallow water the height relates to energy in a wave and depth of the water column. As the water shallows a wave will increase in height until it begins to break and the height decreases. A relationship between depth and maximum wave height was seen in *Figure 2.7*. As the tide flooded the  $\frac{1}{2}$  maximum wave height increased and as it ebbed it decreased. Ultimately wave height will not provide an ecologically meaningful estimate of wave energy because short waves may still be powerful despite their size. The depth of water limits the

height of the wave. The broken, high energy waves would appear longer in length and shorter in height in a wave signature. Length is a component not incorporated into the wave height index.

A residual index essentially measures the area under a curve. This is directly proportional to the energy in a wave or series of waves. Energy is usually imparted to the surface of the sea water initially in the form of wind. Sufficiently large waves will propagate this energy large distances through deep water. As a wave reaches shallow water it climbs in height but narrows in length owing to the change in speed at the front of the wave. Consequently the area under a wave form remains constant until the point at which it starts to break when energy is lost as heat and noise. A wave will break when the ratio of wave height to water depth approaches a specific value, as defined by the function  $B_b$ . As the tide rises over a specific littoral site on a shore the depth above the site is increased and so fewer waves will have broken. Therefore it would be expected that the waves passing over a site fully immersed at high tide would contain more energy and will have a greater cross sectional area. A residual index such as  $A_{veDev}$ ,  $A_{rms}$  or the quadratic should demonstrate this change in residual value throughout a tidal period.

*Figure 2.7* demonstrates that for two of the residual indices ( $A_{veDev}$  and  $A_{rms}$ ) a decrease in the energy of wave action is observed at the deployment site during shallow periods of the tide. This could be further exacerbated due to topographic details such as boulders and gullies which ‘protect’ the site from wave crash up to a critical tidal depth after which the wave action floods over the obstacle and a sudden increase in wave action may be observed. This was not evident at the location in the present study.

The quadratic index did not demonstrate a decreasing residual value with decreasing submergence depth on the flooding tide, it showed an increased residual value. Because contemporaneous, replicate data sets were used to compare between the indices the lowest residual values for these indices would demonstrate the most appropriate fit to the data. It appears therefore that the index loses accuracy towards the end of the tide (*Figure 2.7*).

*Figure 2.5* explains the increased value of the quadratic index. The underlying model has underestimated the sea water level for the final period before exposure following the tide. This is seen when compared to the residuals predicted using the 2 min average (*Figure 2.6*). From these plots it can be seen that the wave signature derived from the quadratic residuals has been shifted above the mean sea water level and this explains the higher than expected residual average. Because the index predicts mean absolute residuals the same would be true if the quadratic underestimated the mean sea water level.

The quadratic index was probably an ineffective estimator of wave action for two reasons. First, subtle differences in the accuracy between the sensors multiply over a tidal period and result in slight differences in the height of the tidal curve. This means that the underlying model of tidal fluctuations predicted by the quadratic regression calculated from a complete tidal period does not make similar predictions of mean sea water levels between the sensors. Although this is barely noticeable across a 6 h scale, over short time scales relevant to wave frequencies this can be a problem. In *Figure 3.6* the variance between the three sensors for the quadratic index increased markedly as the tide progressed. Discrepancies in the units of pressure calculated by the transducers would affect the quadratic prediction of the tidal curve.

These departures explain the differences seen between sensors for the index at small scales relevant only to wave frequency.

Second, it is unlikely that a tidal fluctuation will follow a perfect curvi-linear model such as a polynomial function (quadratic) or a sine wave. Tidal surge due to wind action, interference from wave action, atmospheric pressure systems, general weather conditions and storm surge all can influence the rates at which a tide ebbs and floods. The AveDev and  $A_{rms}$  indices are able to constantly adapt to fluctuations in tidal height likely to be seen in the empirical data due to the short period over which they fit the underlying tidal model. These two indices produced lower residual values from the data than did the quadratic index, suggesting they are fitting the sea water level better and providing a more accurate and repeatable measure of wave action throughout an entire tidal period.

The  $A_{rms}$  has the lowest residual values suggesting this is the best determines the deviation from the sea water level. Crucially, the variance between sensors is high for  $A_{rms}$  relative to AveDev (*Figure 2.7*). It appears that the  $A_{rms}$  is sufficiently accurate that any difference due to calibration errors between the sensors is increased for this index, making it inappropriate as an index of wave action for these sensors.

Due to its low between-sensor variation and ability to predict an accurate and empirically based tidal curve relevant at frequencies required for the estimation of wave forces, the AveDev index is the most appropriate index for measuring wave action between shores and days with these sensors. In addition its application to large volumes of data is extremely efficient using Excel, a software package available on the majority of personal computers. Having imported the text file into an Excel spreadsheet using the VBA macro the user simply enters the =AVEDEV() function at the bottom of the first column of two minute data and then drags along the row. A

major advantage to the use of the AveDev index is its ability to provide within- and between-tide information on wave action, which can be applied to ecological functions within and between spatial and temporal scales of shores and tides.

## Chapter 3 – Passive Settlement Traps

### 3.1 INTRODUCTION

#### 3.1.1 Supply Side Ecology

Benthic organisms often exhibit a planktonic larval distribution phase as part of their life history. Dependent on the duration of the larval phase and the extent of passive hydrodynamic dispersal, larvae can disperse across considerable distances. A closed system exists where propagules are distributed within the confines of the local population and it is therefore possible to predict subsequent population demographics from the reproductive output of the adults. In relation to the degree of larval dispersal of an organism, which in some instances can be considerable, many benthic populations can be considered demographically open. Demographically open populations are distinct from genetically open populations. Genetically open populations are concerned specifically with the supply of recruits from outside the local population. If recruitment rate is independent of the local population size then the population is considered demographically open (Johnson 2005). Recruitment in open demographic communities cannot rely upon the same predictive mechanisms as closed populations because successive recruitment is usually disconnected from a measures of adult fecundity (Caley *et al.* 1996). Open population recruitment is made up of three components.

1. The number of larvae initially supplied.
2. The rate of settlement of those larvae.
3. The rate of mortality of those that settle up to the point of recruitment as defined by the experimenter (Lewin 1986; Minchinton and Scheibling 1991; Bertness *et al.* 1992; Grosberg and Levitan 1992).

This field of research is broadly known as supply side ecology.

Larval supply experienced a flush of interest in the field of ecology in the 1980s (Lewin 1986) after realisation of the historical bias towards studies emphasising post settlement processes as driving community structure. Largely this bias arose from the pioneering work on competition by Connell (1961a; 1961b; 1972) and predation by Paine (1974). These post-settlement processes were emphasised due to their effect on assemblage structures common on intertidal shores such as zonation. However a reappraisal of the literature on the subject by Connell (Connell 1985) and some influential studies on the coast of California (Gaines and Roughgarden 1985; Roughgarden *et al.* 1985; Gaines and Roughgarden 1987) led to a revision of this emphasis. These studies demonstrated that supply side processes become important when larval input levels are low and recruits do not saturate the adult populations.

Studies that have attempted to incorporate planktonic larval supply have often used instantaneous measures of larval abundance such as pump samples or net tows. These limit the scale of a study through practical and logistical restriction of the equipment. The requirement to suitably replicate forces the experimenter to limit research across temporal and spatial scales. Instantaneous measures of larval supply such as net tows often will suffer from fouling or physical damage due to waves. Pumps are often cumbersome, expensive and, like nets, require manual operation hence limiting study to single easily accessible locations such as piers or from boats in open water away from the intertidal zone.

### **3.1.2 Quantifying larval supply**

Attempts have been made to integrate larval supply over time without requiring instantaneous measurements or limiting the deployment of the equipment to open waters. Setran (1992) describes a horizontal trap designed for deployment in the

rocky intertidal that can sample larval input to the substratum in mild to significant wave crash conditions (wave height was less than 1.2m). Setran designed the trap as a result of other larval traps being restricted to low or unidirectional flow common to environments such as bays and inlets, tropical reefs and lagoons or freshwater lakes. The intertidal environment offers a more complex flow regime where tidal and wind driven currents interact with wave crash and topographic heterogeneity to produce complex flow regimes. The main body of Setran's intertidal plankton trap was built from an 18 l rigid plastic food container. A Nitex mesh was positioned at one end of the food container that had been cut away to provide an outlet for the flow of water. The inlet was a one-way flap system that remained closed during periods of seaward surge or slack water. During shoreward surge the flap would open and water would filter through the opening and out through the mesh, trapping plankton inside the device. Setran's results indicate the trap is capable of capturing sufficient numbers of plankton to demonstrate diurnal heterogeneity indicative of diel vertical migration. Wave surge is necessary to the effective functioning of the device; however this is not the only process forcing patterns of larval supply to the substrate. For example the device is limited in its application in quantifying tidally-driven variations and therefore insufficient for monitoring regular delivery of larvae to the intertidal.

Yan *et al.* (2004) built a trap design incorporating a mesh filter and flow-through system conceptually similar to Setran for use in the wave-swept rocky intertidal. Flow through the trap is made possible by the inclusion of outlets in the lower side of the trap bounded by mesh netting and an inlet in the top of the trap. The inlet is held vertically and is stoppered by a valve design. The valve design consists of a positively buoyant plastic ball that closes the inlet during slack water or reversed flow through the trap, preventing washout of the sample. Once again, this trap

demonstrates significant capture of a wide range of near-shore plankton species in proportions similar to those sampled by net trawls in a separate study of the same area. However this trap must be unable to record larval input relevant in flow environments less than those restricted by the closure of the one way inlet during “slack” water as does Setran’s trap.

Hannan (1984) describes the use of a passive sediment trap for identifying the rates of initially deposited larvae in soft sediment communities. Her goal was to explain the variability in deposition of benthic species over scales ranging from tens of metres to kilometres. Her premise was that although larval settlement was driven largely by larval choice across small scales, large-scale variability may be driven by passive deposition of larvae in the same manner as passive deposition of sediment particles of a similar fall velocity. Hannan (1984) defined “initially settled” as the period of settlement behaviour leading up to the decision to settle or not, making an important distinction between deposition of larvae and Scheltema’s (1974) clearly-defined “settlement choice”. Hannan used the biased sampling properties of sediment traps to assess whether the rank order of capture for two different designs deployed alongside each other would be similar for larvae and sand particles. If they were, then larvae would clearly be supplied as passive particles to the traps in the same rank order as sand particles. Flume studies showed that one design – a gallon jar whose inlet was less than the jar diameter – caught significantly more glass beads and dead larvae than did a second standard cylinder design. These designs were then compared in the field for both larval and sediment capture rates. Larvae were caught in significantly higher numbers in the jars than in the cylinders, even though the jars had similar heights and mouth diameters. Unfortunately, Hannan was unable to explicitly rule out the possibility that larvae had actively selected the gallon jars over the

cylinders. However her experiment highlighted the concept of passive larval flux as a measure of larval delivery to the substratum and the possibility of quantifying this process with sediment trap designs.

Yund *et al.* (1991) developed a larval trap containing a dense formalin killing solution to use alongside settlement and recruitment counts. Their cylindrical sediment trap had a high aspect ratio (height to width) and was filled with formalin that as well as killing larvae that entered the trap, acted to damp internal flow in the tube and limit resuspension of larvae. They discussed aspects of bias in sediment traps in light of their use as larval sampling devices. Traps may over- or under-estimate true levels of particle supply. They argue, however, that as long as tube traps are required only for measures of relative larval flux (as would be the case in large scale or comparative studies) this will not be a problem for larval biologists. They point out that different trap designs perform with different biases across different flow regimes and for this reason comparisons cannot be made between studies using different designs. Sediment traps collect particle sizes in different proportions as a result of variation in their collection efficiency. This is due to the differences in fall velocity of different sized particles. In order to capture a particle that particle must drop through the eddy that develops at the top of the tube. Owing to their lower fall velocity, small particles are less likely to achieve this than will particles with higher fall velocities. Consequently the efficacy of tube traps to sample the total plankton community is weak because different larval stages and species often vary a great deal in their fall velocity. Relating supply across larval types is therefore limited when using cylinder traps, which may therefore show strong species bias.

Importantly Yund *et al.* (1991) stressed that capture rates may vary according to flow conditions, which can affect trap collection efficiency in two ways.

Resuspension of captured particles can occur due to the eddy created near the inlet of the trap. Subsequently this requires the traps to have a relatively high aspect ratio to avoid this bias. This limits the depth of the inlet eddy from reaching the captured particles in the bottom of the tube. Analysis of the depth of dilution of the formalin layer in their trap suggested an aspect ratio of  $\geq 12$  was required to eliminate resuspension under the flow conditions experienced by them. The process by which traps can be biased when measuring particle capture in their susceptibility to the effect of horizontal flow on capture rates was highlighted as fundamental to larval trapping. Increased flow will increase the rate of capture over time. To a sedimentologist interested in only the vertical flux of particles this is a problem. As a result traps will measure a degree of continual flux rather than simply sampling concentration, as in the case of a net or pump sample. Yund *et al.* (1991) argue this is a very important aspect of time-integrated larval samplers, such as a sediment trap, and distinguish this aspect as a fundamental advantage to their use in studies of larval supply. Larval input is a result of flux, which is a product of larval concentration and advection rates to the target area. Instantaneous samplers such as nets and pumps miss this vital process. A passive larval trap successfully integrates these processes.

Bertness *et al.* (1992) noted comments that, historically, studies measuring recruitment had often missed the crucial phases of supply and early settlement mortality of larvae as a result of experimental procedure. Following on from the development of the larval trap concept by Yund *et al.* (1991), they deployed the trap alongside settlement counts on natural substrate immediately adjacent to the traps as well as recording early mortality. The larval traps were deployed in the subtidal zone 15 to 40 m from the intertidal sampling sites at two locations. The Mt. Hope Bay site demonstrated high levels of recruitment relative to Little Compton. The traps were

retrieved approximately every two weeks. From this study Bertness *et al.* (1992) were able to identify an earlier onset of cyprid arrival in the water column at one site over the other. The number of larvae supplied to Mt Hope Bay was also much greater than Little Compton. Rates of settlement were seen to correlate with results from the traps; however numbers of trapped cyprids were very low. Timing and magnitudes of cyprid abundance were similar for the two measures. However, the most significant finding in this work was that although settlement was predictable by supply, the slope of the relationship was dependent on the degree of available space. When space was severely limited, as in the case of Mt Hope Bay, settlement per unit of supply was increased as larvae appeared to settle preferentially at the this site over the other site. One day in the early part of the settlement season demonstrated relative settlement failure for this site. This point fitted well with the relationship between supply and settlement at the low settlement site. This was explained as being due to low levels of saturation of available space at this site at this time, when later in the season larvae demonstrated more of an inclination to occupy available space when they found it. They argue therefore that counts of settlement do not necessarily describe the rates of larval supply to a site. Studies of proportional rates of supply and settlement provide unique insights into the ecology of these systems.

Todd (2003; 2006) discussed the use of a compact, affordable and easily manufactured passive settlement trap modelled on the concept of Yund *et al.* (1991) as an effective means of quantifying larval supply to the rocky shore. The early design was a smaller version of Yund's (1991) design, incorporating internal baffles and a dense urea killing solution. Its size allowed it to be deployed directly alongside settlement assays in the intertidal rocky shore and replicated much as the Yan *et al.* (2004) design. The replacement of formalin with urea precluded the use of

carcinogenic noxious chemicals whilst the baffles and dense solution provided hydrodynamic stability within the trap cylinder. The results demonstrated absolute capture values considerably greater than those of Yund *et al.* (1991) and Bertness *et al.* (1992). The trap was proven to work consistently over tidal and daily time scales despite washout of the dense urea killing solution across a range of wave crash conditions. Data collected for larval supply demonstrated significant temporal and spatial variation within and between sites separated by ~ 50 m across tides as well as days. Results showed consistent within-site variation on small scales, indicating that increased urea washout led to decreased larval capture efficiency (Todd 2003). It was felt, therefore, that further design improvements were required to increase retention of fluid and captured larvae whilst not compromising the influx of larvae. As a result spiral baffles were added to the top section of the trap where the fluid exchange occurred (Todd *et al.* 2006). This had the effect of markedly decreasing urea washout, presumably by stabilising the flow within the trap, whilst maintaining the target area through which the larvae had to pass. Analysis demonstrated no significant improvement of the capture of larvae by the spiral trap design, however the tests were weak due to low larval numbers (Todd *et al.* 2006).

Gardner (1980a; 1980b) demonstrated that different designs of sediment trap affected the relative capture rate of the trap. He used dyes to distinguish flow patterns around and within different designs of traps. His designs included cylinders with various inlets including cones. He found cone designs demonstrated longer residence times of fluids inside the traps than did straight cylinder designs, even though rates of circulation and exchange near the opening were equivalent. Gardner (1980a; 1980b) also demonstrated that there is a direct correlation with trapping efficiency of sediment and the residence time of dyes within the trap. In fact narrow neck, wide-

bodied trap designs caught significantly more particles in absolute terms than expected from predictions of vertical deposition rates. Gardner stated categorically that the capture efficiency of a trap is a function of fluid residence time. Field experiments demonstrated that the increase in residence time observed in trap designs with a narrow inlet and wide body led to an increase in capture of fine particles with lower sinking rates. This reinforces the idea that the longer residence times allow more particles to settle out. Designs without baffles, when compared to baffled designs, again demonstrated a high ratio of large to small particles. Rapid fluid exchange in unbaffled designs led to a decrease in retention of particles rather than an increase in influx of particles and their subsequent capture. This was manifested disproportionately in particles with a low fall velocity.

Hannan (1984) used jars of similar inlet size but different internal diameters and corroborated these findings. The jars with the largest internal diameter relative to the opening caught the largest number of passive particles (sand, glass beads and inert larvae) Further studies in the field demonstrated similar results for numbers of live larvae.

In order to stabilise the flow inside the traps further, therefore, a cone inlet was included in the definitive trap design in Todd (2006) and the present study. This was in order to increase the efficiency of particle retention and therefore larval retention within the trap throughout a range of wave action typical to the rocky intertidal shore.

### **3.1.3 Small-scale hydrodynamics – implications for slot position**

In marine habitats, small-scale topographic heterogeneity influences hydrodynamics to a significant degree. This in turn has been shown to influence the composition of species assemblages on these scales. For example, Cusson and

Bourget (1997) and Guichard and Bourget (1998) examined the influence of different sized boulders on sand grain size, flow rates (using dissolution of plaster of Paris cylinders) and biodiversity (richness and diversity plus density and biomass estimates) at the metre scale. Both papers demonstrate a complex, non-symmetrical, low flow environment that forms on two sides of the boulders depending on the state of the tide. Importantly they demonstrate that hydrodynamic flow, not predation or sediment composition, was important in determining the biodiversity found in the vicinity of a turbulent body. They speculate this result may be due to passive dispersal of larvae or alterations in food availability affecting species assemblages.

Breitburg *et al.* (1995) demonstrated larval aggregations of reef fish in the low flow wake of boulders, which allowed these fish larvae to maintain their position in high flow environments. The fish larvae were observed actively selecting low flow environments throughout a tidal period. Mullineaux and Butman (1991) demonstrated barnacle cyprids actively exploring and settling on tiles with varied flow regimes along their face. They appeared to actively explore and select areas on the tile that could be correlated to specific boundary layer patterns of flow.

Todd (2003) demonstrated consistent slot positional effects across 8 trap positions separated by 10 cm whilst deploying 4 traps in fixed positions for multiple days. The resolution of that trap design is clearly sufficient to detect small-scale variation in larval supply, which itself may link to variation in settlement and subsequent recruitment at these scales. Whether or not these differences outweigh the importance of post-settlement factors in determining adult abundance has yet to be determined.

The aim of the present study was to further improve on the capture rates of the passive settlement trap described by Todd (2003, 2006) to aid in the deployment of

the trap across increasing fine temporal and spatial scales. The addition of spiral designs as described in Todd (2006) was shown to be of limited benefit to cyprid capture despite significantly improving urea retention. This study will investigate the addition of a coned inlet on the premise that it will increase retention times of particles such as larvae and provide an opportunity to increase trap capture rates.

## 3.2 METHOD

### 3.2.1 Trap design and construction

The design for the spiral baffled traps with a cylindrical inlet was conceived by Professor Chris Todd of the Gatty Marine Lab prior to the beginning of this study. The addition of the conical entrance was formulated through group discussions that took place at the beginning of the study in 2003.

The traps were built from 50 ml polypropylene, specimen tubes (Greiner Cellstar, product no. 210270; [www.greinerbioone.com](http://www.greinerbioone.com)). They have a 2.8 cm diameter and are 11.5 cm tall. A cylindrical skirt extends 1.5 cm from the tube wall surrounding a conical base.

The trap design can be described in three sections. The bottom section is the collection chamber. This was built from a complete specimen tube. The conical bottom was cut away with a scalpel that was first heated in a Bunsen burner flame. The plastic melted away easily with very little disfigurement of the tube walls; however the hot blade was kept a safe distance of a few mm from the wall and the skirt so that the heat from the blade did not warp or texture the surface. Once the cone had been cut and pushed out of the tube the excess cone plastic could be carefully shaved with a sharp scalpel blade until it was flush with the internal walls of the tube. The threaded cap end of the tube trap then provides a watertight seal for closing the trap.

The second section of the trap was composed of two sections of baffles. These limited turbulent washout within the trap thereby encouraging the sinking of passive particles and improved retention of the killing solution. The lower part of that section consisted of full baffles. Tubes were cut 3 cm from the skirt end so that they included only the skirt, the cone and some of the tube wall. A lathe was used to produce a

clean, straight cut. A metal ruler was then gently scraped around the cut end to remove any remnant plastic and clean the edge. This was important so as to provide a neat joint with the adjacent section. The conical end of the part was cut down with a lathe to leave a 0.5 cm hole at the end of the cone flush with the skirt. Four bleed holes were created at equidistant points on the baffle with a thick paper clip wire (approximately 1 mm) that had been heated in a Bunsen flame. These were made as close to the skirt wall as possible whilst not disfiguring the wall. They allowed air to bubble up through the baffles whilst the trap was re-filled, ensuring a constant volume of killing solution amongst days. Pairs of 3 cm full baffles were aligned in sequence above the collection chamber. A third full baffle was included above these two parts, however this baffle aperture was only 1.7 cm, including only the skirt and cone. To accurately cut the part close to where the tube wall ended, and the skirt and cone began, the lathe cut slightly above the join and then a circular sander was used to grind the plastic to the desired point. The baffle and bleed holes were created in the same manner as the large full baffles.

The upper part of the baffled section was constructed of parts similar to the smaller full baffle. However, rather than creating a hole at the base of the cone, three quarters of the cone were removed with a hot scalpel blade leaving a triangular 'flap' or quarter baffle. The triangular baffle was cut over a piece of graph paper to aid the accurate repetition of the baffle shape. Four baffles were arranged in a progressively 90° offset pattern creating a clockwise descending spiral of quarter baffles.

The third section was the inlet. Multiple designs of inlet were deployed throughout this project. They were essentially constructed in one of two ways. The first and earliest design was the same as the 3 cm part used in the large baffles. The

cone was removed using the same method as the collection chamber. This left a 5 cm<sup>2</sup> cylindrical inlet to the trap.

The second design type was a coned inlet. The end of a coned, skirtless 50 ml specimen tube (Greiner Cellstar, product no. 210261; [www.greinerbioone.com](http://www.greinerbioone.com)) was cut 3 cm from the tip of the cone. The design of the inlet could be modified according to requirements by carefully grinding the tip with a circular sander to produce the desired inlet area. Traps of 0.25 cm<sup>2</sup>, 0.5 cm<sup>2</sup>, 1 cm<sup>2</sup> and 2 cm<sup>2</sup> aperture area were built and deployed throughout this project. Grinding was done after casting the traps in resin. Because the inlet must be produced with a degree of accuracy it made the coned end easier to handle. Henceforth, these coned traps are referred to as 0.25 cm<sup>2</sup>, 0.5 cm<sup>2</sup>, 1 cm<sup>2</sup> and 2 cm<sup>2</sup> traps.

The trap parts were taped together with sellotape to hold them in place. The sellotape was stretched because it was wrapped around each part to produce a tight fit. It was important at this point to ensure the trap does not bend. Rolling over a flat surface indicated whether this occurred and it was easy to identify any mis-aligned parts.

The trap was then set in a hard resin. Before this was possible it was necessary to construct a mould in which the resin would be poured around the trap. A 25 cm long section of 4 cm polypropylene waste pipe was used as a model trap to create the silicon mould. Two sets of three 1 cm wide shrink-wrap plastic collars (two layers each) with two 0.7 cm gaps between each of the three collars were positioned at the top and bottom of the model trap. The trio near the inlet end of the tubes was spaced 8 mm from the model trap inlet end; the lower set of collars began 11 mm from the cap end. For the 5 cm<sup>2</sup> trap moulds, the end of a skirted Greiner tube was attached to the inlet end of the trap mould. The Greiner tube was wrapped with sellotape 1.4 cm from

the start of its skirted end so that it would fit snugly inside the plastic pipe with the unwrapped cone section protruding. For the coned inlet mould the end of a skirtless Greiner tube was used in exactly the same manner. At the cap end of the model trap a Greiner tube was inserted into the waste pipe with the cap end protruding. Again tape was applied, this time 27mm from the capped end to seal the thinner centrifuge tube against the waste pipe.

1 kg of Flexil-S RTV-30C silicon and standard 5 % green catalyst (Jacobson Chemicals; [www.jacobsonchemicals.co.uk](http://www.jacobsonchemicals.co.uk)) was used to produce a trap mould from the model trap. 500 g of liquid silicon were initially mixed and fully degassed in a vacuum pump. The trap model was laid in a 10 x 10 x 25 cm plastic tub. The tub was half filled with the liquid silicon and left to go-off around the model trap. A mould release agent (J-Wax, Jacobson Chemicals; [www.jacobsonchemicals.co.uk](http://www.jacobsonchemicals.co.uk)) was liberally applied to the surface of the first half of the mould and then the second half of the mould was poured on top following the same protocol. Once the silicon had set the two halves were pulled apart and the model trap removed. The cap end of the mould was neatly cut away with a scalpel before the thicker part of the mould formed by the 4cm waste pipe began. Notches were cut into the mould down to the widest part of the trap mould. This would allow the resin to be poured in when the sellotaped trap parts were arranged in the mould.

To set the trap in resin the mould was sprayed with J-Wax and the taped trap parts were laid in the mould. The mould was taped together firmly between two wooden boards to ensure it stayed rigid and no leakage could occur between the mould halves. 165 g of epoxy (Robnor Resins Ltd., Product no. PX771C/NC; [www.robnor.co.uk](http://www.robnor.co.uk)) was mixed with 55 g of hardener (Robnor Resins Ltd., product

no. HY1300GB) and degassed in a vacuum pump. The resin was poured into the mould around the trap parts and the mould was left overnight to set.

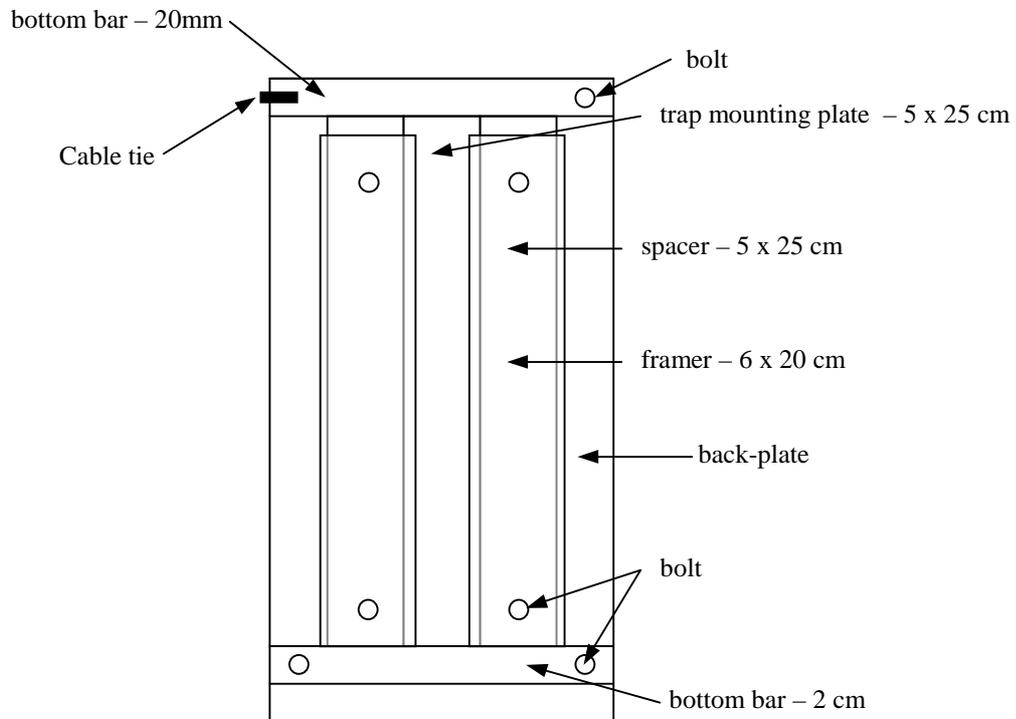
### **3.2.2 Trap mounting and deployment in the field**

Individual traps were mounted on a 5 x 25 cm strip of acrylic. 8 holes were drilled in the acrylic strip corresponding to either side of the 4 grooves on the traps. Two pieces of foam pipe lagging were cut into 2 x 4 cm squares and were mounted behind each of the groups of collars on the trap. Cable ties secured the traps behind the acrylic strips. The female end of the tie was held against the back of the acrylic strip and pliers were used to pull the cable tie tight. This ensured a strong grip on the trap to reduce movement during wave crash or rotation of the traps through the season.

The traps were deployed in the field attached to acrylic back-plates. The size and shape of the back-plate depended on the experimental design required; however the mounting mechanism for the traps was standard throughout and can be described here. This mechanism was conceived following discussions amongst colleagues working on this project. Majority credit should be given to Chris Andrews (technician) for the development of the following design.

The back-plate consisted of a continuous acrylic sheet. 20 cm strips of acrylic 6 cm wide were arranged along the back-plate, spaced 4 cm apart. These were the framers that held the trap in place. A spacer placed under the framers allowed the trap mounting plate to slide under the lip of the framers. The spacers are 5 x 25 cm strips of acrylic - the same as the strip on which the traps were mounted. A 2 cm thick bar of acrylic was bolted along the bottom of the slots on the back-plate to stop the traps slipping out the bottom of the slots. A second bar was bolted at one end along the top

of the slots. The other end was cable tied. The cable tie could be snapped allowing the bar to pivot on the single bolt to remove, replace or rearrange the traps when necessary (*Figure 3.1*).



*Figure 3.1. Schematic diagram of a single trap mount detailing acrylic components.*

In the field, rope lattices were tied around rocks to create fixing points for instruments and equipment. 0.6 cm nylon rope was tensioned onto the rock to provide a net-like frame. Pairs of 5 cm nylon “cod end” rings were attached to either end of the twine and spare twine was wrapped three or four times between the two cod end rings. This twine could then be pulled tight and fixed with half hitches. The rope lattice design must be adapted to the specific rock shape but as a general rule a top and bottom rope were tensioned around the rock and then counter tension ropes were tied to stop these ropes moving up or down. Cracks or buttresses provided opportunities to further secure the ropes. This method did not require bolts and therefore left the rock relatively undamaged. Additionally, elasticity in the twine

helped absorb some of the shock of wave crash on the acrylic back-plates making them less likely to crack or snap.

Barnacle larvae, like many invertebrate larvae, have the ability to swim in still water therefore it was necessary to use a killing solution of concentrated urea in seawater so they could not swim out of the trap. Urea in solution is a relatively safe chemical so the traps could be deployed in close proximity to bathing beaches. Urea is common in the intertidal environment, produced as a waste product by the majority of organisms on the shore. Because it was so concentrated and denser than seawater it did not dilute a great deal. It also provided a stabilising influence on the hydrodynamics inside the tube. At servicing of the traps after two tides a clear seawater volume was detectable at the trap entrance and there was a very abrupt interface to the blue-dyed urea. This indicates washout, but minimal mixing of urea by seawater.

240 g of 98% urea crystals (20888-4; Sigma-Aldrich co. ltd.) were diluted per 1 litre of seawater. The solution was left overnight to fully dissolve. 1 g of bromophenol blue (BPB) per 1000 ml of distilled water was made up according to the required volume of urea and allowed to dissolve overnight. This was then added to the urea solution, once both solutions were fully dissolved, at 1% by volume. BPB urea solution was made up in bulk at the start of the experimental period. Before being used each time it was stirred well otherwise condensation can develop inside the storage vessel and a halocline may result.



*Plate 3.1* Three passive larval traps deployed as part of a supply-settlement array. The traps are filled with urea solution, which is dyed blue with BPB. The array is attached to a rope lattice on a concrete block at Tentsmuir.

### **3.2.3 Trap collection and lab analysis**

Traps can be serviced tidally or daily (every two tides). To empty a trap *in situ*, a sealable plastic pot was held underneath the trap and the screw cap loosened. The majority of the fluid in the trap was carefully drained into the pot whilst still holding the cap in place on the trap opening (outlet). If the cap is removed too quickly the trap contents will empty too fast and may spray out the side of the pots. A small amount of the trap sample was rinsed through the trap into the pot with a plastic pipette to dislodge any material that may have settled on the baffles. The cap was washed thoroughly in the trap sample and then in a clean rock pool before re-threading; this removed any sand from the cap threads, which would cause a leakage of the urea solution. A hypodermic needle could be used to apply a small amount of Vaseline around the threads of the cap each day after it had been screwed back onto the trap base to ensure there was no leakage. To refill a trap with concentrated urea, a flexible plastic tube with a funnel attached to the top was pushed down the inside of the trap to the cap of the collection chamber. The tube end was cut to a point and tubing twisted, whilst inserting downwards, through the baffles. The selection of pipe

was important. Too soft and it would not push past the spiral baffles. Too stiff and it was hard to twist and bend through the spiral baffles at the top. The urea solution was poured into the traps through the pipe so that air bubbles were less likely to become trapped whilst it was being refilled. It is important to maintain a continuous flow of liquid into the funnel because stopping and starting will allow air through the tubing, resulting in a large air bubble in the trap. The traps were topped up to the top and left for a few minutes to see if the fluid level dropped. If it did so significantly there was likely to be a leak at the cap. The cap must be removed, cleaned and replaced and the trap refilled because the leak would continue throughout the deployment of the trap and bias the result.

Following deployment there was inevitably some washout of the urea solution at the inlet of the trap. Samples of the retrieved and mixed trap solution were taken to assess the degree of washout of urea solution. Once returned to the laboratory the particulate matter in the samples was allowed to settle out for an hour and then a 1.2 ml aliquots are taken from the trap sample pots with a Gilson pipette. Samples were taken close to the surface so that none of the sand, algae or larvae was taken up through the pipette. The samples were frozen in 1.5ml eppendorf microtubes for storage throughout the experimental period. After the fieldwork was complete each sample was run through a Shimadzu UV-1601 spectrophotometer set to read absorbency at 594nm. A filtered sea-water blank was used as a reference control and the samples were analysed in bulk so the same blank was used. Seawater dilutions of 10% increments of the original BPB urea solution were made up and a calibration curve was plotted against the same seawater blank. The percent washout of each trap was determined from this calibration curve.

Each trap sample was filtered through a 100  $\mu\text{m}$  sieve with tap water and then solid contents of the sample were washed into a large glass petri dish. The sample was swirled to lift out the less dense organic content, which could be gently decanted into a smaller glass petri dish. Large algal fragments were removed by eye with a pair of needle nosed tweezers. Larvae were sorted, identified and counted under a dissecting microscope and preserved in 50% methanol.

### **3.2.4 Trap design comparisons**

Multiple trap arrays were constructed and deployed at three sites in two years so that comparisons of inlet designs could be made in the field. Data for cyprid capture of two species of barnacle *Semibalanus balanoides* and *Balanus crenatus*, proportional trap urea retention and volume of sand capture were routinely recorded. Traps were re-filled after two tides, for as long as day light conditions permitted. When retrieval was restricted by daylight the deployment was extended for a third tide and servicing resumed the following morning. These three-tide deployments were approximately fortnightly and are not included in the analysis here as no study was done to examine the effectiveness of the traps beyond two tides.

In 2004 multiple trap design arrays were deployed at Fife Ness and Tentsmuir. At Tentsmuir a 9-trap array was deployed with 0.5cm<sup>2</sup> and 1cm<sup>2</sup> coned trap designs and a cylindrical 5cm<sup>2</sup> unconed spiral design. Triplicate traps for each of the three designs were deployed. At Fife Ness in 2004 a 12-trap array was deployed. This array consisted of 2 sets of 6 slot positions either side of an artificial settlement substrate array because it was part of the mesoscale study described in Chapter 5. 0.5cm<sup>2</sup>, 1cm<sup>2</sup> and 2cm<sup>2</sup> coned designs and 5cm<sup>2</sup> unconed design was deployed in triplicate.

In 2005, the 9-trap array was moved to Reres Wood. Coned and spiral trap designs with 0.5cm<sup>2</sup> and 1cm<sup>2</sup> inlets and a 5cm<sup>2</sup> unconed spiral design were deployed in triplicate once again. Part way through the season the 5cm<sup>2</sup> unconed spiral design was replaced by a 0.25cm<sup>2</sup> coned and spiral design because it was apparent that the 0.5cm<sup>2</sup> design was still able to capture large numbers of cyprids despite its small aperture size. In 2005, the 12-trap array was re-deployed at Fife Ness in the same location as in 2004. On this occasion, 10 slots were used, 5 either side of the artificial substrate array. 0.5cm<sup>2</sup> coned and spiral trap designs and a 5cm<sup>2</sup> unconed spiral design were deployed in triplicate once again. 1cm<sup>2</sup> traps were deployed in quadruplicate due to their involvement in the 2005 mesoscale study (Chapter 5). Part way through the season the 5cm<sup>2</sup> unconed spiral design was replaced by a 0.25cm<sup>2</sup> coned and spiral design as at Reres Wood.

Analysis of the data was undertaken using ANOVA. Todd (2003) demonstrated the original trap design resulted in detectable slot positional effects, possibly due to consistent small scale hydrodynamics across a trap array. Due to the possibility of consistent positional effects, trap positions were randomised prior to each daily deployment in the field. Following Mauchly's test for sphericity, the existence of positional effects for each array, in each year, was determined by balanced repeated measures design ANOVA on data for *Semibalanus balanoides* and *Balanus crenatus* cyprid capture, urea retention and sand capture. The amount of sand and *Balanus crenatus* larvae was insufficient at Fife Ness to perform the analysis for this site on these dependent variables.

Data for cyprid capture of both species and urea retention was transformed where appropriate to normalise the data. Sand was measured only if >1ml was recorded. If data for the dependent variable, cyprid capture rate, was less than 1 for

any slot position on any day that day was removed from the analysis because it would provide no relative information on the performance of that slot position. For the urea analysis any leakage of a trap meant the exclusion of these data for that day from the analysis.

Differences in trap designs and between days were examined with balanced factorial ANOVA because this is the most robust method when data fail the assumptions of ANOVA. Bonferroni multiple pairwise comparisons were performed on design types when a significant difference was found from ANOVA. Transformed data for *Semibalanus balanoides* cyprid capture, urea retention and sand capture were used when appropriate.

At Tentsmuir and Reres Wood it was necessary to adjust urea retention for sand capture. Due to the density of concentrated urea there is a steep seawater/urea gradient resulting in a recognisable interface between the killing solution and the washout zone in a trap whilst it was deployed in a field environment. Therefore urea concentration was not judged to be distributed in a uniform manner throughout the trap and the crucial factor linking urea concentration to cyprid capture was judged to be contact with a sufficiently concentrated urea solution rather than total trap concentration. Sand capture displaces urea at the bottom of the tube forcing the more concentrated urea solution towards the inlet. As a result it was necessary to determine the volume of washed out urea rather than retained urea. The volume of sand was subtracted from the total volume of the trap. This was multiplied by the proportion of urea washed out. The volume of washed out urea was then divided by the total volume of the trap and this was expressed as proportion of urea retained. Data for percentage urea retained was then arcsine transformed into degrees.

### 3.3 RESULTS

#### 3.3.1 Slot positional effects

##### 3.3.1.1 *Semibalanus balanoides* cyprid capture

Fife Ness 2004 alone failed Mauchly's test for sphericity and subsequent degrees of freedom were adjusted in the repeated measures ANOVA for *Semibalanus balanoides* cyprid capture (see Table 3.2).

Site	Days deployed	Number of array slot positions
Tentsmuir 2004	32	9
Fife Ness 2004	43	12
Reres Wood 2005	28	9
Fife Ness 2005	31	10

Table 3.1 Slot positional affects were tested for four sets of data prior to conducting design analysis. Multiple trap arrays were required. Days were removed from the slot analysis when no relative capture data was recorded for any one slot on the array.

Site	Within Subjects Effect	Mauchly's W	Approximate Chi-Square	df	Sig.	Greenhouse-Geisser ( $\epsilon$ )
Tentsmuir 2004	SLOT	.225	41.953	35	.201	
Fife Ness 2004	SLOT	.075	98.781	65	.005	.715
Reres Wood 2005	SLOT	.268	31.729	35	.636	
Fife Ness 2005	SLOT	.186	45.028	44	.441	

Table 3.2. Mauchly's test for sphericity across slot positions for  $\log(x+1)$  transformed *Semibalanus balanoides* cyprid capture at four site conditions.

Site	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Tentsmuir 2004	SLOT	.206	8	.026	1.430	.184
	Error	4.461	248	.018		
Fife Ness 2004	SLOT	.490	7.864	.062	.910	.507
	Error	22.606	330.304	.068		
Reres Wood 2005	SLOT	.646	8	.081	1.767	.085
	Error	9.863	216	.046		
Fife Ness 2005	SLOT	.216	9	.024	.472	.893
	Error	13.717	270	.051		

Table 3.3. One-way Repeated Measures ANOVA. Slot = repeated measure. Day = independent variable. Data is for  $\log(x+1)$  transformed *Semibalanus balanoides* cyprid capture at four site conditions. Degrees of freedom were adjusted where appropriate according to Mauchly's test for sphericity.

No significant slot affects were found for  $\log(x+1)$  transformed *Semibalanus balanoides* cyprid capture at the four sites. Accordingly it is concluded that

randomisation of trap positions throughout deployment effectively removed any positional effects in further analysis of trap performance (*Table 3.3*).

### 3.3.1.2 Urea retention

Site	Days deployed	Number of array slot positions
Tentsmuir 2004	34	9
Fife Ness 2004	58	12
Reres Wood 2005	36	9
Fife Ness 2005	37	10

*Table 3.4.* For slot positional analysis of urea retention, days were removed from the slot analysis when no washout data was recorded for any one slot on the array.

Site	Within Subjects effect	Mauchly's W	Approximate Chi-Square	df	Sig.
Tentsmuir 2004	SLOT	.342	32.302	35	.605
Fife Ness 2004	SLOT	.307	62.759	65	.561
Reres Wood 2005	SLOT	.218	48.967	35	.061
Fife Ness 2005	SLOT	.182	55.819	44	.114

*Table 3.5.* Mauchly's test for sphericity across slot positions for arcsine transformed proportional urea retained (degrees) at four site conditions. Data has been adjusted for sand capture where appropriate.

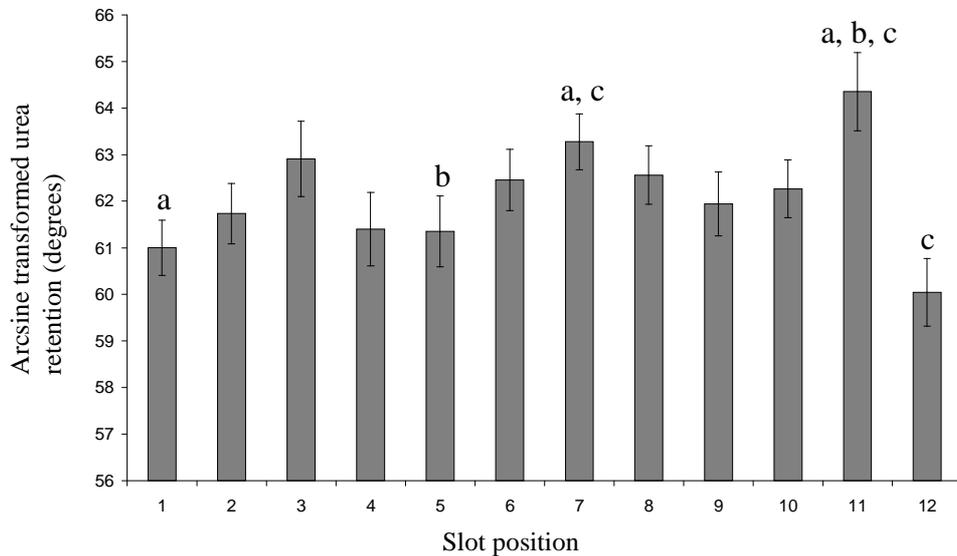
All sites conformed to Mauchly's test for sphericity across slot positions for urea retention (*Table 3.5*) so no adjustments were required in the repeated measures ANOVA.

Site	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Tentsmuir 2004	SLOT	221.982	8	27.748	.570	.802
	Error	12859.197	264	48.709		
Fife Ness 2004	SLOT	818.854	11	74.441	3.873	< 0.001
	Error	12050.281	627	19.219		
Reres Wood 2005	SLOT	183.025	8	22.878	.594	.783
	Error	10788.896	280	38.532		
Fife Ness 2005	SLOT	359.024	9	39.892	1.333	.219
	Error	9694.576	324	29.922		

*Table 3.6.* One-way Repeated Measures ANOVA. Slot = repeated measure. Day = independent variable. Data is for arcsine transformed proportional urea retained (degrees) at four site conditions.

Significant slot effects were found for urea retention at Fife Ness in 2004 (*Table 3.6*). *Post hoc* analysis using pairwise comparisons for estimated marginal means, after applying a Bonferroni confidence interval adjustment (Field 2005),

showed five of the slot positions demonstrating significant differences from other slot positions across 58 d of the settlement season. Significantly different pairs of slot positions are summarised in *Figure 3.2*.



*Figure 3.2* Mean urea retention for 12 slot positions at Fife Ness in 2004. Error bars show one standard error. Letters denote significant differences between slot positions for urea retained according to Bonferroni adjusted estimated marginal means.

### 3.3.1.3 Sand capture

Site	Days deployed	Number of array slot positions
Tentsmuir 2004	25	9
Reres Wood 2005	10	9

*Table 3.7.* For slot positional analysis of significant sand capture, days were removed from the slot analysis when sand was recorded at less than 1 ml for any one slot on the array.

As a result of the unique intertidal and subtidal substrate of each site in the study, sand was caught only at the beach sites of Tentsmuir and Reres Wood (*Table 3.7*).

Site	Within Subjects Effect	Mauchly's W	Approximate Chi-Square	df	Sig.	Greenhouse-Geisser ( $\epsilon$ )
Tentsmuir 2004	SLOT	.000	213.821	35	<.001	.301
Reres Wood 2005	SLOT	.000	95.741	35	<.001	.374

*Table 3.8.* Mauchly's test for sphericity across slot positions for significant sand capture (ml) at two sites.

Adjustment of degrees of freedom was required for both sites due to failure of Mauchly's test for sphericity (Table 3.8). Greenhouse-Geisser estimates of epsilon provided appropriate value adjustments.

Site	Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Tentsmuir 2004	SLOT	64.856	2.410	26.907	1.444	.243
	Error	1077.882	57.850	18.633		
Reres Wood 2005	SLOT	978.026	2.996	326.477	.618	.609
	Error	14251.278	26.961	528.583		

Table 3.9. One-way Repeated Measures ANOVA. Slot = repeated measure. Day = independent variable. Data is for significant sand capture (ml) at two sites.

Following adjustment no significant slot positional effects recorded were recorded at Tentsmuir or Reres wood for sand capture (Table 3.9).

### 3.3.1.4 *Balanus crenatus* cyprid capture

Site	Days deployed	Number of array slot positions
Tentsmuir 2004	25	9
Reres Wood 2005	10	9

Table 3.10. For slot positional analysis of *Balanus crenatus* cyprid capture, days were removed from the slot analysis when no comparative capture data was recorded for any one slot position in the set.

Sufficiently high abundances of *Balanus crenatus* larvae were recorded only at the sand beach sites of Tentsmuir and Reres Wood (Table 3.10).

Site	Within Subjects Effect	Mauchly's W	Approximate Chi-Square	df	Sig.
Tentsmuir 2004	SLOT	.412	21.416	35	.967
Reres Wood 2005	SLOT	.030	38.923	35	.344

Table 3.11. Mauchly's test for sphericity across slot positions for log(x+1) transformed *Balanus crenatus* cyprid capture at two sites.

Both sites conformed to Mauchly's test for sphericity across slot positions for *Balanus crenatus* cyprid capture (Table 3.11) so no adjustments were required in the repeated measures ANOVA.

Site	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Tentsmuir 2004	SLOT	1.405	8	.176	1.753	.088
	Error	21.633	216	.100		
Reres Wood 2005	SLOT	.964	8	.121	1.248	.278
	Error	10.818	112	.097		

Table 3.12. One-way Repeated Measures ANOVA. Slot = repeated measure. Day = independent variable. Data is for log(x+1) transformed *Balanus crenatus* cyprid capture at two sites.

No significant slot effects were found for log(x+1) transformed *Balanus crenatus* cyprid capture at the two sites following daily randomisation of trap positions throughout the deployment season (Table 3.12).

### 3.3.2 Trap design comparisons

Although appropriate transformations were performed to limit the violation of assumptions of ANOVA, tests for normality and homogeneity of variance repeatedly failed. However ANOVA is robust to both non-normality and heterogeneity of variances for large data sets with adequate numbers of levels within the factors (Underwood 1997) so the analysis was continued.

### 3.3.2.1 *Semibalanus balanoides* cyprid capture.

Source	df	Mean Square	F	Sig.
Design	2	.176	5.89	.004
Day	35	2.05	146	<.001
Interaction	70	.030	2.07	<.001
Error	216	.014		

Table 3.13a

Source	df	Mean Square	F	Sig.
Design	3	21.875	36.081	<.001
Day	42	75.437	197.997	<.001
Interaction	126	.606	1.593	.001
Error	344	.381		

Table 3.13b

Source	df	Mean Square	F	Sig.
Design	2	1.271	28.862	<.001
Day	15	.853	20.310	<.001
Interaction	30	.044	1.060	.402
Error	96	.042		

Table 3.13c

Source	df	Mean Square	F	Sig.
Design	2	.138	2.810	.079
Day	13	4.020	89.333	<.001
Interaction	26	.049	1.089	.373
Error	84	.045		

Table 3.13d

Source	df	Mean Square	F	Sig.
Design	2	.649	19.645	<.001
Day	14	1.372	36.861	<.001
Interaction	28	.033	.921	.584
Error	90	.036		

Table 3.13e

Source	Df	Mean Square	F	Sig.
Design	2	.618	21.724	<.001
Day	14	2.269	58.179	<.001
Interaction	28	.028	.731	.825
Error	90	.039		

Table 3.13f

Tables 3.13. Factorial ANOVA for  $\log(x+1)$  transformed *Semibalanus balanoides* cyprid capture across multiple design arrays for three sites over two years. *a.* Tentsmuir 2004; *b.* Fife Ness 2004; *c.* Fife Ness 2005 5cm<sup>2</sup> deployment; *d.* Reres Wood 5cm<sup>2</sup> deployment; *e.* Fife Ness 2005 0.25cm<sup>2</sup> deployment; *f.* Reres Wood 0.25cm<sup>2</sup> deployment.

Significant differences for Day were seen for all data sets however these were trivial as they were expected due to the temporally variable nature of larval supply (Caffey 1985; Todd 2003). Significant Design (= trap design) effects were seen for all sites except for Reres Wood during the 5cm<sup>2</sup> trap deployment.

Significant interactions between Design and Day were found at both Tentsmuir and Fife Ness in 2004. The interaction effects were as a result of amplitudinal effects, rather than a crossing over of the relative performance of traps on different days (Tables 3.13a-f).

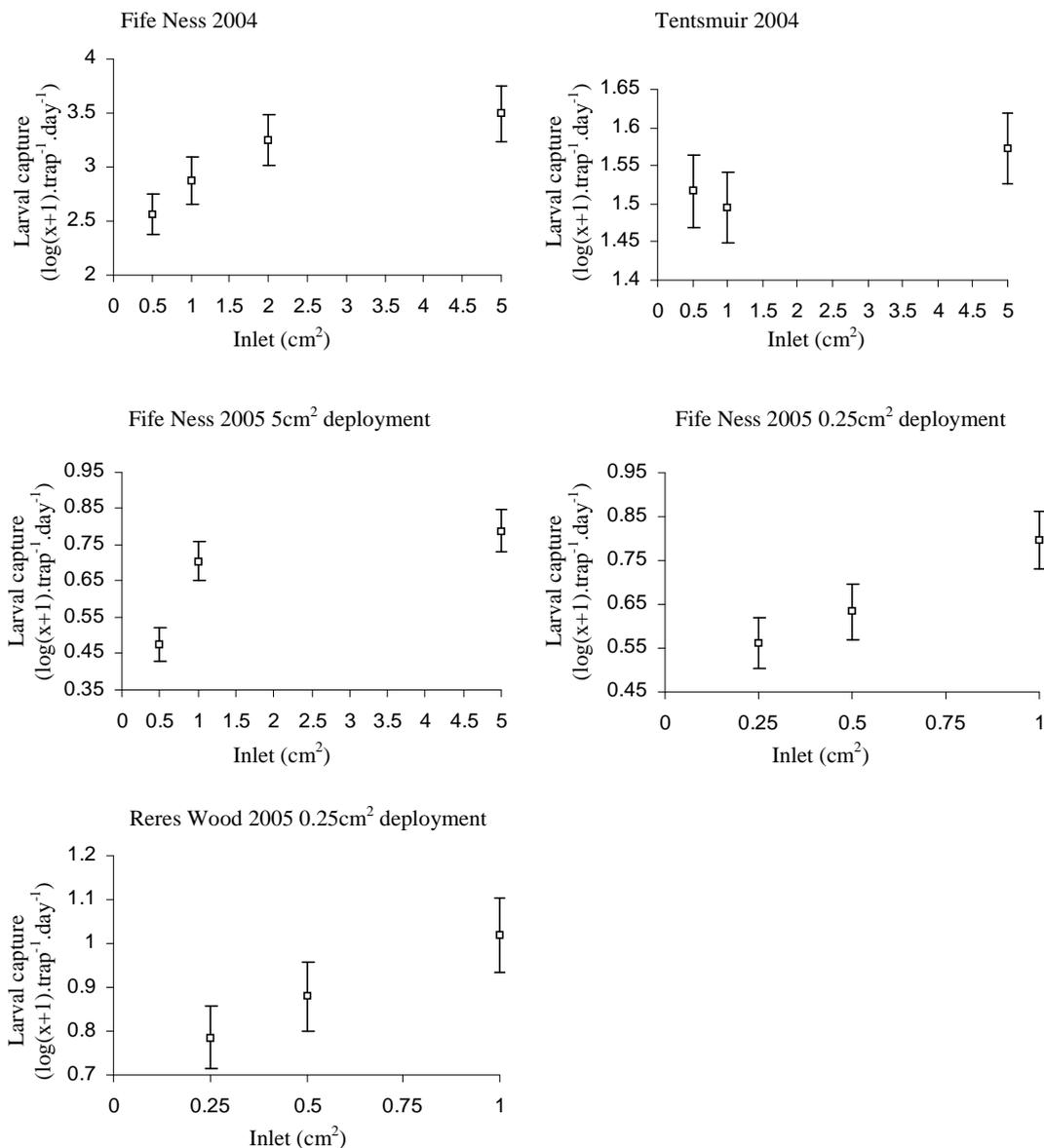


Figure 3.3 Mean  $\log(x+1)$  *Semibalanus balanoides* cyprid capture per trap per day plotted against inlet area for the period of deployment.  $\pm 1$  standard error of the mean shown.

Across almost all the sites, increasing trap inlet area increased absolute capture rates of larvae. No significant difference was seen between the 0.25cm<sup>2</sup> and 0.5cm<sup>2</sup> inlet design at Fife Ness or Reres Wood. Significant differences were seen between all other trap combinations across all the sites in one set or another. Due to the lack of significant design differences, post hoc analysis of the 5cm<sup>2</sup> design deployment at Reres Wood 2005 has not been undertaken (Figure 3.3).

### 3.3.2.2 Urea retention

Source	Df	Mean Square	F	Sig.
Design	2	6572	510.9	<.001
Day	38	167.4	29.90	<.001
Interaction	76	12.86	2.297	<.001
Error	234	5.599		

*Tentsmuir 2004*

Source	Df	Mean Square	F	Sig.
Design	2	1091	107.3	<.001
Day	22	51.50	10.99	<.001
Interaction	44	10.17	2.169	<.001
Error	138	4.687		

*Fife Ness 2005 5cm<sup>2</sup> deployment*

Source	Df	Mean Square	F	Sig.
Design	2	414.3	119.0	<.001
Day	13	23.57	19.41	<.001
Interaction	26	3.480	2.867	<.001
Error	84	1.214		

*Fife Ness 2005 0.25cm<sup>2</sup> deployment*

Source	df	Mean Square	F	Sig.
Design	3	2639	275.8	<.001
Day	66	132.2	15.45	<.001
Interaction	198	9.567	1.118	.165
Error	536	8.558		

*Fife Ness 2004*

Source	Df	Mean Square	F	Sig.
Design	2	2917	50.26	<.001
Day	19	292.7	43.97	<.001
Interaction	38	58.03	8.717	<.001
Error	120	6.658		

*Reres Wood 2005 5cm<sup>2</sup> deployment*

Source	df	Mean Square	F	Sig.
Design	2	926.7	484.6	<.001
Day	15	19.00	8.173	<.001
Interaction	30	1.913	.822	.724
Error	96	2.325		

*Reres Wood 2005 0.25 cm<sup>2</sup> deployment*

Table 3.14. Factorial ANOVA for arcsine transformed percent urea retention across multiple design arrays for three sites over two years.

Highly significant differences between Days were seen for all data sets.

Significant Design effects were seen for all sites. Significant interactions were found at all the sites. These were attributed to amplitudinal variation, rather than crossing over of the relative performance of the trap Design on different Days (Table 3.14).

Bonferroni corrected pairwise multiple comparisons revealed significant differences for all pairings of trap designs at all sites for urea retention so these are not presented here.

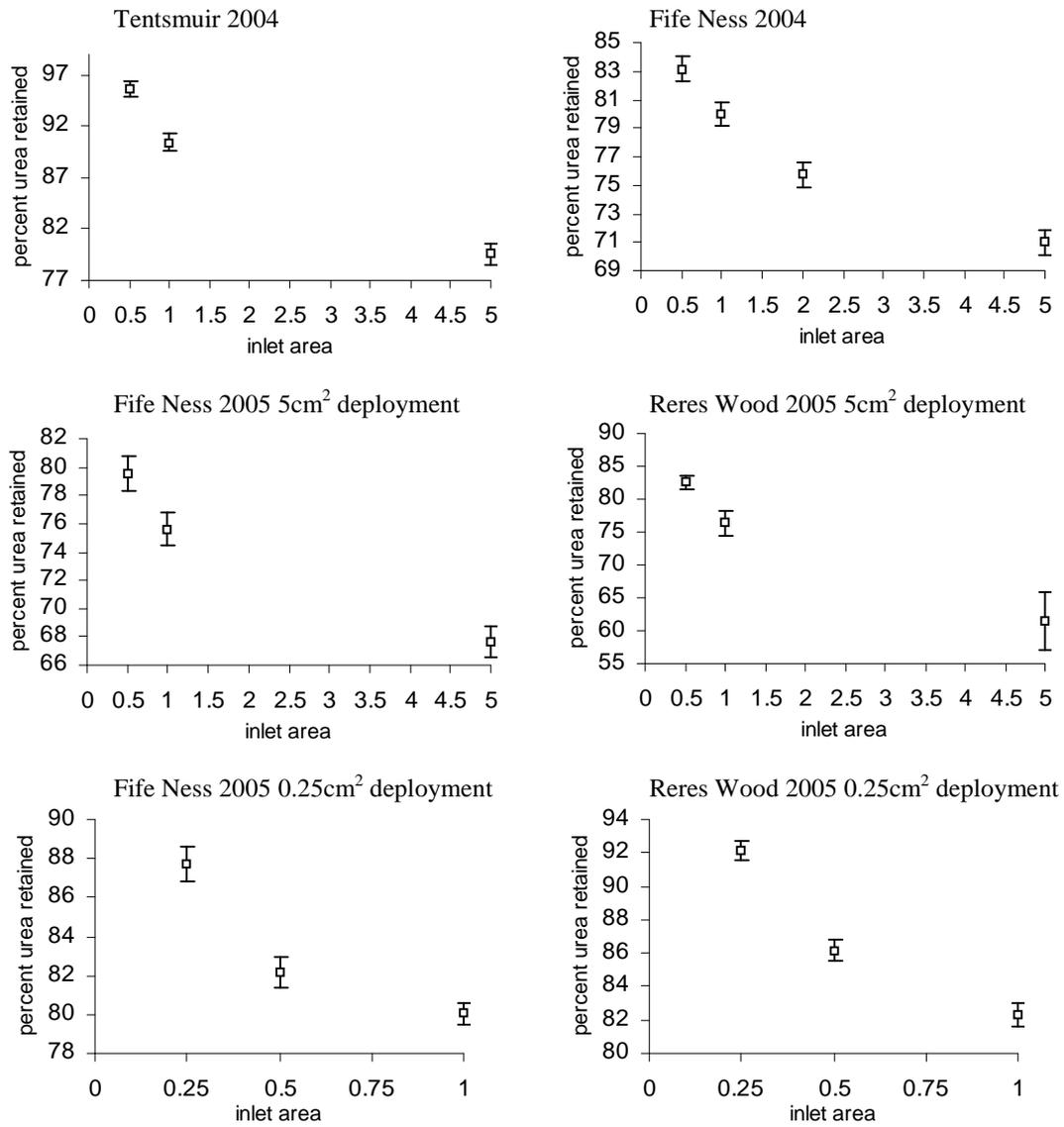


Figure 3.4. Mean and 95% c.i. for percentage urea retained (data has been back transformed from arcsine transformed percent urea retained) for six periods of deployment plotted against the area of inlet.

Figure 3.4 demonstrates the relationship between retained urea and inlet size.

The 95 % confidence intervals are very small relative to the mean urea retained demonstrating highly significant differences between inlet designs for urea washout.

The relationships are consistently negatively correlated.

### 3.3.2.3 Sand capture

Table 3.15a.

Source	df	Mean Square	F	Sig.
Design	2	221.7	15.74	<.001
Day	26	47.92	252.2	<.001
Interaction	52	14.09	74.30	<.001
Error	162	.190		

Table 3.15b.

Source	df	Mean Square	F	Sig.
Design	2	4281	19.27	<.001
Day	8	1244	21.59	<.001
Interaction	16	222.1	3.855	<.001
Error	54	57.62		

Tables 3.15 Factorial ANOVA for significant sand capture (ml) across multiple design arrays for two sites. *a.* Tentsmuir 2004; *b.* Reres Wood 5cm<sup>2</sup> deployment.

Significant differences were found across both sites for Day and Design.

Significant interactions were also observed (Table 3.15). The interactions were due to amplitudinal variation between trap Design over Day. They were not due to crossing over of the relative Design capture rate on different Days.

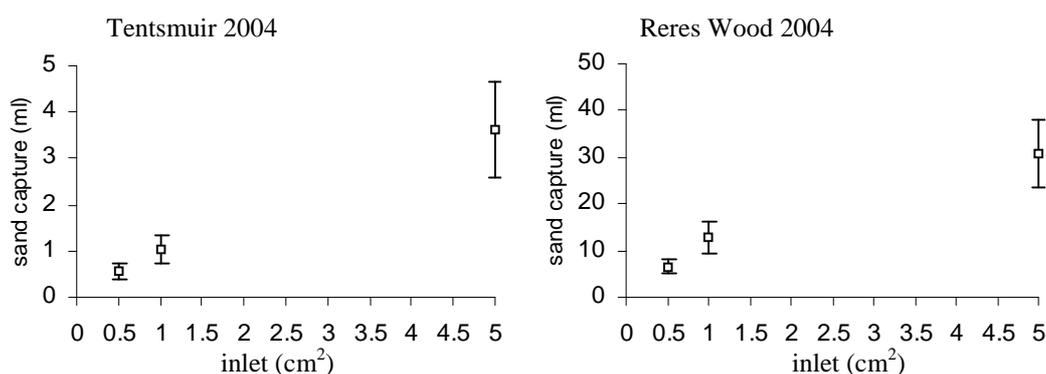


Figure 3.5 Mean and 95% c.i. for relevant sand capture (ml) against area of inlet for the period of deployment.

Significant differences were seen between all the trap designs at both sites according to Bonferroni adjusted multiple pairwise comparisons (Figure 3.5).

### 3.3.2.4 *Balanus crenatus* cyprid capture.

Table 3.16a.

Source	df	Mean Square	F	Sig.
Design	2	10.36	241.4	<.001
Day	36	1.807	95.11	<.001
Interaction	72	.043	2.268	<.001
Error	222	.019		

Table 3.16b.

Source	df	Mean Square	F	Sig.
Design	2	3.134	56.49	<.001
Day	15	.664	18.44	<.001
Interaction	30	.055	1.540	.060
Error	96	.036		

Table 3.16c.

Source	df	Mean Square	F	Sig.
Design	2	1.249	13.81	<.001
Day	7	.258	4.607	.045
Interaction	14	.090	1.610	.111
Error	48	.056		

Tables 3.16 Factorial ANOVA for  $\log(x+1)$  transformed *Balanus crenatus* cyprid capture across multiple design arrays for two sites over two years. a. Tentsmuir 2004; b. Reres Wood 5cm<sup>2</sup> deployment; c. Reres Wood 0.25cm<sup>2</sup> deployment.

Significant differences in Day and Design were seen across all three sets.

Interactions between day and design were seen at both sites for periods of deployment of the 5cm<sup>2</sup> design (Table 3.16).

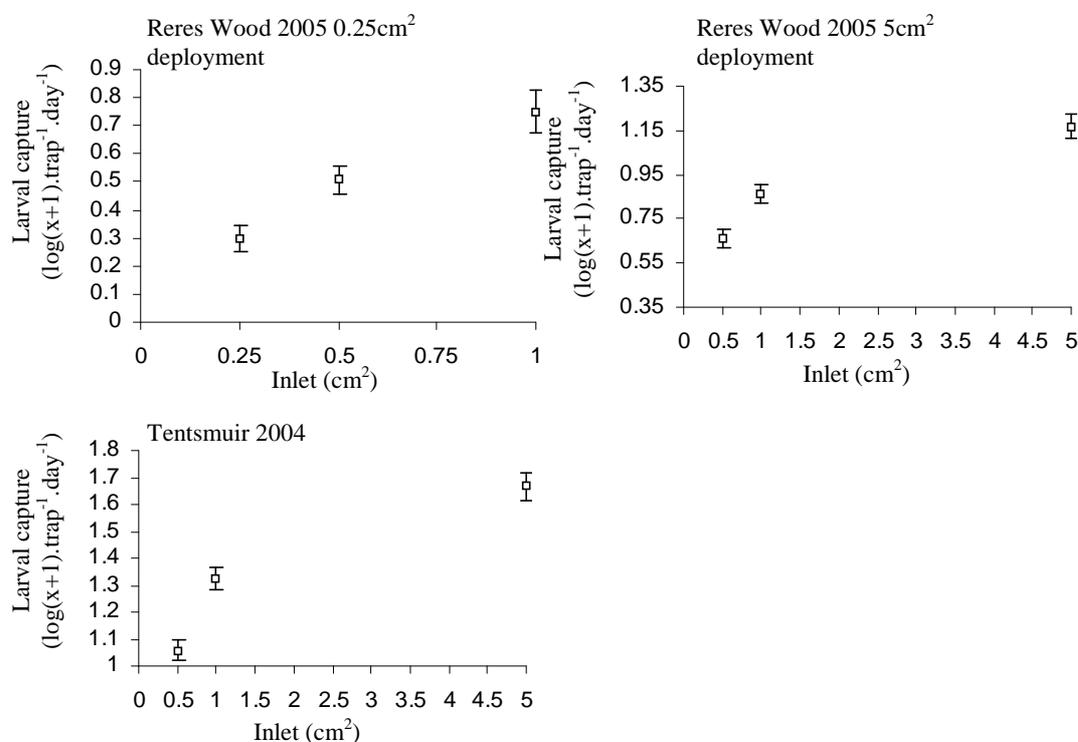


Figure 3.6 Mean  $\log(x+1)$  *Balanus crenatus* cyprid capture against area of inlet for the period of deployment.  $\pm 1$  standard error of the mean shown.

Significant differences were observed between all the trap designs at all the sites for *Balanus crenatus* capture (Figure 3.6).

Slot positional analysis was not possible for Fife Ness because significant numbers of *Balanus crenatus* were not observed at this site to ensure sufficient days when all the slot positions captured cyprids of this species. Thereby it is not possible to explicitly differentiate differences driven by trap design for this species at this site from those that may be driven by slot position. However, analysis of slot positions for *Semibalanus balanoides*, sand capture and, to a large degree, urea suggests that the daily randomisation of trap designs across the arrays eliminated small-scale spatial variations (sections 3.3.1.1; 3.3.2.3; 3.3.1.2 respectively). The design comparison studies conducted at Fife Ness are included here under the assumption that experimental variation due to significant slot effects will be negligible due to randomisation of the traps throughout the total period of deployment.

Table 3.17a.

Source	df	Mean Square	F	Sig.
Design	3	0.237	9.443	<.001
Day	17	0.388	18.33	<.001
Interaction	51	0.025	0.519	.996
Error	144	.048		

Table 3.17b.

Source	df	Mean Square	F	Sig.
Design	2	0.788	22.13	<.001
Day	21	1.603	3.083	<.001
Interaction	42	0.036	0.684	.922
Error	132	.052		

Tables 3.17 Factorial ANOVA for log(x+1) transformed *Balanus crenatus* cyprid capture across multiple design arrays for Fife Ness over two years. a. Fife Ness 2004; b. Fife Ness 2005 5cm<sup>2</sup> deployment.

Significant differences were seen at Fife Ness for trap design and day and no significant interactions were observed.

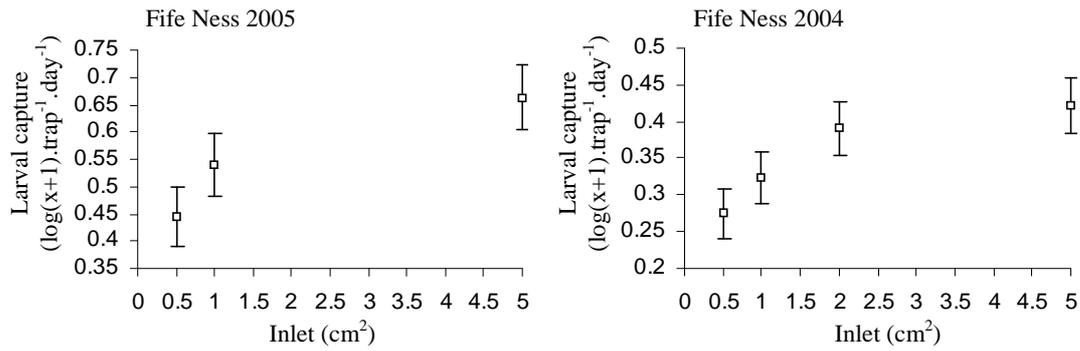


Figure 3.7 Mean log(x+1) *Balanus crenatus* cyprid capture against area of inlet for the period of deployment.  $\pm 1$  standard error of the mean shown.

Significant differences were found between 5cm<sup>2</sup> and 0.5cm<sup>2</sup> trap designs in both years. Significant differences seen at Reres Wood and Tentsmuir between all the trap designs were likely due to the significantly higher capture rates observed at the sand sites than at Fife Ness (Figure 3.7).

### 3.4 DISCUSSION

#### 3.4.1 Positional effects

Todd (2003) demonstrated clear and consistent overall positional effects on trap capture performance among traps separated by 10 cm. He suggested that these positional effects were most likely due to real, fine-scale, hydrodynamically driven variations in larval supply to the heterogeneous substratum. These traps were deployed in an array sequence similar to the arrays deployed here to assess differences in design of the passive settlement traps. This fine scale variation around boulders has been discussed in other studies (Hannan 1984; Cusson and Bourget 1997; Guichard and Bourget 1998b). Variation in larval abundance can be driven by passive particle dispersion across a range of scales (Butman 1987).

The implications for comparing different designs on a fixed array are clear. The shape of boulders to which the arrays are attached, or even the structure of the arrays themselves, may generate hydrodynamic patterns that have a real effect on the supply of propagules to the trap positions. Randomisation of the positions of the different trap designs on a daily basis appears to have rendered almost all positional effects across the arrays non-significant. The only exception found was for urea retention on the 12 trap array at Fife Ness in 2004. This was the largest array deployed. Its size, combined with its specific location, may explain this result. *Post hoc* analysis suggested that, apart from positions 11 and 12, differences between pairs of traps occurred for traps that were a long way apart on the array (see *Figure 3.2*). Despite this position 12 demonstrated the highest level of washout and position 11 the lowest level of washout. It is likely that this effect is driven by a hydrodynamic structure linked to the specific shore topography surrounding the rocky substrata. Water may be funnelled past either end of the boulder causing high degrees of

flushing in position 12 whilst swirling around the rock and leaving position 11 relatively undisturbed. Trap position 1 shows the next highest mean washout suggesting this may occur at both ends of the array.

Randomisation of traps amongst slot positions is clearly important when testing the design of the passive settlement traps on a multiple design array. The modifications to the Todd (2003) array described in this study made this possible. Although randomisation of trap designs will not always guarantee successful independence from small-scale positional effects, it is important to limit this factor as much as possible. Deploying over a large number of days, such as demonstrated in this study, will also limit the chances of small-scale variation in flux biasing trap design capture rates.

### **3.4.2 Trap design**

The traps show evidence of selectivity in their trapping ability. Very rarely were nauplii found in the traps whereas cyprids were plentiful. Net tows and pump samples (*pers. obs. and those of Adrian Gude*) showed an abundance of nauplii in the water column during the season so these clearly were available to the traps. Other intertidal passive larval trap designs have demonstrated their ability to capture a wide range of suspended intertidal larvae. Yan *et al.* (2004) reported capture of a wide range of planktonic larvae in their mesh trap when deployed in the intertidal. They also caught very large numbers of nauplii relative to the number of cyprid larvae. They gave no indication as to whether these values are representative of typical cyprid/nauplius proportions in the water column. In relation to the present results the proportion of nauplii to cyprids was reversed, suggesting a strong bias in favour of cyprid stage barnacle larvae of the two species examined here.

In order for these passive traps to capture larvae there has to be fluid exchange in the upper section of the trap. Video footage of dye injected into and around the tube under unidirectional flow in a flume suggests the velocity of fluid flow after it enters the trap is severely decelerated with the addition of the coned inlet design (*pers. obs.*). Passive particles transported into the trap will be decelerated, allowing them to drop out of the eddy in the top of the trap and pass into the still body of fluid beneath the fluid exchange zone. Observation of the traps in the field and in a laboratory flume suggests the urea washes out very quickly from the top of the trap section to a constant depth according to the trap design and prevailing flow conditions. A distinctive halocline was observed between the dyed urea solution and this section of fluid exchange in the top of the trap during the flume studies. Once a particle has passed through the urea barrier there is no reason to suggest it would be washed out again as the fluid is no longer exchanging with the flow outside the trap. Assuming the larvae cannot swim out of the trap as a result of the effective killing solution it will remain trapped in the collection chamber. Personal observations of larvae in urea demonstrate they are able to survive and swim at least momentarily in urea solution; however, undoubtedly the traps capture a significant proportion of these larvae. *Semibalanus balanoides* larval capture rates of similar trap design from other studies caught significantly less larvae than the average capture rates observed in present study. Bertness *et al.* (1992) saw a maximum daily average over a fortnight of 9 cyprids. This was equivalent to 0.2 cyprids per 1 cm<sup>2</sup> inlet diameter. In the present study on the 29<sup>th</sup> April 2004 at Fife Ness the average cyprid capture amongst 12 traps of four inlet designs was 216 cyprids per 1 cm<sup>2</sup>. On that day a 0.5 cm<sup>2</sup> trap caught 364 cyprids in one day, equivalent to 728 cyprids per 1 cm<sup>2</sup> inlet diameter.

Todd (2003) suggests increased urea retention may indicate increased larval capture. Consequently, traps subject to increased washout may capture larvae with reduced efficiency. The addition of the spiral baffle design to the old traps certainly increased urea retention but did not improve the larval capture efficiency of the traps. This may have been because the traps were deployed at the end of the season. Larval abundance was low and therefore the test was weak. Clearly urea retention plays an important role in ensuring captured cyprids do not escape the collection chamber of the trap once they have passed the fluid exchange eddy near the inlet of the trap. In fact the inclusion of brine has been shown to alter the aspect ratio of cylinder traps and decrease their trapping efficiency (Gardner and Zhang 1997). They suggest the halocline generated at the interface between brine and eddy flux creates a false bottom, effectively reducing the aspect ratio for the duration of fishing time. For this reason improving particle retention rather than fluid retention may be the key factor in improving passive larval trap designs. Fluid exchange must occur for cylinder traps to function because they do not capture larvae in the manner of Yan *et al.* (2004) or Setran (1992) that rely on flow through the trap.

Trap urea retention was increased significantly by the addition of successively smaller coned inlets at all the sites. Plots of urea retained against inlet area demonstrate the relationship is possibly curvi-linear (*Figure 3.4*) and urea is disproportionately retained with decreasing coned inlet. Many factors may be contributing to this result. The addition of the cone inlet appears to affect fluid exchange in a complex manner only partly explained by inlet area. In all likelihood the relationship between fluid exchange and inlet design is determined by a combination of aspect ratio and Reynolds number as suggested by Yund *et al.* (1991).

Increasing urea retention by the addition of the coned inlet led to a significant decrease in the absolute number of cyprids of both species captured by each trap. However, this is to be expected because the target area (the size of the inlet) is decreased. The important point is that the relationship is not a straight line (*Figures 3.3; 3.6; 3.7*). As the area inlet is increased the rate of improved capture relative to the area of the inlet decreases. Therefore, although the inlet area has been decreased the coned traps are capturing more cyprids per unit area of inlet. This result is not seen at Tentsmuir for *Semibalanus balanoides* because there the mean capture for 0.5cm<sup>2</sup> traps was greater than 1cm<sup>2</sup>. This is likely to be due to random variation, and Bonferroni pairwise comparisons showed no significant difference for this pairing.

At Tentsmuir and Reres Wood a straight-line relationship appears to exist between increasing inlet size and sand capture with increasing inlet area leading to increased capture (or retention) of sand (*Figure 3.5*). The form of the relationship suggests a difference in the capture performance for the sand particles. As the proportion of inlet size to trap diameter is increased (i.e. coned inlet area is decreased) the rate of sand capture does not increase in the same manner as for the cyprids. More larvae are caught relative to sand per area of inlet as a result. This is also true of algal debris and other detritus at all of the sites. Due to the considerable variation in particle size of algal fragments found throughout the trap samples it would have been difficult to quantify these components in a meaningful way. Therefore, whilst they are acknowledged their quantity was not included in the analysis. However it demonstrates a major logistical advantage to decreasing the inlet size of the trap. As long as sufficient larvae are captured by a smaller inlet design the processing time of the sample is greatly reduced when there is less material in the trap sample through which larvae must be sorted from and identified. In addition, the 5 cm<sup>2</sup> cylindrical

traps were occasionally subject to clogging due to dead limpet and littorinid shells and hermit crabs. The choice of trap design is a subjective decision to some extent based on the shape of the relationship demonstrated in *Figure 3.3; 3.6; 3.7* and the numbers of available cyprids.

Capture rates for cyprids demonstrates that smaller coned inlets generally captured more passive particles per unit area of inlet. The addition of a coned inlet significantly improves the capture rate per unit area inlet for the passive settlement trap. Addition of an entrance cone and decrease of the inlet size increased the proportional capture rate for cyprids, suggesting the cone design offers an improvement in larval retention efficiency not seen in the spiral design with a cylindrical entrance. In addition, the mean daily cyprid capture values for passive settlement traps demonstrated here are considerably greater than any values reported in the literature (Gaines and Roughgarden 1985; Yund *et al.* 1991; Bertness *et al.* 1992). High abundances increase the precision and reliability of the measurement of larval supply. The significantly high capture rates observed in these traps across the settlement season in Fife demonstrate an opportunity to study the relationship between supply and settlement with a level of resolution across suitably small scales previously unavailable to ecologists.

## Chapter 4 – Artificial Settlement Substrates

### 4.1 INTRODUCTION

The colonisation of habitats by benthic marine organisms with planktonic larvae involves three phases. Initially, larvae are dispersed in the water column as they develop and grow. Second, upon contact the larvae will test a substratum for suitability of settlement. Finally the larvae will settle and metamorphose if the site is suitable. Sessile invertebrates such as barnacle larvae undergo permanent attachment and rapid metamorphosis as part of the final stage. As a result, larvae of sessile organisms such as the barnacle must select a settlement location on the basis of ensuring the survival of the individual through to successful recruitment in the adult population. A suitable habitat may be distinguished as having limited competition with organisms that may capitalise on adaptations to a specific habitat whilst being suitable to the organisms own survival. Additionally important to many sessile marine invertebrates is the need to settle near to conspecifics in order to successfully cross-fertilise and reproduce. The ability to perceive its environment prior to settlement and metamorphosis is a behaviour exhibited by many dispersive larval invertebrates (Crisp 1965).

There is a trade off between settling close to a large number of conspecific with which sexual reproduction is possible and mutual repulsion as a result of competition for limiting resources. This tension exists for almost all organisms but is particularly evident in barnacles as a result of their spatially fixed adult form. Unlike the aggressive behaviour exhibited by active animals, the territory of established animals restricts late arriving sedentary animals. A distance of ~2 mm is maintained between recruits during settlement, however as populations reach specifically high densities this distancing does not remain constant. Cyprids were observed exploring

for longer periods following contact with conspecifics if densities were high whilst they sought out less dense areas of substrate. Crisp suggests additional space allows the barnacle more room to become established before contacting another adult's base (Crisp 1961).

#### **4.1.1 Barnacle settlement.**

Whilst in the plankton, cyprids are attracted to light and tend to congregate at the surface layers. Later they reverse this behaviour and will swim down to seek a suitable surface for settlement. Having once contacted a surface the larva becomes relatively indifferent to light and begins exploring the surface. This exploratory stage requires the use of temporary adhesive cement that allows the larvae to attach the surface. It is thought that hairs similar to those found on insects are applied very close to the surface and a small quantity of sticky secretion is used to attach the cyprid with these hairs during the exploratory stage (Yule and Crisp 1983). Provided certain conditions are met the cyprid will undergo settlement and metamorphosis into the adult barnacle form. Permanent attachment to the substrate occurs by means of the antennules. A secretion is poured out from the cement glands, which open on the penultimate segment of the antennules. This cement hardens and anchors the cyprid permanently to the substratum. The organism undergoes some orientation movements of its body following the formation of a peduncle appendage. The bivalved larval shell is then shed along with some posterior body parts and the cyprid pulls itself down onto the rock, forming an almost spherical shape. The first traces of the compartments and opercular valves appear. At this point the animal is no longer a cyprid and is recognised as a spat. This process takes 24 to 30 h following attachment. The whole basal area is then cemented down from the original point of attachment of

the antennules (Stubbings 1975). It is believed that the cement secreted by a juvenile within the first 35 d is different to that of the adult cement, which demonstrates a greater adhesive strength (Yule and Walker 1984a).

Settlement is an active process presumed to be under nervous control. Larvae can reject substrata following exploratory behaviour indicative of impending settlement, making it sometimes hard to distinguish the point at which settlement occurs. As a result of clear evidence from early laboratory studies on the active response of larval settlement, an emphasis has been placed on this active response. Butman (1987) notes observations by Thorson advising caution when applying results of laboratory experiments in still water to the field. In the field, larvae will not have a similar opportunity to compare substrata. It is far more likely that the 'choice' to settle is more likely to be an 'acceptance' of substrata. This is because larvae have little means of exploring substrata over the scales of kilometres that may offer sufficient variation to include the ideal conditions recognised in a comparative laboratory experiment (Butman 1987).

In both experimental and theoretical ecology an important distinction must be drawn between settlement and recruitment (Keough and Downes 1982). Settlement is a biologically defined phase in the larval stage of an organism's life cycle. It occurs following the movement of the larvae from the pelagic to the benthic environment. This is unlike recruitment in the sense that recruitment is a parameter defined by the examiner. An organism becomes a 'recruit', as defined by Keough and Downes (1982), when the experimenter actually observes and records the individual. Early mortality of settlers can be severe, so any study using counts of recruits as a proxy for settlement will miss mortality at this early stage. Patterns in recruitment may arise either from differences in the rates of settlement or differences in the rates of

immediate post-settlement mortality. Distinguishing between these two causes of variation in settlement and subsequent recruitment may have important implications in recognising active or passive factors that determine patterns of abundance.

#### **4.1.2 Active Settlement Cues**

##### **4.1.2.1 Flow**

A slight increase in the flow of water across the substrate around a cyprid will stimulate settlement. This can be observed by manually sucking cyprids into a pipette. They are seen to temporarily attach to the walls of the pipette and will then release once the flow stops. The response also can be observed when pouring liquid from a beaker containing cyprids. The majority of the cyprids will be found on the inside of the beaker (Pyefinch 1948). Settlement of *Semibalanus balanoides* cyprids in flow was studied extensively by Crisp (1955). Observations were made of maximal swimming speeds in flow which were estimated at  $\sim 4\text{-}5\text{cm}\cdot\text{s}^{-1}$ . Attachment was observed to be limited to a shear stress of  $\sim 100\text{ sec}^{-1}$  on flat substrata. Monitoring settlement in glass tubes, Crisp (1955) found that increasing the rate of flow led to a marked increase in the rate of attachment of the cyprids above a shear of  $\sim 50\text{ sec}^{-1}$ . Decreasing the flow led to an increase in detachment of the recently settled cyprids, suggesting the reaction to flow is temporary. When exploring the substratum, cyprids seemed capable of considerable motion against the prevailing mid stream flow. This was as a result of boundary layer conditions considerably decreasing the hydrodynamic forces experienced by the cyprids. Calculations suggest increased drag occurs, as a result of the dynamics of vortices and eddies, on cyprids that are attempting to crawl along a flat surface compared to those in a tube. Settlement activity was observed at higher shear values in glass tubes than on a flat surface. This

is discussed by Crisp as an explanation for their preference to settle in pits and cracks as the drag forces experienced by the cyprids will be far less, offering greater opportunity to explore (Crisp 1955).

The settlement response to flow is of obvious adaptive significance to organisms such as barnacles that require sites with strong tidal currents or wave-wash to enhance post-larval filter, or suspension feeding. This would explain the observation of greater settlement in areas of moderate exposure than in shelter. Areas of high flow such as headlands and narrow passageways with strong currents often demonstrate richer assemblages of larger individuals. This may be as a result of a high supply of food and decreased incidence of silting.

#### **4.1.2.2 Substrate type**

Work as far back as the 1920s has shown that the presence of suitable substrate is important in the rate of settlement of many invertebrate larvae. For example *Mellita sexies-perforata* (sand dollar) required sand to settle in a laboratory tank during the critical stages of larval development for them to settle successfully (see review by Crisp 1965).

Caffey (1982) examined settlement on four rock types common to four different shores along the east coast of Australia. The rock types demonstrated obvious differences in their response to heat as well as being different in surface texture, colour and contour. The boulders were cut into regular rectangular plates and embedded flush to the rock at each of the shores. His experiment was designed to demonstrate whether variations in recruitment to the four shores were driven by their unique geology. The findings of that study indicated that geology did not significantly affect recruitment between shores.

Other studies have demonstrated that rock type can affect rates of settlement of cyprids. Studies of shale and sandstone demonstrate selection of substrate by larvae, even when specific characteristics of the rock such as colour have been altered. The causes of patterns of settlement on rock are hard to pinpoint, as there are many potential characteristics. These can include roughness, colour, and friability of the surface or even its chemical composition (James and Underwood 1994).

In general, marine invertebrate larvae settle more readily on rough than on smooth surfaces (Crisp 1974). However some species have demonstrated a response to smooth surfaces over those of roughened surfaces (Crisp and Ryland 1960). Clifford *et al.* (1992) demonstrated clear settlement preferences of fresh-water stream invertebrates to biofilms on rough over smooth textured tiles. These factors were linked to flow characteristics that influenced biofilming on the different surface textures. The subsequent quantitative differences were explained as influencing settlement of macrofauna as a result of an increase in food availability (Clifford *et al.* 1992).

#### **4.1.2.3 Rugosity**

Crisp and Barnes (1954) were the first to coin the term “rugophilic” to describe the tendency for barnacle larvae to settle and accumulate in grooves and cracks on the substratum. Wood and Bakelite surfaces possessing a linear grain, channelling, or pits of different diameters were exposed to different angles of light because this had previously been shown to influence the settlement of larvae. The majority of individuals were orientated parallel, or nearly parallel, to the grooves independent of the direction of light; larvae showed similar patterns of orientation in the pits. Different types of grooves were examined and it was shown that orientation

was strongly influenced by the depth of the grooves. The ability to acquire a better purchase on the rock is provided by a grooved surface as well as offering protection to a young barnacle from a heavy wave crash environment. The authors less easily explained orientation to the direction of a small groove. It was thought that this may arise from a strong response to larger grooves, as a result of exploratory behaviour in an attempt to find a larger groove or due to an increased adhesion following cementing (Crisp and Barnes 1954).

Crisp (1974) made an important distinction between substratum texture and contour. He described topographic detail as being surface characteristics that are large relative to the cyprid. He noted, however, that the exact boundaries between contour and texture are hard to define. A rough texture was shown by Wisely (1959) to increase settlement, particularly when on black Bakelite as opposed to any other colour.

Wethey (1986) demonstrated that larvae would settle repeatedly in the same locations on replicated casts of rock structures. He demonstrated that these locations tended to be areas of low shear. Flume studies showed increased sedimentation at these positions. The patterns observed demonstrated that small-scale topographic variation produced consistent patterns of settlement in larvae and passive deposition of particles in flow. The combined effect of eddies and still water zones created by pits may collect larvae and enhance settlement rates alongside responses to flow and other behavioural cues.

#### **4.1.2.4 Biofilms**

Left in sea water, any well-cleaned surface is rapidly colonised by a surface film of macromolecules and microscopic organisms. This biofilm has been

demonstrated to act as a settlement cue to many invertebrate larvae. The response may not necessarily be positive, or even a pre-requisite to a response; however the literature abounds with examples of larvae responding specifically to the presence or lack thereof of biofilms (Crisp and Ryland 1960; Wiczorek and Todd 1997).

Neal *et al.* (1996) demonstrated for *Elminius modestus* cyprids that exploration of filmed surfaces was enhanced compared to biologically clean surfaces. Increasing the shear rate encouraged attachment of larvae to all the surfaces examined. Additionally, the nature of the biofilm produced differential effects. Cyprids were seen to detach less often from surfaces where biofilms had been grown in high shear conditions than in low shear conditions or on clean surfaces. The total number of attaching cyprids was higher on high shear-acclimated biofilmed surfaces than any other. It was suggested that the ability of larvae to indirectly sense the general hydrodynamics of a site through a specific biofilm composition would circumvent the need to recognise simultaneous ambient hydrology, not necessarily related to the general site conditions (Neal *et al.* 1996).

Surfaces left to develop biofilms for different periods of time and at different field sites demonstrated substantial variation in subsequent settlement. A preference for more heavily filmed surfaces that had been exposed for longer periods was explained by differential degrees of filming. Variation in settlement due to differences in sites at which the tiles were filmed was thought to be as a result of different degrees of filming at these sites rather than biofilm compositions specific to those sites (Todd and Keough 1994; Keough and Raimondi 1996).

Individual species components of biofilms demonstrate different capacities for inducing settlement amongst species of fouling organisms. For example Neal and Yule (1994) demonstrated a positive settlement response to *Deleya marina* biofilm by

*Elminius modestus* cyprids. They showed, however, that this relationship reversed with age of the biofilm. The related differences were independent of the surface wettability of the filmed substrates (Neal and Yule 1994).

The influence of biofilms on settlement is not due to a simple physical effect on the surface. Physico-chemical or chemical factors are involved in this cue response. Biofilm development can vary according to spatial and temporal differences and these can have a real effect on larval settlement rates and subsequent recruitment (Crisp 1974).

#### **4.1.2.5 Surface chemistry**

The inherent chemical properties of a surface such as critical surface energy can influence settlement of macrofouling larvae. Larval behaviour, adhesive strength, settlement and metamorphosis of larvae all can be influenced by properties of the substrata. Following early work on the effects of conditioning surfaces with biofilms and bacterial attachment, silane reagents have been used to modify surface energy of substrates and its subsequent effects on settlement and metamorphosis (Rittschof *et al.* 1998).

For example, Roberts *et al.* (1991) altered smooth clear glass rods by a process of silanisation to affect the surface properties of the substrate. Barnacles and bryozoans were shown to be affected by surface chemistry of the glass rods; hydrozoans were not. High surface energy encouraged the settlement of barnacles and not bryozoans, which appeared to prefer low surface energy. Studies on the adhesive properties of barnacle cement suggest that high wettability may increase surface adhesion. The surface composition may be detectable by cyprids and an increase in adhesion may be as a result of an active response (Roberts *et al.* 1991).

#### **4.1.2.6 Colour**

James and Underwood (1994) selected boulders from above the high water mark on the shore that had presumably not been fouled by conspecifics. Shale and sandstone boulders were selected as they offered a favourable and unfavourable substratum respectively for the settlement of spirobid larvae. They were interested in the effect of colour on settlement because there was a clear distinction in colour of the rock types. Using dark grey and light yellow paving paint they altered the colour of slate and sandstone boulders to that of the opposing rock type and compared these to rocks that were painted the same colour as the respective rock type. It was stated that the paint did not substantially alter the texture of the rock, however it is possible that the different paint type may have altered the biofilm that developed on the rock. They found natural or experimentally darkened boulders had more recruits than natural or experimentally lighter boulders (James and Underwood 1994). Wisely (1959) demonstrated a preference for black Bakelite when these were deployed in Sydney harbour.

#### **4.1.2.7 Gregariousness**

As a result of their fixed or sedentary adult form, many invertebrate larvae demonstrate gregarious settlement behaviour. This is a fundamental necessity if successful cross-fertilisation of adults is to occur, as well as carrying the additional advantage of offering a potentially suitable environment in which to live.

The gregarious nature of barnacles was extensively investigated by Knight-Jones (1953). He obtained live net samples of cyprids of three species of barnacles from the field and from temporary attachment to furoid algae. He studied their

relative settlement rates to determine whether they were disproportionately inclined to settle in the presence of adults of the same and different species. Individuals were seen to be stimulated to settle in the presence of settled adults or even on substrate where adults had been rubbed away leaving their bases intact. He concluded that even after barnacles had been cleaned from the underside of boats the presence of adult base plates alone can stimulate settlement of larvae. A new coat of paint is sufficient to halt any further stimulus from the bases.

By placing small shells or stones covered in barnacles inside porous silk bags and suspending these in water in dishes (Knight-Jones 1953) demonstrated that the gregarious response to settle in barnacle cyprids required contact rather than by means of a water-borne chemotactic response. Comparisons were made between dishes that contained *Mytilus* valves with recently detached adult bases and valves that did not. Significantly increased settlement was observed where contact with prior adult material was possible. Where contact was precluded, settlement occurred at rates similar to dishes that had no barnacle-filled silk bags. Following a sequence of biochemical manipulations he concluded that the compound was a possible quinone-tanned protein present in the epicuticle of the adult barnacle and in the cement in the base.

#### **4.1.2.8 Chemical settlement cue**

Crisp and Meadows (1962; 1963) treated slate surfaces that were not readily settled by barnacles with an aqueous solution of whole adult barnacles. Cyprids were observed settling in numbers similar to those in areas where conspecifics were present, thus simulating the gregarious behaviour seen in the field. This study demonstrated that a compound present in the tissue of barnacle adults was responsible

for this behavioural aspect of larval settlement. The response was seen to greater degrees when newly-moulted individuals were used and there was a clear species-specific preference. Barnacle species have also been shown to respond to solutions of extracts from a number of related species, including other arthropods and some species of sponge. Even vertebrate (fish gut) extract offered a significant settlement preference; however these unrelated species were found in an area thought to favour recruitment success in barnacle so these species were not wholly unrelated (Crisp 1965). Soaked and unsoaked slates were dipped in a solution of the 'arthropodin' compound and settlement was shown to be preferential on the soaked slates. This result matched Knight-Jones' finding that the response required the larvae to contact the substrate on which the substance had been absorbed. There was no evidence of chemotaxis in this study. This early work therefore laid the foundations for extensive investigation into the biochemical processes involved in gregarious responses in fouling organisms, particularly among cirripedes.

Extensive examination of the chemistry of the settlement-inducing compound was conducted by Gabbot and Larman (1987) following the early work on 'arthropodin' by Crisp, Knight-Jones and colleagues (Knight-Jones 1951; Knight-Jones 1953; Crisp and Meadows 1962). A brief review can be found in Clare and Matsumura (2000).

Some early evidence pointed towards responses in the swimming behaviour of cyprids when exposed to potential waterborne pheromones. It is suggested that a water-soluble factor may emanate from the adsorbed protein prior to cross-linking of cuticular proteins of newly metamorphosed juvenile barnacles. Partially purified seawater following conditioning with adult barnacles provided the first direct evidence of a waterborne pheromone. The response seen was, however, only of

temporary induction of adhesion to a surface. The compound has been isolated and described. The elicited response to a waterborne pheromone is less significant relative to the degree of response seen to the settlement-inducing protein complex described broadly as 'arthropodin' by early papers (Clare and Matsumura 2000).

According to Langmuir's principle of independent surface action, the number of molecular layers required to produce a given field of force is very small, only one or two. Crisp (1974) argued that a cyprid will not be able to distinguish between a thin or thick film of extract. He demonstrates this with a comparative study of different monolayers of arthropodin.

The aims of this chapter are two-fold. First, to investigate the degree to which natural topographic variation alone effects settlement. Rugophilia is a long recognised phenomenon in settling larvae, particularly *Semibalanus balanoides* however no study to date has successfully investigated the patterns of settlement on isolated natural rock topography. A novel method for casting rock topographies in the field and then generating replicable tiles in the laboratory of uniform physical and chemical properties is demonstrated. Replicate tiles within blocks are examined with a latin square design for four topographic mimics to determine whether there is significant variation in settlement driven by natural topography alone, independent of differences in larval supply, shown to vary over very fine scales (Todd 2003).

Second, a series of artificial settlement substrates will be developed for use as part of a supply settlement array (Chapter 5) in a mesoscale study. These substrates must be affordable, repeatable and easy to deploy in the field. Crucially, they must provide a suitably desirable substrate so as to produce sufficient daily resolution to distinguish the characteristically high variation in larval settlement throughout the settlement season. For this reason they will be painted with adult extract, coloured

black and maintain a 3-dimensional component to their design. Machined Perspex and ceramic bathroom tiles will be investigated as suitable materials for artificial settlement substrates. Two designs of machined Perspex and a pitted ceramic bathroom tile will be examined for daily settlement per  $\text{cm}^2$  in an attempt to produce the highest settlement per unit area and most suitable resolution in daily settlement variation. This will allow for the deployment of the tiles along a large stretch of coastline so as to distinguish variation in larval settlement daily and across metre and kilometre scales (Chapter 5).

In addition to these aims, settlement behaviour will be examined on the three dimensional surfaces of the ceramic tiles. The surface structure of the ceramic tiles provides three distinct areas within which settling larvae may explore and attach. Therefore preferences in settlement response can be investigated for larvae and observed over the season as a whole. Insufficient settlement was observed on the Perspex tiles for any behaviour to be investigated.

## 4.2 METHOD

### 4.2.1 Topographic diversity and positional effects

In an attempt to address the complexity of accurately measuring and replicating realistic textured surfaces Hills *et al.* (1998) developed a method of replicating surface texture whilst maintaining the consistency of other confounding variables such as surface chemistry or colour. They used polyvinylsiloxane dental impression material to cast four textured surfaces. The surface textures were achieved by sieving four different sizes of sand particles onto pieces of flat wood covered in glue and leaving them to set. The casts were then used to manufacture replicate panels from green polyester resin that would contrast well with the brown of the cyprids during image analysis (Hills *et al.* 1998). A similar method is used here to examine the effect of natural rock topography removed of adult barnacles on the settlement of *Semibalanus balanoides* barnacle cyprids.

Multiple layers of 5mm thick Perspex were clamped together and cut into 13x13cm squares. Two 1.5cm borders were drawn on the squares so that the second border was 3 cm in from the edge. The squares were then cut diagonally into triangles. The outside border was then cut away, followed by the inside border.

1cm thick square frames were constructed from pairs of L-shaped Perspex borders by abutting the diagonal corners of each pair and then layering on top of a second pair. Each square's abutting corners were arranged in opposition to the layer beneath. Using Araldite glue and clamping the layers as they set ensured a strong bond and a solid square frame 1cm thick.

Two sets of squares were produced, one having an external square edge of 10 cm and an internal square edge of 7 cm. The second larger square had an internal square edge of 10 cm and an external edge of 13cm. The larger squares were made

from four layers of Perspex, the smaller only two, which meant that when fitted back together, the internal square sat recessed by 1cm.

Before the smaller squares were assembled 1mm grooves were cut across one side of the L-shape half way along. When glued together into a square shape two lengths of 1mm microfilament nylon were laid in opposing grooves to form a cross in the centre of the square. The grooves ensured there was no gap between the layers caused by the nylon wire.

Four roughly 11x11cm areas of natural rock were scraped clean of adult barnacles, washed and dried. All four areas came from a single large boulder at Fife Ness (*Plate 4.2*). The smaller frames were placed onto the clean clearances and sealed to the rock by pushing water-soluble play-dough putty around the edges (*Plate 4.3*). Polyvinylsiloxane dental impression material (Type 3; low consistency – light bodied. Sds Kerr UK Ltd., Product # 28418) was applied to the rock surface inside the sealed frames to a depth that would cover the monofilament cross (*Plate 4.4*). Once the impression material had hardened the play-dough could be removed from the edge of the frames and the frames carefully lifted off the rock. The dental impression material was held within the frames by the monofilament and friction along the sides of the frames. This result was a negative copy of the specific rock topography (*Plate 4.5*).

In the laboratory the small frames containing the four casts of natural rock topographies were placed inside the large square frames after the inside edges of the large frames had been coated with J-wax. More J-wax was applied to the cast surface and the small frames. Unstained filled polyester resin (FC702PA Trylon; [www.trylon.co.uk](http://www.trylon.co.uk)) mixed with liquid catalyst hardener (product # 1010171; Trylon) was poured into the large moulds over the top of the cast and the small frames and left to set overnight in a fume cupboard (*Plate 4.6*). The casts were then turned out

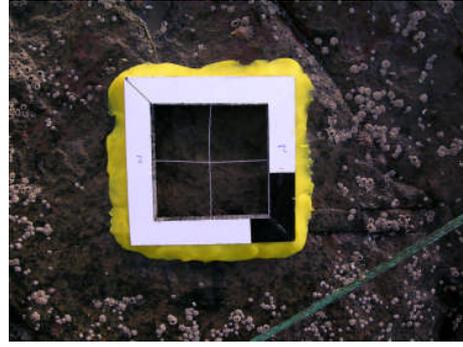
producing a positive cast of the specific rock topography. The resin casts were cut down into 5x5cm squares (*Plate 4.7*).

Each small frame containing the polyvinylsiloxane was carefully disassembled, removing the monofilament from the natural rock casts (negative) whilst making sure they were kept intact. An 8x8x1cm box was placed bottom-up within a 10x10x5cm square plastic tub. The 5x5cm resin cast (positive) was placed, topographic detail face up, on the upside down box. The polyvinyl rock cast (negative) was fitted to the resin cast (positive). (*Figure 2.1a*)

Flexil-S RTV-30C silicon and standard 5 % green catalyst (Jacobson Chemicals; [www.jacobsonchemicals.co.uk](http://www.jacobsonchemicals.co.uk)) was poured into the tub containing the rock casts and box. This was left to set overnight in a fume cupboard. The following day the silicon was removed from the tub and the upturned box and resin 'plug' were removed from the silicon (*Figure 2.1b*).



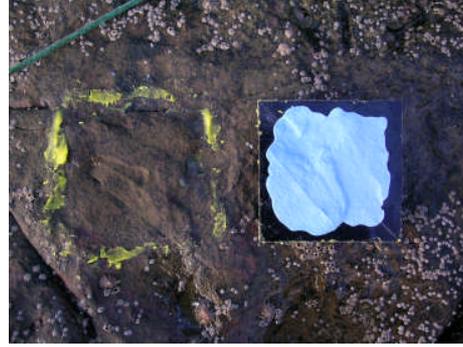
*Plate 4.2*



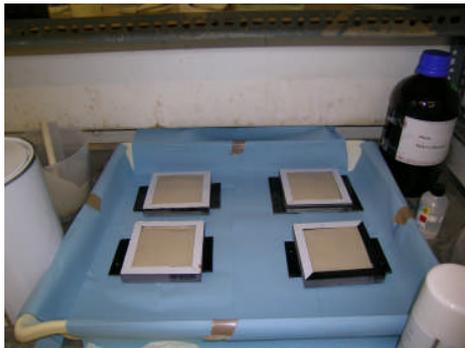
*Plate 4.3*



*Plate 4.4*



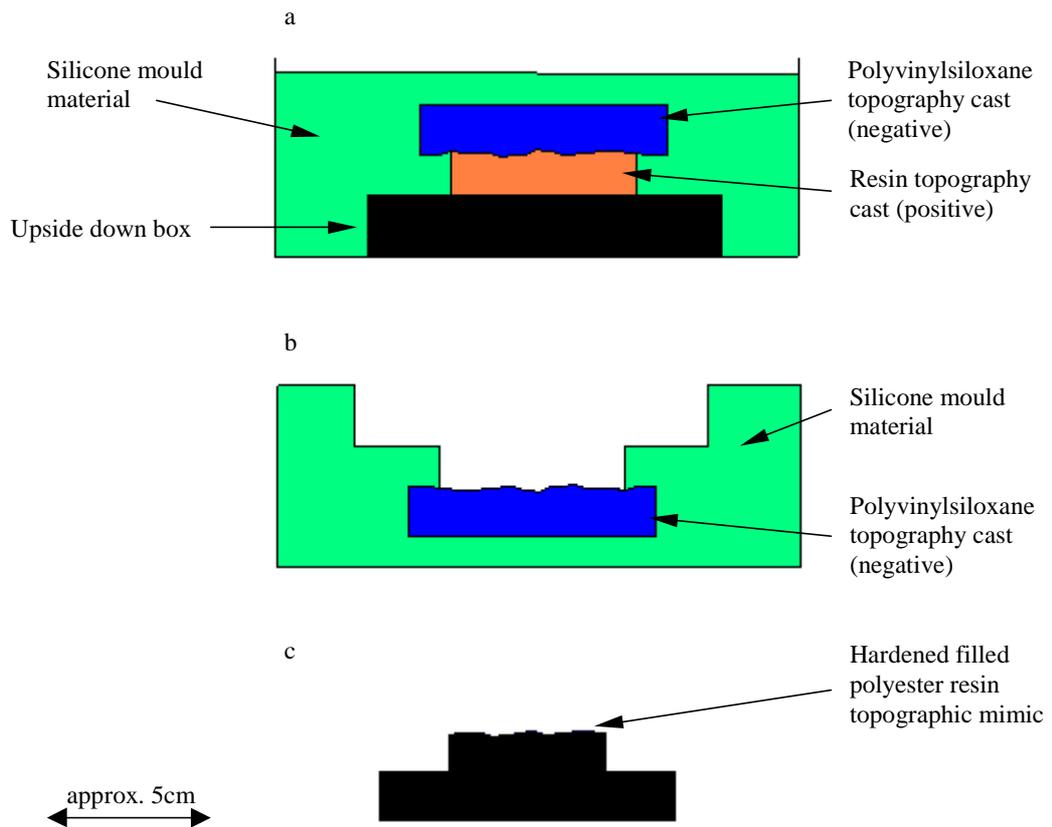
*Plate 4.5*



*Plate 4.6*



*Plate 4.7*



*Figure 4.1* Schematic diagram of the mould casting process. (a) shows the set-up after liquid silicon has been poured into the square tub. (b) shows the mould after it has been turned out of the tub. (c) shows the finished tile after it has hardened and been turned out of the mould. The drawings are not to scale.

The silicon mould was covered in J-wax and filled with polyester resin mixed with liquid catalyst hardener and standard black dye (product # 1010706; Trylon) was poured into the T-shaped square hole of each topographic mould. This was left to set for 3 h and then the tiles can be turned out and left to finish the curing process in a fume cupboard whilst more tiles were poured (*Figure 4.1c*). 4mm holes were drilled into two opposing sides on the border of the tiles once they had fully cured. The tiles were then soaked and leached in running tap water for 1 week.

For deployment in the field two pairs of 70x70cm Perspex sheets were used so that 16 tiles (four replicates of each of four topography casts) could be attached to it in a randomly allocated Latin square each day. At each of the 16 positions four pairs of holes were drilled parallel to the axis of the tile so that orientation of the tiles also

could be allocated randomly. Cables secured the tiles to the Perspex mounting boards. Two rock blocks were selected at Fife Ness in 2005. A rope lattice (Todd 2003) was secured at both blocks so that the Perspex boards could be secured on the top surface of the blocks, lying horizontally. Each day the Perspex boards were replaced with a new set of cleaned tiles in a different Latin square formation. The settled tiles were taken back to the laboratory and *Semibalanus balanoides* cyprid densities per tile were recorded. The settlement of *Balanus crenatus* was very low (<1% of total) and so the presence of these cyprids was not recorded. When counted the tiles were scrubbed rigorously in tap water with a stiff bristle toothbrush to remove any trace of cyprids.

#### **4.2.2 Artificial settlement substrate (ASS) selection.**

Although artificial settlement substrata infrequently result in comparable larval densities to natural substrata their ability to provide repeatable degrees of shape and size outweigh these costs. Natural rocks are harder to quantify in terms of their texture and surface area (Clifford *et al.* 1992). In addition fouling of natural rocks by other organisms has been shown to affect settlement at different sites. Differential fouling between sites would bias estimates of relative settlement between sites if not properly controlled for. A clean tile also eliminates the possibility of gregarious responses to adults or remnant adult bases that are hard to remove from natural rock. Crucially, counts of natural rock do not control for topographic heterogeneity between replicates - both surface texture and contour - which have been demonstrated to act as powerful cues for settlement.

As part of the long term monitoring of larval settlement along the Fife coast it was necessary to develop an artificial settlement substrate that could be deployed

alongside the passive larval traps as a contemporaneous measure of active settlement of cyprids. The main goal of the development of an artificial settlement substrate (ASS) was to increase the per unit area settlement of barnacle cyprids perhaps to levels as on natural surfaces. This would allow greater resolution in comparative studies over temporal and spatial scales for settlement and could be compared to measures of passive larval supply from the traps.

Three designs of artificial settlement substrata (ASS) were used to determine daily cyprid settlement throughout the project. The first design of Perspex panel was deployed during the 2003 and 2004 *Semibalanus balanoides* settlement season. Acrylic sheets were cut into 8 x 13cm panels. Black acrylic was used because this colour has been demonstrated to be especially attractive to cyprids. These were sanded to remove the shiny surface. Grooves were milled into the panel. The vertical grooves (long grooves) were 0.2cm deep and 0.5cm wide. They were 0.5cm apart. So that there are maximum edges the vertical grooves start and end 0.25cm from the vertical edges of the panel. Six vertical grooves ran the length of the panels, three either side. There were two pairs of 5.5cm vertical grooves that ran from the top and bottom edges to the middle. 0.5cm wide horizontal grooves were milled every 0.5cm. These were 0.1cm deep. Two sets of 6 horizontal grooves were milled from the top and bottom edge. Two pairs of horizontal grooves were milled between the full grooves with a 2mm ridge separating them from the sets of six horizontal grooves. This gave a total of 114.6cm of edge space. A 2.5 x 2cm square was left un-milled in the middle of the panel. A 0.6cm hole was drilled through for attaching the panels to an acrylic backboard with a nylon nut and bolt (Product no. 707-0822, Farnell, 6mm wingnut no. 292-619, RS Components). This design is demonstrated in *Figure 4.2*.

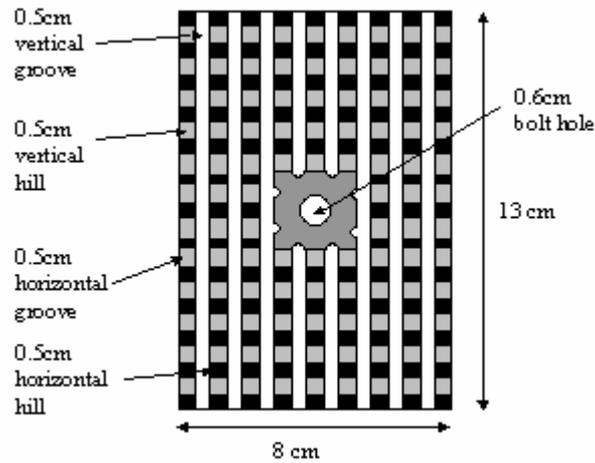


Figure 4.2 Schematic diagram of old panel design. Deep vertical grooves are 0.2cm deep. Horizontal grooves are 0.1cm deep.

The second design of panels were deployed during the *Semibalanus balanoides* settlement season in 2004. They were made from the same black acrylic panels as the old panel design. The acrylic was cut into 7.2cm squares. 0.5cm grooves were separated by 0.2cm hills. The vertical grooves were 0.1cm deep and the horizontal grooves were 0.05cm deep. This gave a total of 188cm of edge space. 0.6cm holes were drilled for nylon bolts to attach the panels to acrylic backboards (Figure 4.3).

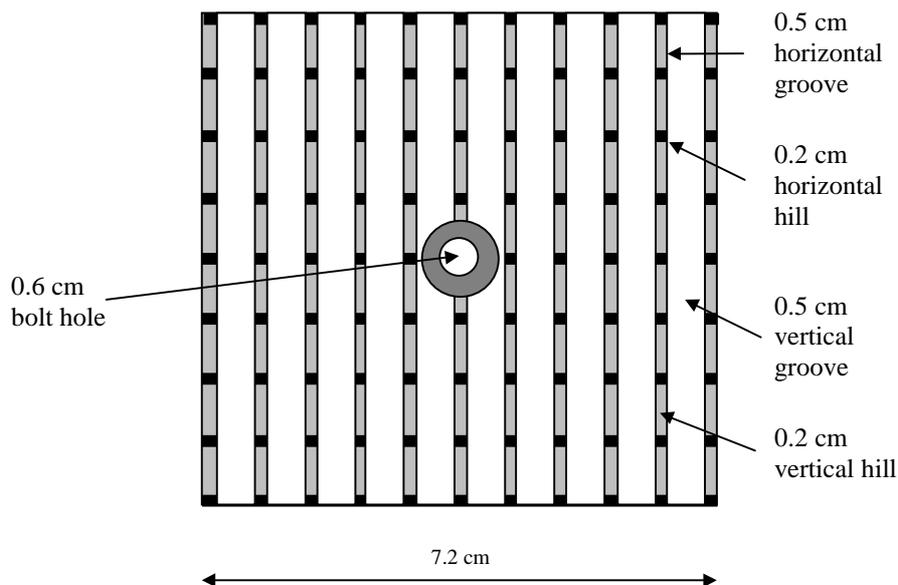


Figure 4.3 Schematic diagram of new panel design. Deep horizontal grooves are 0.1cm deep and horizontal grooves are 0.05cm deep

Ceramic tiles were deployed as the third design of ASS through the *Semibalanus balanoides* settlement seasons in 2004 and 2005. 9.8 x 9.8cm domestic terracotta ceramic tiles (BCT Ltd. [www.bctltd.co.uk](http://www.bctltd.co.uk); Candy Tile Company, England. Product no. CAN32835) were drilled through the middle with a 0.6cm hole. The same wing nut as the panels were used for attaching this ASS to acrylic backboards. The unglazed reverse surface of the panels were used as the settlement substrate. It consisted of a stippled hard substratum and 58 irregular pits. The pits were approximately 0.1cm deep and consisted of a slightly rounded perpendicular wall. The pattern of pits was essentially identical amongst replicate tiles because the tiles were all manufactured from the same mould design. Perimeters of the pits ranged in size (0.35 – 10.50cm; median = 1.08cm) and area (0.05 – 6.74cm<sup>2</sup>; median = 0.38cm<sup>2</sup>). The total area of pits was 45.1cm<sup>2</sup>. The ceramic tiles were darkened with a black permanent marker to increase the settlement of barnacle cyprids (*Plate 4.1*).



*Plate 4.1* Unglazed reverse surface of a domestic terracotta ceramic tile. The tile has been coloured black with permanent dye and was used as an easily repeatable artificial settlement substrate once painted with crude barnacle extract.

Barnacle extract was used to enhance settlement on all the ASS. 230 g of fresh adult barnacles was scraped from rocks on the intertidal and ground in distilled water

with a mortar and pestle. The ground material was mixed with distilled water, made up to a volume of 1000ml and left overnight at 10°C. The mixture was then well mixed and the fluid poured off into centrifuge tubes. These were spun for 30 min at 14 300 x g in a Beckman, JA10 rotor. The clear, slightly pink/orange supernatant was pipetted into individual storage containers and frozen at -20°C. When required the individual containers of adult extract were defrosted in a fridge overnight and well mixed before being painted onto the panel or tile with a paint brush. The first layer was allowed to dry on before the second coat was applied on top. Approximately 0.5ml was required for each ASS.

Comparisons between designs were made in two phases. In 2004, 4 replicates of old and new Perspex panels were deployed on an 8 slot array at Boarhills. Positions of the two designs were randomised daily for 8 days.

In 2004 ceramic tiles were deployed alongside new Perspex panels at four sites. The tiles were deployed in fixed positions in triplicate replication. The Perspex panels were deployed in quadruplicate replication in fixed positions on the same array. Tile and panel arrays were retrieved and replaced with freshly cleaned and painted ones daily and settlement of *Semibalanus balanoides* was assessed.

Settled ceramic tiles were returned to the laboratory where the number of *Semibalanus balanoides* cyprids settled in three locations was noted:

1. Against the pit walls.
2. In the middle of the pits, away from the pit walls.
3. Outside of the pits on the raised surface of the tile.

### **4.2.3 Statistical analysis**

#### **4.2.3.1 Topographic variation**

A three factor ANOVA was used with topography (TOPOG) a fixed factor and array (BLOCK) and day (DAY) as random factors. Levene's test for homogeneity of variance failed for both the raw data and  $\log(x+1)$  transformed data. The data were also non-normal (Kolmogorov-Smirnov) prior to and following  $\log(x+1)$  transformation. ANOVA is robust to non-normality and heterogeneity of variance (Underwood, 1997). Data remained un-transformed for the analysis. Post hoc analysis was undertaken on topographic variances using Bonferroni multiple pairwise comparisons.

#### **4.2.3.2 Artificial settlement substrate**

A two way ANOVA was undertaken to distinguish differences between old and new Perspex panels deployed simultaneously in 2004 at Boarhills. Panel type was fixed, day was random. Assumptions of normality and homogeneity of variance again were not met for transformed ( $\log(x+1)$  and Box-Cox) and untransformed data. Data were left as absolute numbers per unit area of panel.

The ceramic tiles were deployed together with the new panels in 2004 at Fife Ness, Cambo Farm, Caiplie and Tentsmuir. An independent ANOVA with two factors was used to determine significant differences between panels and tiles at each site. Substrate was fixed and day was random. The dependent variable was un-transformed *Semibalanus balanoides* cyprid settlement.cm<sup>-2</sup>. Once again assumptions of ANOVA were tested with Levene's test and the Kolmogorov-Smirnov test. These assumptions were violated for transformed ( $\log(x+1)$  and Box-Cox) and un-transformed data alike.

## 4.3 RESULTS

### 4.3.1 Topographic variation

Source	SS	df	Mean Square	F
TOPOG	15008.57	3	5002.86	23.767**
BLOCK	1751.85	1	1751.85	6.25
DAY	295858.62	14	21132.76	72.659**
TOPOG * BLOCK	241.37	3	80.46	0.38
TOPOG * DAY	12848.58	42	305.92	1.45
BLOCK * DAY	3927.12	14	280.51	1.33
TOPOG * BLOCK * DAY	8840.78	42	210.50	0.72
ERROR	104707.25	360	290.85	

Table 4.1 Three factor independent ANOVA for topography (fixed), block and day (random). Data is absolute values of settlement of *Semibalanus balanoides* cyprids per tile. \*\* is  $p < 0.001$

There were the expected significant differences between Days, but this result was considered trivial. Topography demonstrated significant differences in settlement on the tiles. There were no significant differences between blocks. Topographic differences were consistent at both blocks and across different days. No significant differences were found between different topographies at different blocks on different days i.e. no significant second order or third order interactions (Table 4.1).

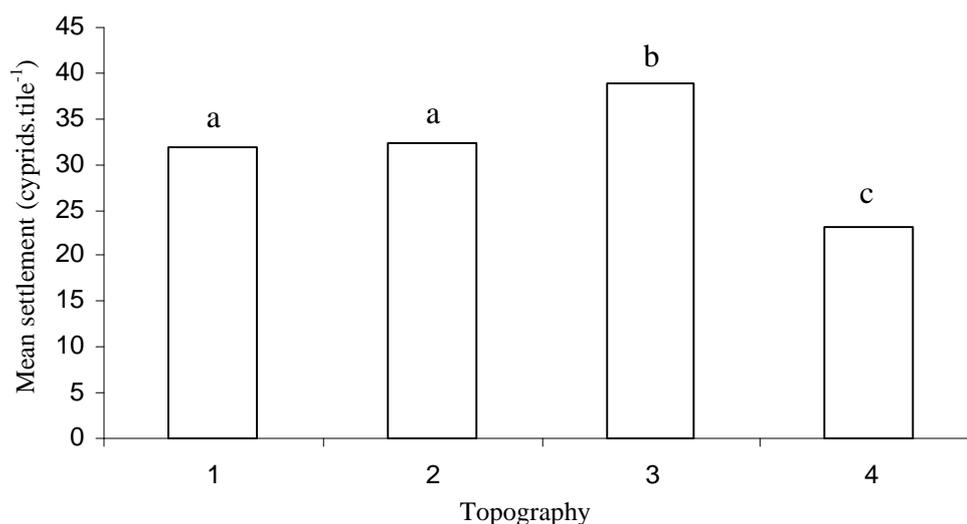


Figure 4.4 Bonferroni multiple pairwise comparisons of the four topographies. Letters denote the groups to which each topography belongs.

Two topographies showed no significant differences from one another, however significant differences were observed amongst the other topographic casts (*Figure 4.4*).

### 4.3.2 Artificial settlement substrate selection

#### 4.3.2.1 Perspex panel design

Source	SS	df	Mean Square	F
DAY	15.372	7	2.196	38.05**
PANEL	4.789	1	4.789	2.59
DAY * PANEL	12.945	7	1.849	32.04**
ERROR	2.770	48	.058	

*Table 4.2* Two way independent ANOVA for day (random) and panel design (fixed). Data was for per area *Semibalanus balanoides* cyprid settlement. 4 replicate panels of each design (old and new) were deployed over 8 days.

Two way ANOVA showed that there was a significant effect for Day but not Panel. There is also a significant interaction between Day and Panel (*Table 4.2*).

*Figure 4.5* demonstrates a large departure from the relative values of per area settlement for the 6<sup>th</sup> day in the sequence for old panels against new, demonstrating the interaction was as a result of an amplitudinal effect.

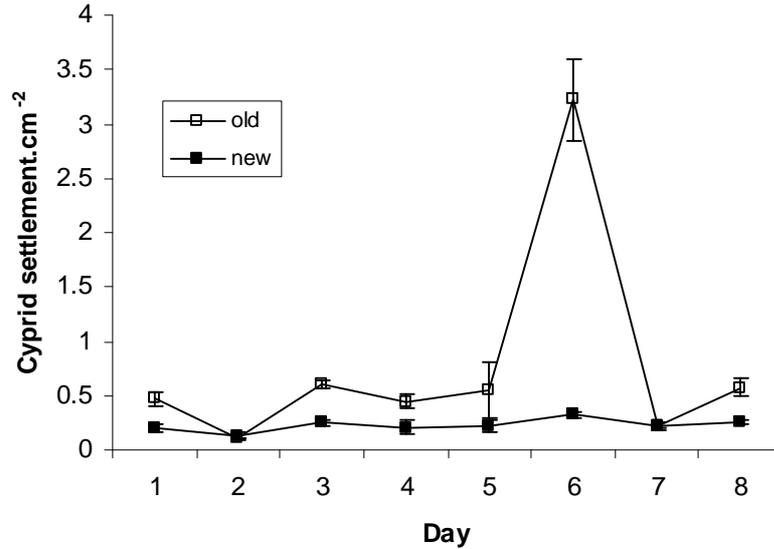


Figure 4.5 *Semibalanus balanoides* cyprid settlement per cm<sup>2</sup> for each day. Two panel designs were compared. Old panels with deep grooves shown as the open squares. New panels with more grooves per area are shown with the filled squares. The mean  $\pm$ 1 s.e. are shown.

The ANOVA was re-run following removal of this day and the results are in Table 4.3. There were significant differences between Days and Panel designs and no significant interaction between Day and Panel demonstrating only one Day caused the interaction.

Source	SS	df	Mean Square	F
DAY	.594	6	.099	3.70**
PANEL	.625	1	.625	23.34**
DAY * PANEL	.312	6	.052	1.94
ERROR	1.124	42	.027	

Table 4.3 Two way independent ANOVA for Day (random) and Panel design (fixed). Data was for per area *Semibalanus balanoides* cyprid settlement. 4 replicate panels of each design over 7 days following the removal of one day due to an interaction between the two independent variables.

### 4.3.2.2 Ceramic tile design

Source	SS	df	Mean Square	F	Sig.
Substrate	14359418	1	14359418	96.926	<.001
Day	20800144	34	611769	4.129	<.001
Substrate * Day	5037025	34	148148	39.511	<.001
Error	656164	175	3750		

Table 4.4a

Source	SS	df	Mean Square	F	Sig.
Substrate	3055477	1	3055477	35.621	<.001
Day	3079769	30	102659	1.197	<.001
Substrate * Day	2573305	30	85777	98.939	<.001
Error	134380	155	867		

Table 4.4b

Source	SS	df	Mean Square	F	Sig.
Substrate	1106952	1	1106952	83.502	<.001
Day	268176	19	14115	1.065	.446
Substrate * Day	251874	19	13257	7.571	<.001
Error	175097	100	1751		

Table 4.4c

Source	SS	df	Mean Square	F	Sig.
Substrate	8061537	1	8061537	56.816	<.001
Day	5694805	30	189827	1.338	.215
Substrate * Day	4256620	30	141887	58.950	<.001
Error	373072	155	2407		

Table 4.4d

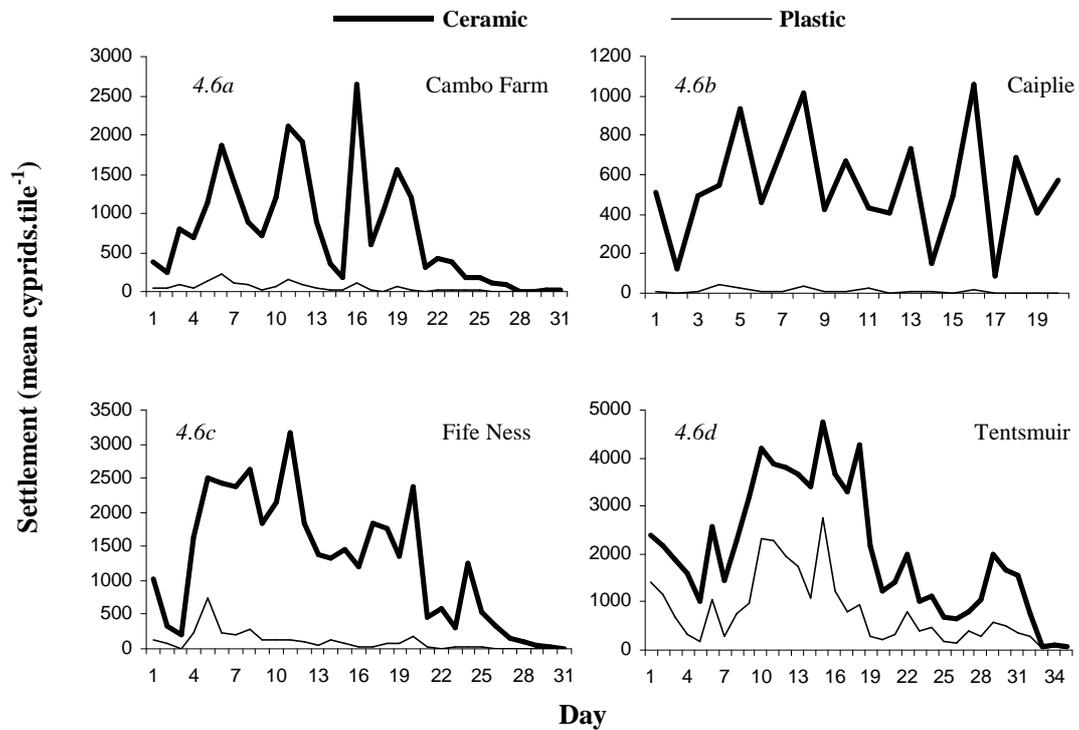
Table 3.4a-d Two way independent ANOVA for Substrate (new panel design and ceramic tiles) and day at four sites in 2004. Day is a random factor, Substrate is fixed. Data is average settlement per area. a. Tentsmuir; b. Cambo Farm; c. Caiplie; d. Fife Ness

When two way independent ANOVA was run for each site (Substrate fixed, Day random) significant day effects were seen at Tentsmuir but at none of the other sites (Tables 4.4a-d). Substantially more settlement was seen at Tentsmuir than the other sites (Figure 3.4).

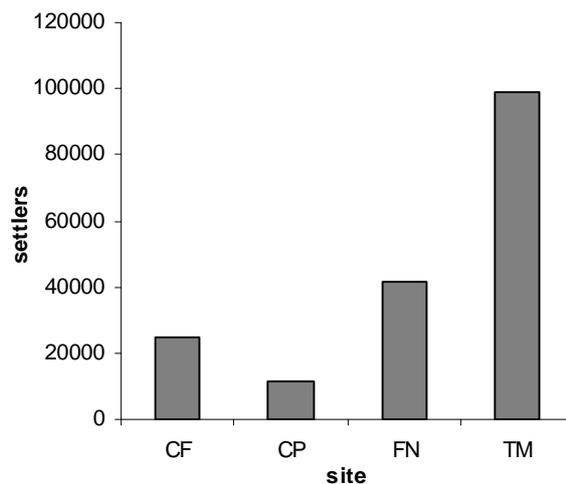
Significant interactions were seen between substrate and day at all the sites (Table 3.4a-d) indicating that differences between the substrates were not comparable

each day. However plots of substrate by day for each site demonstrates that these interactions are as a result of amplitudinal effects (*Figure 4.6a-d*).

Differences in settlement for both substrates each day were seen at Tentsmuir (*Table 4.4a*; *Figure 4.6d*) despite a first order interaction (*Table 4.4a*).



*Figure 4.6a-d.* Total settlement per day for two substrates. The thick line is daily settlement on ceramic tiles. The thin line is daily settlement on the new plastic panel design. *a.* Cambo Farm; *b.* Caiplie; *c.* Fife Ness; *d.* Tentsmuir



*Figure 4.7* Total *Semibalanus balanoides* settlement (tiles and panels combined) at each site for 2005.

### 4.3.3 Settlement behaviour.

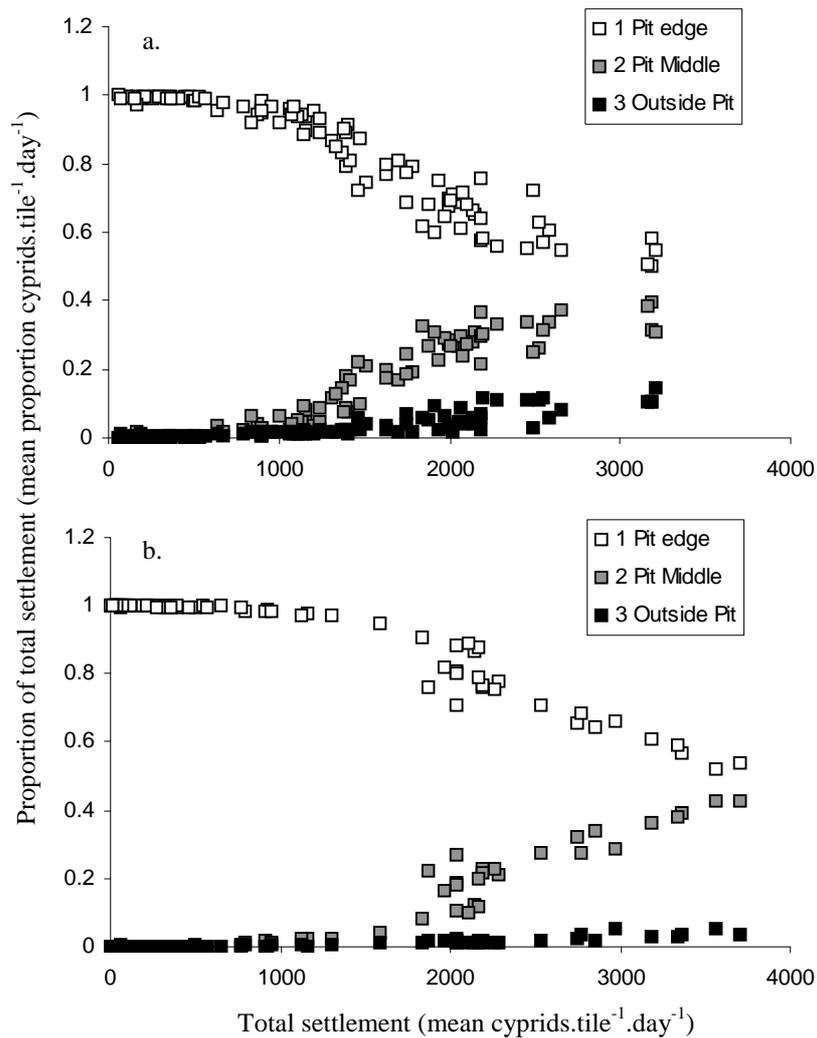


Figure 4.8 Settlement as a proportion of total settlement for three features specific to the pitted ceramic terracotta tiles. Proportion settled on 1 (the pit edges), 2 (the middle of a pits) and 3 (outside the pits). Two sites were examine. a. Tentsmuir in 2005; b. Reres Wood 2005.

Settlement against the edges of the pits constitutes effectively 100% of the settlement on the tiles for the lower third of the range of settlement densities observed at the two sites (*Figure 4.8*). The total settlement on the tiles increases beyond a mean of approximately 1000 cyprids per tile, the proportion of settlement against the pit edges declines and the proportional settlement in the middle of pits increases. Settlement outwith the pits also increases but is significantly less than the proportion of cyprids settling in the pits, both against the edge and in the “middle”.

## 4.4 DISCUSSION

### 4.4.1 Topographical variation

Two general processes affect the settlement of many balanomorph barnacles. Oceanic and tidal currents determine the distribution and abundance of larvae across temporal and spatial scales. Secondly the barnacles settle according to cue-driven settlement behaviour, largely centring on the detection and selection of the substratum (Hills *et al.* 1998).

Significant variation in settlement each day was consistent between blocks for the 15 days over which the Latin squares were deployed, demonstrating significant temporal variation in the settlement of cyprids on the rock casts. A number of authors have demonstrated that these characteristic peaks in settlement are driven by transport of the larvae from the larval pool to the shore by oceanic and tidal features (Hawkins and Hartnoll 1982; Caffey 1985; Pineda 2000).

According to *Table 4.1* all four Topographies exhibited consistent differences independent of Block demonstrating that natural differences in topography alone result in consistent differences in settlement in the field, independent of rates of larval supply at broader scales between blocks. The recognition that topographic heterogeneity is an important factor in determining settlement at small scales is well-documented in the literature, but many of these studies involve the use of artificially uniform topographic features (Crisp and Barnes, 1955; Hills *et al.*, 1998). Very few experiments have investigated ecologically real, natural topographic heterogeneity (for exception see Wethey, 1986). The present study also demonstrates topographic variation in settlement is consistent over temporal and spatial scales and differential rates of supply at scales relevant in the field.

*Figure 4.4* shows that topography 3 had a significantly greater settlement density and topography 4 had a significant lower settlement density. Topography 1 and 2 demonstrated an intermediate settlement density and were not significantly different from each other. Topography 1 and 3 both demonstrated a deep ridge ~ 1-2 mm deep with a gently sloped wall. Larvae were observed settling in these ridges and on the lower side of these features on both these tiles. No obvious features were observed on topographic casts 2 and 4. Topography 3 demonstrated consistently greater densities however it is not immediately clear why there was greater settlement on this topography relative to topography 1 as the latter demonstrated a more extensive ridge structure. Topography 4 may have exhibited particularly low settlement due to the slightly convex nature of the tile. The patterns of settlement that were observed demonstrate the complex variability in settlement natural topographic detail alone can produce in settlement studies. For this reason, standardised artificial settlement substrate are paramount to controlling for this variation in studies comparing rates of settlement between and within shores and even rock blocks.

#### **4.4.2 Artificial settlement substrate selection**

Yule and Walker (1984b) showed that abrading Perspex surfaces increased the forces required to detach a cyprid. When abraded Perspex was combined with a treatment of barnacle extract an even stronger bond strength by the cyprids resulted. Differences were also seen in the attachment forces of cyprids on light and dark Perspex panels. The darker panels demonstrated an increase in the force required to detach the cyprids. It is unlikely that the latter demonstrates an increase in the opportunity to bond with the substrate and suggests the cyprids are able to control the degree of their attachment according to preferences.

Trials of artificial settlement substrates here in 2003 suggested settlement of barnacle cyprids on the old panel design was as a result of settlement against an edge in the deep vertical grooves. It was felt that to improve the settlement densities on the artificial settlement substrate, increasing the grooves per unit area would lead to an increased rate of settlement per unit area in the 2004 deployment. This led to the second ('new') design of panel that had a far greater number of grooves per unit area. Following the comparison tests of the two panel designs, however, it was apparent that there was a significant decrease in settlement on the new design. It is clear that this was due to a difference in the groove design. Due to the increase in the number of grooves it was not possible to cut as deeply as for the original design because it would have significantly weakened the acrylic panel. A loss of 1mm of groove height appears to have rendered the new design significantly less desirable to cyprid settlement. This may be due to the altered turbulence of flow across the surface of the new panel. Mullineaux and Butman (1991) demonstrated a difference in settlement of *Balanus amphitrite* cyprids on plain polycarbonate panels of differing thickness and polycarbonate panels with a 5 mm plate attached vertically at the downstream end of the plate. This was as a result of boundary-layer flows affecting the rates of contact and subsequent exploratory behaviour of the cyprids. These factors may influence the settlement of larvae on the Perspex panels in the present study, with active increased substratum rejection because of a less favourable boundary layer condition on the new panels, or increased rates of passive contact on the deeper grooved tiles due to greater depth of the turbulent boundary layer. The variation in settlement observed on the natural topographic casts also demonstrated in this study highlight this effect. Topographic variation can produce unpredictable variation in settlement as a result of passive and active processes.

The deployment of the ceramic tiles lead to a marked increase in the rate of settlement of cyprids compared to the new panel design. The proportion of cyprids on the tiles compared to the new panel design was close to 6:1 in favour of the tiles. Comparisons of the two Perspex panel designs showed settlement was 4 times higher on the old design. This demonstrates the tiles perform better in terms of absolute settlement than either of the acrylic panel designs and the ceramic tiles can be expected to perform 1.5 times better than the best Perspex panel design. It is assumed that this is due to a combination of deep grooves and the unique texture of the tiles. Acrylic, even when sanded and milled is a relatively smooth surface and appears to show low wettability (*pers obs.*). The porous, rock like nature of the tiles seems to be a more preferable surface for the attachment of the cyprids.

Besides Tentsmuir, where particularly high values of settlement were observed, the new panel design did not have sufficient settlement to discriminate daily variation in cyprid settlement (*Figure 4.6a-d*). The ceramic tiles demonstrated sufficient settlement for temporal variation to be distinguished. This is an important factor in selecting a settlement substrate for use in mesoscale analysis of temporal and spatial variation in settlement of barnacle cyprids within and between sites.

#### **4.4.3 Settlement behaviour.**

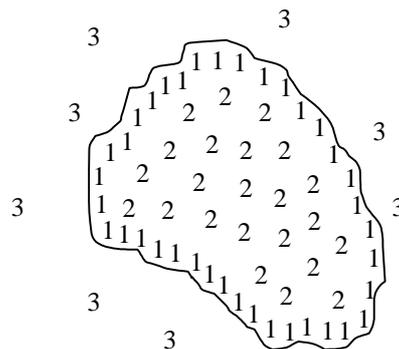
At relatively low settlement densities the cyprids settled against the edges of the pits. rugophilic settlement behaviour is well recognised in the literature and this drives the initial phase in the settlement preference cascade seen here. Once the pit edges are sufficiently full with cyprids that newly arrived cyprids cannot find any space against the wall during its exploratory phase, the cyprid individual will be forced to settle on less desirable surfaces; in the middle of the pits and finally, outside

the pits on the surface of the tile. *Plate 4.8* demonstrates typical patterns of settlement on the ceramic tiles for days of moderate and high settlement.



*Plate 4.8* Observed settlement patterns of *Semibalanus balanoides* cyprids on the blackened, ceramic tiles painted with crude adult extract. At moderate densities cyprids settled against the pit walls (*left*). At high densities they filled the pits. Some cyprids would settle outside the pits on the high relief areas (“top”) of the tile (*right*).

*Figure 4.8* demonstrates that the cyprids occupy the middle of the pits essentially only once the pit edges are saturated. Some settlement is observed outside of the pits on the high relief parts of the tile but this is clearly less preferable to the cyprids than in the pits themselves, whether against the pit walls or in the middle of the pits. From these data it is apparent that cyprids demonstrate a preference cascade when settling in increasing densities onto the pitted ceramic tiles.



*Figure 4.9* Schematic representation of a cascading settlement response in *Semibalanus balanoides* cyprids on pitted ceramic tiles.

Prime settlement areas become more densely occupied and areas on the tiles that are less preferable to settlement begin to fill up. Pre-emption of space by other cyprids limits suitable settlement of larvae. Space pre-emption by other species or

adults is a common density-dependent process affecting settlement (Morgan 2001).

Larvae that cannot settle are forced to delay metamorphosis whilst searching for more suitable substrate.

Settlement 'choice' is crucial to adult survival but presumably also larval survival because cyprids are lecithotrophic. As a result, extended delay of metamorphosis can lead to settlement failure due to depletion of the lipid stores used for energy by the cyprid during this phase (Pechenik 1990). A cyprid may, therefore, become increasingly or suddenly "desperate" to settle (Marshall and Keough 2003). This may alter the shape of, or even overpower, a cascading settlement preference, most likely at high settlement densities or late in the season (Gribben *et al.* 2006).

## **Chapter 5 –Larval Supply and Settlement**

### **5.1 INTRODUCTION**

#### **5.1.1 Models of community dynamics**

Underwood and Denley (1984) drew attention to the fact that historically models and generalisations of intertidal community structure were biased towards the effects of mortality on potentially dominant species due to physical factors, predation, competition and interactions between these processes. Manipulative studies concerning community structuring on competition and on predation (Connell 1961b; Paine 1974) had set a precedent for ecology and established post-settlement processes as the paramount structuring forces defining community dynamics. Underwood and Denley (1984) suggested that testing assumptions with alternative hypotheses was fundamental to accurately describing models of communities and identifying new directions for research that may be overlooked if experimental testing only reinforces previously accepted views. These empirical studies and others that followed them neglected to examine variation in settlement as an alternative hypothesis for community structuring.

Connell (1985) revisited some of this early work, including that of his own and colleagues, and concluded that in populations with low recruitment levels individual mortality was independent of densities of settlement and recruitment. As a result variation in adult abundance was driven by spatio-temporal larval abundance patterns, rather than post-settlement mortality in low density, open demography communities.

### 5.1.2 Demographically open marine populations and larval delay

Broadly defined, recruitment is the addition of new individuals to a population. A simplification of a closed demographic population describes the upward regulation of the population by the birth rate of that population as the juveniles are directly recruited into the population. A great deal of animal and algal species that comprise the marine intertidal communities have a dispersive larval phase terminating as a sessile, space-limited adult (Thorson 1950; McEdward 1995). The distance larvae can disperse will depend on the speed and direction of water currents transporting the larvae and the length of time that individuals spend in the larval stage. The length of dispersal time is highly variable between species (Pechenik 1990) and many populations can disperse their larvae great distances (Gaines and Roughgarden 1985).

For species with an extended larval dispersal phase, juveniles are dispersed away from the adult population and any recruitment to the adult population is as a result of larval immigration from distance or perhaps a different section of the shore. As a result birth rates and recruitment to a local population may be decoupled for the adult population. Populations regulated by immigration are subject to interaction between biotic and abiotic processes acting on both recruitment and post-recruitment survival (Caley *et al.* 1996).

Gaines and Roughgarden (1985) demonstrated that *Balanus glandula* populations were regulated by post settlement mortality, such as predation from starfish, only at a location where the population achieved high levels of settlement. At low levels population abundance and density were independent of predator-induced mortality. An oscillatory pattern indicative of an equilibrium community with a time-related interference mechanism in population size over time is seen at the high settlement shore. It is suggested that the delayed destabilising effect is growth of the

recruits. When recruits obtain a critical density that can no longer be supported by their environment, density dependent mortality markedly reduces the population size and primary space becomes available once again. New settlers re-saturate the substrate and the process begins again. However at low settlement sites such density dependent mortality is not observed and so there is no oscillation in population size over time. Variation in population size is regulated directly by variation in the immigration of juveniles. Mortality remains independent of population density, because the population never reaches a density whereby significant per capita mortality occurs. Intermediate populations were observed switching between the two processes. For years where settlement remained low the population remained stable. However, if settlement reached a sufficiently high level, oscillatory patterns were observed.

Studies have reported the large variation that occurs in settlement across a broad range of temporal and spatial scales of months to years and metres to kilometres within a similar habitat (Caffey 1985). Clearly if variations in recruitment processes are large the population must be understood in the light of both pre- and post-settlement variation.

### **5.1.3 Distinguishing supply and settlement**

For benthic organisms with planktonic larvae, recruitment has three components (1) water column larval supply; (2) the settlement patterns of competent larvae; (3) the survivorship of settlers to the age of initial census.

Larvae may contact the substrate frequently whilst in the plankton (Denny and Shibata 1989) and this allows them ample opportunity to repeatedly test the substrate for settlement. There is a behavioural component to the success or failure of

individual larval settlement and this has received considerable attention in the literature (Crisp 1974; Rittschof *et al.* 1998).

If inferences about the effects of recruitment on the population dynamics of adult organisms are based on observations made following the influence of early settlement events, these studies will have no means of assessing the relevance of these early processes. Explaining patterns of recruitment and how these relate to patterns of adult distribution and abundance requires early quantification of larval abundance and settlement densities and the processes that influence these densities (Keough and Downes 1982).

Settlement can be distinguished from supply (“delivery to the substratum”) in *Semibalanus balanoides* as the point at which a cyprid cements itself to the rock. After this point the individual cannot detach and migrate away, unlike mobile benthic invertebrate species (Moksnes and Wennhage 2001). Larval supply is generally considered to be a passive process, particularly in the often turbulent rocky intertidal shore (Butman 1987; Denny and Shibata 1989).

Some studies suggest that considerable variation in settlement can be explained by variation in larval supply (Bertness *et al.* 1992; Miron *et al.* 1995). However, Keough and Downes (1982) demonstrated that small-scale variation in larval recruitment on the rocky shore in California of a number of invertebrate species was determined by preferential settlement in cracks and predation by fish. Either one or both of these factors determined the distribution of recruits after three weeks. Therefore early post-settlement mortality and behavioural factors were more important in explaining the distribution of settlers measured at this point than was larval supply. If the observer is to infer larval supply by using a measure of settlement

it is essential to obtain very early settlement distributions on a uniform substrate before post-settlement processes can interact.

The relative importance to population and community structure of larval supply, settlement, recruitment, or post-recruitment mortality is not yet fully characterised in marine communities (Levin 2006). However, whether patterns of settlement - of which much is known - are influenced by patterns of supply of larvae can be addressed only if appropriate methods of quantifying larval supply are used (Underwood and Keough 2001).

#### **5.1.4 Integrating supply over time**

In many studies planktonic larval abundance (= concentration) is used as a proxy for larval supply or availability of larvae to the substrate (Gaines *et al.* 1985; Miron *et al.* 1995). Pumps and net samples - the most common method for recording this variable - provide a snapshot of larval distributions in the plankton. Larval supply, however is a dynamic flux. There is a great degree of temporal and spatial variation in larval supply that will remain undetected if there is insufficient integration of measures of larval abundance over time (Gaines and Bertness 1993; Moksnes and Wennhage 2001). Because it is a flux, measures of larval supply must combine larval concentration with variation in the rates of advection to the substrate over time. To understand variation in larval supply across large temporal and spatial scales an integrated measure of larval abundance over time is therefore paramount (Gaines and Bertness 1993; Todd 2003).

The importance of spatial variation to instantaneous measures of larval supply was amply demonstrated by Miron *et al.* (1995). They described variation in the vertical distribution of *Semibalanus balanoides* larvae influencing the rate of

settlement at different heights on a pier. They suggested that this may be a common feature in other neustonic species such as *Balanus glandula* whose adults are found high on the shore the larvae of which are similarly high in the water column.

Variation in height of settlement on the shore driven by supply of larvae may be missed if larval samples from a single depth in the plankton are integrated across vertical height of the intertidal.

Another problem with instantaneous measures of larval supply is the compromise required between sampling frequency necessary to adequately integrate larval supply over time, and the extent of spatial scale across which empirical observations can be made. Studies involving larval sampling across large spatial scales necessarily preclude the quantification of temporal variation. Studies with adequate temporal sampling have been restricted to work at only a few sites (Gaines and Bertness 1993).

Whitlach and Osman (1998) developed an automated device that was capable of exposing hard or soft settlement substrate to larvae in the field at different times, whilst maintaining previously-fouled settlement communities within the device. This allowed them to examine settlement dynamics within almost any predefined temporal scale. Although the device was cumbersome and relatively expensive to make they demonstrated within-tide variation in settlement and variation due to photoperiod of a number of both epifaunal and infaunal species larvae. Despite this, multiple devices would be required to examine the patterns simultaneously at other shores.

Moksnes and Wennhage (2001) described the use of fixed pairs of nets facing in opposite directions and perpendicular to the inlet of a bay. They trapped decapod larvae that migrated into and out of nursery areas within the bay. They compared this measure of larval supply to temporal variation in abundance of settlement and

recruitment both outside and within the bay area. A higher concentration of early stage larvae outside the bay was observed from net tows; however correlation with settlement onto artificial settlement substrates was poor due to the lack of integration over time of this instantaneous method. A high degree of correlation with larval supply in the fixed net traps and settlement on artificial settlement substrates was observed over short time scales. At longer time scales the migratory nature of the species meant that the number observed on the artificial settlement substrates was poorly correlated with trap counts. These fixed net traps were deployed in calm, wave protected bay conditions and at some distance from the settlement substrate and recruitment sites, restricting their ability to integrate over spatial scales. The traps were accessed by boat, which is impractical for an intertidal environment. They were also quite large in size and therefore would be subject to damage due to fouling by drift algae and rubbish or wave crash.

A simple way to solve the problem of instantaneous, indirect and damage-prone measures of larval supply such as pumping and netting is to use a passive tube trap (Hannan 1984; Yund *et al.* 1991; Metaxas 2004). Bertness *et al.* (1992) used four large larval sampling tubes (Yund *et al.* 1991) with high aspect ratios and deployed sublittorally approximately every fortnight 15-40m offshore in 4-5m of water. Traps were deployed adjacent to the shoreline settlement sampling quadrats at two shores, which differed in terms of adult and larval population densities. These shores were separated by 30 km of coastline. Shoreline settlement quadrats had combinations of the following conditions; presence or absence of adult conspecifics, differing degrees of wave exposure, different heights on the shore and presence or absence of algal crusts. Daily observation of settlement allowed the authors to quantify spatial and

temporal variation in settlement within and between shores. Settlers were cleared each day from the substrate once settlement densities had been recorded.

Between the sites, differences in larval supply explained a significant proportion of the variation in settlement, recruitment and ultimately adult abundance for these two sites. It was observed that although the supply of larvae was four times greater at Mt Hope Bay than Little Compton, the rate of settlement at Little Compton was an order of magnitude greater. At both sites larval supply was highly correlated with settlement. Early in the settlement season, remarkably low settlement was observed relative to larval supply at Mt Hope Bay. It is suggested by Bertness *et al.* (1992) that the discrepancy in settlement response of the larvae between the sites was due to pre-emption of suitable settlement space at Mt Hope Bay and therefore intensification of settlement on the cleared quadrats at this site later in the season. Early in the season settlement rates were similar at both sites, because similar amounts of suitable primary settlement space was available at both sites.

Gaines and Bertness (1992) studied larval supply with tube traps and settlement to the rocky shore for two distinct populations of cyprids in the Narragansett Bay area, Rhode Island. They demonstrated that in years when there was a high river input (short flushing time) in Narragansett Bay there was low supply and low settlement of the distinctive large “bay” cyprids within the bay. Supply and settlement of bay cyprids was apparent on the adjacent open coastline during these years. In years of low river input to the bay, “bay” cyprids were rarely identified outwith the bay on the open coastline. A bimodal size distribution of recruits in years with short flushing times was observed in the open coast population. The use of larval tube traps in that study demonstrated that patterns of recruitment and characteristics of the adult population in these two areas was due to processes affecting larval supply.

Passive larval traps provide a unique method of integrating supply over time whilst being cost efficient and requiring no moving parts. In addition they provide autonomous continual records of abundance and this is a major advantage to monitoring areas that are difficult to access. Metaxas (2004) deployed larval traps similar in design to Yund *et al.* (1991) on oceanic volcanic ridges accessible only by deep sea submersibles. These environments contain rich assemblages of invertebrate marine species. The sites were at depths of between 1500 and 2200 m. The traps were deployed for 7-10 days, then retrieved by capping the traps with the submersible's 'claw' before returning them to the surface. Captured larvae could then be identified and enumerated to characterise the supply of invertebrate larvae typical to these biologically rich deep ocean communities. Temporal variation in larval supply was evident between years but not between sites within years. That study, although obviously limited to some extent by logistics at this depth, demonstrates the useful properties of time-integrating passive larval traps to sampling in remote or inaccessible environments.

### **5.1.5 Desperate Larvae**

The larval energy reserves for *Semibalanus balanoides* cyprids are not finite. This phase of the free swimming larvae of the barnacle does not feed and relies on energy reserves obtained from the plankton prior to metamorphosis into this terminal planktonic stage. These energy reserves must last beyond metamorphosis into the adult stage for up to four days before adult feeding can commence. It is suggested that the competency to settle may last less than 5 weeks at 10°C (Lucas *et al.* 1979).

Variability in larval settlement behaviour seems to be the rule rather than the exception in many species of marine invertebrates. This may be due to the nutritional

state of larvae (Marshall and Keough 2003). Decreased energy reserves often leads to decreased discrimination of settlement cues. This is known as the “desperate larva hypothesis” (DLH) and was first described by Knight Jones (1951; 1953) and Wilson (1953). It has been demonstrated that delaying metamorphosis has important costs to both post-larval growth rates and development (Marshall *et al.* 2003).

Gribben *et al.* (2006) demonstrated that older larvae of two species of bryozoans changed their response to settlement cues and settled in much higher numbers on natural antifouling paints developed from algal extracts than younger larvae. Larval settlement response was dynamic, with larvae rejecting and accepting settlement cues based upon the extent of their energy reserves. Larvae were capable of settlement across the full range of laboratory-induced delay periods. Botello and Krug (2006) demonstrated that un-fed obligate lecithotrophic larvae of *Alderia* sp. became increasingly responsive to dissolved settlement cues with age. The sensitivity of fed larvae was unaffected with age. These larvae also demonstrated a variable component in settlement response according to parentage suggesting a heritable component to the degree of settlement response.

Marshall and Keough (2003) suggest that there is a link between larval size and the extent of larval longevity. Larger larvae stay active longer than do smaller larvae. They demonstrated that larger larvae of three marine species delayed settlement longer than their smaller larval cohorts both in the laboratory and in the field. They suggest that the variation in larval size produced by adults of these species would lead to differences in the length of time before a larva settles. This would allow species with dispersive larvae to produce larvae with a range of dispersal potential simply by altering the size of the larvae (see Chapter 1; section 1.1.3.2 for further discussion).

### 5.1.6 Aims of the present study

The aim of this chapter is to characterise the relationship between larval supply and settlement of *Semibalanus balanoides*. Larval supply determines the abundance of larvae available to settle across continuous spatial and temporal scales. Passive larval traps provide a means of measuring integrated larval supply. The traps used in this study (Todd 2003; Todd *et al.* 2006) can be sufficiently replicated within- and between-shores to obtain an accurate description of the relationship between supply and settlement.

Larval supply and settlement are highly variable processes (Caffey 1985; Todd 2003; Todd *et al.* 2006). Abundances can vary between hours, days and years and within- and between-shores. Whether supply of larvae can fully explain the variation in settlement at these spatial and temporal patterns can be determined only with sufficiently scaled sampling of the process. In light of the historical focus on cue-based settlement response (Crisp 1974; Rittschof *et al.* 1998) the present study aimed to provide an insight into the importance of rates of larval supply to patterns of settlement.

Wave action is considered a potential source of variation in settlement response (Connell 1985). Many larval species have mechanosensory abilities that allow them to sense vibrations. Low frequency vibrations from wave action may act as a cue to the proximity of a shore, or even the properties of a shore such as sand or rock. Rittschoff *et al.* (1998) demonstrated that vibrations related to the effect of waves breaking on sand beaches inhibited settlement of barnacles.

Wave action was measured here with autonomous data logging pressure transducers (Chapter 2). A composite measure of wave action over two tides will be examined as a potential force driving settlement rates per unit of larval supply. This

will not be an examination of the increase in the rates of supply with wave action because no measure of larval concentration was available (Gaines and Bertness 1993). Rather, the focus of the study was to accurately describe the relationship between daily supply and settlement across multiple years and sites characteristic of the British Isles intertidal environment. A method for examining behaviourally-driven settlement responses relative to a direct measure of larval supply will be demonstrated.

## 5.2 METHOD

### 5.2.1 Supply data

The supply of larvae was recorded using the modified passive larval trap described in Todd *et al.* (2006). 1cm<sup>2</sup> conical inlet trap designs were selected for deployment across a number of sites due to their ability to reliably capture larvae at all prevailing levels of supply whilst still offering a high absolute abundance of larval captures on any one day.

### 5.2.2 Settlement data

The unglazed reverse surface of domestic terracotta ceramic tiles (Chapter 4) that had been coloured black with permanent dye was used as an easily replicated artificial settlement substrate. The tiles were painted with two coats of adult barnacle extract prior to each (daily) deployment and were known to provide a very favourable settlement substrate for barnacle cyprids.

Settlement preferences of cyprids appeared to follow a three stage hierarchical cascade on these pitted ceramic tiles;

1. Settlement against the pit wall.
2. Settlement in the “middle” of the pits.
3. Settlement outside the pits on the high relief (“top”) areas of the tile.

See Chapter 4; section 4.4.3 for a discussion and photographs of the tile pits and the *S. balanoides* larval settlement preference cascade.

For this investigation only settlement of cyprids against the pit walls (preference 1.) is used as a measure of settlement. Settlement against the pit walls was recorded when a cyprid was either in contact with, or clearly aligned parallel and positioned within half a cyprids body width of a pit wall so as to be essentially against

it. This settlement preference was chosen because it would potentially demonstrate a consistent and extensive relationship between supply and settlement across the range of larval supply measured by the passive larval traps in Fife.

### **5.2.3 Wave data.**

In addition to the traps and tiles, wave transducers were deployed to measure the wave action experienced at a limited number of blocks on the shores. The wave transducers are described in detail in Chapter 2. The wave sensors were set to cycle 2 minutes on and 13 minutes off, recording pressure fluctuations due to water depth above the sensor at 10 Hz. The AveDev index (Chapter 2) was calculated for each 2-minute immersion period. The mean AveDev index for two full tides corresponding to the appropriate daily trap and tile deployment was used as a measure of wave action for each sample period.

### **5.2.4 Location and array design**

Data for this study were collected over consecutive years at a number of sites in Fife along the east coast of Scotland. In 2004, multiple trap and panel arrays were deployed on single blocks at three shores; Cambo Farm (CF), Fife Ness (FN) and Tentsmuir (TM). In 2005, pairs of blocks were used within each shore. Block 2 at Fife Ness in 2005 was the same as the block at Fife Ness in 2004. New pairs of blocks were also used at Caipie (CP), Tentsmuir and Reres Wood (RW) in 2005 (*Figure 5.1*). The traps required daily service during an ~ 4 hr period of low tide. Due to the distances between sites several investigators assisted in the collection and collation of the data used in this study.

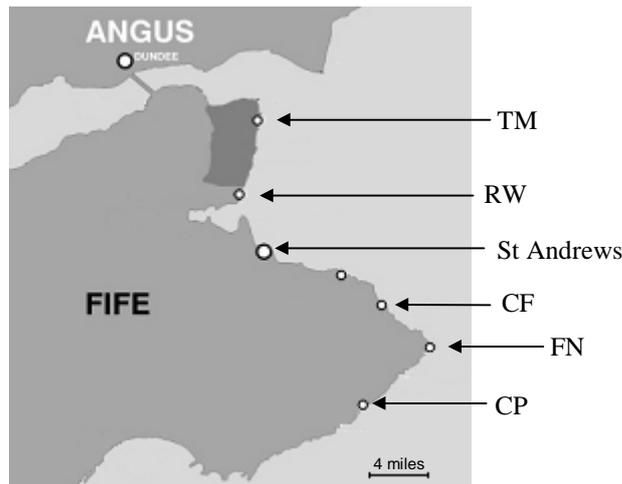


Figure 5.1 Map showing the East Neuk of Fife. Five shores were used over two years in this study. TM = Tentsmuir; RW = Reres Wood; CF = Cambo Farm; FN = Fife Ness; CP = Caiplic.

Multiple trap and tile arrays were deployed at the various sites. The Perspex rack arrays comprised six trap slot positions designed so that the occupied trap positions could be randomised daily (Chapter 3; section 3.2.2). Three trap slots flanked each side of a tile backboard (Figure 5.2). Randomising the trap positions was undertaken daily to ensure there was no positional effect of trap placement (Todd 2003; 2006).

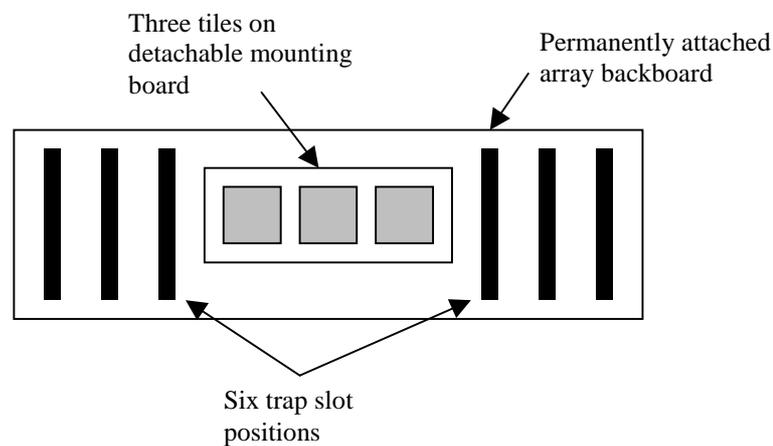


Figure 5.2 Typical supply-settlement array. Six trap slot positions allow for daily randomisation of three (2004) or four (2005) traps. Three tiles are attached to a mounting board and were exchanged daily, following settlement, with freshly painted unsettled tiles attached to a replacement mounting board. Arrays at Fife Ness were extended outwards to accommodate additional traps (see text).

In 2004 triplicate  $1 \text{ cm}^2$  traps were deployed and these were randomly allocated slot positions amongst the six slots each day. The exception to this in 2004 was at Fife Ness when three  $1 \text{ cm}^2$  traps were randomised daily across a twelve-trap

array as part of the trap aperture size comparison experiment described in Chapter 3. At each block in 2005 four replicate 1cm<sup>2</sup> traps were randomised daily across six positions. The exception to this was at Fife Ness where four 1cm<sup>2</sup> traps were randomised daily across ten slot positions, again as part of the second year of trap comparison studies described in Chapter 3. Over both years three replicate tiles were deployed on each array at each block. Traps and tiles were serviced after two tides (see Chapters 3 and 4 for descriptions of deployment and servicing of the traps and tiles and quantification of larval abundance).

### 5.2.5 Modelling

Initial interpretation of the relationship between supply and settlement of *Semibalanus balanoides* cyprids involved inspection of scatter plots of mean daily settlement of larvae against tile pit walls against mean daily supply per trap for each block (APPENDIX 1 – Scaled and Unscaled Scatter Plots). These plots confirmed a non-linear shape to the supply-settlement relationship at almost all the sites. Two non-linear models – a form of a logistic model and the Michaelis Menten asymptote model (MM) - were selected for investigation in the model fitting process.

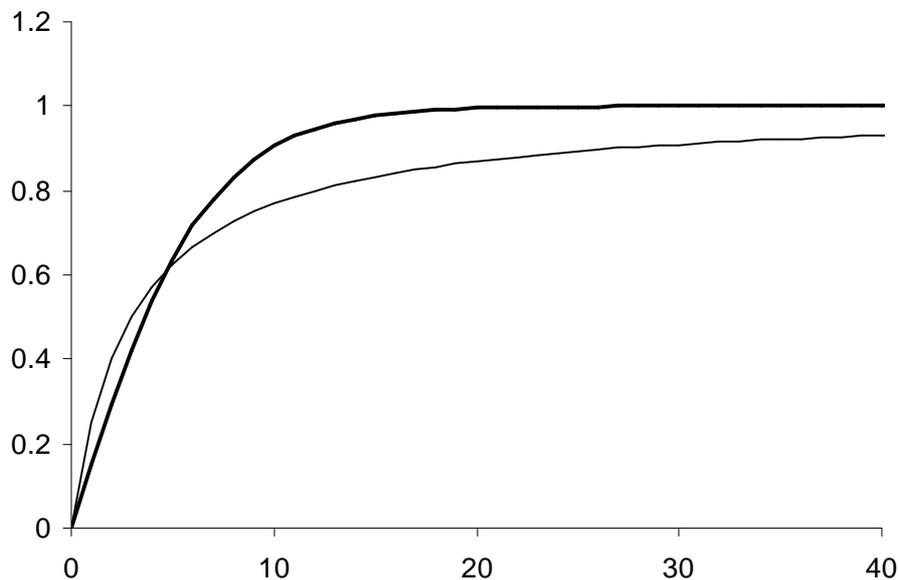
The logistic model. A 2 parameter model with a single negative inflection that passes through the origin (*Figure 2.1*):

$$Y = \frac{2A}{(1+e^{-BX})} - A$$

The MM model (Ratkowsky 1983). A 2 parameter asymptotic model that also passes through the origin (*Figure 2.1*):

$$Y = \frac{AX}{(B+X)}$$

All models were chosen to intercept the origin. Models forced through the origin were chosen on the assumption that if there is no supply there can be no settlement so an intercept value for  $Y$  in the model would be illogical. In all the models  $X$  is the mean daily supply of cyprids per trap and  $Y$  is the mean daily settlement of cyprids against the pit walls of a single tile. For both models the asymptote is determined by the constant  $A$  and the rate of increase towards the asymptote determined by the constant  $B$ . The Michaelis Menten model gradually approaches the asymptote rather than the sharper negative inflection of the logistic (*Figure 5.3*). Therefore the logistic would suggest a relatively constant relationship between supply of larvae and settlement towards saturation. The MM model demonstrated a more consistent decrease in settlement towards saturation of the substrate.



*Figure 5.3* The two non-linear models used to describe the relationship between supply and settlement of *Semibalanus balanoides*. The thick line is the logistic model ( $A = 1, B = 0.3$ ). The thin line is the MM model ( $A = 1, B = 3$ ).

Early attempts at fitting three parameter logistic curves (including an additional positive inflection point) to investigate a possible aggregate response in

cyprid settlement demonstrated there was insufficient resolution in the data to determine such a process at low levels of supply.

Comparisons also were made against a simple linear model intercepting the origin. This model took the form:

$$Y = BX$$

This model assumes that settlement increases as a direction function of the supply of larvae and a constant  $B$ . This value is conceptually similar to the parameter  $B$  in the two non-linear models demonstrating the rate of settlement.

The data revealed that there were very low levels of settlement on the tiles for days where there was no cyprid capture in the traps. There were undoubtedly cyprids available to settle that were not caught in the traps. As a result, positive mean values for settlement when supply was zero were confirmed as being due to limitations of replication, and of the capture efficiency of the traps at extremely low larval concentrations. To remove the effect on the models of positive values for settlement when supply was zero, days when trap captures were either 0 or 1 cyprid per block of traps (mean cyprid trap capture  $< 0.5 \text{ cyprid.trap}^{-1}.\text{day}^{-1}$ ) were removed.

Before the models were fitted, normality of populations from which the daily mean supply and settlement were obtained was tested with plots of average daily supply on median daily supply and average daily settlement on median daily settlement. Daily supply and settlement data both demonstrated heterogeneity of variance. If normally distributed, the relationship between the mean and median should be closely linear with the median equivalent to the mean ( $y = x$ ). Skewed distributions would produce a consistent and large difference from 1 for the slope. Mean:median ratios of daily supply and daily settlement were very close to 1 and

therefore they were assumed to be distributed normally around the mean (APPENDIX 1.2 – Normality of Supply and Settlement).

Examination of the arithmetic model residuals demonstrated a positive variance to mean ratio. Cube root transformations of settlement on each tile prior to obtaining the mean daily settlement homogenised the residuals around the models (Quinn and Keough 2002). Examination of the mean:median ratio suggested that conformation to normality of the daily settlement data was either improved, or remained unaffected, by the transformation (See Appendix 1.2 – Normality of Settlement).

Appropriate models were selected using the AIC model selector. A modification of the original formula - termed  $AIC_c$  - is recommended when sample sizes are  $< 40$  per parameter (Burnham and Anderson 1998). Both of these selectors adjust for  $n$  samples and  $k$  parameters. The mean square residual can also be used to select between non-linear models (Quinn and Keough 2002). Models were chosen on the basis of the  $AIC_c$  alone (APPENDIX 1.3 – Model Fitting Summary Table).

The models were fitted in SPSS using a Gauss-Newton iterative algorithm and least squares estimation of the parameters of the pre-specified linear, logistic and MM models.

## 5.3 RESULTS

### 5.3.1 Model selection

AIC<sub>c</sub> comparisons between a linear model, a logistic model and the MM model showed the latter to be the most accurate representation of the relationship between supply and settlement demonstrated at 10 of the 11 sites (APPENDIX 1.3 - Model Selection Summary Table). This model was therefore deemed most appropriate for description and further investigation of the relationship between supply and settlement. The models were plotted individually for each site and their corresponding residuals plotted against predicted values for Y (see APPENDIX 1.4 - Michaelis Menten models).

### 5.3.2 Outliers

Plots of standardised residuals for each block demonstrated outliers from the model. At three blocks > 5% of the standardised residuals lay outwith  $\pm 2$  z-scores. These blocks are demonstrated in *Figure 5.8* and the plots of the residuals are demonstrated in *Figure 5.9*.

May 16 2005 is highlighted as being exceptional at two sites. At Reres Wood, block 2 settlement was significantly higher than expected from the model estimate. At block 1 at Fife Ness settlement on this day also was anomalous; however the relationship here was reversed due to settlement being lower than predicted by the model (*Table 5.1*).

Site	Sample Size	Date	Standardised Residual
Tentsmuir block 1 2005	47	8 <sup>th</sup> May	-2.230
		24 <sup>th</sup> May	-2.062
		25 <sup>th</sup> May	2.092
Reres Wood block 2 2005	38	15 <sup>th</sup> May	2.081
		16 <sup>th</sup> May	2.935
		18 <sup>th</sup> May	-2.490
Fife Ness block 1 2005	43	11 <sup>th</sup> April	-5.171
		16 <sup>th</sup> April	3.868
		21 <sup>st</sup> April	2.066
		10 <sup>th</sup> May	2.211
		16 <sup>th</sup> May	-3.750

*Table 5.1* Blocks where > 5% of the residuals lay outwith 2 standard deviations of the distribution of the model residuals. Grey highlighted days are outwith the expected number of residuals (> 5%).

### 5.3.3 Parameters

Parameter *A* gives the asymptote for the MM model (Ratkowsky 1983). This value can be raised to the power of 3 to back-transform to the estimated hypothetical mean arithmetic value for the number of settling cyprids at which no more cyprids will settle against a tile's pit walls for that block in that year. These values ranged considerably for the nine blocks across five shores and two years in *Figure 5.4*.

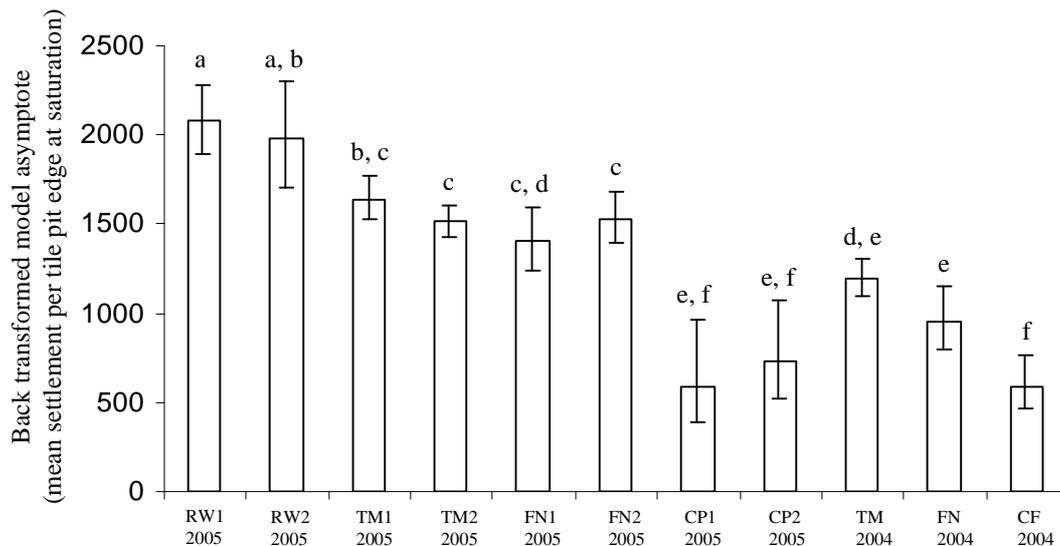


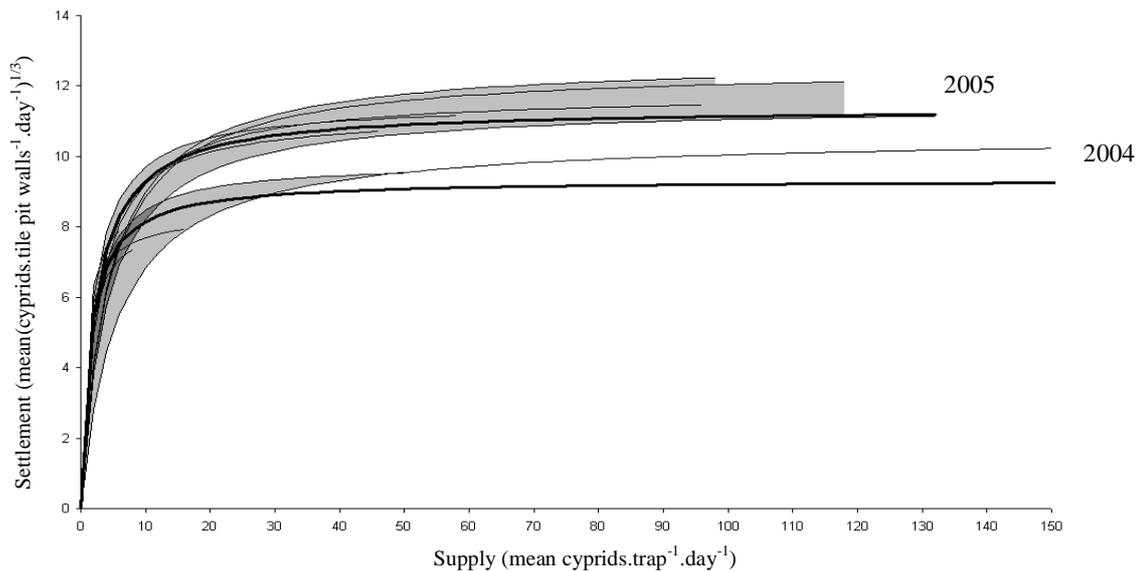
Figure 5.4 Bar chart showing the back-transformed arithmetic asymptote  $\pm 1$  standard error from a MM model of cube root transformed settlement on arithmetic supply. The model asymptote is equivalent to the maximum predicted mean settlement against the pit walls for a terracotta tile. Letters denote groups of blocks where the standard errors of the estimated asymptotes overlap indicating a significant difference at the 5% level. RW = Reres Wood; TM = Tentsmuir; FN = Fife Ness; CP = Caiplie; CF = Cambo Farm.

Both blocks at Reres Wood (RW) in 2005 demonstrated the highest levels of saturation of the tile pit walls. These were closely followed by Tentsmuir (TM) and Fife Ness (FN). Caiplie (CP) demonstrated the lowest saturation level in 2005. In 2004 Tentsmuir had a slightly higher saturation level than Fife Ness but the blocks at the two shores were within 2 s.e. of each other. Cambo Farm showed the lowest saturation levels in 2004. This site demonstrated saturation levels equivalent to those of Caiplie in 2005. Groups a, c and e/f appeared to be distinct from one another. Groups b and d contained a single block from each of the consecutive groups a, c and e/f (Figure 5.4).

At all shores in 2005, predicted mean saturation values between blocks within shores were within 2 s.e. of each other. Despite this there were differences between shores. The exception was between Tentsmuir and Fife Ness, which demonstrated no

significant differences in asymptotes but qualitative differences consistent between years.

Year class composite models were estimated and differences in saturation between year class were examined using a z-test (Fowler *et al.* 1998). The difference between mean settlement saturation for 2005 ( $11.382 \pm 0.369$  cyprids.tile pit wall<sup>-1</sup>.day<sup>-1</sup>)<sup>1/3</sup>) and mean settlement saturation for 2004 ( $9.336 \pm 0.476$  cyprids.tile pit wall<sup>-1</sup>.day<sup>-1</sup>)<sup>1/3</sup>) was highly significantly different ( $z = 3.399, p > 0.01$ ). The composite models are plotted in *Figure 5.5*.



*Figure 5.5* Line plot of the asymptotic MM models. The thin lines represent the models of each block. The shaded areas represent the range between those models for each year class. The thick lines are the estimated composite models for each combined year class of shores. No model is plotted beyond its maximum observed value of mean daily supply.

Parameter *B* gives the rate of settlement at a site. Although the rate of settlement may vary between shores and years, the value of this parameter is dependent on the saturation value, because it represents the larval supply when settlement is half that of the saturation point ( $1/2A$ ). Subsequently, analysis of the rate of settlement is presented here for blocks only, because no significant variation in saturation was observed between blocks.

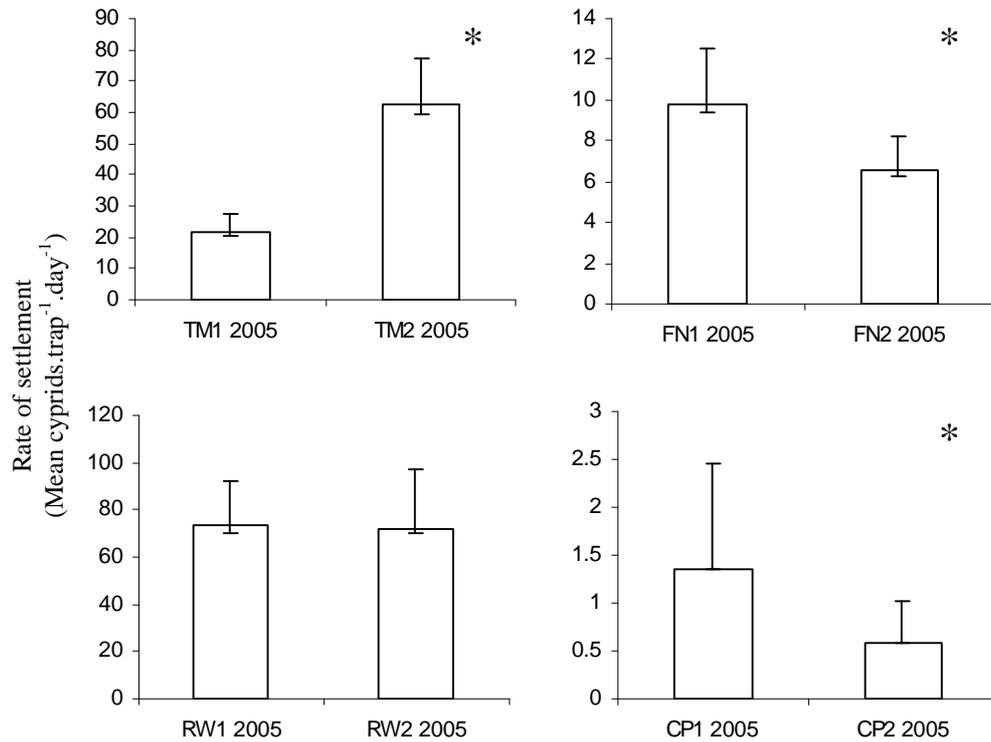


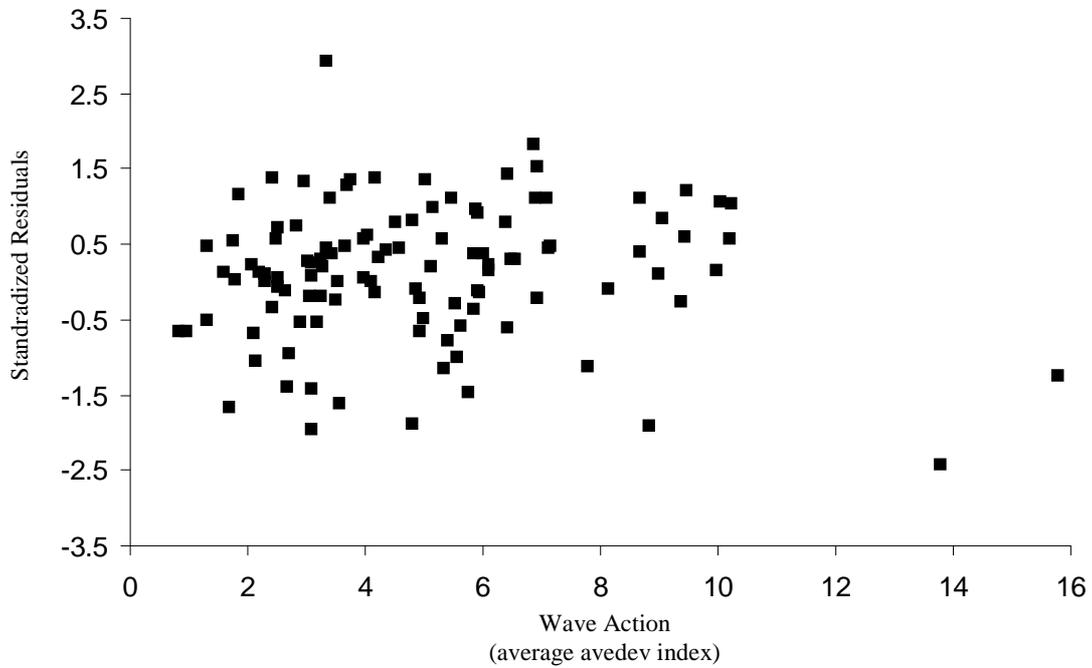
Figure 5.6 The rate of settlement predicted by the MM model (parameter  $B$ ). This value is the mean daily larval supply.trap<sup>-1</sup> at which settlement is ½ the maximum settlement predicted by the model, and is equivalent to the rate of settlement prior to saturation. Error bars are ±1 s.e. of the estimate. Starred plots (\*) demonstrate significantly different values for  $B$  between blocks within the shore.

Significant differences in the rate of settlement prior to achieving saturation were observed in three of the four sites where duplicate block arrays were deployed. At Reres Wood there was no significant difference in the rate of settlement (Figure 5.6).

### 5.3.4 Wave Action.

Standardised residuals for the MM supply-settlement relationship at five sites were plotted against the mean AveDev wave crash index (Chapter 2), corresponding to that deployment period (Figure 5.7). Regression of the standardised residuals against wave action showed no significant effect of wave action on the standardised residuals ( $F_{(1,109)} = 0.002$ ,  $p > 0.05$ ,  $R^2 = 0.00$ ). Neither were there site-specific responses (APPENDIX 1.5 – Standardised Residuals against Wave Action (AveDev))

per Block). The residual scatter was randomly distributed for AveDev values < 11. Above this value only two days were recorded. Both these days demonstrate negative residuals. These are 30<sup>th</sup> April and 1<sup>st</sup> May at Fife Ness in 2004.



*Figure 5.7* Scatter plot of standardised residuals of the MM models for the supply-settlement relationship of five blocks against wave action measured on the block. The blocks were CP1 2005, RW1 2005, CF 2004, FN 2004 and FN1 2005. Wave action is measured as the average AveDev index for 2-minute samples taken every 15 minutes throughout the corresponding tidal immersion periods at the block each day.

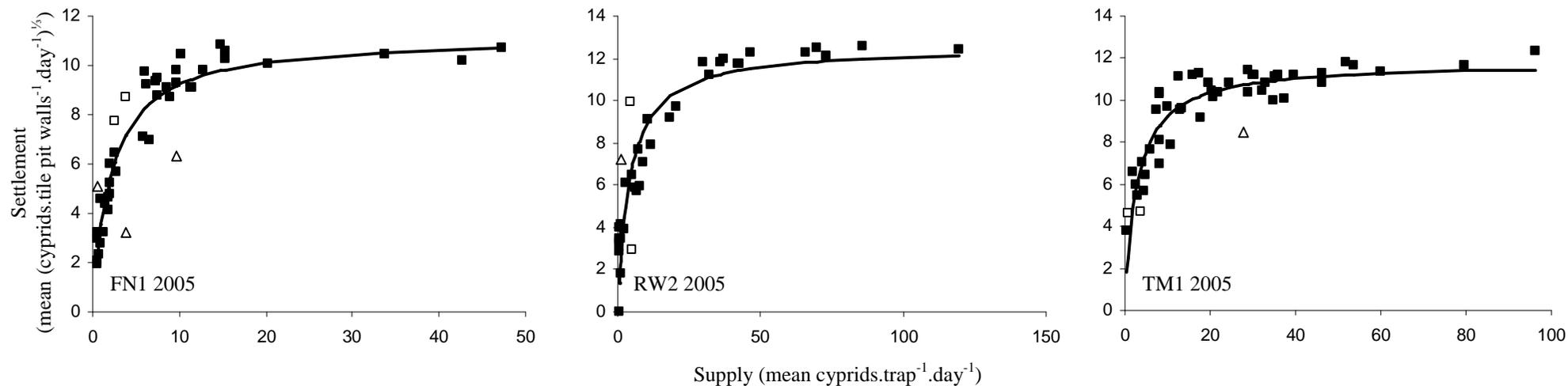


Figure 5.8 Plots of Michaelis Menten models overlaid onto measures of mean daily supply per trap and settlement per tile pit walls (cube root transformed) for three blocks. Empty squares are outlier days expected as a proportion of the total residuals. Empty triangles are outlier days outside the 5% probability of an outlier occurring.

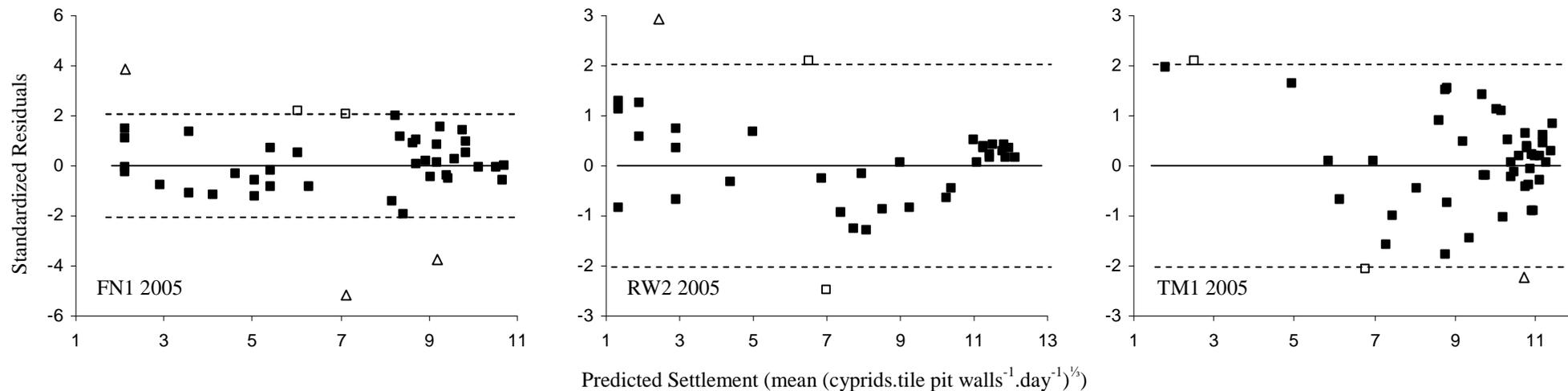


Figure 5.9 Plots of the standardized residuals for the Michaelis Menten models for three blocks. Dotted lines show the 95% confidence intervals for the residuals. Empty squares are the 5% of the standardized residuals expected as outliers. Empty triangles are the standardized residuals outside the 5% probability of an outlier from the model occurring.

## 5.4 DISCUSSION

### 5.4.1 Models

Gaines and Bertness (1993) examined the relative importance of variation in larval abundance and supply to settlement of larval cyprids. Instantaneous plankton samples from pumps and nets alone explained only a small proportion of the variation in larval settlement. The correlation was improved when larval abundance was combined with a measure of flow, obtained by dissolution of dental chalk. Passive larval traps deployed for two-week intervals explained up to 98% of the variation in larval settlement. The fortnightly trap deployment meant that daily variation would have been lost in that study. A large amount of the variation in settlement was explained by variation in supply; however the numbers of larvae trapped were very low, at  $\sim 9$  cyprids.trap<sup>-1</sup>.day. In addition their traps were deployed tens of metres distant and in the sublittoral, demanding the assumption that larvae were uniformly distributed vertically and horizontally in the water column. This is rarely the case in planktonic distributions. Miron (1995) demonstrated that variation in the vertical distribution of *Semibalanus balanoides* cyprids occurred in the plankton and this determined the distribution of settlers vertically on a pier. Hence the deployment of the traps by Gaines and Bertness (1993) up to 40 m away from the settlement sites would have also increased the error variance in that study.

The larval traps deployed in the present study were positioned in very close proximity to the natural and artificial settlement substrate and accessed daily. Even when measured at this small spatial and temporal scale, before the substrate becomes saturated, the relationship between supply and settlement was closely correlated. Once saturation occurs, however, there is little or no correlation between the two processes and variation in supply cannot be used to explain variation in settlement.

Barnacle larvae have been observed settling in high densities over a very short period of time. This high settlement rate sometimes leads subsequently to the phenomenon of hummocking of juvenile barnacles. Hummocking barnacles were described in detail by Barnes and Powell (1955). In the spring of 1949 in the Firth of Clyde barnacle adults grew in dense coralline hummocks consisting of a cluster of elongated individuals. The elongation was particularly pronounced towards the centre of the hummock. The calcareous parts of the barnacles were described as 'fragile' and easily detachable. Hummocks that developed on the rocky shore began to break off in large patches in July through to the start of winter. Clearly there was a significant cost to the individuals that settled at these high densities as the year progressed. This would suggest a mechanism that would select for an avoidance response to dense aggregations of settlers.

Crisp (1961) studied patterns and behaviour of settlement in the larvae of *Semibalanus balanoides* and observed that on a uniform surface or groove at high settlement densities the pattern of settled larvae is non-random. Late larvae were observed spacing themselves from earlier arrivals. Crisp described this response as analogous to territorial behaviour in mobile organisms. It allows recently metamorphosed individuals to grow and establish themselves before contacting other individuals and directly competing for space. He also observed that as the population density rose, the spatial separation between settlers decreased, presumably in response to the limitations of suitable space.

The MM model indicates a gradual decrease in settlement response. If the point of saturation was reached when space was limited by the average size of a cyprid, or the size of a fixed cyprid territory, then rapid change in the rate of settlement of larvae indicative of the logistic model would have been observed (see

*Figure 5.3*). A logistic model that allows for a sharp negative inflection point at saturation would better describe such a pattern of supply and settlement but this was not supported by the data.

#### **5.4.2 Saturation and settlement rate**

An asymptotic relationship between supply and settlement was observed with maximum settlement independent of daily larval supply. However, the point at which saturation occurred varied between shores and years (*Figures 5.4; 5.5*). It appears that larval settlement densities were not limited by space alone and therefore rejection of available substrate by larvae must have occurred at high larval densities (Berntsson *et al.* 2004). Based on comparisons of pairs of blocks within shores, variation in significant levels of saturation within shores is not significantly different (*Figure 5.4*).

In 2005 at Tentsmuir, Fife Ness and Caiplie the duplicate blocks within shores showed different rates of settlement prior to saturation (*Figure 5.6*) suggesting that variation in the rate of settlement between blocks may occur at low levels of supply. Saturation levels were potentially independent of rates of settlement. Despite saturating at similar levels at Fife Ness and Tentsmuir the rate of settlement prior to saturation at Fife Ness was significantly less than at Tentsmuir.

It appears that within shores (between blocks) there may be significant variation in the rate of settlement between blocks, despite similar levels of saturation. This may be due to variation in settlement preference of different blocks. Because repeatable settlement substrata were used at all the blocks and replaced daily this eliminated the possibility that variation could be due to responses to biofilm or other substratum cues. The variation in rates of settlement must have been due to variation in the hydrodynamics experienced between the blocks. Alternatively, saturation may

not be determined by small-scale settlement cues distinguished between blocks but by factors occurring across larger scales between shores such as the “desperation” of individuals making up the larval population dispersed to that shore that day.

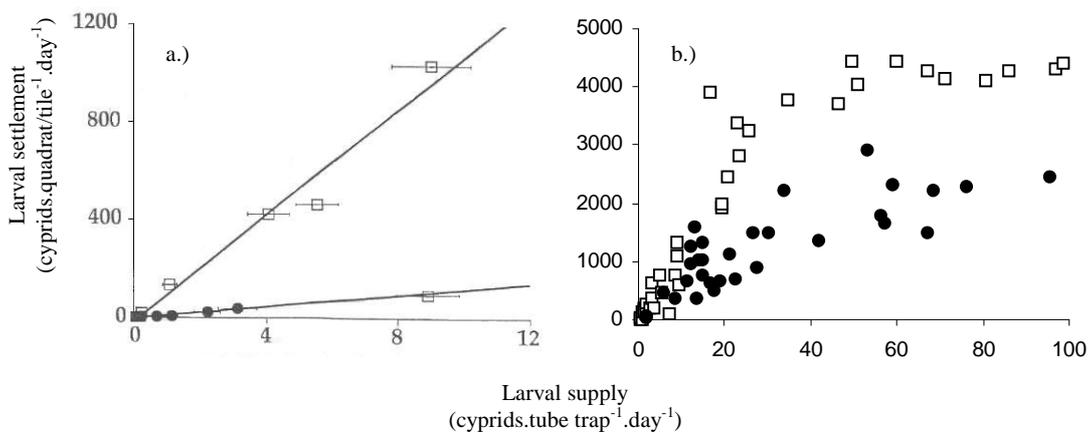
Differences in the rate of settlement over time have been demonstrated in previous studies. Connell (1985) described increased settlement early in the season on uncleared quadrats. Later in the season there was a shift in preference to cleared quadrats due to high densities of individuals on the uncleared quadrats. Hunt and Scheibling (1996) observed enhanced settlement of mussels in protected tide pools at sites where there were ice-scoured rocks and explained this as intensification of settlement on suitable substrate due to the proximity of unsuitable ice scoured-rock. Variation in availability of competent larvae was not a factor that explained the differential rates of settlement. The larvae rejected ice-scoured rock and settled in far higher numbers in tide pools at the same site. That study highlighted important considerations required when using artificial settlement substrate as a measure of larval supply.

Denny and Shibata (1989) modelled the rate of contact of the substrate for larvae in a turbulent environment. Under wave/turbulence conditions typical of rocky shores their model predicted that larvae would contact the substratum frequently on scales of tens of seconds to minutes. Settling larvae may, therefore, have considerable opportunities to sample and select between settlement substrates. Settlement density can also vary according to the availability of suitable settlement substrate (Pineda and Caswell 1997). This may be attributable to an increase in available larvae due to pre-emption elsewhere on the shore and in increased per capita rates of settlement (Knight-Jones 1951; Wilson 1953; Pechenik 1990).

In the present study, at Reres Wood and Tentsmuir, supply and settlement experimental arrays were deployed on blocks surrounded by expansive tracts of sandy shore. Larvae arriving at the sites would have passed over very little suitable settlement substrate as a result of the soft sediment environment extending for kilometres. High and consistent larval supply was observed at both these sites, presumably due to an intervening lack of available substrate (Pineda 1994). High rates of settlement per unit of supply were observed at these sites. This increased settlement response may be due to larval desperation following extensive dispersal within an environment offering no hard substrate. In addition there was considerable supply of larvae to Reres Wood and Tentsmuir as stage VI nauplius larvae will moult on to the cyprid stage and increase the number of already delaying cyprids in the plankton. This results in an increased concentration of larvae and an increased proportion of delaying cyprids.

Bertness *et al.* (1992) also deployed passive larval traps *in situ* and measured larval supply. They examined two sites: Mt Hope Bay's rocky intertidal shore faced seaward and demonstrated consistently high larval supply and settlement densities. Little Compton was a leeward facing rocky shore and consistently demonstrated low larval supply and settlement densities. They demonstrated that, despite a four fold increase in supply of larvae to Mt Hope Bay relative to Little Compton, there was an order of magnitude increase in settlement. This resulted in a far greater rate of settlement at Mt Hope Bay relative to Little Compton. Despite this Bertness and Gaines *et al.* (1992) were unable to sample at the fine spatial and temporal scales achieved in the present study due to the logistical constraints of their trap design and deployment. Their traps were deployed in the sublittoral for a period of up to 14 days before retrieval yet they observed settlement daily. It is likely that saturation of

natural substrates was not observed relative to a measure of supply because the data were integrated over a fortnight to produce mean supply and settlement per day. Temporal variation in supply and settlement can be very large. The daily settlement of larvae is likely to be skewed for daily temporal scales and the daily supply and settlement averaged over a fortnight will provide insufficient information about this variation (Caffey 1985; Todd 2003; Todd *et al.* 2006). In addition to a lack of temporal variation it is also likely that some of the spatial variation in supply of larvae was missed, because the traps are deployed no closer than 15 m and in depths far greater than the settlement substrate. This assumes that larvae are evenly dispersed throughout the plankton, despite previous reports from pump sampling suggesting this may not be the case (Miron *et al.* 1995; Gude *unpubl.*). *Figure 5.10* compares the empirical plots of the supply-settlement relationship obtained in that study with two sites obtained from the present study utilising daily collection of larval supply and settlement obtained within very close proximities. Clearly the number of larvae captured by the passive larval traps used in the present study catch a far higher number of larvae per unit area settlement despite the quadrats used by Bertness *et al.* (1992) being half the size of the ceramic tiles used in the present study.



*Figure 5.10* Comparison of the supply-settlement relationship for *S. balanoides* cyprids observed empirically by a.) Bertness *et al.* (1992) at Mt Hope Bay (open squares) and Little Compton (closed circles) and b.) the present study at Reres Wood 2005 block 2 (open squares) and Tentsmuir 2004 (closed circles). Differences in the rate of settlement were observed in both studies however saturation was only observed in the present study.

Despite the decreased rates of settlement at Fife Ness, settlement density at this shore approached high levels of saturation, equivalent to the sand shores. Fife Ness is a relatively exposed shore and many of the barnacle adults are large and well established (*pers. obs.*) suggesting a high post-recruitment survivorship at this site. Larvae use a suite of biotic and abiotic settlement cues to identify settlement sites within and between shores and it may be that they are more inclined to settle in high numbers at Fife Ness as a result of detecting settlement cues specific to this shore, such as microbial biofilms (Todd and Keough 1994; Keough and Raimondi 1995).

#### **5.4.3 Outliers**

Larval settlement failure and over settlement were observed on a small number of days at a number of sites (*Table 5.1; Figures 5.8 and 5.9*). These may have occurred due to the effects of larval delay. The extent to which larvae are capable of delaying metamorphosis in the field is largely unknown (Pechenik 1990). This is as a result of the difficulties associated with (1.) identifying the onset of competency and (2.) identifying the final point at which delay of metamorphosis occurs before competency is lost. Competency may be lost through death or reversal of the competent capacity of the larvae as a result of stress such as starvation. When observing larval supply in studies of species with varying competency to settle, a measure of competency may often be important (Licius *et al.* 1990).

Measuring passive supply using traps containing a killing solution such as formalin (Yund *et al.* 1991; Metaxas 2004) or dense urea (Todd 2003; Todd *et al.* 2006) may suffer from problems related to the competency of larvae trapped. A difficulty with using passive settlement traps that retain larvae by use of a killing solution is that there is no way of directly testing competency in captured larvae.

*Semibalanus balanoides* larvae are usually competent to settle shortly after metamorphosis from the final nauplius VI stage into the cyprid stage (Stubbings 1975). However for other larval species including coral planulae (Vermeij *et al.* 2006), pre-settlement history can be important in determining settlement response for example.

Moksnes *et al.* (2001) were able to test competency of trapped larvae to settle because their net trap design did not require a killing solution. However an individual net design requires continuous unidirectional larval flux to capture and retain larvae. This suited the organism and environment they studied because larval flux was due to migration in and out of a bay area. Relevant flux of the larvae was in two directions so this required only a pair of net traps facing the entry and exit to the recruitment site. For capture of sessile intertidal larvae such as barnacles on a wave-swept intertidal shore, this design would be difficult to deploy. The turbulent nature of the environment requires a record of multidirectional larval flux. Other trap designs have utilised a flow-through design, but they required a stopper system to limit larval washout due to back-flow. This may either restrict capture of larvae below certain flows or have limited retention time of larvae due to their behaviour (Yan *et al.* 2004).

Some studies have demonstrated that larval size is a good indicator of competency (Marshall and Keough 2003) and it may be possible to measure the size or lipid content of larvae captured during settlement failure events such as that noted on 11<sup>th</sup> April at Fife Ness block 1 in 2005 (*Table 5.1; Figures 5.8 and 5.9*). Settlement failure events may also be caused by abiotic factors such as increased flow (Crimaldi *et al.* 2002). These are discussed in section 5.4.4.

Departures from the model that are not due to lack of competency pose new and important ecological questions. These may constitute important events that can

drive over-settlement or settlement failure of competent larvae and have major implications to recruitment of populations that show high variation in larval supply. How important settlement failure events are in light of the highly variable temporal larval supply is largely unknown (Underwood and Keough 2001).

#### **5.4.4 Wave action**

According to *Figure 5.7* rates of larval settlement were not affected by wave action. When a larva is competent to settle it must cross the boundary layer in order to reach the substrate. This near-bottom layer is a region of great importance to the general ecology of benthic organisms because it can determine nutritional levels, suspension of propagules and delivery rates of available larvae (Butman 1987; Mullineaux and Butman 1991; Butman and Grassle 1992).

Within the lower section of the boundary layer the velocity gradient is logarithmic so the larvae are likely to experience limited effects from the turbulence once the boundary layer is penetrated (Denny 1988). This may explain why there is no settlement response to wave action within blocks.

Crimaldi et al. (2002) demonstrated that the ability to anchor quickly in highly turbulent environments was crucial for *Potamocorbula amurensis* (Asian clam). Instantaneous turbulent events were correlated with resuspension of larvae rather than mean turbulence over a given period in flume studies. At larger temporal scales increased turbulence due to wave action on the rocky shore may lead to settlement failure as was observed on April 30 and May 1 at Fife Ness in 2004 (*Figure 5.7*).

It should be noted that although no variation within blocks for per capita settlement was explained by wave action, different shores demonstrated different wave regimes and significant differences in rates of settlement and levels of substrate

saturation. Exposure-related cues such as biofilm communities indicative of the seasonal wave regime at a shore might offer an explanation for between site variation in the supply settlement relationship.

Presented here is a tool to reliably quantify larval supply to a wave-swept intertidal shore. This was combined with an easily replicated and favourable artificial settlement substrate for the settlement of barnacle cyprids. The pitted surface provided multiple environments within which a simple model of the cascade of settlement preferences exhibited by *Semibalanus balanoides* cyprids could be demonstrated. As a result of the high rates of larval supply and settlement attained in the present study, the relationship between daily supply and settlement of larvae has been characterised and an analytical model (the Michaelis Menten model) developed for the analysis of the supply-settlement relationship. The resulting analysis demonstrates the following factors concerning the nature of the supply-settlement relationship:

1. Rates of larval settlement can vary between sites within and between shores, independent of substrate cues; larvae can actively reject available primary substratum.
2. Rates of larval supply can be sufficient to saturate available primary substratum within a single day.
3. Saturation is attained gradually, suggesting the rate of rejection increases with settlement density.
4. Saturation levels can vary between years and between shores within years but do not appear to vary within shores within years, suggesting saturation levels are not determined by the same processes as rates of settlement.

5. Settlement failure and over-settlement may occur. There is no conclusive explanation from the current study, however wave action may determine the upper limits of settlement rates, as in other laboratory-based studies (e.g. Crimaldi *et al.* 2002).

## Chapter 6 - Discussion

### 6.1 Summation

#### 6.1.1 Wave sensor

A major source of physiological stress in the intertidal environment is the potential periodic exposure of organisms to air that occurs diurnally. This causes desiccation and heat stress and has been demonstrated as a major structuring influence in many intertidal environments (Lewis 1964; Bertness *et al.* 2006). Typically, exposure to desiccation and heat is estimated from tidal charts, providing a measure of expected immersion/emersion for a known tidal height across a region. However, observations of tides may differ significantly from those predicted from tidal charts. This may be due to variation in topography, changes in barometric pressure and wind or wave conditions (Venegas *et al.* 2002). In addition, large boulders and gullies can alter the onset of immersion/emersion within a shore by retaining water during the ebb tide or restricting flow during the flood tide. Therefore measuring the timing of immersion/emersion accurately at a specific location on the shore is necessary when empirically testing models of desiccation and heat stress on the rocky intertidal shore.

The effects of wave action on the structuring of intertidal communities is well recognised (Lewis 1964; Menge 1976). Wave action can vary horizontally and vertically within a shore as a result of topography (Denny *et al.* 2004). Categorical definitions of wave exposure are commonly used in studies of wave exposure (Hunt and Scheibling 1996; Pannacciulli and Relini 2000; Jenkins and Hawkins 2003) and this limits generalisations on the effects of wave action to community dynamics (Lindegarh and Gamfeldt 2005).

The wave sensors developed in the present study provide a means of measuring (1) immersion time, (2) depth of immersion and (3) wave action at scales

that enable quantitative comparison with independently measured ecological factors (e.g. larval supply, settlement) directly in the field. A number of devices can record these properties at local scales, similar to the wave sensors (e.g. turbulent mixing - Muus 1968; instantaneous maximum wave force - Bell and Denny 1994; immersion time - Venegas *et al.* 2002) but are unable to distinguish all three processes simultaneously. Demonstrated here is a simple, versatile and accurate device for the accurate characterisation of “wave exposure” within and between shores.

### **6.1.2 Experimental supply-settlement array**

Previous studies demonstrated the effectiveness of passive larval traps for quantifying the supply of larvae to the benthos (Hannan 1984; Butman 1987; Yund *et al.* 1991; Bertness *et al.* 1992; Metaxas 2004), however these studies required the deployment of large passive larval traps some distance (3 - 40 m) from the adult benthic population or community. Patterns of larval supply can vary consistently over time (days - years) and across spatial scales (cm - km). Therefore, in order to accurately sample rates of larval supply in relation to larval settlement, a passive larval trap that can be both replicated and deployed within close proximity to the substrate at multiple sites and serviced daily is necessary. A scaled down trap design based on the passive larval traps in Yund *et al.* (1991) which maintained a high aspect ratio (inlet diameter:trap depth ratio) and included chambers and spiralled baffles allowed for the successful deployment of the trap across a range of wave action typical to the Scottish east coast (Todd 2003; Todd *et al.* 2006).

The present study involved the assessment of the addition to the original trap design (Todd 2003) of a conical inlet with the explicit aim of increasing the reliability of the trap to sample larval supply in a wave-swept environment. The addition of an

increasingly smaller conical inlet combined with the spiralled internal quarter baffles resulted in a decrease in the absolute trap capture of cyprids, but an increase in the per unit inlet area capture of cyprids. It was concluded that the decreased inlet area relative to the internal trap diameter increased larval retention as a result of decelerating flow inside the cylinder, whilst maintaining sufficient particle and fluid exchange. The 1 cm<sup>2</sup> conical inlets were selected as they combined a high retention of larvae across a range of wave action with a sufficient absolute capture rate at low planktonic larval flux (Todd *et al.* 2006).

The effects of natural topographic variation on larval choice of settlement substrate were demonstrated with casts of natural rock replicated within a shore. Significant differences in settlement due to topographic variation in natural substratum will obscure the supply settlement relationship if settlement on natural rock is used. The trap design, therefore, was combined with a standardised artificial settlement substrate that provided three distinct small-scale environments within which a cascade in settlement preference was observed, potentially within a two-tide period. This allowed for the characterisation of the daily supply-settlement relationship.

### **6.1.3 Analytical tools**

The ability to replicate measures of supply and settlement across multiple spatial scales (within and between shores) and integrate over time, whilst sampling at a high frequency (daily) throughout the settlement season allowed for the accurate description of the supply-settlement relationship. Least squares regression was applied to the operationalised data and models of the supply-settlement relationship were fitted so that generalisations could be made across spatio-temporal scales.

The Michaelis Menten (MM) model best described the supply-settlement relationship, demonstrating a gradually decreasing rate of settlement per unit supply for *Semibalanus balanoides* cyprids. Settlement densities approached saturation within a single day (two tides); however the point at which potential daily saturation of the tiles occurred varied significantly between shores and between years but not within shores. The rate of larval settlement to the tiles (the supply at which settlement was half the estimated saturation value) varied within shores.

In addition to variation in the supply-settlement relationship across temporal and spatial scales, the effect of wave action on the supply-settlement relationship was assessed. An index of wave action integrated for the complete immersion period was used to assess daily wave action corresponding to supply and settlement. The wave sensors were deployed within close proximity to the experimental array so as to ameliorate any horizontal variation in hydrodynamics as a result of topographic variation (Denny *et al.* 2004). No significant variation in the supply-settlement relationship was observed with variation in wave action however there was some evidence of settlement failure at high levels of wave action.

## **6.2 Implications and future direction**

### **6.2.1 Supply-settlement relationship**

Following arrival, larvae may accept or reject substratum based on a number of cascading responses. The rate of settlement of larvae in the field may vary as a result of either larval supply to the substratum or settlement behaviour. Therefore in order to determine what drives patterns of settlement, measuring rates of larval supply alongside variation in settlement is paramount.

Hunt and Scheibling (1996) demonstrated that settlement of mussel larvae on artificial settlement substrates was enhanced by active rejection of ice-scoured natural rock relative to the un-scoured substratum. Pineda (1994) also demonstrated increased rates of settlement at sites where there was limited suitable settlement substrate. In neither of these studies was a direct measure of larval supply obtained. Therefore, it was unclear as to whether differences in the rates of settlement were due to increased larval supply (either increased planktonic larval abundance, increased flux of larvae due to hydrodynamics or both) or settlement behaviour.

Several studies have successfully quantified larval supply and compared this to rates of settlement at relevant spatial scales, demonstrating variation in rates of settlement as a result either of rejection of substratum or differential larval supply (Bertness *et al.* 1992; Miron *et al.* 1995; Berntsson *et al.* 2004). The present study provides qualitative empirical evidence that daily rates of larval settlement may vary within a shore. In addition this study has identified that daily variation in the supply of larvae is not always reflected in variation in settlement as space can frequently become saturated. This has important implications for studies that empirically test models of arrival of larvae to the shore from the plankton (Pineda 2000). Larval settlement may not accurately predict variation in supply.

The reproductive success of sedentary and sessile animals depends upon a trade-off between proximity to reproductively viable cohorts and intraspecific competition for resources. As a result territorial responses at the time of settlement are common in a number of phyla. The extent of territorial spacing varies with population density and length of delay period (Crisp 1961; Knight-Jones and Moyses 1961). This territorial response manifests, in the supply-settlement relationship, as a gradual decay in the rate of settlement towards the maximum settlement density achievable on the

substrate (= saturation), as indicated by the fit of the MM in preference to the logistic model in the present study.

Variable rates of settlement and saturation within and between sites was demonstrated but not explained in the present study. Two hypotheses could be tested as possible explanations of the variation in settlement rates and saturation.

(1) Cyprids responded to low availability of suitable settlement substrate in the proximity of the experimental array.

Kent *et al.* (2003) studied settlement of *Semibalanus balanoides* cyprids in relation to adult population densities and demonstrated that gregarious responses were not evident on a high density shore. At that location there was a negative settlement response to increased abundance of adult barnacles. This was identified as cyprids switching behaviour from a gregarious response to searching for available free space. Crucially, the settlement behaviour of cyprids was determined by the detection of densities of adults on adjacent substratum.

This response to the availability of space of the immediate shore environment could be reflected in variation in the supply-settlement relationship between shores. Denny and Shibata (1989) suggest the frequency of contact with the substrate can be sufficiently high in a turbulent environment that a larva can repeatedly sample local substrate. By manipulating the available substrate on concrete blocks deployed in the middle of sandy beaches, and then characterising the rate of settlement on artificial settlement substrate relative to larval supply as described in the present study, an empirical test of this hypothesis could be attained. However the nature of beach environments may, in itself affect the nature of the supply-settlement relationship. Therefore an alternative hypothesis must be tested;

(2) The rate of settlement and saturation varies as a result of specific trait responses.

Larvae have been demonstrated to become increasingly “desperate” with age (Gribben *et al.* 2006). This is largely thought to arise from depletion of energy reserves (Lucas *et al.* 1979) and can be reflected by the size of the individual larva (Marshall and Keough 2003). At sand beach sites there is the potential that high rates of settlement and willingness to saturate at high densities is as a result of the high proportion of “desperate” larvae nearing the end of their facultative delay period. Size and lipid content of trapped larvae could be measured to quantify the proportion of “desperate” larvae being supplied and settling at a site. Larvae could be removed from traps and tiles for analysis of the relative proportions supplied and settled. This would determine if the proportion of extensively delayed settlers, particularly at high levels of saturation, reflected the proportion supplied to the substrate, or if recently metamorphosed larvae rejected dense aggregations of conspecifics on the tiles. Shores could be compared for the proportion of “desperate” larvae caught in the traps. Sand beach shores may demonstrate a high proportion of old larvae. This could have important implications for harbours and ships moored close to expansive tracts of sand such as the Tay estuary.

### **6.2.2 Larval supply and population dynamics**

Understanding the behaviour of larvae when they settle is important in integrating models that describe local larval supply (e.g. Pineda 1991) with models of the abundance of settled organisms; from larvae (e.g. Keough and Downes 1982), through to adults (Jenkins 2005).

Modelling dispersal of larvae requires appropriate physical oceanographic models combined with an understanding of the behaviour of the larvae in the plankton. For example, studies have demonstrated the effects of behaviour of planktonic larvae on retention ability of crustaceans in estuaries (Tankersley and Forward 1994). These models require validation in the field using direct measurements of currents and mechanisms that drive the transport of larvae together with measures of larval abundance in the water column. Patterns of larval supply and settlement have been linked to wind (Hawkins and Hartnoll 1982; Bertness *et al.* 1996; Todd 2003), upwelling and internal wave propagation (Pineda 1991; Pineda 1994), and bay flushing time (Gaines and Bertness 1992). Settlement rate depends, in part, on the rate of larval supply to the substrate and this can be highly variable between days (Caffey 1985; Todd 2003) and years (Barnes and Powell 1955; Hansson *et al.* 2003). The testing of sufficient alternative hypotheses concerning the rates of settlement requires a measure of larval supply. The assumption that variation in settlement is a reflection of larval supply may be inappropriate if saturation of the substrate occurs.

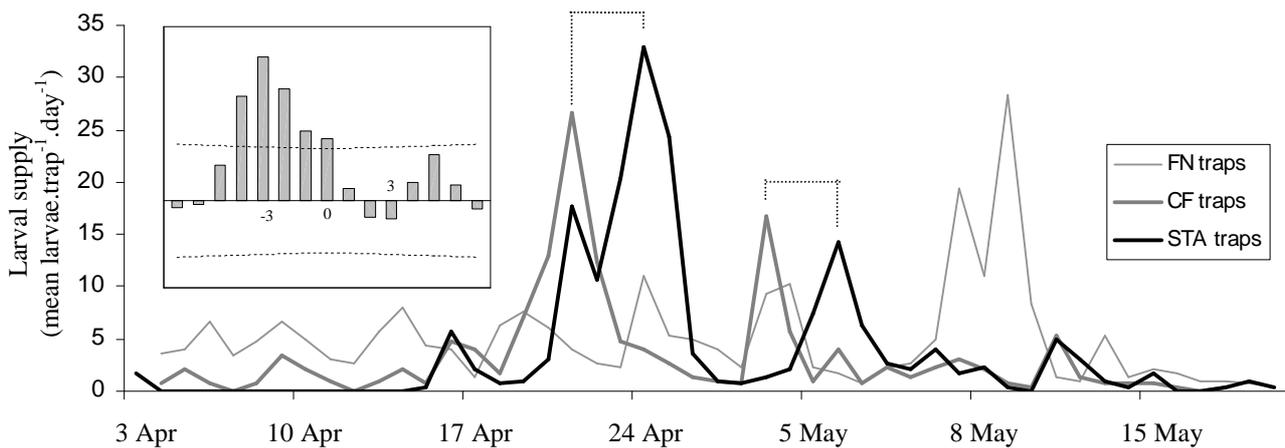
### **6.2.3 Applications**

Morgan (2001) suggests that more thorough studies should be conducted to elucidate the complexity inherent in communities with species that exhibit multiple life history stages such as marine benthic communities in order to better describe stock-recruitment models. Yoshioka (1986) demonstrated that this was possible, at least for organisms with annual recruitment patterns, sessile adult phase and a short dispersal period. He quantified abundance at a series of life stages from adult stock size through larval abundance and recruitment back into the population. By examining

factors that exerted influence on each stage, Yoshioka was able to explain important amounts of the variation in a stock-recruitment model with a single study.

Passive larval traps enable the investigator to gather integrated data with a high degree of resolution at a crucial transitional stage in the life history of benthic marine organisms. Larval supply - defined as the larval abundance combined with rates of delivery to the substrate – could be easily incorporated into studies monitoring recruitment and mortality across broad spatio-temporal scales and in models of population structure such as Yoshioka (1986). This would provide an additional step in highly variable stock-recruitment models of highly dispersive species such as *Semibalanus balanoides*.

Peaks in larval supply were observed at consecutive sites in 2003 with significant lag times (*Figure 6.1*). Larval traps may provide a tool for observing horizontal transport of aggregated planktonic larvae in years where saturation of the substrate occurs and dense planktonic aggregations are not depleted (e.g. Gaines *et al.* 1985).



*Figure 6.1* Time series of larval supply (mean larvae.trap<sup>-1</sup>.day<sup>-1</sup>) for three sites in Fife in 2003 demonstrates that peaks in supply occurred at the three sites examined that year; FN – Fife Ness, CF – Cambo Farm, STA – St Andrews Bay. Cross correlation analysis demonstrate a significant lag of between 2 and 4 days is seen between larval supply at Cambo Farm and St Andrews Bay (inset chart) suggesting northerly progression of aggregations of planktonic larvae along the coastline.

Larval traps are readily deployed in relatively inaccessible environments such as wave swept rocky intertidal shores (Todd 2003; Todd *et al.* 2006) or deep ocean volcanic ridges (Comtet *et al.* 2000; Khripounoff *et al.* 2000; Metaxas 2004) allowing for high resolution temporally integrated data on larval abundance patterns across mesoscales.

Due to considerable spatial and temporal variation in community characteristics and recruitment, describing coastal community dynamics requires studies that cover broad temporal and spatial scales. Studying representative habitats within a region over a long period of time is the only way to obtain an appropriate perspective on factors regulating these communities. Small scale manipulative experiments provide a means of testing specific larval responses outside of the laboratory; however integrating them over considerable spatial and temporal scales is the only way they can be related in importance relative to other interacting mechanisms in the field and this can be extremely labour intensive. This study demonstrates the advantages of an experimental array that requires low levels of maintenance and ease of deployment across large scales on intertidal shores.

The future of work on supply-side ecology will be focused on identifying its relevance to current issues in ecology and environmental research. Extensive changes in the marine communities of Great Britain have been observed in studies of past records of abundance and distributions of organisms. These have been related to large scale processes such as rising sea temperatures for example (Southward *et al.* 1995). Increased global warming can alter wind patterns favourable to coastal upwelling, resulting in decreased larval retention near to the coast and therefore decreased recruitment, diminishing these fish stocks (Bakun 1990).

Many scientists, managers and policy makers consider the establishment of marine reserves or protected areas as a primary means of sustainable management of fisheries and marine environments. Amongst other factors, protected areas must include the larval source for a population as well as corridors through which areas are linked by larval transport (Peterson and Estes 2001). For example Bode *et al.* (2006) demonstrated that larval transport between two regions in the Great Barrier Reef was primarily north to south. Only a few “gateway reefs” were able to transport larvae from a sink subregion (in the south) to a source subregion (in the north) and maintain the connectedness of the metapopulation. When conditions were recruitment-limited the southern region depended on larval supply from the northern region. Protection of larval sources and larval transport corridors between sources and sinks are vital in maintaining and sustaining healthy marine habitats and communities.

Overexploitation of marine species, destruction of coastal habitats, introduction of exotic species, pollution of inshore waters and potential effects of global warming and changing sea level means that ecology is an important facet of modern environmental science. Relating the importance of supply-side processes and incorporating them into models to inform decisions on management of resources, commercial or otherwise, is vital to maintaining the relevance of ecology within modern society. (Khripounoff *et al.* 2000)

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## APPENDIX 1.1

### Unscaled Scatter Plots

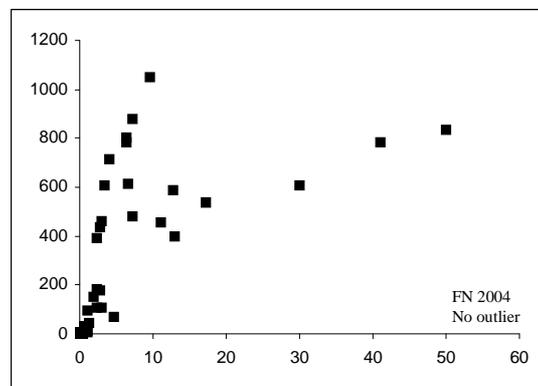
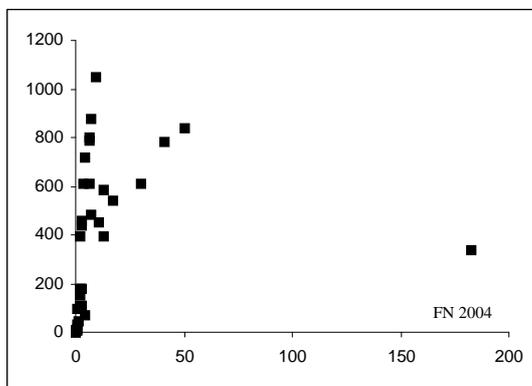
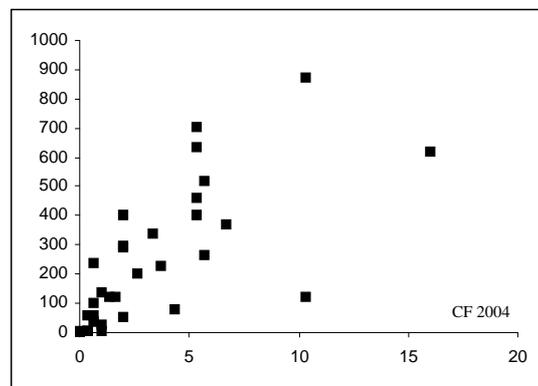
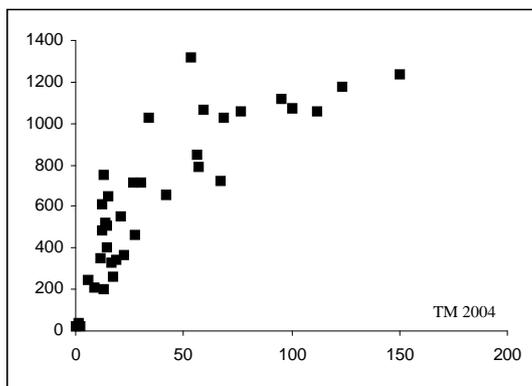
$X = \text{Supply (mean cyprids.trap}^{-1}.\text{day}^{-1})$ .

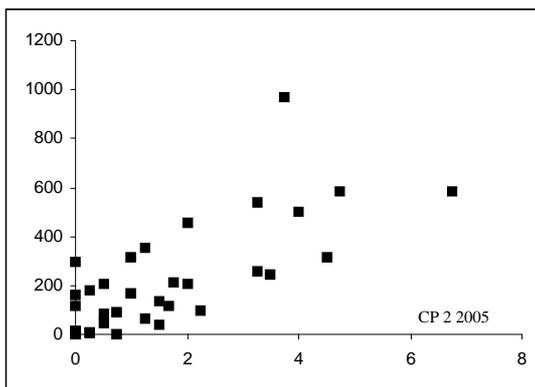
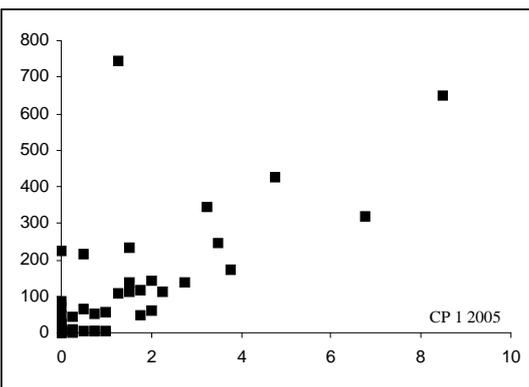
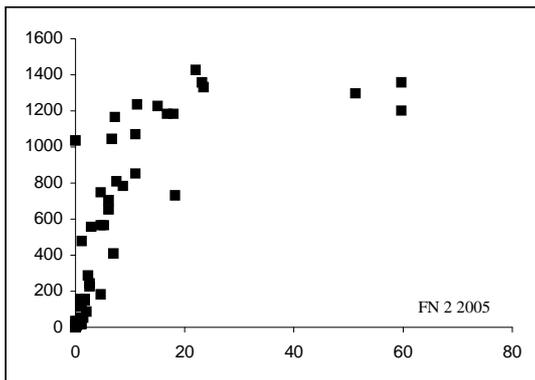
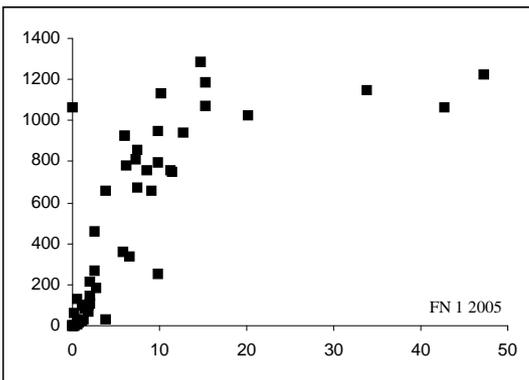
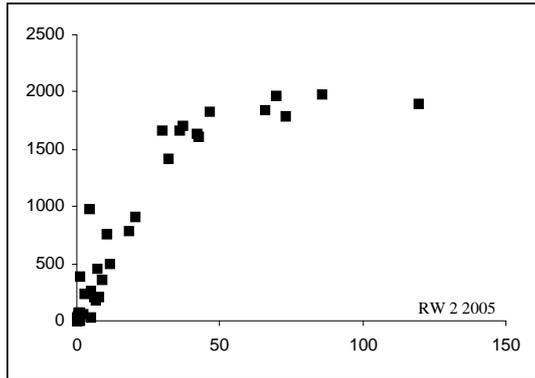
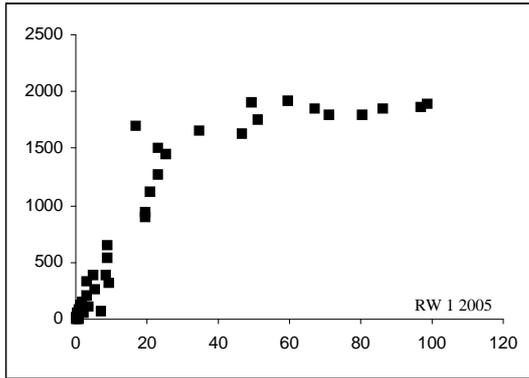
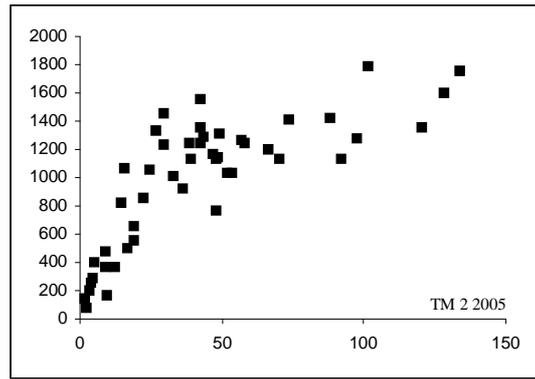
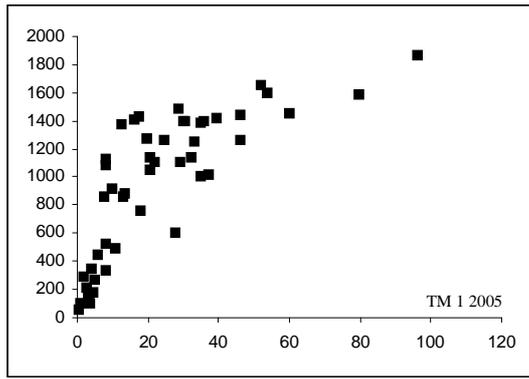
For 2004  $n = 3$ .

For 2005  $n = 4$

$Y = \text{Settlement (mean cyprids.tile pit edge}^{-1}.\text{day}^{-1})$

$n = 3$

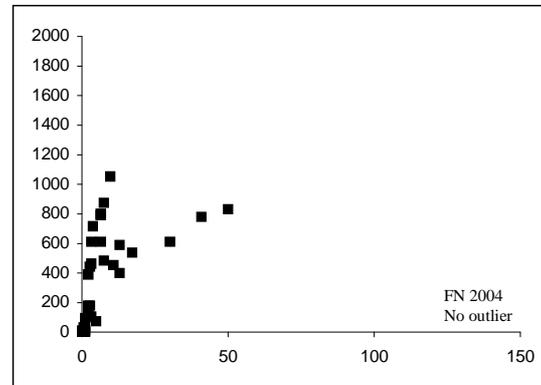
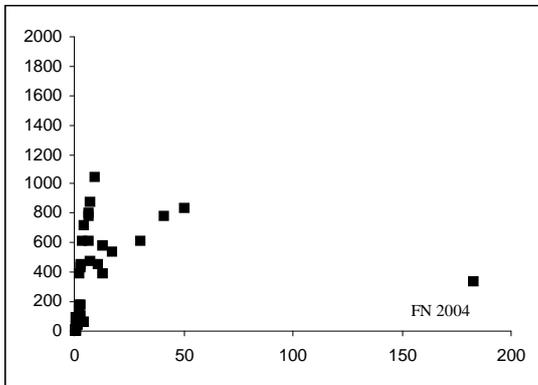
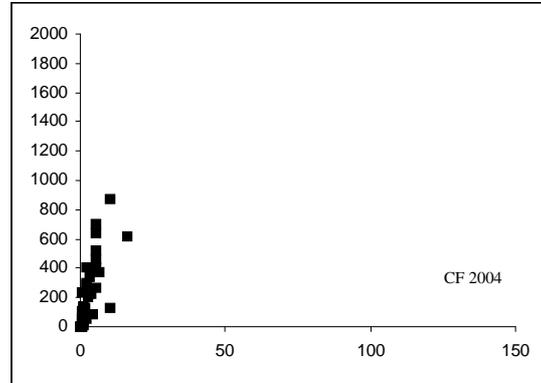
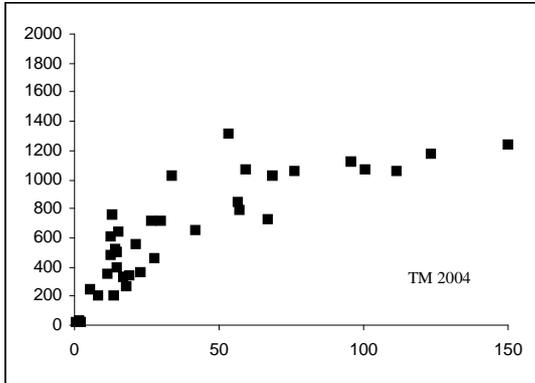


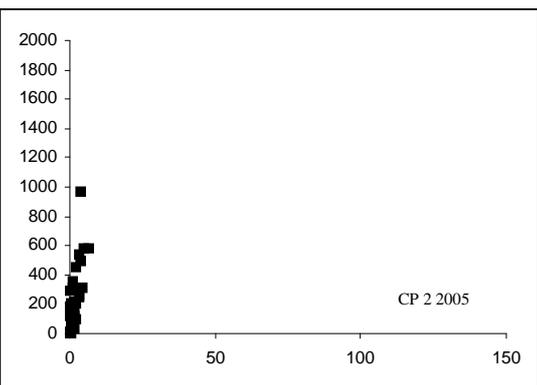
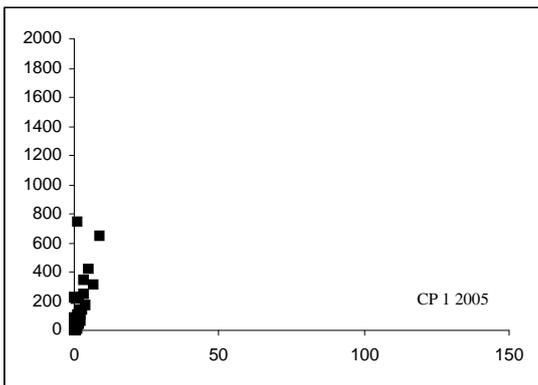
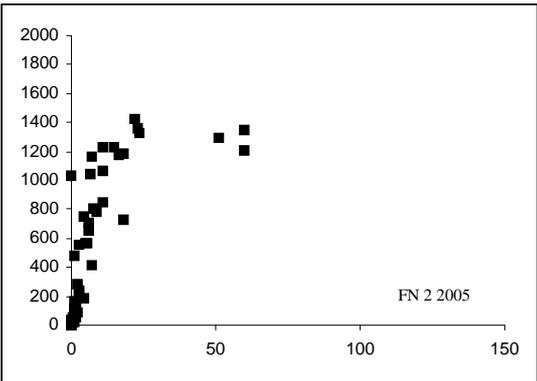
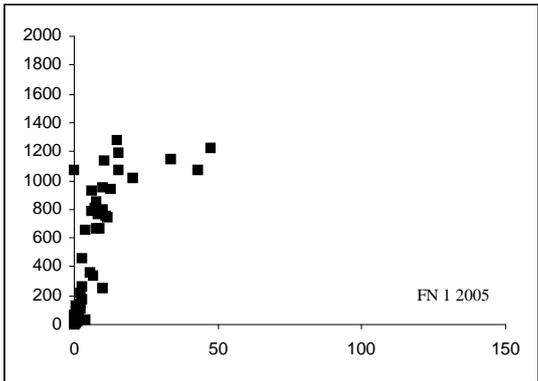
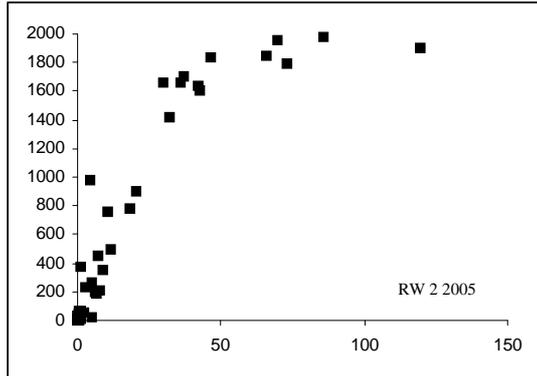
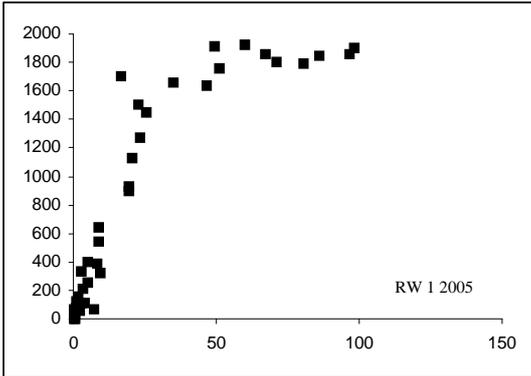
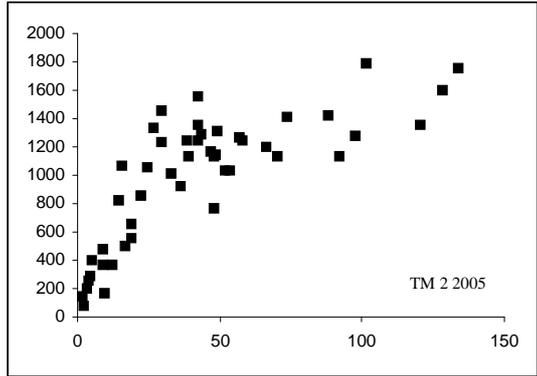
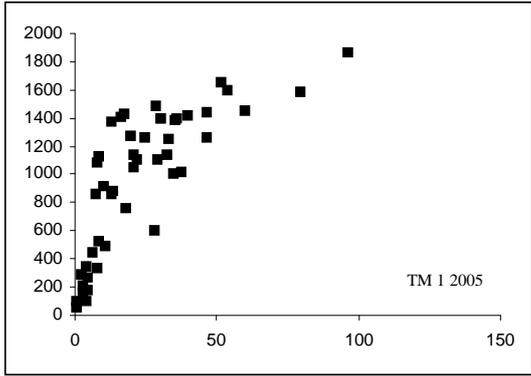


**Scaled Scatter Plots**

**X** = Supply (mean cyprids.trap<sup>-1</sup>.day<sup>-1</sup>)

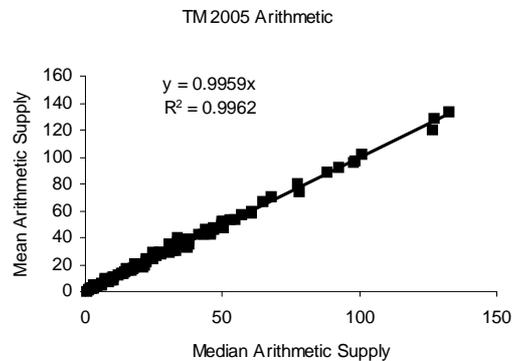
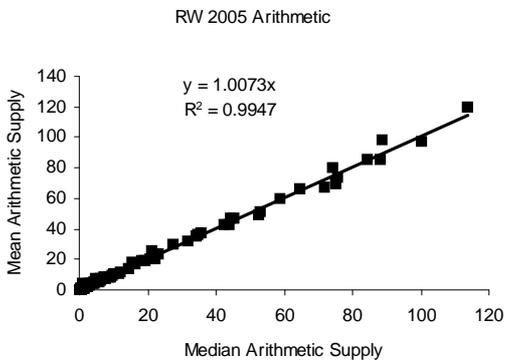
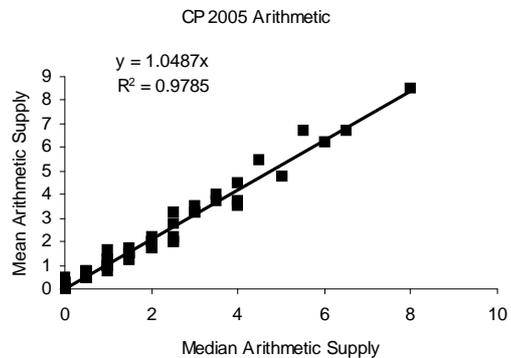
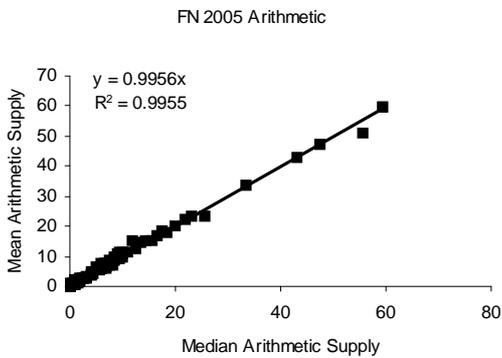
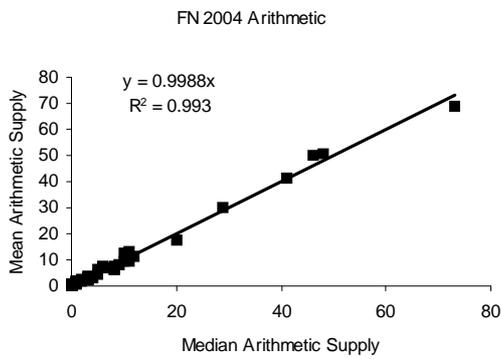
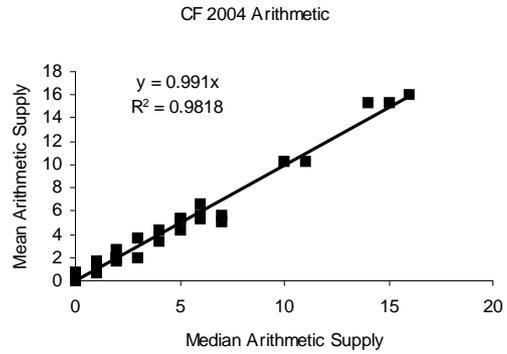
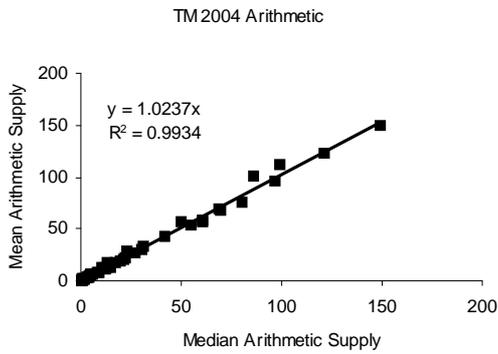
**Y** = Settlement (mean cyprids.tile pit edge<sup>-1</sup>.day<sup>-1</sup>)



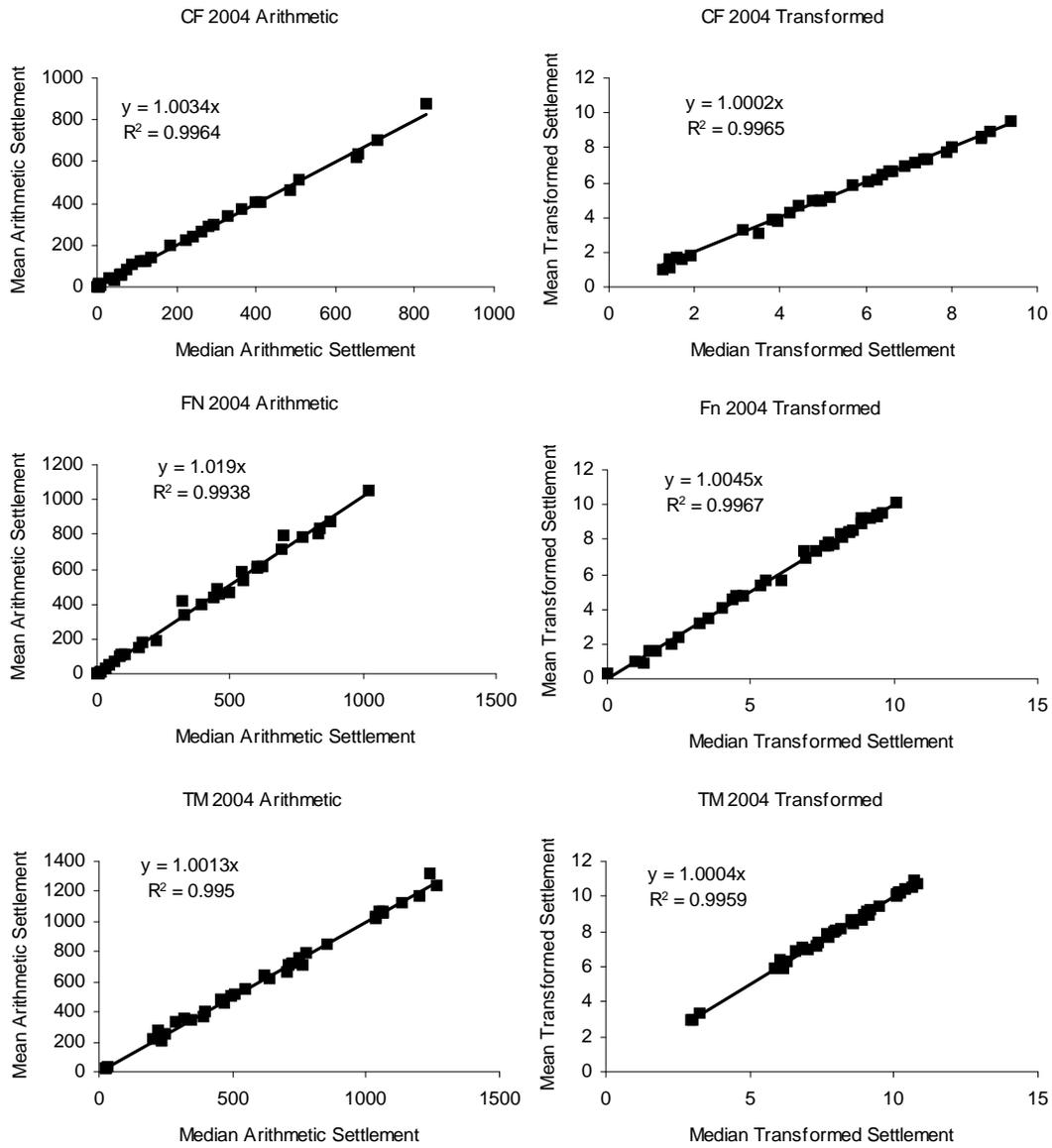


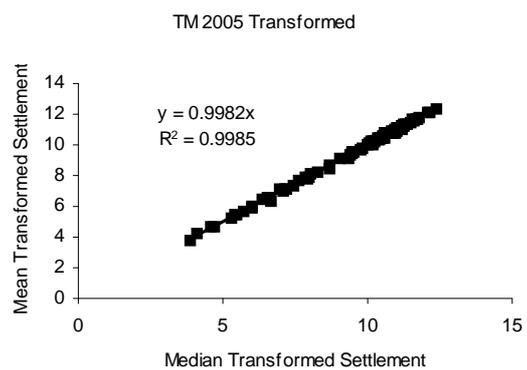
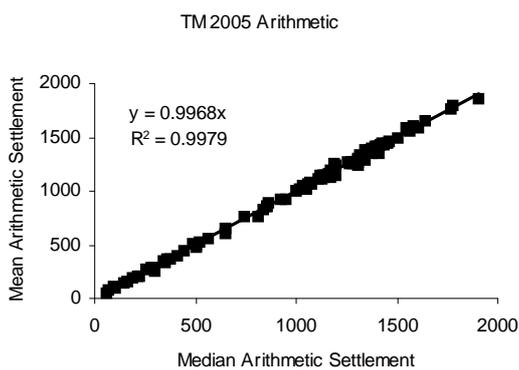
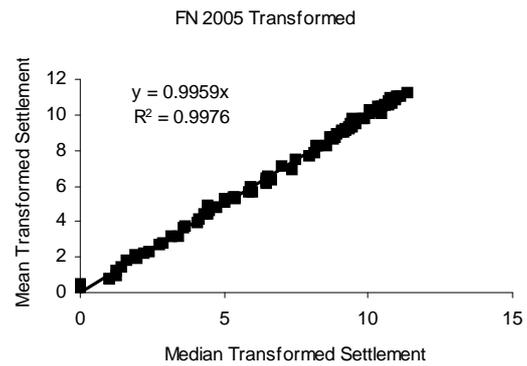
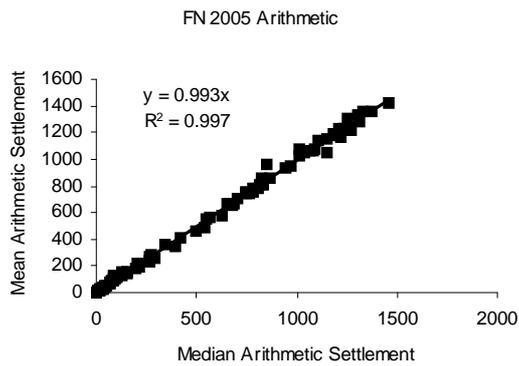
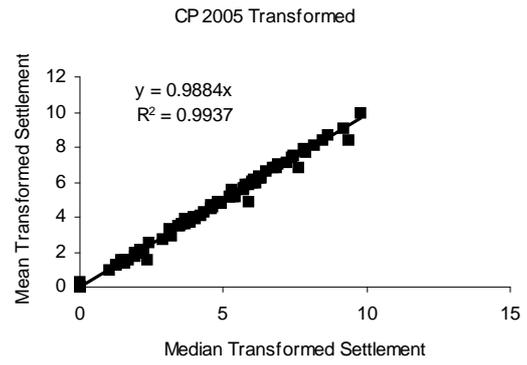
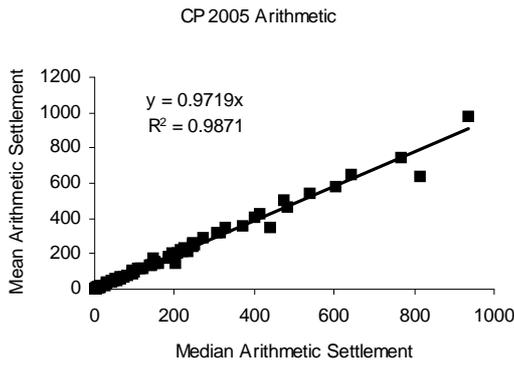
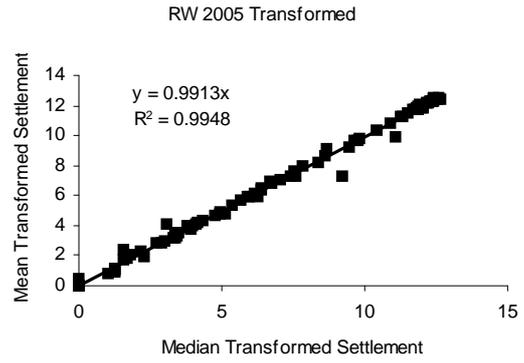
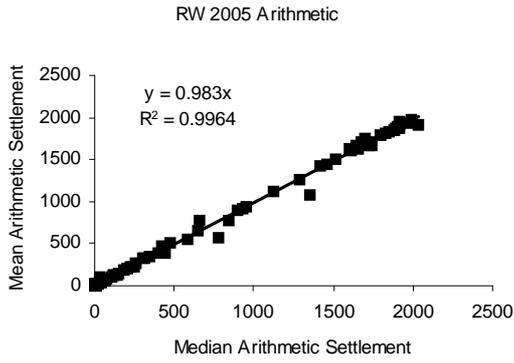
APPENDIX 1.2

**Normality of X – supply**



## Normality of Y – settlement





## APPENDIX 1.3

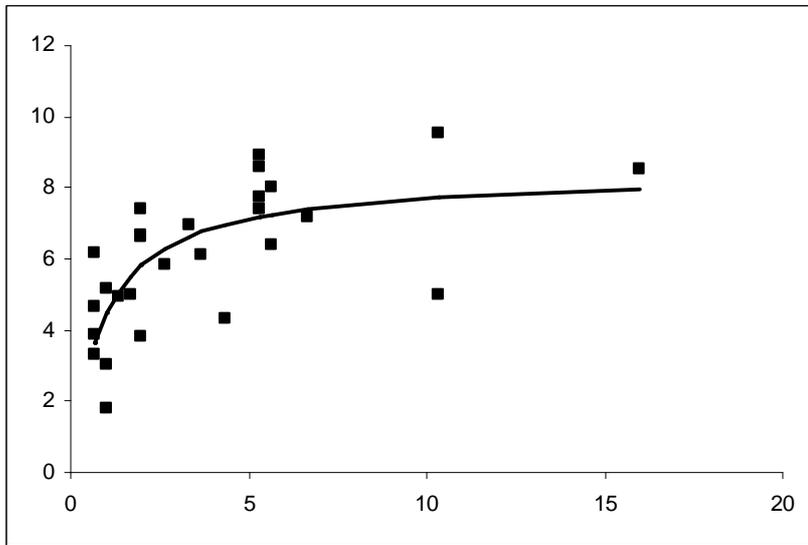
**Model fitting summary table**

Site	Model	AIC	AICc	MSresidual	n	k	b1	b1 lower	b1 upper	b2	b2 lower	b2 upper
CF 2004	Linear	31.66612	31.82612	12.55294	27	1	1.543	1.151	1.936			
CF 2004	MM	11.53485	12.03485	1.90138	27	2	8.369	6.952	9.786	0.873	0.284	1.461
CF 2004	Logistic	13.14932	13.64932	2.18204	27	2	7.19	6.319	8.062	1.354	0.829	1.88
CP1 2005	Linear	23.99599	24.1699	7.582972	25	1	1.543	1.151	1.936			
CP1 2005	MM	13.39097	13.93642	2.37486	25	2	8.385	5.732	11.039	1.104	0.133	2.076
CP1 2005	Logistic	14.67116	15.21661	2.67206	25	2	6.532	5.137	7.927	1.375	0.672	2.078
CP2 2005	Linear	23.05003	23.23185	7.535021	24	1	2.035	1.605	2.464			
CP2 2005	MM	11.46734	12.03877	2.04710	24	2	9.007	6.811	11.203	0.837	0.169	1.505
CP2 2005	Logistic	13.42325	13.99468	2.46965	24	2	7.347	6.161	8.532	1.548	0.86	2.236
FN 2004	MM	10.88937	11.35091	1.72808	29	2	9.828	8.59	11.066	1.594	0.818	2.369
FN 2004	Logistic	9.39443	9.85597	1.53467	29	2	8.613	7.86	9.365	0.715	0.531	0.899
FN1 2005	MM	9.07801	9.37801	1.31248	43	2	11.202	10.266	12.137	2.137	1.478	2.795
FN1 2005	Logistic	14.79054	15.09054	1.78214	43	2	9.446	8.823	10.07	0.624	0.488	0.761
FN2 2005	MM	3.96070	4.27649	0.99780	41	2	11.513	10.785	12.242	1.876	1.416	2.335
FN2 2005	Logistic	11.83660	12.15239	1.55288	41	2	9.98	9.389	10.57	0.669	0.539	0.798
RW1 2005	MM	7.95377	8.29663	1.27071	38	2	12.756	11.963	13.549	4.188	3.087	5.29
RW1 2005	Logistic	19.59143	19.93429	2.57218	38	2	11.479	10.671	12.286	0.257	0.194	0.32
RW2 2005	MM	19.87301	20.22595	2.68535	37	2	12.548	11.291	13.806	4.161	2.446	5.875
RW2 2005	Logistic	26.78228	27.13523	4.12798	37	2	11.561	10.404	12.718	0.223	0.155	0.291
TM 2004	MM	-1.78930	-1.41430	0.68327	35	2	10.601	9.963	11.239	5.525	3.828	7.222
TM 2004	Logistic	7.67213	8.04713	1.27326	35	2	9.526	8.95	10.103	0.165	0.129	0.2
TM1 2005	MM	4.15812	4.43085	1.00778	47	2	11.789	11.202	12.375	2.789	1.987	3.551
TM1 2005	Logistic	12.05098	12.32371	1.48353	47	2	10.697	10.235	11.159	0.336	0.273	0.4
TM2 2005	MM	-4.18051	-3.90779	0.66980	47	2	11.483	11.051	11.915	3.968	3.006	4.93
TM2 2005	Logistic	10.61151	10.88424	1.38252	47	2	10.447	10.038	10.856	0.256	0.201	0.311

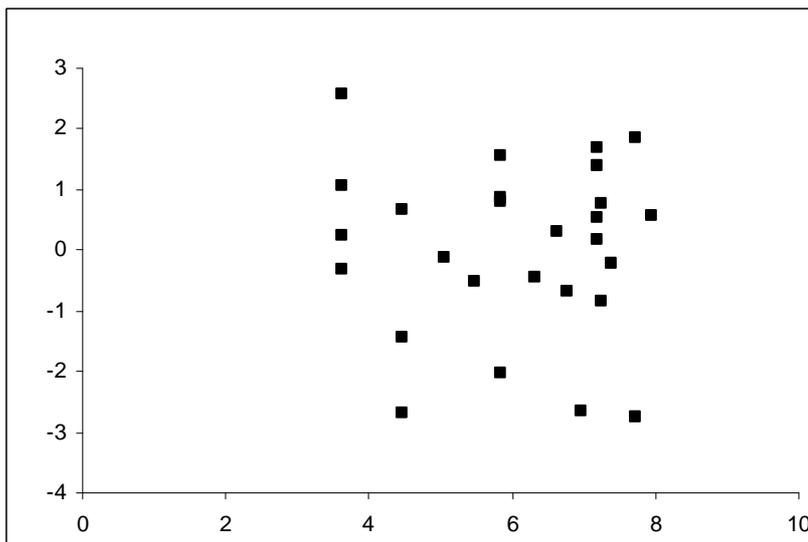
APPENDIX 1.4

**Michaelis Menten models**

CF 2004 no x less 0.5 MM

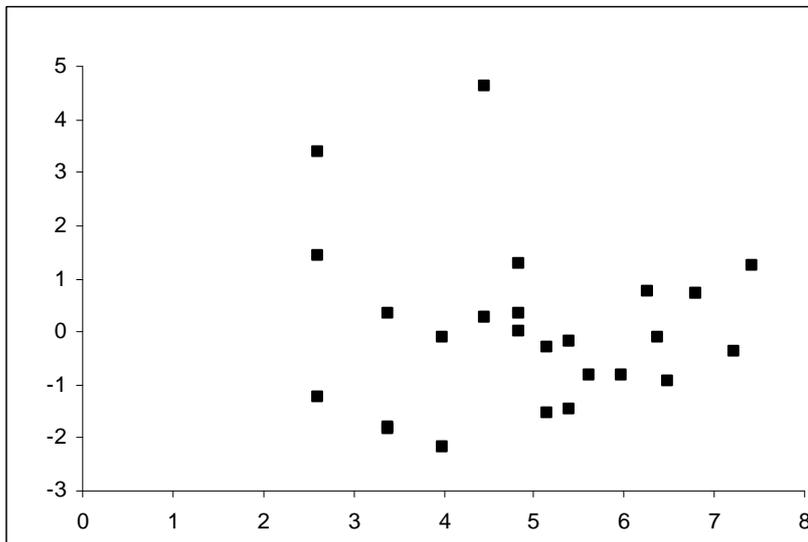
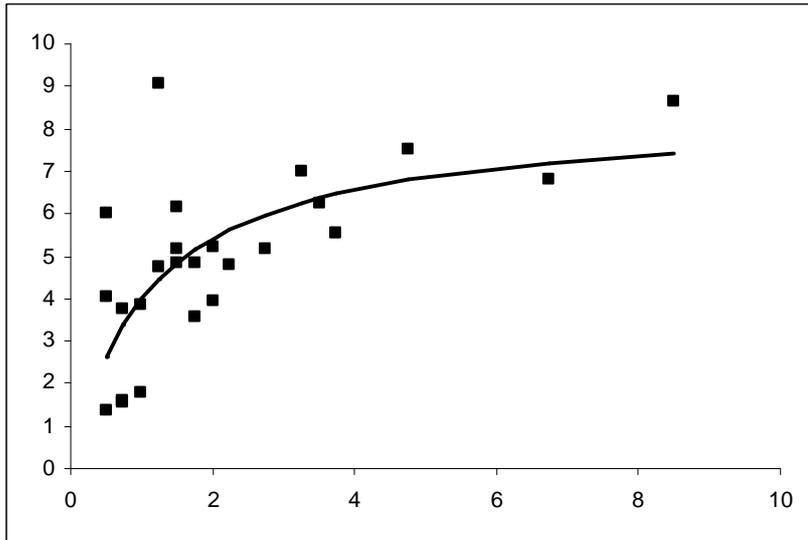


Model plots demonstrating supply (x axis = mean *S. balanoides* cyprids per trap per day) and settlement (y axis = mean *S. balanoides* cyprids per tile per day<sup>3</sup>). Solid black squares are the observed data. Solid black line is the estimated MM model for that block. All values where larval supply was less than 0.5 cyprids were removed from the data set)

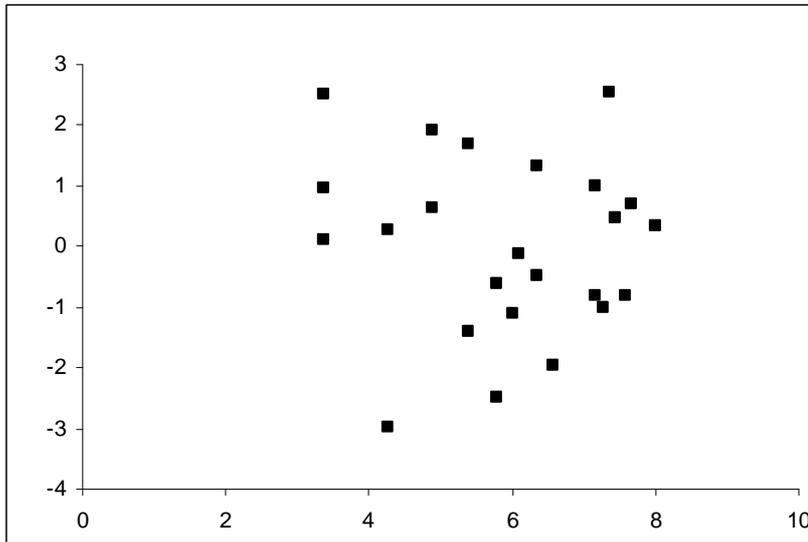
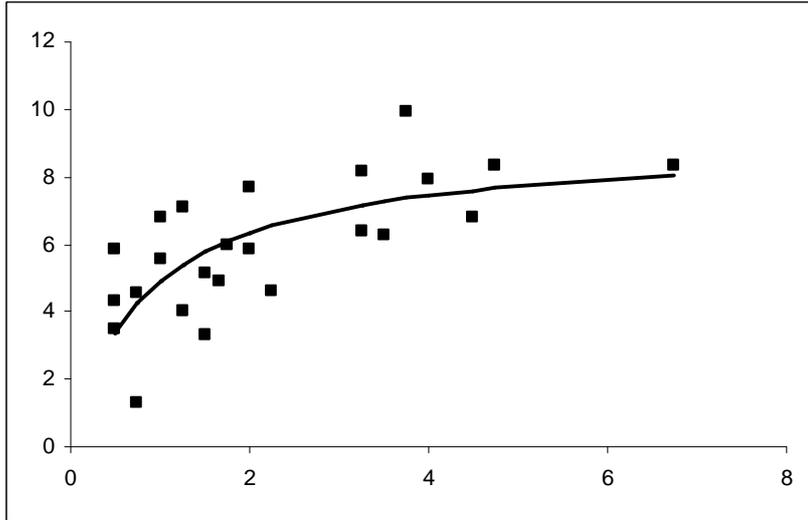


Standardised residuals from the corresponding model plots. X axis is the predicted value from the model for settlement. Y axis is the observed difference from Y for that point.

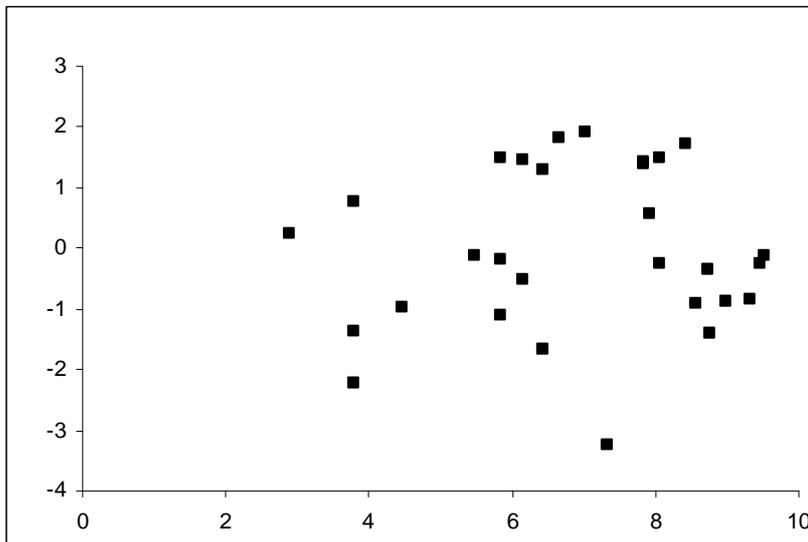
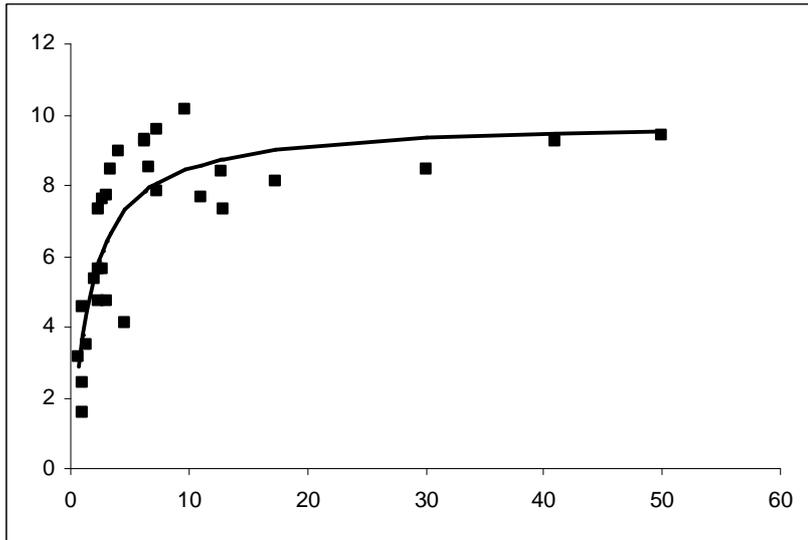
CF1 2005 no x less 0.5 MM



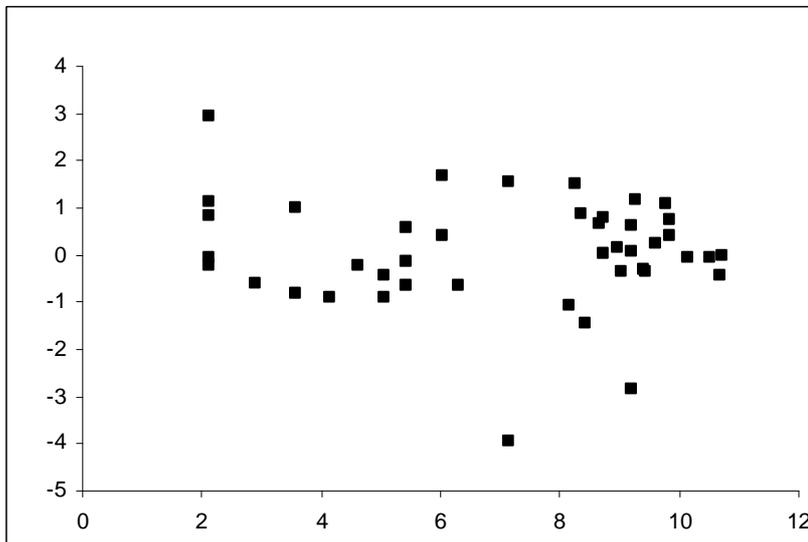
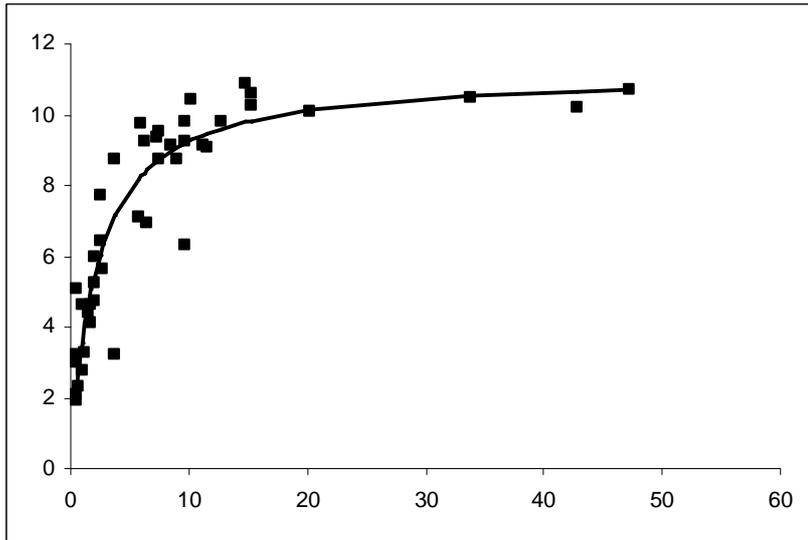
CP2 2005 no x less 0.5 MM



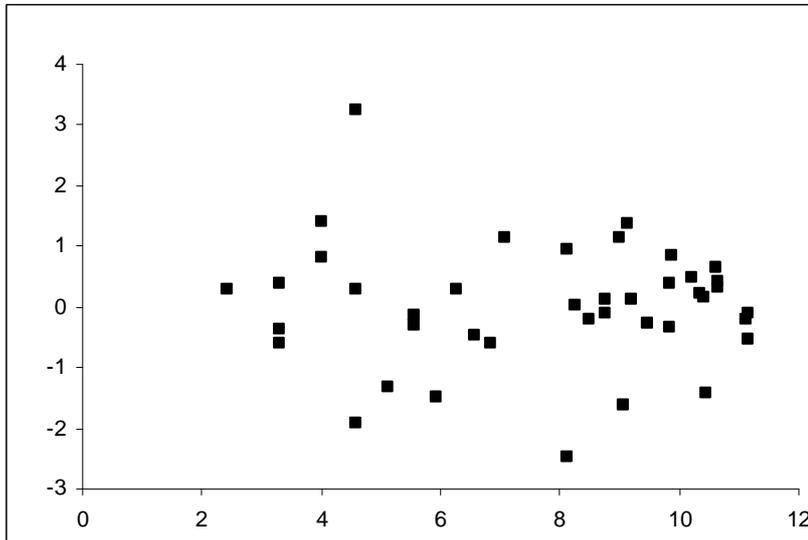
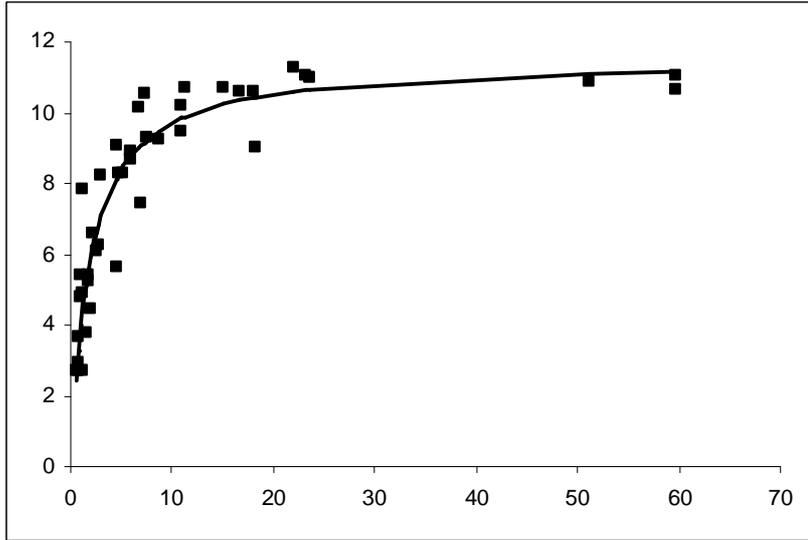
FN 2004 no x less 0.5 MM



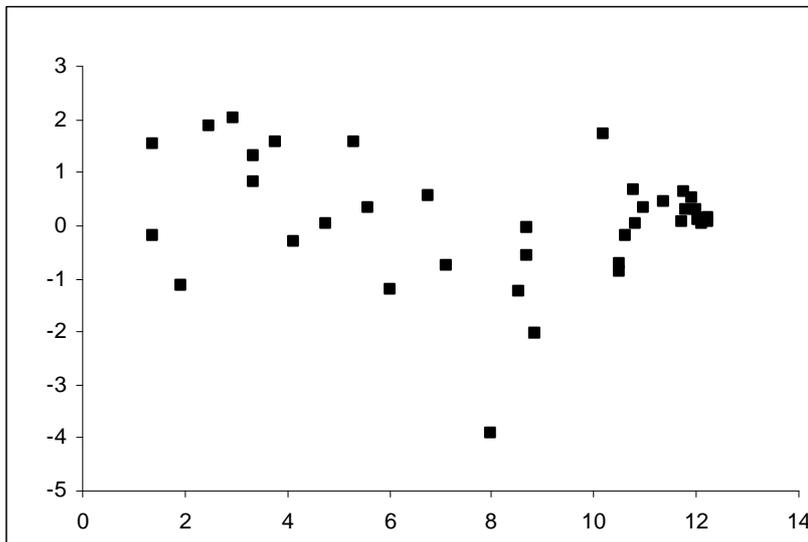
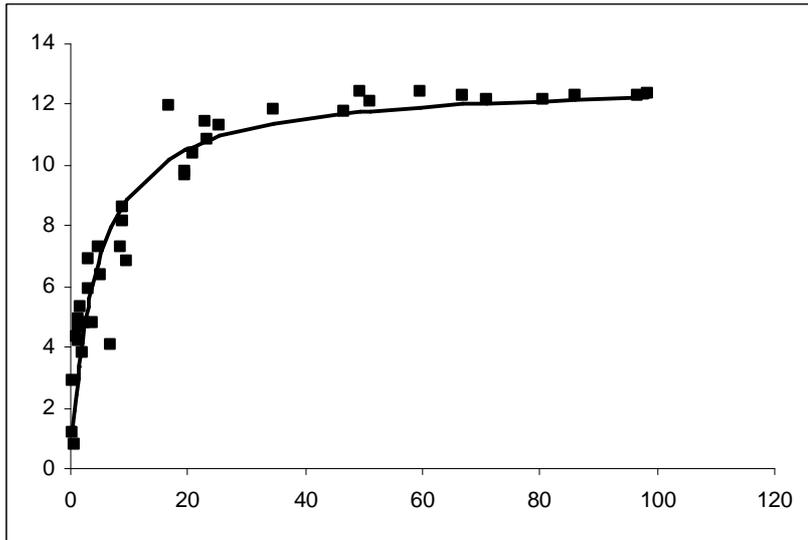
FN1 2005 no x less 0.5 MM



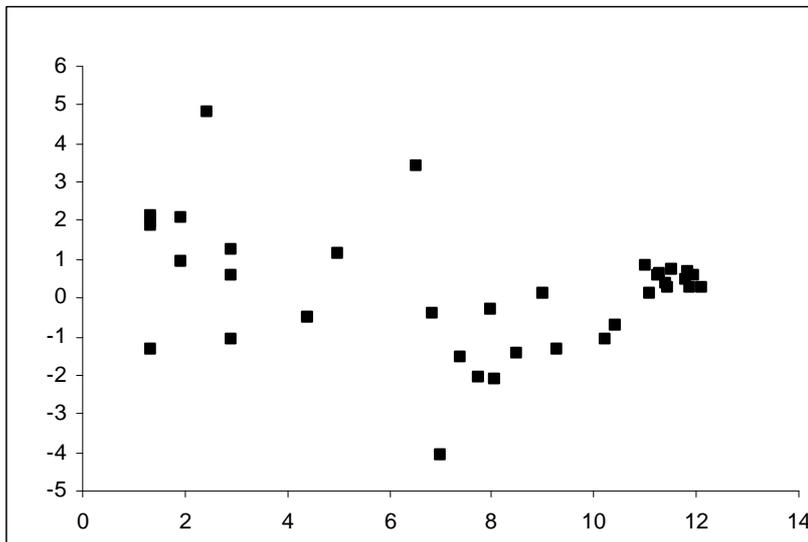
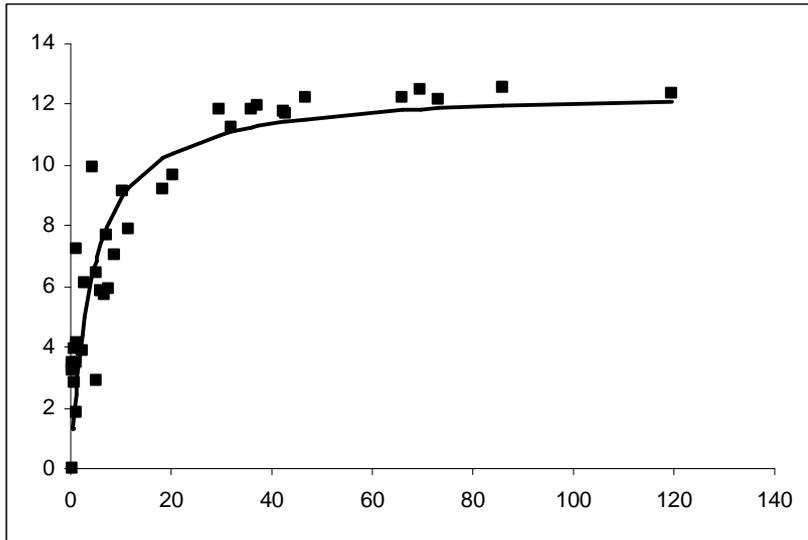
FN2 2005 no x less 0.5 MM



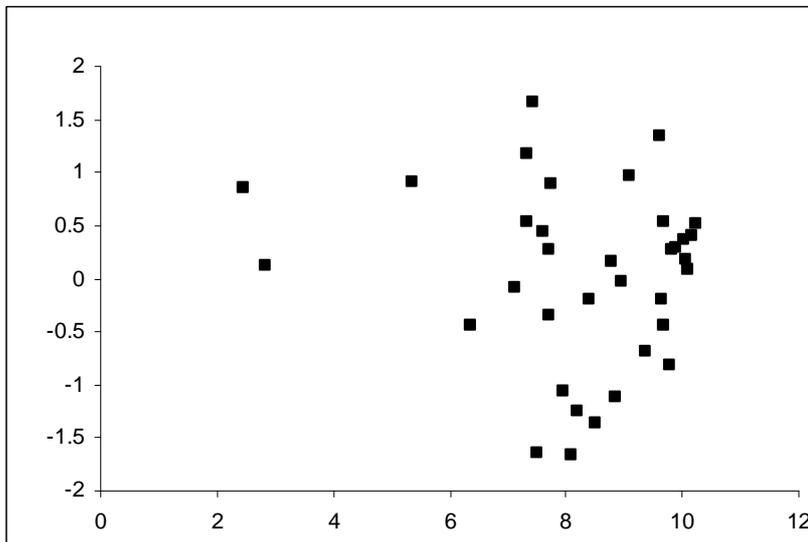
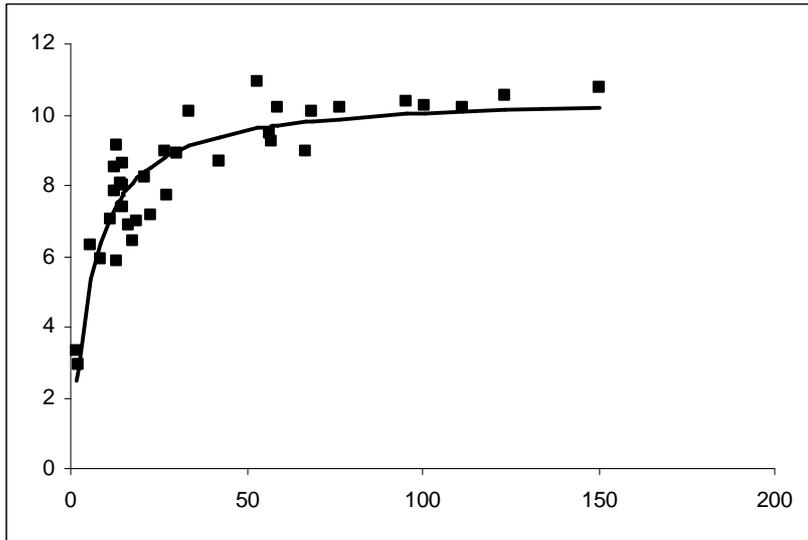
RW1 2005 no x less 0.5 MM



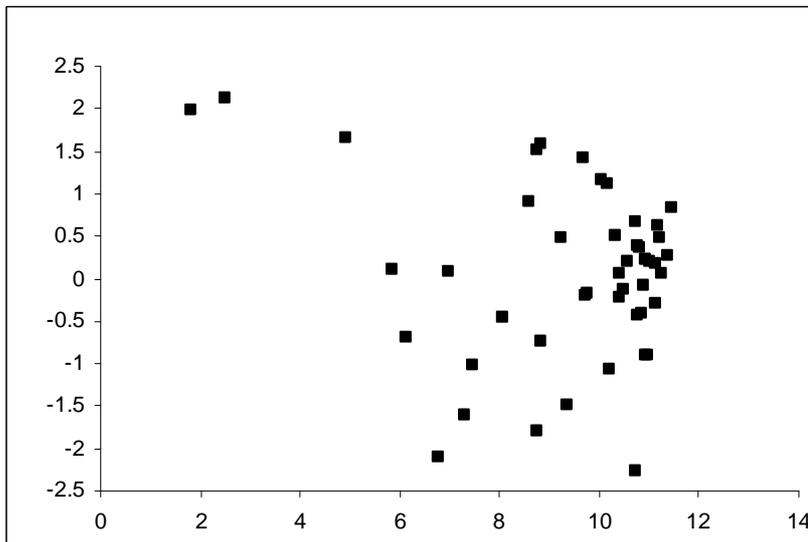
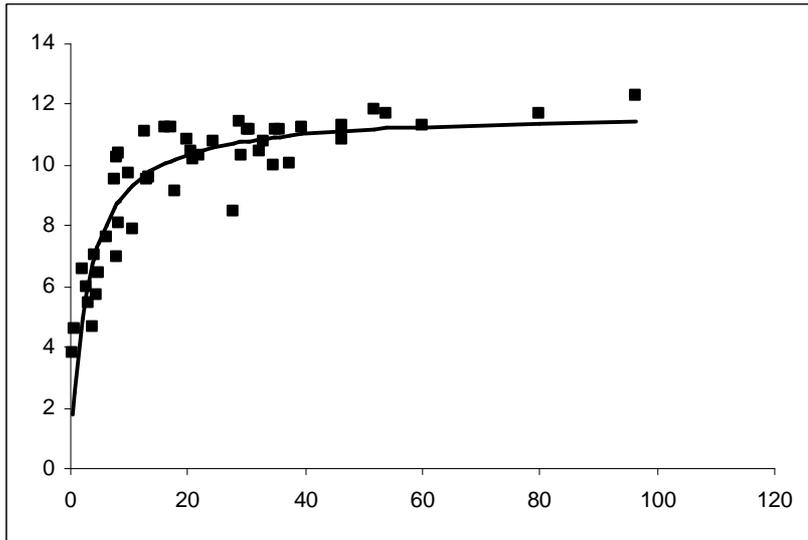
RW2 2005 no x less 0.5 MM



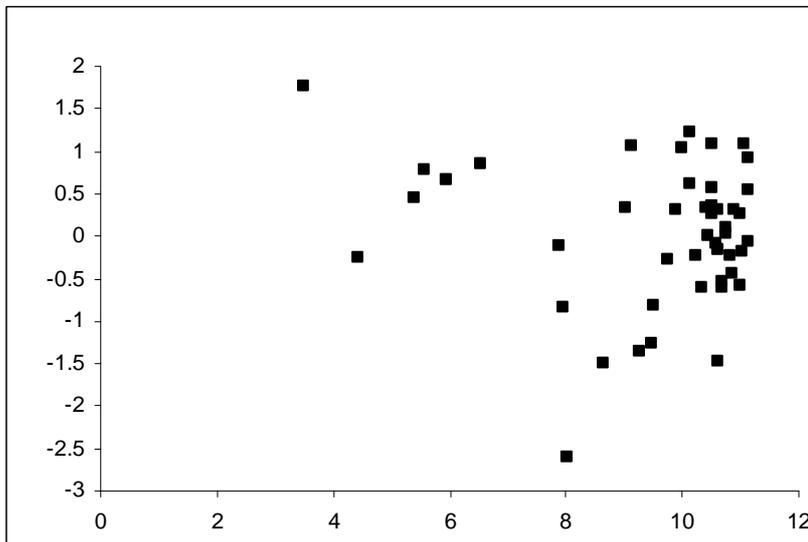
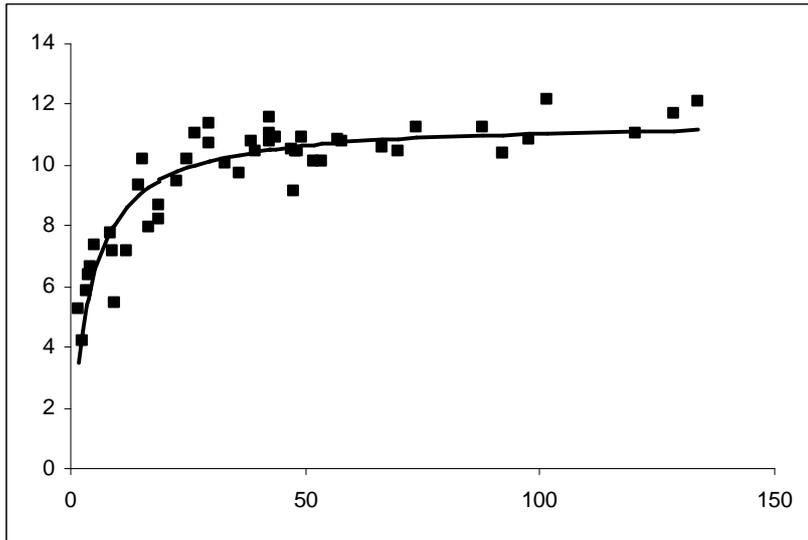
TM 2004 no x less 0.5 MM



TM1 2005 no x less 0.5 MM



TM2 2005 no x less 0.5 MM



APPENDIX 1.5

**Standardised residuals against Wave action (AveDev) per block**

X = AveDev (Wave Index – Chapter 2)

Y = Standardized Residuals from MM model

