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# An evaluation of the relative influences of elevated temperature and ocean acidification on processes influencing the distribution of intertidal barnacles

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**An evaluation of the relative influences of elevated temperature  
and ocean acidification on processes influencing the distribution  
of intertidal barnacles**

By

**HELEN SARAH FINDLAY**

A thesis submitted to the University of Plymouth  
in partial fulfilment for the degree of

**DOCTOR OF PHILOSOPHY**

**School of Marine Science & Engineering  
Faculty of Science**

In collaboration with  
**Plymouth Marine Laboratory**

**January 2010**

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Helen S. Findlay

**ABSTRACT**

Ocean acidification and climate change are occurring as a result of anthropogenic release of carbon dioxide (CO<sub>2</sub>) into the atmosphere. Changes in climate and ocean chemistry are occurring concomitantly and at a faster rate than previously recorded in Earth's history, yet little is understood about how they will influence the population dynamics and ecology of many marine organisms. The barnacle *Semibalanus balanoides* is a major space occupier on rocky shores in northern Europe and hence changes in its population ecology can have a broad influence on other species. An intertidal high CO<sub>2</sub> microcosm system was developed in order to determine how temperature and CO<sub>2</sub> interact to affect *S. balanoides* egg development, nauplii development and cyprid development. Changes in abundance and viability of the early life stages impacts the supply of larvae arriving in the intertidal and post settlement mortality determines the number of individuals reaching reproductive age. Laboratory experiments indicated that elevating CO<sub>2</sub> and temperature slows the metamorphosis of cyprids thereby increasing their exposure to desiccation. Increased temperature and CO<sub>2</sub> had greatest impact on smaller individuals prior to metamorphosis with poor survival being linked to slow growth and ability to calcify. Embryo development rates were also reduced significantly by elevated CO<sub>2</sub>, while the survival of brooding adults was lowered. These experimental data were then incorporated into a population model used to predict the changes in population abundances over the coming century. At the southern edge of its geographic range, *S. balanoides* is predicted to be most significantly impacted by temperature, although in colder years CO<sub>2</sub> has a significant influence. A conceptual model developed using these empirical data suggests that at the northern edge of its geographic range, *S. balanoides*, appears more likely to be impacted by ocean acidification than temperature, particularly through changes in resource allocation and changes in life history.

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*"I do not know what I may appear to the world; but to myself I seem to have been only like a boy playing on the seashore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me."*

*Isaac Newton, 1642-1727*

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Relevant scientific seminars and conferences were regularly attended at which work was often presented; external institutions were visited for consultations purposes and several papers prepared for publication (the relevant publications are listed at the beginning of each chapter).

Presentation and conferences attended:

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## **CHAPTER 1. GENERAL INTRODUCTION**

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## 1.1. INTRODUCTION

Over the past decade, increasing awareness of global climate change, both through natural variability and anthropogenic impacts, has produced a huge variety of studies investigating what impacts climate change may have on the environment (IPCC 2007; Brierley & Kingsford 2009; Heller & Zavaleta 2009). Until recently, the majority of studies highlighted the direct and indirect effects of rising temperature, as a result of increased greenhouse gas levels, predominantly atmospheric carbon dioxide (CO<sub>2</sub>), as potentially the most disruptive change on the environment relative to its natural state (Ramanathan & Feng 2008). Increasingly however, studies are demonstrating that CO<sub>2</sub> also has a direct and potentially significant impact on the marine environment through ocean acidification (Caldeira & Wickett 2003; The Royal Society 2005; Orr et al. 2005; Hoegh-Guldberg et al. 2007; Le Quéré et al. 2007; Guinotte & Fabry 2008). Within the past five years there has been increased investigation into the effects that elevated CO<sub>2</sub> (lowered pH and consequent changes in carbonate chemistry) might have on marine organisms and the ecosystem. Despite the obvious evidence that climate change and ocean acidification are both occurring at the same time, there are currently few studies that have investigated the relationships between temperature and ocean acidification and how they might interact to affect individual animal physiology and ecology (Orr et al. 2009). Until recently there has been a tendency to investigate the variables independently. Nevertheless new results on interactions are continually emerging (Metzger et al. 2007; Martin & Gattuso 2009; Parker et al. 2009; Gooding et al. 2009) and show mixed results concerning the relative importance of temperature and CO<sub>2</sub>, which will be described in more detail in section 1.3. Temperature is understood to be an important controlling factor of biogeographic distribution (Hutchins 1947; Southward 1958); hence organism and population response to temperature and ocean acidification will be important when determining whether species are able to shift their distribution to follow changes in the environment.

Rocky shores occur at the interface between the oceans and land throughout the world. They span through every latitude and constitute a major proportion of the littoral habitat on wave-exposed shores, as well as occurring along many enclosed and sheltered coastlines. They are one of the most studied marine ecosystems due to their accessibility and socio-economic importance, yet they are also one of the most environmentally variable and complex marine habitats (Paine 1994). The distribution and diversity of rocky shore species varies widely on both global and local scales as a result of complex interactions between abiotic and biotic processes (Lewis 1964). Intertidal communities living at the edge of their geographic range have been shown to fluctuate with changes in climate; several studies have investigated changes in community and populations with respect to temperature (Southward 1967; Southward 1991; Southward et al. 1995; Hawkins et al. 2003; Helmuth et al. 2006; Mieszkowska et al. 2006) however, although several studies have investigated the impact of ocean acidification on intertidal organisms (e.g. Gazeau et al. 2007; Bibby et al. 2007; Miles et al. 2007; Spicer et al. 2007; Bibby et al. 2008; Beesley et al. 2008; Hauton et al. 2009), these experiments have all been carried out under simulated subtidal conditions. Only one study has attempted to correlate the changes in community structure on the rocky shore with changes in pH (Wootten et al. 2009) but there is still much uncertainty as to whether the changes occurred because of declining pH, increasing temperature or biological interactions. Therefore, it is interesting to investigate whether these intertidal species, which experience a naturally variable environment, would be susceptible to chronic ocean acidification and changes in temperature.

This chapter will summarise what changes are occurring with respect to climate change and ocean acidification and the overall implications of these changes (section 1.2). Furthermore, this chapter will look at how these changes in CO<sub>2</sub> and temperature will impact (or have impacted) marine organisms (section 1.3) and more specifically rocky shore populations (from the geographic range edges) and the processes that influence the

distribution of species within the rocky shore (section 1.4). It will describe the tools used for analysing the environment and for making future predictions (section 1.5). Finally, in section 1.6, the aims and objectives for the rest of this thesis will be presented.

## 1.2. INCREASING ATMOSPHERIC CARBON DIOXIDE (CO<sub>2</sub>) LEVELS

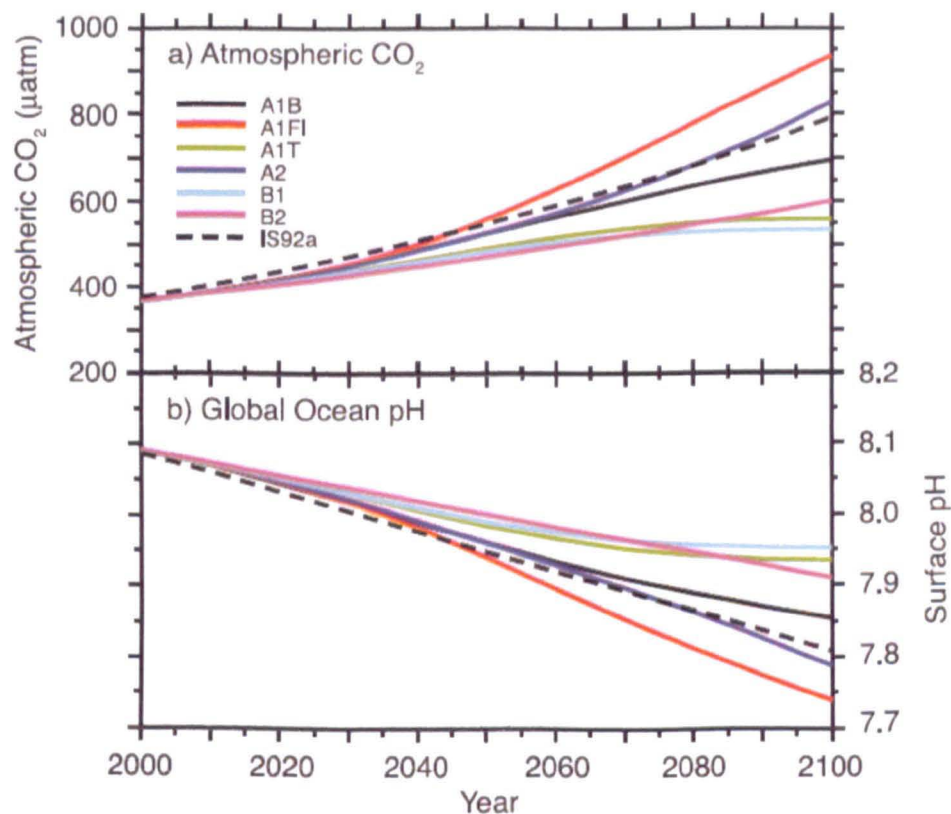


Figure 1.1: Changes in global average surface pH and saturation state with respect to aragonite in the Southern Ocean under various SRES scenarios. Time series of (a) atmospheric CO<sub>2</sub> and (b) projected global average surface pH for the six illustrative SRES scenarios and the IS92a scenario. Modified from IPCC (2007).

CO<sub>2</sub> is a one of several greenhouse gases, which act to maintain a warm temperature within the Earth's atmosphere by trapping solar radiation (Thomson 1997) and without which the Earth would be uninhabitable (Tuckett 2009). However, atmospheric CO<sub>2</sub> concentrations are increasing as a result of anthropogenic activities (Keeling 1970) leading to concern about rising temperatures and resultant changes in climate. The CO<sub>2</sub> concentration in the atmosphere has risen sharply from about 280 ppm before the industrial revolution in the

mid 18<sup>th</sup> Century to year 2007 levels of about 385 ppm (IPCC 2007). Post-industrial revolution human activities such as the combustion of fossil fuels, increased deforestation, agriculture and cement production now release over 7.8 billion tonnes of carbon per year into the atmosphere (Raupach et al. 2007). The magnitude of CO<sub>2</sub>-induced chemical change is unprecedented at least over the past 25 million years (Turley et al. 2006), and the pre-industrial atmospheric CO<sub>2</sub> concentration has not been exceeded for the last 420,000 years (Petit et al. 1999). The Intergovernmental Panel on Climate Change (IPCC 2007) fourth assessment presented CO<sub>2</sub> future projections based on two sets of models; both included representations of the ocean and terrestrial climate feedbacks and were run under a variety of scenarios based on different CO<sub>2</sub> emission rates. The models predict that atmospheric CO<sub>2</sub> will continue to rise over the next century, reaching levels from 540 to over 970 ppm (IPCC, 2007) unless CO<sub>2</sub> emissions can be drastically reduced (figure 1.1a).

#### **1.2.1. What does increased CO<sub>2</sub> mean for Earth's atmosphere and oceans?**

Atmospheric CO<sub>2</sub> concentration correlates strongly with atmospheric temperature; increased CO<sub>2</sub> in the atmosphere enhances the greenhouse effect and hence there have been increases in both sea and air temperature beyond pre-industrial levels (IPCC 2007; Levitus et al. 2005; Mackenzie & Sciedek 2007). Since the period 1850-1899, global surface temperature has increased by an average of 0.76 °C (ranging between 0.57 and 0.95 °C) (IPCC 2007). Observations have shown that, as a result of anthropogenic forcing (Barnett et al. 2001), over the last 40 years around 84 % of the total heating of the Earth system (oceans, atmosphere, continents and cryosphere) has gone into warming the oceans (Barnett et al. 2005). The annual sea surface temperature (SST) around UK coastlines, for example, has increased by 0.5 °C between 1871 and 2002 (DEFRA 2005). Models estimate that a doubling of CO<sub>2</sub> over the next century will result in an increase in average global temperature of between 1.5 - 4.5 °C (IPCC 2007).

As a result of atmospheric and oceanic warming there has been, and will continue to be, consequences for the rest of the Earth system; for example, an increase in the rate of sea-ice melting in the Arctic Ocean (Holland et al. 2006; Stroeve et al. 2007; Gillett et al. 2008) and around the Antarctic (de la Mare 1997). Additionally there has been an increase in the rate of glacial melting (IPCC 2007) and the melting of land ice (glaciers and ice caps) will generate an increase in sea-level worldwide (Vaughan 2009); furthermore, melting of ice will also cause freshening in certain areas of the ocean, such as the Arctic and in coastal shelf regions (Steinacher et al. 2009). As the oceans become warmer and fresher there will be changes in the salinity and thermal properties of the various water masses that drive large-scale circulation patterns which, in turn regulate the ocean and atmospheric climates at a global scale (Kuhlbrodt et al. 2007). Any changes to the temperature and salinity of these various water masses could impact on the overturning circulation rates and patterns, resulting in altered climatic conditions (Hansen et al. 2001; Bryden et al. 2005; Hansen & Østerhus 2007). Although there is speculation as to the changes that might be occurring in these large-scale ocean circulations, it is currently unclear what the implications might be and more observations are needed (Kanzow & Visbeck 2009).

Not only are there likely to be major ecological consequences of climate changes, but a report by Stern (2006), commissioned by the British Government, has demonstrated climate change will also have a large economic cost. The Stern Report reviews the risks from all the possible aspects of climate change. The report recommends that the cost of early action to mitigate climate change will be less than the costs associated with future impacts of continued emissions, which will damage the economy if nothing is done. Subsequent comments following the Stern Review (2006) have suggested that the true cost of climate change, because change is happening faster than predicted, may actually have been underestimated by the initial review.

Until recently it was believed that climate change impacts caused by anthropogenic CO<sub>2</sub> emissions were the only ones of consequence to the Earth system. Although CO<sub>2</sub> was observed to be increasing within the oceans as early as the 1970s (Brewer 1978), it was believed that seawater chemistry would not be altered as a result of uptake of CO<sub>2</sub> because of the capacity of the oceans' buffer reserves. In 2003 a paper by Caldeira & Wickett brought to light the fact that the pace at which anthropogenic carbon was being added to the atmosphere, and thus to the ocean, was not (and is not) being matched by the oceans' ability to buffer the resulting increase in hydrogen ions. This has brought about a rapid response in the scientific community to research the impacts that ocean acidification will have on marine communities.

### **1.2.2. CO<sub>2</sub> in the marine environment**

The oceans have the capacity to absorb large amounts of carbon dioxide (CO<sub>2</sub>) because CO<sub>2</sub> dissolves and reacts in seawater to form bicarbonate (HCO<sub>3</sub><sup>-</sup>) and protons (H<sup>+</sup>) (see Appendix 1 for further details). Between a quarter and a third of the CO<sub>2</sub> emitted into the atmosphere from the burning of fossil fuels, cement manufacture and land use changes has been absorbed by the oceans (Sabine et al. 2004). Over thousands of years, the changes in pH have been buffered by bases, such as carbonate ions (CO<sub>3</sub><sup>2-</sup>); however, the rate at which CO<sub>2</sub> is currently being absorbed into the oceans is too rapid to be buffered sufficiently to prevent substantial changes in ocean pH and CO<sub>3</sub><sup>2-</sup> and as a consequence, the relative seawater concentrations of CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup> and pH are changing. Since pre-industrial times the oceans pH has decreased by a global average of 0.1 units (Kleypas et al. 2006) and pH will continue to decrease as long as CO<sub>2</sub> emissions continue. The most recent IPCC (2007) report, using IS92a CO<sub>2</sub> emissions scenarios, predicts that the pH of the surface ocean will decrease by as much as 0.4 units by the year 2100 (figure 1.1b) and 0.77 units by 2300 (Caldeira & Wickett 2003). These decreases in pH are long-lasting as it will take tens of thousands of years for these changes in ocean chemistry to be buffered through



neutralisation by calcium carbonate sediments, and the level at which the ocean pH will eventually stabilise will be lower than it currently is (Tyrrell et al. 2007).

Ocean acidification is now well documented in ocean time series. These series show an increase in seawater pCO<sub>2</sub> parallel to the atmospheric increase (Brewer et al. 1997) and show a decrease in pH (Table 1.1), although the rates of pH decline are slightly different at each site as a result of local conditions. Observations of decreasing pH have also been found in upwelling (Feely et al. 2008) and coastal waters (Wootten et al. 2008) in the NE Pacific.

*Table 1.1: Rates of declining pH at different locations across the world's oceans*

Name	Location	Rate of pH decline (units yr <sup>-1</sup> )	Reference
Hawaiian Ocean Time-series (HOT)	Sub-tropical North Pacific	0.0019 ± 0.00025	Brix et al. 2004
European Station for Time-series in the Ocean, Canary Islands (ESTOC)	Sub-tropical (East) North Atlantic	0.0017 ± 0.0004	Santana-Casiano et al. 2007
Bermuda Atlantic Time-Series (BATS)	Sub-tropical (West) North Atlantic	0.0012 ± 0.0006	Bates & Peters 2006
Iceland Sea	Polar (Nordic Seas) North Atlantic	0.0024 (winter pH)	Olafsson et al. 2009

The change in carbonate ion (CO<sub>3</sub><sup>2-</sup>) is also an important consequence of ocean acidification. The concentration of CO<sub>3</sub><sup>2-</sup> in seawater directly influences the saturation, and consequently the rate of dissolution, of calcium carbonate (CaCO<sub>3</sub>) minerals in the ocean. At the year 2008 global average pH level, ~8.1, the surface ocean is super-saturated with respect to CaCO<sub>3</sub>. There are three main mineral forms of CaCO<sub>3</sub>, which are, in order of least soluble to most soluble, calcite, aragonite and high magnesium-calcite (Mg-calcite). Due to differences in the mineral properties and the oceanic environment (for example, as salinity increases the saturation state increases but as temperature increases the saturation state decreases) the saturation profiles of calcite, aragonite and Mg-calcite vary with latitude and ocean basin, with the Arctic and Southern Oceans having the lowest levels of

saturation (Orr et al. 2005). Increasing atmospheric  $p\text{CO}_2$  will cause the saturation states to decrease, as has been occurring since pre-industrial times, with recent model projections predicting that the Arctic Ocean will become undersaturated with respect to aragonite by the year 2030 (Steinacher et al. 2009).

### **1.2.3. Biological influence on carbonate chemistry**

Animals and plants living in the ocean can have a strong influence on carbonate chemistry. For example, in temperate and sub-polar regions there is a strong seasonal cycle of  $\text{CO}_2$  in the oceans and shelf seas. During spring and summer the dissolved inorganic carbon (DIC, see appendix 1) concentration decreases as a result of phytoplankton and algae fixing carbon, through photosynthesis, (Gislefoss et al. 1998; Brostrom 2000; Miller et al. 1999; Slagstad et al. 1999). Increased thermal stratification and decreased wind strength in the summer period additionally prevents the reintroduction of DIC from below the seasonal thermocline (Gislefoss et al. 1998). In autumn and winter turbulent mixing destabilises the water column allowing DIC concentration to be replenished in the surface layers (Gislefoss et al. 1998; Findlay et al. 2008). Benthic organisms have also been shown to significantly alter the water chemistry *via* photosynthesis and respiration (Smith & Key 1975; Gattuso et al. 1998b), while sediment-derived alkalinity also can alter the carbonate chemistry in the overlying water (Sarmiento & Gruber 2006; Andersson et al. 2006).

An additional biological process that substantially impacts the dynamics of the carbonate chemistry is the biological precipitation of calcium carbonate ( $\text{CaCO}_3$ ) by calcifying organisms such as coccolithophores (one group of phytoplankton), molluscs and corals (Smith & Key 1975).  $\text{CaCO}_3$  is formed by the reaction of calcium ions with bicarbonate ions, releasing water and  $\text{CO}_2$  as by-products (see appendix 1).  $\text{CaCO}_3$  formation not only binds dissolved carbon into particulate carbon therefore reducing DIC, but it also lowers alkalinity, and additionally feeds back to influence the flux of  $\text{CO}_2$  between the atmosphere

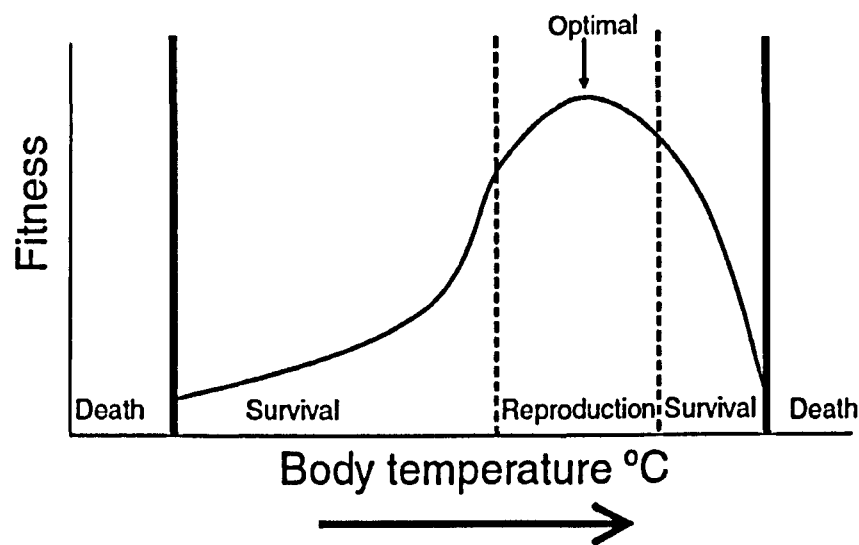
and the ocean (Sarmiento & Gruber 2006). Moreover, it was initially believed that calcifying organisms require conditions which are super-saturated with respect to the  $\text{CaCO}_3$  minerals in order to calcify (Riebesell 2000). This led to the assumption that ocean acidification, which will cause many areas of the ocean to become under-saturated, would severely impact calcifying organisms. Furthermore, the processes of calcification may result in the build up of hydrogen ions which must be exported out of the organism (Pörtner et al. 2005). In a more acidic environment this may become more difficult because pumps will have to work against a greater proton gradient.

### **1.3. IMPACTS OF $\text{CO}_2$ ON MARINE ORGANISMS**

To understand an organism's biogeography scientists must understand its physiology and the effects of temperature on that physiology. While each species has a tolerance range within which it can survive, the effect of changing temperature begins long before lethal limits are reached (figure 1.2). Any deviation from optimal conditions will cause a physiological response and may result in a reallocation of resources in an attempt to maximise fitness (Sibly & Calow 1986).

Many physiological processes are regulated by temperature. In poikilotherms metabolic rate, for example, generally increases with increasing temperature (Prosser et al. 1950). Under a constant energy intake, an increase in temperature will result in a decrease in energy surplus available for growth and reproduction (Sebens 1982). Experimental and observational studies on temperature effects have led to the formulation of models, which can predict how an individual will allocate resources in a given environment, how rapidly an individual will grow, when it will reproduce, and how many offspring it will produce (e.g. Sebens 1982). Other modelling techniques make use of this knowledge of life-history traits to examine population dynamics in response to changing temperature (e.g. Svensson et al. 2006). Laboratory and field observations underpin these modelling studies, and these

techniques have been used to explain biogeographic patterns (e.g. Hutchins 1947; Caughley et al. 1988 Pörtner 2002b) as well as species responses to a changing climate (e.g. Pörtner 2001; Pörtner et al. 2001; Pörtner 2002a; Helmuth et al. 2006; Poloczanska et al. 2008; Herbert et al. 2009).



*Figure 1.2: A thermal performance curve representing a fitness component (e.g. survival, growth, development, reproduction, movement potential) as a function of body temperature. Around the optimal temperature fitness is high and reproduction can occur, as the fitness decreases the organism survives but can not reproduce, at the temperature extremes death occurs. Adapted from Kearney & Porter 2009.*

Rocky shore species, and intertidal barnacles in particular, are often used as long term environmental monitoring tools as they show clear shifts in their distribution and population dynamics in parallel with changes in temperature (Southward 1967; Southward 1991; Southward et al. 1995; Hawkins et al. 2003; Helmuth et al. 2006; Mieszkowska et al. 2006). For example, northern hemisphere studies have shown that in warmer periods animals living towards the northern edge of their distribution have increased in abundance and extended their ranges northwards, whereas cold-water species have declined or retreated, and *vice versa* for colder periods (Southward et al. 1995). Other studies have

shown changes in abundance of macro-invertebrate species and whole community structure on the coast of California as a response to a warming climate (Sagarin et al. 1999). In the study by Sagarin et al. (1999), southern species appeared to increase in abundance, while most northern species decreased, correlating with the long term increase in ocean shoreline temperature by 0.79 °C during the 60 year study period (Sagarin et al. 1999). Seemingly it is the changes at the edges of a species range that are most important, and of most interest, for making future predictions about the impact of physico-chemical conditions on the overall distribution of a species (Caughley 1988).

It is also important to acknowledge that environmental factors often interact to influence the survival, and hence distribution, of species (Brett 1956; Kinne 1971). As a result of the understanding that often there is more than one controlling factor, studies of multiple environmental factors have been carried out. These include experiments using temperature and salinity (e.g. Costlow et al. 1962; Harms 1986; Dimock & Groves 1975); temperature, salinity, and oxygen (e.g. Mcleese 1956; 1959; Aldrice & Forrester 1971); temperature, salinity and ultraviolet radiation (e.g. Przeslawski et al. 2005); temperature and food supply (e.g. Sanford et al. 1994; Desai & Anil 2004); and temperature and pollution (e.g. Buxton et al. 1981; Lannig et al. 2006). These studies show that elevated temperature may cause increased susceptibility to additional stresses even if organisms are not directly impacted by warming itself. Temperature can also affect interactions between species, such as through competition (Svensson et al. 2006) and predation (Sanford 1999) or through the timing of ontogenetic transitions and spawning (Edwards & Richardson 2004; Philippart et al. 2003). Biological interactions have a major role to play in defining an individual's ability to survive, and a population to persist, in a particular location, even when abiotic conditions are optimal. While it is widely believed that by altering species ranges at independent rates, new biological interactions will occur, which may prevent survival, such interactions will be difficult to predict and require further research. Furthermore, the rate of

present and projected warming, as a result of increasing atmospheric CO<sub>2</sub>, may be much greater than the rate at which species can shift their distribution to more favourable conditions and/or adapt to locally rapid environmental change. Hence understanding the impacts of climate change and ocean acidification on processes involved in dispersal is also important.

The basic homeostasis of organisms requires that they maintain a dynamically stable internal intra- and extra-cellular pH (Davenport 1974). The mechanisms for short-term acid-base regulation vary according to the complexity of the organism and range from very little control to complete regulation (reviewed by Truchot 1981; Heisler 1986). It is less well understood how much the surrounding environment influences internal pH and how quickly internal pH can recover from a lowering of pH (acidosis) when pH in the external environment is lowered for long periods of time. Furthermore, the addition of CO<sub>2</sub> (hypercapnia) has its own direct effect on organism physiology (Cameron 1978; Lindinger et al. 1984; Burnett 1997). Hence ocean acidification has the potential to expose organisms both to hypercapnia and acidosis. Again, little is known about the response of organisms to relatively small, but long-term, changes in CO<sub>2</sub> and pH in the marine environment. Furthermore, there is uncertainty surrounding the relationships and interactions between CO<sub>2</sub>, pH and temperature (Pörtner et al. 2005).

The additional decrease in carbonate ions as a result of ocean acidification will result in calcified organisms living in a less favourable environment for reproduction and maintenance of calcium carbonate structures. Further energy and resources required to maintain a costly shell or calcified structure will put additional strain on resource supply, especially if hypercapnia, acidosis and temperature are already impacting on other physiological processes. It is not surprising then, that initial ocean acidification research was carried out on calcifying organisms and primarily on the process of calcification.

Indeed, most calcifying organisms studied to date, representing the major marine calcifying groups (coccolithophores, pteropods, foraminifera, corals, calcareous macroalgae, mussels, oysters, echinoderms and crustacean), showed reduced net calcification rates in response to short periods of elevated CO<sub>2</sub> (reviewed by Kleypas et al. 2006; Haugen et al. 2006; Guinotte & Fabry 2008). However, the explanation proposed by these studies, that the process of calcification is disrupted by ocean acidification is simplistic and probably unrealistic, as many of these organisms are still able to calcify in a more acidic environment. It seems more likely that the direct process of calcification is not impacted by ocean acidification, but by a combination of altered physiological processes (see Table 1.2) resulting in a reallocation of resources combined with increased dissolution rates, is the cause of mortality and decreased individual fitness (Wood et al. 2008).

*Table 1.2: Examples of other aspects of an individual's biology and ecology that have been shown to be impacted by ocean acidification.*

Area studied	References
Physiology	Miles et al. 2007; Spicer et al. 2007; Michaelidis et al. 2007; Wood et al. 2008
Embryonic development	Dupont et al. 2008; Kurihara et al. 2009; Parker et al. 2009; Eglisdottir et al. 2009; Ellis et al. 2009
Larval development	Arnold et al. 2009; McDonald et al. 2009; O'Donnell et al. 2009; Munday et al. 2009
Organism health	Beesley et al. 2008
Immune response	Bibby et al. 2008
Behavioural activities	Bibby et al. 2007
Ecosystem function	Widdicombe & Needham 2007; Bellerby et al. 2008; Dashfield et al. 2008; Widdicombe et al. 2009; Wood et al. 2009

There have been no attempts in the literature to include the impacts of hypercapnia and acidosis into either physiological models or further into population models. However, a global long-term change in chemical conditions of the marine environment is occurring, and understanding where individuals and species are most at risk is fundamental to predicting where they will be able to survive in the future. Thus it is vital that experiments investigating the response of organisms to both temperature and ocean acidification are

carried out with the aim that models are then developed in order to make predictions about future impacts.

Investigations on the combined effects of temperature and elevated CO<sub>2</sub> on marine organisms are, to date, limited to just a handful of studies (Metzger et al. 2007; Martin & Gattuso 2009; Parker et al. 2009; Gooding et al. 2009). Metzger et al. (2007) focused on short-term physiological implications of CO<sub>2</sub> acting on thermal tolerance and demonstrated that high levels of CO<sub>2</sub> (1 %) enhanced heat sensitivity and led to a narrowing of the thermal tolerance range of crabs. Martin & Gattuso (2009) demonstrated that temperature and elevated CO<sub>2</sub> acted synergistically on coralline algae to reduce net calcification and survival. Parker et al. (2009) found that oyster embryo development was most severely affected by both elevated temperature and CO<sub>2</sub> levels expected for the end of the century. Conversely, Gooding et al. (2009) showed that temperature and CO<sub>2</sub> appeared to enhance growth in starfish. Hence few generalisations can be made about the relative impact of temperature and CO<sub>2</sub> on marine organisms and much more research is required.

Recent research of climate change has been biased towards studying the temperature impacts on organisms and their environment; however in the past decade ocean acidification has been recognised as an important additional stressor in the marine environment. Warming and acidification of the oceans are happening simultaneously and hence if policy makers and coastal managers are to make predictions about the future status of the marine environment it is vital that the interaction of the two processes is understood and quantified. It is not possible to research the impacts of ocean acidification and temperature on every marine organism and hence key organisms or processes are required as useful models. This thesis focuses on an area of the marine environment which has yet to be investigated with respect to ocean acidification – the rocky intertidal. Additionally, it is the impacts on populations across geographic scales that are of most interest, as many



marine organisms have complex life histories which will influence whether a population is sustained in the future. Pre- and post-settlement processes may be crucial in determining the adult population depending on the population type and location and so are important processes that need to be investigated in relation to temperature and ocean acidification. Abiotic factors set the climate space within which a species can survive, yet biological factors may ultimately control whether a species occupies that space. Biological interactions are most influential towards the mid-range, while abiotic conditions act more strongly on populations living at the range edges (Herbert et al. 2009). Therefore one approach to understanding the relative influence of temperature and ocean acidification on overall geographic distribution of a species will be to investigate the populations living at the range edges.

#### **1.4. ROCKY SHORE ORGANISMS AND THEIR ENVIRONMENT**

Although rocky shores account for a small proportion of the world's oceans they are easy to access, sample and experiment on, and hence are a useful model system for ecologists. Patterns of community change may be mirrored subtidally (Southward 1995) and hence have broader monitoring value. Barnacles are major space occupiers on many of the upper and mid-shores worldwide (Stephenson & Stephenson 1972) and around the UK the main intertidal species are *Semibalanus balanoides*, *Chthamalus* spp., *Balanus* spp. and *Elminius modestus*. On sheltered shores algae become more common, particularly species of fucooids, while on exposed shores mussels, such as *Mytilus edulis*, become the major space occupiers (Lewis 1964). The small number of competitively important species therefore makes it easier to formulate predictions about rocky shore assemblages than, for example, sub-tidal sediment. Such modelling is supported by a vast background in manipulative experiments. *Semibalanus balanoides* life-history (Rainbow 1984; Barnes 1989; Klepal 1990; Barnes 1992; Anderson 1994), its distribution and population dynamics (Crisp &

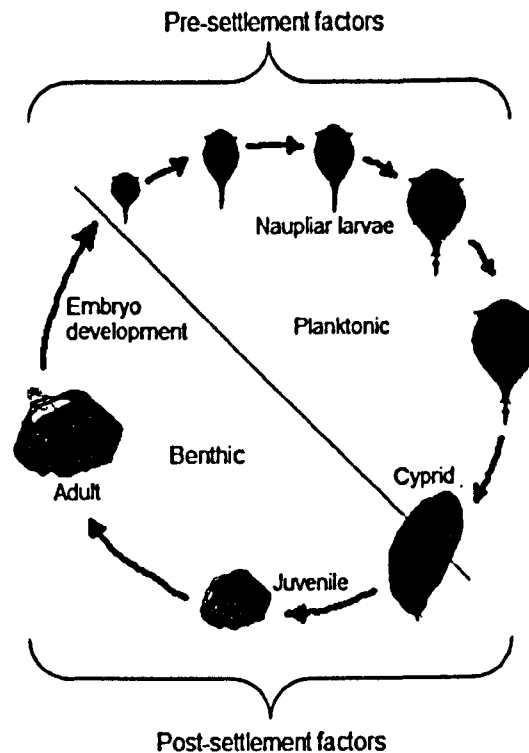
Southward 1958; Southward 1991; Southward et al. 1995) have been well studied and hence it provides an ideal model intertidal species.

#### **1.4.1. *Semibalanus balanoides* distribution and life-history**

*Semibalanus balanoides* is a common boreo-arctic barnacle species found around the UK. Its effective southern limit occurs around the south-west UK but small populations persist as far south as northern Portugal (Barnes 1958). The southern limit of *S. balanoides* has been proposed to be set by a combination of hot summers (> 25 °C) killing recruits and adults on the shore but also warm winters (> 10 °C) preventing gonad development and maturation (Barnes 1957; Lewis 1976). *S. balanoides* is present across both sides of the Atlantic as far north as Greenland, Canada, northern Norway and Svalbard (Petersen et al. 1966; Weslawski et al. 1993; Barnes 1999), and is believed to parallel the summer ice-pack and is limited by ice-abrasion (Barnes 1957) and by late spawning and protracted larval development (Petersen 1962).

Many invertebrate benthic species, including the barnacle *Semibalanus balanoides*, have a two-phase life cycle (figure 1.3) consisting of a pelagic larval phase and an adult benthic phase (Roughgarden et al. 1988). There has been much debate over whether it is the supply of larvae or the post-settlement survival that is more important for determining the abundance of adult populations (Hunt & Scheibling 1997; Todd 1998). More recently it has been recognised that both the processes contribute with a balance depending on the location of the population and the dominant environmental conditions (Hunt & Scheibling 1997; Burrows et al. 2009). If a population is situated in a location where larvae always reach the shore (e.g. bays and estuaries) then supply is not limited and it will be post-settlement processes that have most influence on the population dynamics. However, when supply is at least periodically limited (e.g. open coasts, where wind and circulation may

prevent larvae from returning to the shore) the processes involved in pre-settlement dynamics will be most influential to the population.



*Figure 1.3: Semibalanus balanoides life cycle, starting with embryo development within the adult mantle cavity, releasing naupliar larvae into the plankton, cyprid larvae settles onto the shore and metamorphosis into a benthic sedentary juvenile barnacle, which grows and matures into an adult form. Pre-settlement factors act on the larval planktonic phase, while post-settlement factors influence the benthic stages. Adapted from Desai & Anil (2005).*

Pre-settlement processes include oceanographic and hydrographic influences (Barnes 1970; Kendall et al. 1978; Jackson & Strathman 1981; Alexander & Roughgarden 1996; Crimaldi et al. 2002; Shanks et al. 2003), larval quality and behaviour (Lucas et al. 1979; Jarrett 2003; Emlet & Sadrow 2006) and predation on planktonic larvae (Bullard et al. 1999). Post-settlement processes include successful settlement (Gosselin & Qian 1996; Pineda et al. 2002; Jenkins 2005), successful metamorphosis (e.g. Pechenik et al. 1993), predation (Connell 1961; Barnett 1979), intra-specific competition (Barnes & Powell 1950;

Crisp 1961; Lewis 1964; Todd 1998), inter-specific competition (Southward & Crisp 1956; Crisp 1958; Barnett et al. 1979; Hawkins 1983) and physical disturbance (Barnes 1957; Harley & Helmuth 2003). These processes are all influenced by the physico-chemical environment and hence populations can be affected by the direct influence of the environment on individual physiology and behaviour but also indirectly by the processes described here.

#### **1.4.2. Present conditions at the *Semibalanus balanoides* range edges**

Intertidal organisms, in comparison to those living subtidally, have been thought to be relatively tolerant to stressors such as temperature or pH, because they experience larger variations in their abiotic environment (Newell 1979; Somero 2002). This is certainly the case for temperature (body temperature range, in some locations, exceeds 20 or 30 °C, e.g. Harley & Helmuth 2003), but knowledge of the variability in chemical conditions is more limited. Information on coastal shelf seas, the best approximation for the intertidal habitat of *S. balanoides*, indicated that there is a greater range of pH than in open oceans as a result of terrestrial influences such as river run-off and nutrient enrichment as well as large temperature and salinity fluctuations (Hinga 2002, Blackford & Gilbert 2007). Some intertidal systems can experience even greater pH fluctuations, e.g. in rockpools (Morris & Taylor 1983), but knowledge of the chemical conditions directly above the shore during high tide is virtually non-existent (although Agnew & Taylor 1986 provide an example of diurnal fluctuations in pH of 7.5 – 8.5 and Wootton et al. 2008 show a diurnal range of ~ 0.7 pH units and seasonal range ~1 pH unit in the coastal zone).

Present (year 2008) conditions experienced by *Semibalanus balanoides* at the northern and towards the southern edge of its geographic range are shown in Table 1.3. Air temperature fluctuates significantly in both locations throughout the year, while sea surface temperature has a much narrower range, particularly in the Arctic. The wind direction, speed and tidal

range are also important factors for intertidal organisms particularly when considering level of tide and wave exposure as well as dispersal ability. The seasonal cycle of carbonate chemistry at these two locations has yet to be fully resolved. However, Table 1.3 shows that at the northern range edge pH, DIC and alkalinity are lower than at the southern edge, indicating that addition of CO<sub>2</sub> will cause a more severe decline in pH and the saturation states of calcium carbonate minerals in the north.

*Table 1.3: Environmental conditions at example locations at the southern and northern edges of Semibalanus balanoides geographic range on the east side of the Atlantic.*

	Southern Range Edge <sup>1</sup>	Northern Range Edge <sup>2</sup>
Example Location	Plymouth, Southwest UK	Ny Ålesund, Svalbard, Norway
Air Temperature <sup>#</sup>	0.5 to 25.6 °C	- 19.4 to + 8.4 °C
Sea surface temperature <sup>#</sup>	9.4 to 17.6 °C	- 1.8 to + 5.1 °C
Salinity*	34.9 ( $\sigma^2 = 0.32$ )	34.4 * ( $\sigma^2 = 0.07$ )
Carbonate chemistry*	pH = 8.15, DIC = 2130, A <sub>T</sub> = 2330	pH = 8.11, DIC = 2050, A <sub>T</sub> = 2160
Dominant wind direction*	212°	176°
Wind speed*	3.7 m s <sup>-1</sup> (max 11 m s <sup>-1</sup> in Mar.)	4.3 m s <sup>-1</sup> (max 14 m s <sup>-1</sup> in Jan.)
Tidal range	up to 5.5 m	up to 2 m

<sup>1</sup>Data from PML meteorological station [http://www.npm.ac.uk/rsg/projects/pml\\_weather\\_station/](http://www.npm.ac.uk/rsg/projects/pml_weather_station/) and E1 time series data; <sup>2</sup>Data from Ny Ålesund marine laboratory and Ny Ålesund AWIPEV met station; <sup>#</sup>Annual minimum and maximum values; \*annual mean value.

#### 1.4.3. *Elminius modestus* as a comparative model species

*Elminius modestus* is an invasive species brought to Europe around the mid-1900s from Australasia (Bishop 1947). *E. modestus* has similar tolerance levels and habitat requirements to that of *Semibalanus balanoides*, and the two species co-occur in many locations in the south of *S. balanoides* range (Southward 1991; Harms 1999). Unlike *S. balanoides*, *E. modestus* is a warm-water species which does not have a seasonal life-history (Rainbow 1984). Both species have calcified shells and similar physiology (Rainbow 1984). Because *E. modestus* is a warm-water species it would be expected to extend its geographic range in a warming ocean, while *S. balanoides*, a cold-water species, would be expected to retreat. *E. modestus* and *S. balanoides* provide ideal comparative

model species to assess how different warm- and cold-adapted species respond to elevated temperature and ocean acidification. Furthermore, they have different energetic requirements as a result of their varying life-history and hence comparing the two species provides information on the associated changes in resource allocation.

#### **1.4. PREDICTING FUTURE CHANGES**

In order to investigate the relative influence of temperature and ocean acidification, and their interaction, on populations, this thesis used experiments to provide parameter values which were then incorporated into models. These experiments were designed to elucidate the response of each stage of an organism's life-cycle at different locations within its geographic range to present (year 2008) and future (year 2100) conditions, as well as provide an understanding of the mechanisms behind the response. This basic knowledge then allows physiological processes to be incorporated into mechanistic models of species distributions, which may also be applicable to other species.

Population models are used to investigate population dynamics in relation to abiotic and biotic factors. Often these are based on just one or two interacting species or one species and some environmental conditions. Matrix models are often used for demographic analysis as they allow a population to move through various cohorts or size/age structures (Svensson et al. 2004; Poloczanska et al. 2008). They use specific vital rates or conditions to investigate changes at various levels throughout the age structure. These models have been popular tools for understanding the dynamics of populations on rocky shores, particularly investigating the growth of species and interactions between two competing species (Roughgarden et al. 1988; Svensson et al. 2005, 2006; Brooker et al. 2007).

A population model is useful then to assess, test and predict the relative influences of temperature and ocean acidification on local population dynamics. The model used by

Poloczanska et al. (2008) investigates the dynamics of *Semibalanus balanoides* and *Chthamalus* spp. in response to changing temperature. This model provides an ideal framework in which to incorporate experimentally derived data on impacts of a changing marine climate and ocean acidification to predict future population dynamics. However, specific assumptions are made about the modelled population, such as it is open (demographically) and recruitment is proportional to free-space (Poloczanska et al. 2008). Therefore this particular model would not be suitable to represent all populations, for example, populations that are considered 'closed'. Closed populations are those that gain recruits into the population spawned from adults of that same population. Closed populations are therefore opposite to open populations, which gain recruits from other populations and therefore import new genetic material. This model is therefore not applicable across the whole geographic range of a species and cannot predict overall distribution in the future. In order to make predictions across larger scales, it will be necessary to formulate conceptual models based on experimental evidence and ecological understanding, which guide the user to make the correct assumptions about how temperature and ocean acidification will impact a species in relation to its position in the environment. These models can then be applied either to local population models or species distribution models (Kearney & Porter 2009).

## **1.5. AIMS AND OBJECTIVES**

The aims of this thesis are to (1) generate the empirical data to inform realistic conceptual and numerical models that will predict how ocean acidification and climate change might impact populations of rocky shore species, taking care to assess the relative impacts at different stages throughout a barnacle's life-history and (2) run those models and assess their efficacy.

- Chapter 2 describes the intertidal ocean acidification microcosm system designed specifically for the experiments presented in chapters 3 and 4. It was important that natural

regimes for light, tides, feeding, etc. were used for all the laboratory experiments and that the appropriate ocean acidification techniques were used to replicate future CO<sub>2</sub> scenarios for the intertidal zone.

- Chapter 3 details an experiment carried out using adults and developing embryos to test their vulnerability to prolonged ocean acidification when the adults would naturally be subjected to lower saturation states over winter and are reliant on energy reserves. Embryos develop in the mantle cavity of those adults and during the development period may be subjected to reduced oxygen levels and elevated CO<sub>2</sub> levels.
- Chapter 4 details experiments using on post-larvae and compared the response of *Semibalanus balanoides* to elevated CO<sub>2</sub> and temperature with the response of *Elminius modestus*. A comparison of these two species provides information on how specific are the responses to temperature and ocean acidification between similar species.
- Chapter 5 investigates the importance of calcification with respect to other physiological processes. It is important to evaluate whether studying just the process of calcification can be justified or whether research should be carried out on whole organism physiology in order to understand the impacts of ocean acidification on marine calcifying organisms.
- Chapter 6 describes how information from experiments is used to parameterise a population model and this model can then be used to predict future population dynamics as well as understand the relative importance of temperature and ocean acidification in controlling the population abundance.
- Chapter 7 details an experiment carried out at the northern range edge of *Semibalanus balanoides* and compares the responses to the southern range edge experiment to illustrate whether there are difference between populations across the geographic range.



- Chapter 8 brings together the experiments and modelling information to formulate conceptual models and provide understanding of the broad-scale impacts of climate change and ocean acidification.

## **CHAPTER 2. METHODS DEVELOPMENT**

### **A novel microcosm system for investigating the impacts of elevated carbon dioxide and temperature on intertidal organisms**

Aspects of this chapter have been published in:

Findlay HS, Kendall MA, Spicer JJ, Turley C, Widdicombe S (2008) Novel microcosm system for investigating the effects of elevated carbon dioxide and temperature on intertidal organisms. *Aquatic Biology* 3: 51-62

## 2.1. INTRODUCTION

Early experiments investigating physiological responses of marine organisms to various gas mixtures were initially carried out using relatively crude techniques of saturating water with the gas (e.g. CO<sub>2</sub>) and then diluting with normal seawater before adding the test animal (e.g. Fox & Johnson 1934). Experiments were aimed primarily at eliciting a physiological response and not replicating realistic environment conditions, hence CO<sub>2</sub> levels were often very high and accurate monitoring of levels was not of primary importance (e.g. Dale et al. 1970). These methods above and some that followed, involved sealing the experimental containers once the treated water has been added, restricting the size of organisms that can be used because of build up of wastes in the containers and changes in the gas mixtures by respiration and by diffusion. Regulated flow of gases, mixed using precision gas mixing pumps prior to bubbling through seawater, were developed to enable more accurate control of the final gas concentrations (Pörtner 1987; Cameron & Iwama 1987). These pumps are expensive and therefore in relatively short supply, which prevented large numbers of treatments and long exposure times from being used.

As CO<sub>2</sub> and pH became more topical with respect to environmental change, techniques were developed to regulate seawater pH using pH sensors, *via* feedback systems coupled to a regulated source of CO<sub>2</sub>. This greatly improved the long-term reliability and still remains one of the most implemented techniques to date (Riebesell et al. 1993; Pörtner et al. 1998; Green et al. 2004; Kurihara et al. 2004; Kurihara & Shirayama 2004; Michaelidis et al. 2005; Metzger et al. 2007; Widdicombe & Needham 2007). These systems however, can be expensive and still rely on pH measurements for control, and not precise measurements of CO<sub>2</sub>. Adding pulses of CO<sub>2</sub> gives a stable pH on average but the pulsing of CO<sub>2</sub> may alter the equilibrium of the carbonate system and cause variability in both total alkalinity (A<sub>T</sub>) and total dissolved inorganic carbon (DIC). Fluctuations in the carbonate system can

be accounted for by additionally monitoring DIC and/or  $A_T$ . Use of these systems to investigate longer term seasonal variations is more challenging and has yet to be accurately carried out.

An alternative method developed more recently for investigating realistic environmental scenarios is the two-phase system (Delille et al. 2005). The tops of these *in-situ* mesocosms were isolated from the surrounding atmosphere forming tents that covered more than 90% of each mesocosm surface area. The atmospheric concentration of  $CO_2$  in each tent was then controlled by continuous addition of high- $CO_2$  air mixes. The seawater carbonate system was allowed to develop naturally and equilibrate with the atmosphere. This particular set up involved the use of premixed gases; these types of premixed gases have also been used in experiments to bubble into seawater (e.g. Pane & Barry 2007). Premixed gases are readily available but are expensive and rapidly used up making long running experiments costly.

These previous methods have been designed for mimicking subtidal situations. However, the intertidal is a significant component of the shallow-water ocean margin, is important in the global carbon cycle (Gattuso et al. 1998b; Ver et al. 1999; Chen et al. 2003, 2004) and is a key habitat for many calcifying organisms. There is as yet, no published account of experimental apparatus which enables the combined effects of higher seawater temperatures and lower pH on intertidal habitats/organisms to be investigated.

Building on the experience of Widdicombe & Needham (2007) and Spicer (unpublished observations) a flow-through tidal microcosm system was developed which allows the investigator to simultaneously manipulate temperature and  $CO_2$  to simulate a realistic intertidal scenario. The system was designed primarily with the aim of investigating survival, growth and development of larvae, juveniles and adults of species from the

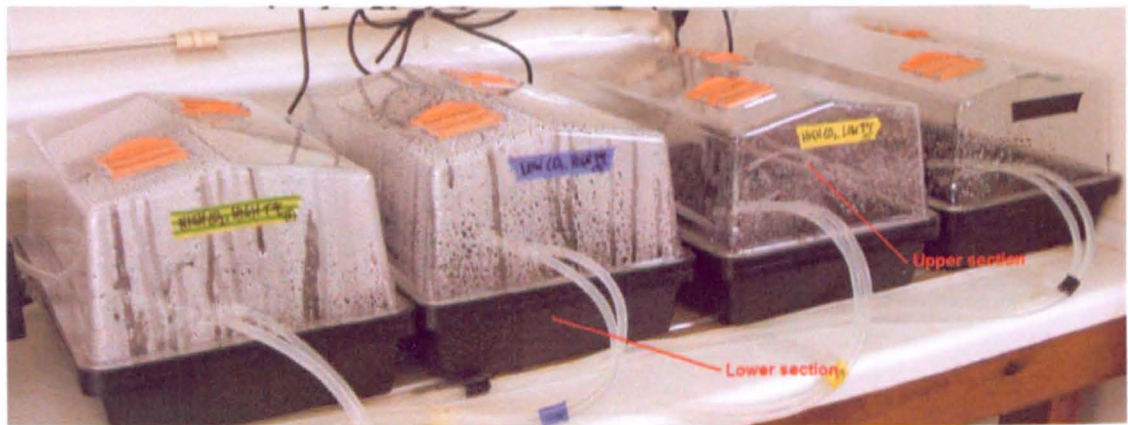
intertidal zone; it is also clearly a suitable experimental tool for tackling a wide range of questions related to the development, settlement and growth of intertidal organisms. The incorporation of a cheap, yet reliable gas mixing system builds on previous gas mixing techniques allowing the investigator to easily control the gas mix over long periods of time (weeks, months). The small scale of the system allows it to be portable between laboratories and flexible with respect to the variety of organisms that can be studied. The stability, precision and reproducibility of this system have been assessed by investigating the response of benthic post-larvae to the experimental conditions created by this system. The target conditions and variability of the system are discussed in comparison to a similar experiment conducted using the mesocosm system of Widdicombe & Needham (2007) and data extracted from the literature.

## **2.2. MATERIALS AND METHODS**

### **2.2.1. The microcosms**

Microcosms were constructed by adapting variable controlled, commercially available (B&Q), electric heated plant propagators (30 x 15 x 20 cm). Each microcosm consisted of two parts: a lower section containing the internal heating elements and an upper section (figure 2.1). The heating elements were connected to an enclosed controllable thermostat which regulated the temperature, *via* a thermocouple, to between ambient (in this case 14 °C) and 40 °C ( $\pm 0.01$ ); the heat was spread evenly throughout the lower section. The upper section contained two adjustable vents, which prevented a build-up of gas that might otherwise have occurred from the constant addition of high-CO<sub>2</sub> air. In each microcosm there was water input and outlet pipes and an air/high-CO<sub>2</sub> air input pipe. The air input pipe had an aquarium air stone attached to maintain fine air bubbling into the bottom of the lower section.

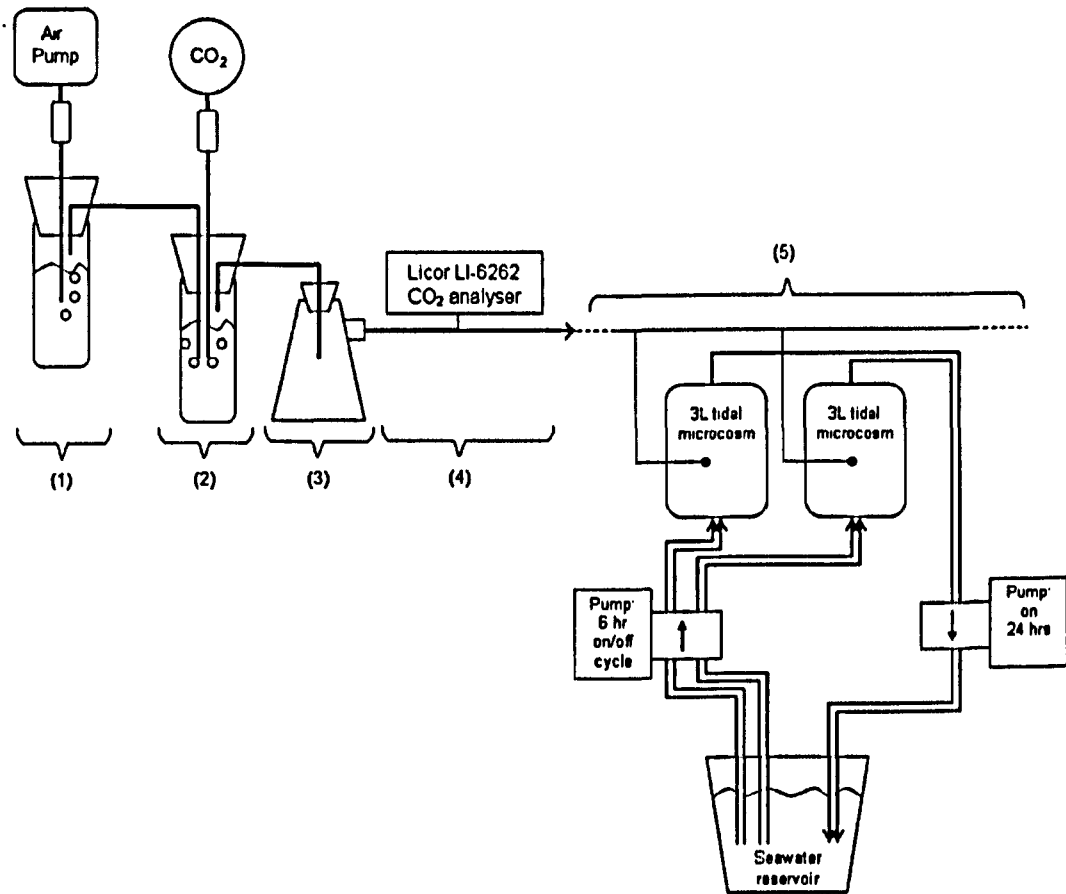
The ambient system discussed here was set up for present (year 2008) southwest UK seawater temperatures, which range from about 8 °C to 15 °C; however, alternative temperatures can be achieved most simply by setting up the experimental system in a suitable controlled temperature/environment room. The use of chillers and heaters (e.g. an Aqua Medic Minicooler) would also be possible, depending on the size of the containers used as microcosms.



*Figure 2.1: Four tidal microcosms showing the upper section and lower section. Also pictured are the water inflow pipes.*

### **2.2.2. CO<sub>2</sub> manipulation**

The concentration of carbon dioxide (CO<sub>2</sub>) flowing into the microcosms was manipulated through an air-CO<sub>2</sub> gas mixing system (figure 2.2). In this system air was pumped through a flow meter ((1) in figure 2.2), into an airtight and pressure resistant bottle (e.g. a 1 l Dreschel mixing bottle) containing 4 % NaOH which both cleaned the air and removed the CO<sub>2</sub>; ((2) in figure 2.2) carbon dioxide (BOC, CP Grade Carbon dioxide 99.995%) was pumped through a second flow meter into a second airtight and pressure resistant bottle (in this case a 1 l Buchner mixing flask) containing distilled water. The cleaned air was also passed into this mixing flask and the use of aquarium air stones, which created fine bubbles of both CO<sub>2</sub> and air, allowed the two gases to mix ((3) in figure 2.2). The mixed high-CO<sub>2</sub> air then fed into a final airtight and pressure resistant bottle (here a 1 l Buchner flask)



*Figure 2.2: Air-CO<sub>2</sub> mixing system consisting of (1) air pumped through a flow meter into a mixing vessel containing soda-lime to remove the CO<sub>2</sub>, (2) pure CO<sub>2</sub> pumped through a flow meter into a mixing vessel along with the CO<sub>2</sub>-free air, (3) mixed high-CO<sub>2</sub> air flows into another vessel to dry the air before (4) passing through a Licor air analyser and on to the microcosms. The tidal system (5) consists of several microcosms connected to the high-CO<sub>2</sub> air or untreated air and seawater, controlled by two pumps: an inflow pump (6 h on/off cycle) and an outflow pump (on 24 h).*

before flowing through a Licor LI-6262 CO<sub>2</sub> analyser ((4) in figure 2.2) and then out to the microcosms. The flow rates of the air and the CO<sub>2</sub> were adjusted to create the final high-CO<sub>2</sub> air mix, for example to create a CO<sub>2</sub> of 1,250 ppm, a flow of 6.25 ml min<sup>-1</sup> of CO<sub>2</sub> needs to be mixed with cleaned air flowing at 5 l min<sup>-1</sup>. The composition of the mixture was monitored using the CO<sub>2</sub> analyser. The high-CO<sub>2</sub> air was bubbled continuously into the upper section of the microcosms using air stones (see above), allowing the seawater CO<sub>2</sub> concentration to reach equilibrium. The control (low CO<sub>2</sub>) microcosms were bubbled

with ambient air pumped using an air pump (Hailea Model V-20). Standard aquarium connectors and valves were used to connect non-porous silicone tubing to the air stones. The bubbling rate was controlled using standard aquarium valves, producing a flow rate of about 50 cc min<sup>-1</sup> into each microcosm. To produce a greater flow rate overall an additional valve was constantly open to release the remaining air flow. The pH (NBS scale) and CO<sub>2</sub> concentration were measured as the water was supplied to the microcosms (see section 2.3.1).

To investigate a wide range of CO<sub>2</sub> concentrations, the CO<sub>2</sub> manipulation set up would need to be replicated for each CO<sub>2</sub> concentration, with the exception of using one Licor CO<sub>2</sub> analyser to periodically analyse all the different CO<sub>2</sub> concentrations. An improvement (although more expensive) to this set up would be to use digital flow meters (e.g. use of a standard solenoid valve coupled to an mV controller), to monitor the pressure and flow regulating the CO<sub>2</sub> and air flow accordingly thus maintaining a precise CO<sub>2</sub>-air mix.

### **2.2.3. Tidal mechanism**

The mechanism used to mimic tidal conditions is presented in (5) in figure 2.2. A peristaltic pump (Watson-Marlow 503S 6 h on/off cycle, manipulated with a commercially available electrical timer) was used to pump seawater from a reservoir (vol. = 15 l, S = 35.7) into each microcosm. A second pump continually pumped the seawater out of the microcosms back into the seawater reservoir. This produced a tidal cycle of low tide (microcosms are empty), flooding tide (pump 1 on, pump 2 on, microcosms take 6 h to fill), high tide (full microcosms) and ebbing tide (pump 1 off, pump 2 on, microcosms take 6 h to empty). Peristaltic pump tubing (Gradko International Ltd 116-0536-18) and silicone tubing (Fisher Scientific FB56471) were used to feed seawater through the system.



The outflow pump was set to produce a flow rate of  $8.3 \text{ ml min}^{-1}$  so as to remove 3 l of water in 6 h; however it remained constantly on, therefore the inflow pump was set to a flow rate of  $16.6 \text{ ml min}^{-1}$  to fill the 3 l microcosms during the 6 h flood, while allowing a continuous flow-through of water. Alternatively digital timers can be used to set the exact tidal regime with the inflow pump turning on and the outflow pump turning off at low tide and *vice versa* at high tide.

## **2.2.4. Evaluation of the microcosm**

### **2.2.4.1. Environmental conditions**

To evaluate the ability of the system to reproduce a high temperature, high  $\text{CO}_2$  atmosphere and equilibrated seawater conditions, an experiment was conducted in which each microcosm was set up with a specific combination of temperature and  $\text{CO}_2$  levels. These experimental combinations enabled an assessment of the system's suitability for mimicking real climate scenarios. The experiments ran for 30 days in a controlled-temperature environment (precision  $\pm 1 \text{ }^\circ\text{C}$ ). Water measurements of  $\text{CO}_2$  concentration (Model GS-136CO-1S micro  $\text{CO}_2$  electrode, Lazar Research Labs), pH (InLab413SG Mettler-Toledo pH meter and combination temperature electrode), temperature and salinity (WTW LF197 Salinity probe) were obtained every second day at high tide. The carbonate system variables, total dissolved inorganic carbon (DIC), total alkalinity ( $A_T$ ), carbonate ion concentration and saturation states of calcite ( $\Omega_{\text{cal}}$ ) and aragonite ( $\Omega_{\text{arag}}$ ), were calculated from measured pH and  $\text{CO}_2$  concentration data using MatLab (version 6.1.0.451) *csys.m* programme from Zeebe and Wolf-Gladrow (2001) ([www.awi-bremerhaven.de](http://www.awi-bremerhaven.de)) using the solubility constants of Mehrbach et al. (1973).

### **2.2.4.2. Suitability for biological experiments**

Several medium-term experiments have now been carried out using this system; these include three barnacle experiments using *Semibalanus balanoides* and *Elminius modestus*

and one limpet experiment using *Patella vulgata*. The initial experiments on *S. balanoides* and *E. modestus* involved placing individuals into each of four tidal microcosms set at year 2008 summer conditions characterised by low temperature (14 °C) and low CO<sub>2</sub> concentration (380 ppm) (Low Temperature Low CO<sub>2</sub> (LTLC) scenario) and year 2100 summer conditions, based on IPCC 2007 A2 scenario of a 4 °C warming and a CO<sub>2</sub> concentration of 1,250 ppm, (High Temperature High CO<sub>2</sub> (HTHC) scenario) (Table 2.1). *S. balanoides* were collected by attaching settlement panels (10 cm x 10 cm tiles) to north-facing rocks mid-shore at Looe, England (50°20'N, 4°27'W), for one week (beginning 30<sup>th</sup> April 2007) during barnacle settlement. On collection the panels contained a mixed age population of barnacles ranging from newly settled cyprids to week-old metamorphs. Three settlement panels were placed in each microcosm. Photographs of each panel were taken at low tide on day 1 and day 30 and every second day in between. A stand was set up so that the camera (FujiFilm A510 FinePix digital camera) and plate were aligned consistently. The photographic images were analysed using image analysis (Image-Pro Plus v.4.5, Media Cybernetics) to estimate both abundance (number of animals per plate) and survival (measured as numbers surviving from one day to the next). The experiment was repeated for settled *E. modestus*.

Table 2.1: Experimental conditions of temperature (*T*), carbon dioxide concentration (CO<sub>2</sub>) and pH (30 day mean values and ±95 % C.I.)

	Low Temp	High Temp
Low CO <sub>2</sub>	T: 14.4 °C (± 0.25) CO <sub>2</sub> : 400 ppm (±17) pH: 8.04 (± 0.027)	T: 19.7 °C (± 0.25) CO <sub>2</sub> : 405 ppm (± 23) pH: 8.06 (± 0.030)
High CO <sub>2</sub>	T: 14.6 °C (± 0.27) CO <sub>2</sub> : 1,100 ppm (± 43) pH: 7.74 (± 0.022)	T: 19.9 °C (± 0.26) CO <sub>2</sub> : 1,103 ppm (± 43) pH: 7.73 (± 0.025)
		IPCC 2100 A2 scenario

The limpet experiment was carried out by collecting adult limpets on small rock chips, which contained the limpet's home scar (6 cm < length < 30 cm) from mid-shore at Looe,

England on 13<sup>th</sup> November 2007 and placing 10 individuals in each microcosm. This experiment was run for 36 days in four microcosms - two replicate control microcosms (T = 12 °C, CO<sub>2</sub> = 360 ppm) and two replicate high-CO<sub>2</sub> microcosms (T = 12°C, CO<sub>2</sub> = 1,250 ppm). Shell measurements were made (shell length, height and width across the apex) and records of numbers surviving were maintained. Light was provided by four lights (Polylux XL 58W, 5,200 Lm) with an 8 h on/ 16 h off cycle.

The third barnacle experiment (*S. balanoides*) involved collecting adults on small rock chips from the mid-shore at Looe, England on 23<sup>rd</sup> November 2007 and placing in excess of 400 individuals in each microcosm. The experiment was run for 91 days in four microcosms – two replicate control microcosms (T = 12 °C, CO<sub>2</sub> = 370 ppm) and two replicate high-CO<sub>2</sub> microcosms (T = 12 °C, CO<sub>2</sub> = 1,250 ppm). Survival of barnacles, as an average for each microcosm, was calculated from photographic images analysed using image analysis, as above. The lights (Polylux XL 58W, 5,200 Lm) was set to within 15 min of the sunrise/sunset times of London, UK on a weekly basis, this ranged from roughly 8 h on/ 16 h off cycle in December, 9 h on/15 h off cycle in January, and 10 h on/ 14 h off cycle in February.

## **2.3. RESULTS**

### **2.3.1. Evaluation of the microcosm**

#### ***2.3.1.1. The tidal cycle***

The tidal cycle was maintained for the duration of each experiment (c. 30 days or c. 90 days). CO<sub>2</sub> concentration varied the most over each tidal cycle (figure 2.3, triangles) but not significantly enough to affect pH and DIC (figure 2.3, circles and crosses).

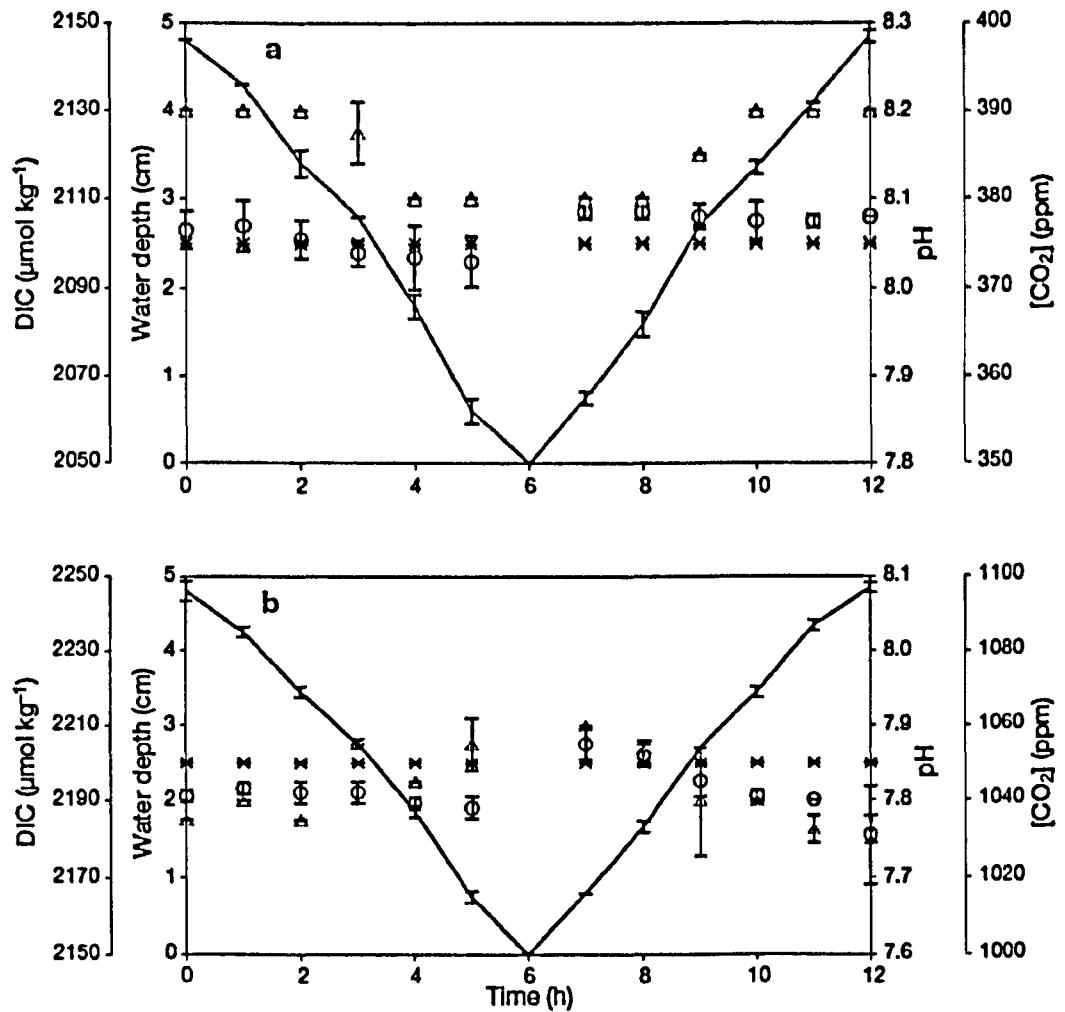


Figure 2.3: pH (circles), DIC  $\mu\text{mol kg}^{-1}$  (crosses) and  $\text{CO}_2$  concentration ppm (triangles) over four tidal cycles (mean values given with error bars representing the 95% C.I.). Also illustrated is the depth (cm) of water throughout the tidal period. (a) low  $\text{CO}_2$  treatment and (b) high  $\text{CO}_2$  treatment.

### 2.3.1.2. Measured values of pH, $\text{CO}_2$ and DIC

In the first two barnacle experiments, only pH and  $\text{CO}_2$  concentration were measured. They showed that conditions could be maintained relatively stable over 30 day periods with only small fluctuations. The atmospheric  $\text{CO}_2$  concentrations were maintained on average at 400 ppm and 1,100 ppm respectively in the low and high  $\text{CO}_2$  scenarios (Table 2.2). The control was higher than the required value (i.e 380 ppm) as result of a slight build-up of ambient  $\text{CO}_2$  within the laboratory. There was greater variation in the high  $\text{CO}_2$  scenarios: 95 % C.I. were  $\pm 9$  ppm and  $\pm 12$  ppm in the low  $\text{CO}_2$  scenarios and  $\pm 18$  ppm and  $\pm 14$  ppm in the high  $\text{CO}_2$  scenarios. The seawater pH was thus maintained at  $8.05 (\pm 0.028)$ ,

8.06 ( $\pm 0.030$ ), respectively in the low CO<sub>2</sub> scenarios and 7.72 ( $\pm 0.021$ ) and 7.73 ( $\pm 0.016$ ), respectively in the high CO<sub>2</sub> scenarios. The pressure of the air and the CO<sub>2</sub> both need to be checked and modified throughout the experimental period to prevent any alterations in the mixing ratios. There was a slight decline in the mixing system air pressure over the experimental time period which caused a change in the pH (increase by 0.03 unit over 30 days) and CO<sub>2</sub> (decrease by ~10 ppm over 30 days) in the low CO<sub>2</sub> scenarios and an opposite effect in the high CO<sub>2</sub> scenarios (pH decreased by 0.04 over 30 days; CO<sub>2</sub> increased by ~ 60 ppm over 30 days).

In subsequent experiments the DIC was measured in addition to CO<sub>2</sub> and pH. This gave a much more stable value for DIC (Table 2.2) and as a result, better calculated values for the carbonate system (see section 2.3.1.4). The CO<sub>2</sub> was prevented from accumulating in the laboratory and was thus maintained reliably at the chosen levels. In the shorter (30 day) limpet experiment the mean  $\pm$  95 % C.I. for CO<sub>2</sub> concentration was 362 ppm ( $\pm 4.4$ ) and 1,258 ppm ( $\pm 120$ ) and for pH levels was 7.94 ( $\pm 0.042$ ) and 7.63 ( $\pm 0.067$ ), respectively. In the longer *S. balanoides* experiment, the CO<sub>2</sub> concentration was 377 ppm ( $\pm 8.5$ ) and ,1270 ppm ( $\pm 92$ ), while the pH was 8.07 ( $\pm 0.068$ ) and 7.7 ( $\pm 0.075$ ), respectively.

### **2.3.1.3. Temperature and salinity**

In the first two barnacle experiments the controlled-temperature room maintained the ambient air temperature at an average of 14°C. The seawater in the low temperature scenario incubators mirrored the ambient air temperature in the upper sections and these sections were maintained at 14.4 °C ( $\pm 0.25$ ) and 14.7 °C ( $\pm 0.28$ ) (mean  $\pm$  95 % C.I.). The seawater in the high temperature treatments were maintained at 19.8 °C ( $\pm 0.25$ ) and 19.7 °C ( $\pm 0.27$ ) reflecting a 5 °C increase in ambient temperature through heating of the base of each microcosm. The seawater used in the experiment had an average salinity of 35.7 ( $\pm 0.33$ ) (Table 2.2).

In the winter experiments, the limpets were maintained at 11.7 °C ( $\pm 0.36$ ) and S = 36.4 ( $\pm 0.96$ ). Over the longer term *S. balanoides* experiment the temperature was slowly increased from 11.1 °C ( $\pm 0.1$ ) to 13.1 °C ( $\pm 0.1$ ) over the 90 day period to reflect and increase in seawater temperature during spring. Salinity was maintained at 35.5 ( $\pm 1.43$ ) over the whole period.

#### **2.3.1.4. Calculated values of carbonate system variables**

Calculated alkalinity ( $A_T$ ) and total dissolved inorganic carbon (DIC) showed greater fluctuations over the initial barnacle experiments and displayed greater variation between treatments than measured pH and CO<sub>2</sub> values (figure 2.4a; mean  $A_T = 2,435 \mu\text{Eq kg}^{-1}$  ( $\pm 98$ ) and mean DIC = 2,554  $\mu\text{mol kg}^{-1}$  ( $\pm 78$ ) in the low CO<sub>2</sub> scenarios and mean  $A_T = 2,554 \mu\text{Eq kg}^{-1}$  ( $\pm 76$ ) and mean DIC = 2,445  $\mu\text{mol kg}^{-1}$  ( $\pm 70$ ) in the high CO<sub>2</sub> scenarios). [CO<sub>3</sub><sup>2-</sup>] was greatest in the high temperature low CO<sub>2</sub> (HTLC) treatment (215  $\mu\text{mol kg}^{-1}$ ) and lowest in the low temperature high CO<sub>2</sub> (LTHC) treatment (95  $\mu\text{mol kg}^{-1}$ ) confirming that both temperature and CO<sub>2</sub> have an affect on the concentration of carbonate ion in the water. The decrease in [CO<sub>3</sub><sup>2-</sup>] from the year 2008 (LTLC) to the A2 2100 scenario (HTHC) is not as large as might be expected if the concentration had been calculated using only an increased CO<sub>2</sub> level and not an increased temperature level. The calcite saturation state ( $\Omega_{\text{cal}}$ ) remained saturated in all treatments although did decrease to a level that may be marginal for dissolution of calcium carbonate; saturation state was over 30 % lower in the HTHC scenario compared to the control. The aragonite saturation state ( $\Omega_{\text{arg}}$ ) is again not undersaturated in any of the treatments, although it is again 30 % lower in the HTHC scenario compared to the control (figure 2.4c).

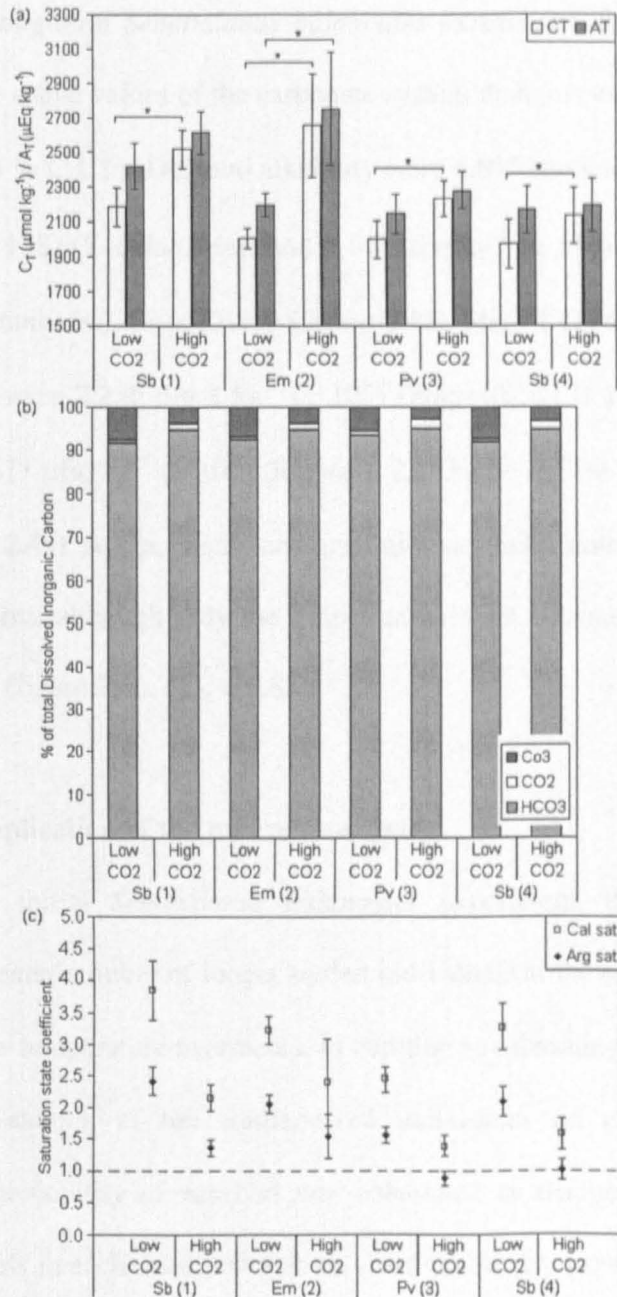


Figure 2.4: (a) Mean alkalinity ( $A_T$ ) and total dissolved inorganic carbon (DIC) concentrations for the low  $\text{CO}_2$  treatments and the high  $\text{CO}_2$  treatments for each experiment (Sb(1) = 30 day *Semibalanus balanoides*, EM(2) = 30 day *Elminius modestus*, Pv(3) = 30 day *Patella vulgata*, Sb(4) = 90 day *S. balanoides*), (error bars represent 95 % C.I.); (b) the composition of the dissolved inorganic carbon in each of the treatments in all the experiments, given as  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$  and  $\text{CO}_2$  percentage of the total dissolved inorganic carbon; (c) mean calcite and aragonite saturation states for each of the treatments in all experiments, error bars represent 95 % C.I. and the dashed line represents the a saturation state of 1, below this line calcite and aragonite will be undersaturated.

In the limpet and long-term *Semibalanus balanoides* experiment, the measured value of DIC produced more stable values of the carbonate system than just using pH and CO<sub>2</sub>. The low CO<sub>2</sub> mean ± 95 % C.I. for DIC and alkalinity were 1,995 μmol kg<sup>-1</sup> (± 106) (limpets), 1,968 μmol kg<sup>-1</sup> (± 138) (*S. balanoides*) and 2,144 μEq kg<sup>-1</sup> (± 114) (limpets), 2,163 μEq kg<sup>-1</sup> (± 135) (*S. balanoides*), respectively (figure 2.4a). High CO<sub>2</sub> mean ± 95 % C.I. for DIC and alkalinity were 2,230 μmol kg<sup>-1</sup> (± 105) (limpets), 2,131 μmol kg<sup>-1</sup> (± 160) (*S. balanoides*) and 2,277 μEq kg<sup>-1</sup> (± 106) (limpets), 2,193 μEq kg<sup>-1</sup> (± 149) (*S. balanoides*), respectively (figure 2.4a). Again, calcite and aragonite saturation states were reduced in the high CO<sub>2</sub> experiments, although only the limpet experiment became undersaturated with respect to aragonite (figure 2.4c, Ω<sub>arg</sub> = 0.88).

### 2.3.2. Biological application of the microcosm system

By chance in the initial *Semibalanus balanoides* experiment, the high temperature treatments had a greater number of longer settled individuals at the start of the experiment compared to the low temperature treatments. In addition to estimating the growth of all the individuals, a sub-sample of ten similar-sized individuals on each panel was also investigated. The probability of survival was calculated as the percentage of the total number of individuals in each treatment that survived the 30 day experiment, multiplied by the percentage of individuals that survived in the sub-sample, to account for relative sizes. It was clear that when simulating 2008 conditions (LTLC), the system was able to maintain almost complete survivorship of barnacles (97 %). In the other treatment combinations, survivorship decreased with each treatment when the size variability was accounted for: LTHC 89 %, HTLC 73 % and HTHC 59 %. In general individuals that were small at the start of the experiment were less likely to survive, particularly in the higher temperature treatments. The increased CO<sub>2</sub> treatments showed more mortality than the LTLC treatment but the difference was not significant. When temperature and CO<sub>2</sub> were both increased there was a significant reduction in cyprid and juvenile survival.



The second barnacle study (*Elminius modestus*) had a more even distribution of new and longer-settled individuals. Survival was highest in the LTLC conditions 80 %, and was reduced slightly 76 %, 70 % and 69 %, respectively in the LTHC, HTLC and HTHC treatments. There was 100 % survival in all limpet treatments, except for in microcosm three (low CO<sub>2</sub> control), one individual died. In the third barnacle experiment (90 days, *S. balanoides*) 69 % survived on average in the controls compared to an average of 48 % surviving in the low pH treatments.

#### **2.4. DISCUSSION**

The system described in this chapter reliably simulates the environmental conditions associated with raised atmospheric CO<sub>2</sub> and temperature under a variety of climate change scenarios. It has been demonstrated that it is possible to incorporate a tidal mechanism into this system enabling experiments to be conducted on intertidal organisms. The success of this system is primarily a result of it being a two phase system in which atmospheric CO<sub>2</sub> was controlled and seawater CO<sub>2</sub> concentration was able to equilibrate with the atmosphere. This provided a good simulation of the natural mechanism of oceanic uptake of CO<sub>2</sub> but on a much smaller scale and faster timescale. Importantly, the system was able to lower and maintain seawater pH without significantly altering the alkalinity of the water.

Temperature was controlled to mimic the heating of rock surfaces during periods of emersion and cooling with the flood tide, thus allowing some thermal relief over the tidal cycle. Previous acidification systems have not included a heating system although such systems have been used in global climate warming experiments (McKee et al. 2000;

Table 2.2: Experimental carbonate system data (mean  $\pm$  95% C.I.) for the four experiments: Sb(1) = 30 day *Semibalanus balanoides*, EM(2) = 30 day *Elminius modestus*, Pv(3) = 30 day *Patella vulgata*, Sb(4) = 90 day *S. balanoides* for each treatment (LC = low CO<sub>2</sub> and HC = high CO<sub>2</sub>). For Sb(1) and EM(2) salinity, temperature, pH and CO<sub>2</sub> data were measured, all other data (A<sub>T</sub> = total alkalinity; DIC = dissolved inorganic carbon;  $\Omega_{\text{calcite}}$  = calcite saturation state;  $\Omega_{\text{aragonite}}$  = aragonite saturation state) were calculated from pH and CO<sub>2</sub> using MatLab *csys.m* from Zeebe & Wolf-Gladrow (2001) ([www.awi-bremerhaven.de](http://www.awi-bremerhaven.de)) and using solubility constant of Mehrbach et al. (1973). For Pv(3) and Sb(4) salinity, temperature, pH, CO<sub>2</sub> and DIC were measured, all other values were calculated from pH and DIC.

	Treatment							
	Sb (1)		Em (2)		Pv (3)		Sb (4)	
	LC	HC	LC	HC	LC	HC	LC	HC
Salinity	35.7 ( $\pm$ 0.30)	35.6 ( $\pm$ 0.32)	34.5 ( $\pm$ 0.33)	34.5 ( $\pm$ 0.30)	36.4 ( $\pm$ 0.97)	36.4 ( $\pm$ 0.96)	35.5 ( $\pm$ 1.43)	35.6 ( $\pm$ 1.44)
Temperature (°C)	14.4 ( $\pm$ 0.25)	14.8 ( $\pm$ 0.27)	14.7 ( $\pm$ 0.23)	14.9 ( $\pm$ 0.20)	11.7 ( $\pm$ 0.36)	11.7 ( $\pm$ 0.35)	11.8 ( $\pm$ 0.42)	11.9 ( $\pm$ 0.44)
pH	8.05 ( $\pm$ 0.028)	7.72 ( $\pm$ 0.021)	7.96 ( $\pm$ 0.022)	7.73 ( $\pm$ 0.051)	7.93 ( $\pm$ 0.030)	7.63 ( $\pm$ 0.040)	8.07 ( $\pm$ 0.045)	7.689 ( $\pm$ 0.055)
DIC ( $\mu\text{mol kg}^{-1}$ )	2185 ( $\pm$ 110)	2517 ( $\pm$ 113)	2003 ( $\pm$ 58)	2652 ( $\pm$ 301)	1995 ( $\pm$ 106)	2230 ( $\pm$ 105)	1968 ( $\pm$ 138)	2131 ( $\pm$ 160)
A <sub>T</sub> ( $\mu\text{Eq kg}^{-1}$ )	2417 ( $\pm$ 133)	2613 ( $\pm$ 124)	2192 ( $\pm$ 69)	2753 ( $\pm$ 329)	2144 ( $\pm$ 114)	2277 ( $\pm$ 106)	2163 ( $\pm$ 135)	2193 ( $\pm$ 149)
CO <sub>3</sub> <sup>2-</sup> ( $\mu\text{mol kg}^{-1}$ )	169 ( $\pm$ 18.9)	95 ( $\pm$ 9.1)	137 ( $\pm$ 9.0)	101.7 ( $\pm$ 23.7)	110 ( $\pm$ 9.0)	63.1 ( $\pm$ 8.2)	146 ( $\pm$ 21.0)	71 ( $\pm$ 14.0)
$\Omega_{\text{calcite}}$	3.84 ( $\pm$ 0.46)	2.15 ( $\pm$ 0.20)	3.22 ( $\pm$ 0.22)	2.40 ( $\pm$ 0.69)	2.44 ( $\pm$ 0.21)	1.39 ( $\pm$ 0.17)	3.27 ( $\pm$ 0.37)	1.58 ( $\pm$ 0.25)
$\Omega_{\text{aragonite}}$	2.42 ( $\pm$ 0.23)	1.36 ( $\pm$ 0.13)	2.06 ( $\pm$ 0.14)	1.54 ( $\pm$ 0.35)	1.56 ( $\pm$ 0.13)	0.88 ( $\pm$ 0.11)	2.09 ( $\pm$ 0.23)	1.01 ( $\pm$ 0.157)

Baulch et al. 2003). Liboriussen et al. (2005) controlled and altered temperature in shallow lake mesocosm system continuously for 16 months. Their flow-through heating system was able to maintain, and vary, the temperature according to seasonal settings with little deviation from the target temperature (the deviation ranged between 0.11 °C and 0.26 °C). The seawater temperature in the system described here displayed larger deviation from the target temperature of 14 °C and 19 °C by 0.80 °C ( $\pm 0.17$ ) and 0.84 °C ( $\pm 0.61$ ) respectively, compared to Librouissen et al. (2005). However, as with Librouissen et al. (2005), the temperature control allows the system to be manipulated on a seasonal basis and is monitored periodically to prevent over or under heating.

The tidal section was created as a flow-through system with a relatively short water residence time (3 h). The tidal system enabled a six hourly semidiurnal tidal cycle to be used, although such a cycle deviates from both the natural general sinusoidal pattern of tidal ebb and flood and from the 6 h 12 min semidiurnal rhythm often seen in nature. The feeding rhythm of many intertidal animals is synchronised with this longer tidal cycle and so over a long period of time animal feeding patterns may become out of synchrony with the system if they are maintained in a constant 6 h tidal system. The advantage of the tidal flow-through system was to prevent evaporation and salinisation in the microcosms, particularly at the higher temperatures. In addition, with the incorporation of appropriate controllers, such as a digital programmable commercially available electronic timer, a 6 h 12 min cycle, or indeed any rhythm, could be achieved.

In the course of these experiments no investigation was carried out on whether there was a significant build up of nutrients or toxins in the system. To date, no tests on nutrients or toxins were carried out because of the small size of the organisms used (barnacles and recently settled limpets) compared to the high turnover rate of the water (average  $0.5 \text{ l h}^{-1}$ ). Although the seawater is recirculated through the system, it is done so for about one week,

and then is replaced with fresh seawater. This change was primarily carried out in order to prevent salinity from increasing, although it should also prevent a major build up of toxins. The reservoir tanks hold nearly twice the amount of seawater necessary to fill the microcosms and the seawater is continually mixed by bubbling with air. If larger organisms are being used, the microcosm size would need to be increased to accommodate them. The husbandry of many common species is routine in marine laboratories and hence it is relatively easy to estimate the necessary turnover rates needed to prevent the organism from being affected by ammonia production, for example. If sediments are to be incorporated into the experimental set up, the investigator needs to carefully consider the flow rate and the volume of the container compared to the volume of sediment, as well as taking into account the sediment depth and nutrient flux from the sediment. To prevent toxin build up or changes in nutrients in larger systems, improvements could be made either using a flow through system where fresh seawater is used every tidal cycle, or incorporating filters into the recirculating system.

One major improvement to the initial barnacle experiments described here, or indeed any acidification system, would be to monitor the carbonate system more rigorously so that more appropriate parameters of the carbonate system are measured. pH and CO<sub>2</sub> concentration are not conservative with respect to changes in state (i.e. temperature and salinity) and thus may not be providing such an accurate account of the carbonate system as could be given by combining these measurements with either alkalinity or total inorganic carbon measurements. The latter two experiments described here additionally measured DIC and gave more stable carbonate system values (Table 2.2). A comparison of the carbonate system results from the two initial barnacle studies described here together with data from the literature and a similar study but using the experimental set-up of Widdicombe & Needham (2007) can elucidate the stability and precision of this set-up. Table 2.3 demonstrates the relative pH values measured at each target CO<sub>2</sub> concentration

in several experiments found in the literature. The pH standard deviations range from as little as 0.01 to 0.11 pH unit. In this study the low and high CO<sub>2</sub> treatments had a pH standard deviation of 0.06 and 0.04 unit respectively, which are within ranges of other experiments described in the literature.

*Table 2.3: pH values ± standard deviation measured for a nominal CO<sub>2</sub> concentration. Values were obtained from the literature to compare with the initial S. balanoides experiment in this study. K04 = Kurihara et al. 2004 (H.p. = Hemicentrotus pulcherrimus (b = before, a = after experiment) and E.m. = Echinometra mathaei), S05 = Shirayama et al. 2005 (1, 2 and 3 represent three replicate tanks, standard deviations not given in literature), Miles07 = Miles et al. 2007, Mich07 = Michaeladis et al. 2007.*

CO <sub>2</sub> (µatm)	This study	K04 (H.p.)b	K04 (H.p.)a	K04 (E.m.)b	S05 1	S05 2	S05 3	Miles07	Mich07
control	8.05 (± 0.06)	7.99 (± 0.10)	7.97 (± 0.16)	8.11 (± 0.00)	7.945	7.937	7.936	7.96 (± 0.07)	8.05 (± 0.02)
560					7.899	7.902	7.897		
860		7.74 (± 0.02)	7.73 (± 0.13)	7.80 (± 0.01)					
1250	7.72 (± 0.04)								
1860		7.59 (± 0.00)	7.56 (± 0.11)	7.68 (± 0.04)					
2340								7.46 (± 0.03)	
3860		7.35 (± 0.04)	7.33 (± 0.08)	7.34 (± 0.02)					
5090									7.3 (± 0.03)
8860		7.03 (± 0.07)	7.16 (± 0.06)	7.13 (± 0.01)					
18860		6.83 (± 0.01)	6.95 (± 0.10)	6.78 (± 0.00)					
22470								6.63 (± 0.11)	

The additional experiments were conducted to repeat the one described here but using *Elminius modestus*, *Patella vulgata* and finally *Semibalanus balanoides*, but over a longer exposure. The conditions remained stable over all experiments and the precision of the first experiment was easily repeated in the others (figure 2.4). The gradual decrease in air pressure seen in the first experiment described above did not occur in the latter

experiments because of awareness of this problem and as such the gas pressures were constantly monitored, *via* the flow rates, and the flow rates were altered as necessary. The confidence intervals shown in figure 2.5 demonstrate that there is much better control (more accuracy) when maintaining the systems at set pH and CO<sub>2</sub> levels using this microcosm system than a large pH feedback system, especially when DIC is additionally measured. It is clear that without measuring DIC or alkalinity, the calculated values are greater than expected and have larger uncertainty than the set up of Widdicombe & Needham (2007).

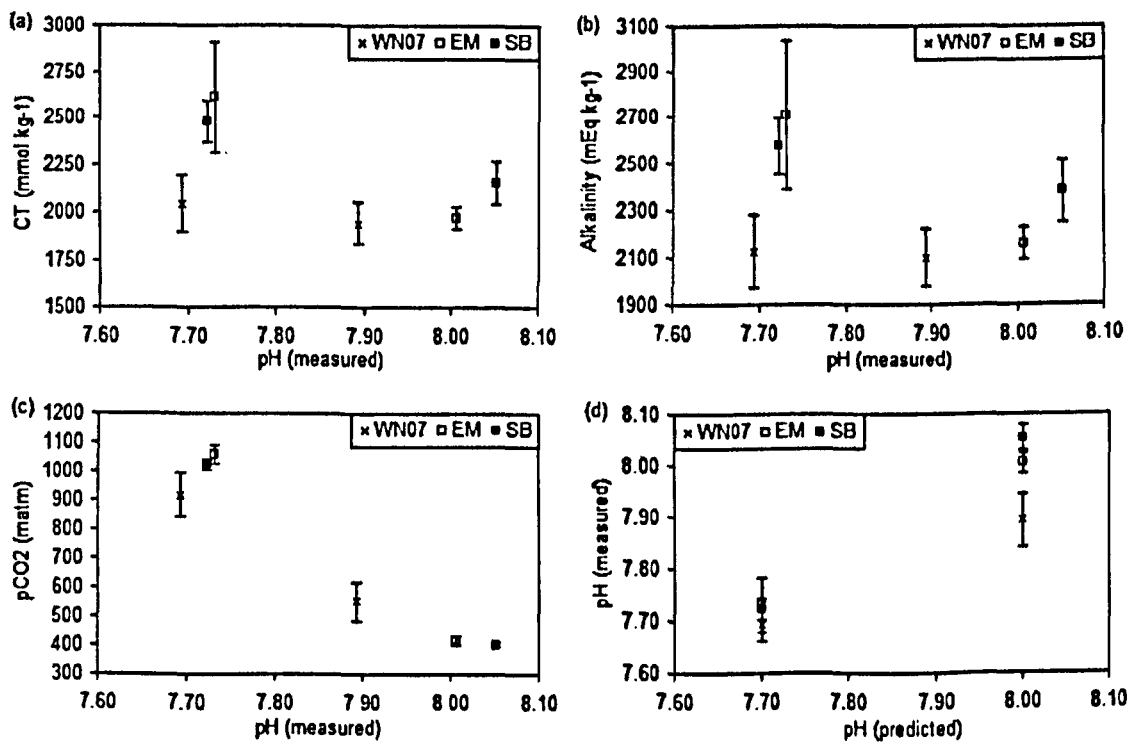


Figure 2.5: Carbonate system values of results from an experiment using Widdicombe & Needham (2007) set-up (crosses, WN07), the initial experiment described here (closed squares, SB) and a second experiment using the same equipment as SB but using a different species (open squares, EM). Values are the mean calculated for the experimental period with error bars showing 95% C.I.. (a) Total dissolved inorganic carbon (DIC) plotted against mean measured pH over the experimental period, (b) alkalinity plotted against mean measured pH over the experimental period, (c) CO<sub>2</sub> plotted against mean measured pH over the experimental period, and (d) pH plotted against predicted/ target pH.

In any application of the system described here it is important also to consider daily and seasonal variability. The conditions were maintained with minimal variability in order to investigate a specific time period within an organism's life-cycle. However, observations have shown that there is a natural seasonal cycle of pH, CO<sub>2</sub> concentration and temperature. pH in the intertidal zone is thought to vary annually by about 1 pH unit, reaching highest levels (~8.5) during winter and lowest (~7.5) in summer as a result of varying levels of biological production and temperature (Hinga 2002); CO<sub>2</sub> concentration also has seasonal and daily cycles as a result of changes in DIC and alkalinity. In the North Atlantic there is a decrease in sea surface CO<sub>2</sub> in summer and an increase in winter predominantly as a result of biological productivity (Sarmiento & Gruber 2006). This system can run for a long period and with minor alterations it would be possible to reproduce seasonal variability in atmospheric CO<sub>2</sub> together with a seasonally varying temperature regime (as demonstrated by the longer-term *S. balanoides* experiment). Such a capability enables investigation of longer-term climate change impacts on whole life cycles of short-lived organisms.

This initial proof-of-concept study has demonstrated that the microcosm system is ideal for small-scale studies and thereby justifies further studies with greater replication. Although it is acknowledged that size matters (Schindler 1998), these microcosm systems allow us to focus on specific conditions and end-point measures in a subject area that is lacking in fundamental data. These microcosms are appropriate for studying organisms from a variety of near-surface environments with a variety of substratum types. The system as described here is relatively small and easy to set up, which makes it adaptable to many locations. Here a controlled-temperature environment was used to maintain ambient conditions and a similar room would be necessary if the *in situ* conditions were highly variable on a daily basis or if the required target conditions were lower than those of the ambient conditions. The ability to move the systems between locations means that the system can reduce long

distance transport of animals and so minimise experimental artefacts resulting from handling and transport.



## **CHAPTER 3. EMBRYONIC DEVELOPMENT**

**Future high CO<sub>2</sub> in the intertidal may compromise adult barnacle (*Semibalanus balanoides*) survival and embryo development rate.**

Some aspects of this chapter have been published in:

Findlay HS, Kendall MA, Spicer JI, Widdicombe S (2009) Future high CO<sub>2</sub> in the intertidal may compromise adult barnacle (*Semibalanus balanoides*) survival and embryo development rate. *Marine Ecology Progress Series*, 389: 193-202

### 3.1. INTRODUCTION

The barnacle *Semibalanus balanoides* is an important space occupier on the rocky shores of northern Europe and America. Its calcareous shell plates provide protection from predators, abrasion, and desiccation (Rainbow 1984). *S. balanoides* is a cross-fertilising hermaphrodite, which develops its egg masses within its shell cavity. Fertilisation in the UK normally occurs by mid-November and the embryos develop over the winter period before hatching between February (near the southern limits of its geographic range, South West England & Northern Portugal) and May (at the northern limits, North UK & Norway) (Barnes 1957; Crisp 1962). Crisp (1959) recorded *S. balanoides in vivo* development times at Brixham (South Devon) and Bangor (North Wales) demonstrating that in early stages there was no appreciable divergence in timing but after stage 8 (as defined by Crisp 1954) development of embryos in Brixham was nearly twice as rapid as development in Bangor. Laboratory studies have shown that the development rate of these embryos is temperature dependent, with a maximum development time occurring in seawater temperatures of ~14 °C, but is also impacted by the availability of oxygen within the egg cavity (Crisp 1959; Lucas & Crisp 1987). Adults of this species undergo a period of lowered metabolism and activity during winter (Rainbow 1984), at which time they carry out oxygenation within the mantle cavity by flushing with seawater during periods of immersion (Barnes et al. 1963). *S. balanoides* offers an opportunity to examine the impacts of ocean acidification on an important space occupier, particularly an ability to focus on the development of eggs and larvae as these stages have been shown in other species to be vulnerable to elevated CO<sub>2</sub>.

Previous studies investigating the impacts of CO<sub>2</sub>-induced acidification on larvae and eggs have shown detrimental impacts on development, growth and survival (Kikkawa et al. 2004; Kurihara et al. 2004; Kurihara & Shirayama 2004; Kurihara et al. 2007; Havenhand et al. 2008; Dupont et al. 2008). However, there have been no specific investigations

regarding the impacts of high CO<sub>2</sub> on embryo development in *S. balanoides* and survival of the adults through this crucial period in their life cycle, when they have minimal food and predominantly rely on lipid reserves for energy. The aim of the present study was to determine whether *S. balanoides*, a species normally exposed to a fluctuating environment, is likely to be impacted by ocean acidification scenarios realistic for the next 100 years; and particularly whether the embryos contained within the calcium carbonate shell cavity, which may be subject to increased dissolution under high CO<sub>2</sub> conditions, would develop normally. The study was conducted using microcosms which simulated immersion and emersion on the shore (Chapter 2) to investigate the effect of elevated CO<sub>2</sub> on (a) the survival of sexually mature adults, during the embryo production and development period, (b) the calcium and magnesium content of the adult shells as a measure of changes in their calcium carbonate shells, (c) the timing of the appearance of different development stages of the embryos within the adults and (d) the timing of the subsequent release of free-swimming nauplii.

## **3.2. MATERIALS AND METHODS**

### **3.2.1. Experimental setup**

*Semibalanus balanoides* adults were collected on small rock chips from the mid-shore at Looe, England (50°20'N, 004°27'W) on 23<sup>rd</sup> November 2007. At least two rock chips were placed haphazardly into each of four microcosms (30 x 15 x 20 cm) within a constant temperature room so that each microcosm contained in excess of 400 individuals (living and dead). Two microcosms were set at control (pH 8.07, CO<sub>2</sub> = 346 ppm) and two were set as high CO<sub>2</sub> treatment (pH 7.70, CO<sub>2</sub> = 922 ppm). The CO<sub>2</sub> concentration, and hence pH level, was maintained in each microcosm using a CO<sub>2</sub> mixing system exactly as described in Chapter 2. pH (NBS scale, Mettler-Toledo pH meter), dissolved inorganic carbon (DIC) (Ciba-Corning 965D Total CO<sub>2</sub> Analyser, Olympic Analytical Service), CO<sub>2</sub> (Licor LI-6262 CO<sub>2</sub> analyser), temperature and salinity (WTW LF197 combination

temperature and salinity probe) were recorded weekly. Total alkalinity, bicarbonate ( $\text{HCO}_3^-$ ), carbonate ( $\text{CO}_3^{2-}$ ), and the saturation states for aragonite and calcite were all calculated from pH and DIC using CO2sys (Pierrot et al. 2006) with dissociation constants from Mehrbach et al., (1973) refit by Dickson & Millero (1987) and  $\text{KSO}_4$  using Dickson (1990). The microcosms worked on a tidal system (Chapter 2) with tide times programmed weekly based on the local Plymouth tide times ( $S = 35$ , water flow  $10 \text{ ml min}^{-1}$ ). Air temperature was set in the controlled-temperature room so that water temperature followed the Plymouth sea surface temperature. Light conditions (Polylux XL 58 W) were set to within 15 min of the sunrise/sunset times for London, UK, on a weekly basis; this ranged from roughly 8 h on/16 h off cycle in December, 9 h on/15 h off cycle in January and 10 h on/14 h off cycle in February. Natural, filtered ( $10 \mu\text{m}$ ), seawater was used in the system and was replenished twice weekly to avoid salinity increases through evaporation. The experiment ran for 104 days.

### **3.2.2. Adult survival and shell mineralogy**

Changes in barnacle abundance on each rock chip were recorded using a digital camera (FujiFilm A510 FinePix) which was maintained in consistent alignment using a stand. The photographic images were analysed (Image-Pro Plus v.4.5, Media Cybernetics) to estimate both abundance and survival. Barnacle survival was estimated from the images taken at the beginning and the end of the experiment by counting living and dead individuals (accounting for individuals removed for sampling). Prior to photography individuals were gently touched to check whether they were able to close their operculum and were counted as dead when the operculum had remained open or the shell was empty.

Adult survival, recorded as a proportion of the total of all rocks within each microcosm, was square root arcsine transformed then tested for normality using a Kolmogorov-Smirnov test and for homogeneity of variances using Levene's test. A one-way nested

ANOVA was used to determine any CO<sub>2</sub> treatment effects, with microcosms nested within CO<sub>2</sub> treatment (n = 2). All statistical analysis was performed using Minitab® 15.1.0.0 (© 2006, Minitab Inc.).

The calcium carbonate composition of the shell was estimated by analysing the calcium ion (Ca) and magnesium ion (Mg) concentrations as a proxy for any changes in calcification or dissolution. Live individuals produce calcium carbonate (calcify) during shell growth, however there may also be some dissolution of the shells; this dissolution, as discussed in the introduction, may be enhanced in high CO<sub>2</sub> conditions. Ca and Mg ions are abundant in seawater and hence are not limiting. Formation of CaCO<sub>3</sub> involves combining inorganic carbon with Ca and often some Mg is also incorporated to form Mg-CaCO<sub>3</sub>. Therefore any observed changes in Ca and Mg should indicate how the shell structure changes over time through calcification and dissolution. The shells of ten individuals were haphazardly selected from each microcosm at the end of the experiment. Shells of ten individuals that were noted as dead at the start of the experiment were also analysed for the concentration of Ca and Mg ions at the end of the experiment. Comparing the concentration in dead animals with the concentrations in live animals provides an estimate of a barnacle's ability to calcify relative to any dissolution effects because calcification will not be taking place in dead individuals. Concentrations of both cations were measured using methods described in Spicer & Eriksson (2003); briefly this involved dissolving the shells in 10 % nitric acid after drying and weighing, then using Inductively Coupled Plasma (ICP) optical emissions spectrometer (Varian 725-ES) to measure Ca and Mg simultaneously. The proportion of Ca and Mg in the shell was calculated knowing the mass of the shell and volume of acid used in the digest.

Calcium and magnesium, recorded as proportions (cation mass [mg]/ total shell mass [mg]), were square root arcsine transformed. Ca/Mg ratio was calculated (mg Ca/ mg Mg) and all

three datasets were tested for normality using a Kolmogorov-Smirnov test and for homogeneity of variances using Levene's test. A two-way nested ANOVA (n = 10) was then used to test for differences between control and high CO<sub>2</sub> and between live and dead barnacles, with microcosm nested within CO<sub>2</sub> treatment.

### 3.2.3. Embryo development

Embryos were maintained within live adult *Semibalanus balanoides* as naturally fertilised broods. On eight occasions during the course of the experiment (days 0 (23<sup>rd</sup> November 2007), 7, 24, 42, 56, 70, 91 and 104) barnacles were removed haphazardly until twenty adults with egg masses were found from each microcosm. After isolating the egg masses in seawater, the embryonic development of 20 eggs from each egg mass at each sample time was determined under low magnification (x40). Developmental stages were assigned using the classification of Achituv & Barnes (1976):

U: Unfertilised

I: Early development from newly laid to few divisions (equivalent stages 1 – 4 in Crisp 1954)

II: Multicellular (equivalent stages 5 – 7 in Crisp 1954)

III: Limb buds developing (equivalent stages 8 – 10 in Crisp 1954)

IV: Nauplius eye apparent (equivalent stages 11 – 12 in Crisp 1954)

IVh: Nauplius hatching (equivalent stage 13 in Crisp 1954); following Crisp (1954) on the two last sample points (day 91 and 104) the embryos were left for five minutes in seawater; if any hatched or they were counted as stage IVh.

Egg development, recorded as the proportion of eggs at each stage (I, II, III, IV and IVh) from 20 barnacles at each sampling time, was square root arcsine transformed and tested for normality using a Kolmogorov-Smirnov test and homogeneity of variances using Levene's test. A repeated measures ANOVA (n = 20) was then performed to determine the

effect of CO<sub>2</sub> treatment and time (day 7, 28, 40, 56, 70 and 104), with microcosm nested within CO<sub>2</sub> treatment.

Development rate was assessed by first calculating the time at which 50 % of the sampled eggs reached each stage, which was calculated by fitting a logistic growth function (as the best model fit) to the embryo stage data and calculating the time of 50 % development. Records of the time taken for each stage to achieve 50 % development were analysed using PERMANOVA (Primer-E) (Anderson 2001) with a nested (replicate microcosms) regression design (development stages I to IV) testing for pH differences. Time at 50 % development for each stage was transformed ( $\text{day}^{0.5}$ ) to produce a linear fit (best model fit with maximum R<sup>2</sup>) whose gradient was taken as the rate of development. Linear regression analysis was then applied to each data set and a 2-tailed regression t-test was used to assess differences between the slopes of the control and high CO<sub>2</sub>.

### **3.3. RESULTS**

#### **3.3.1. Environmental conditions**

The pH was maintained at a mean ( $\pm$  95 % C.I.) of 8.07 ( $\pm$  0.03) and 7.70 ( $\pm$  0.03) in the control and high CO<sub>2</sub> treatment, respectively. Dissolved inorganic carbon (DIC) was on average 1,888 ( $\pm$  90)  $\mu\text{mol kg}^{-1}$  and 2,045 ( $\pm$  83)  $\mu\text{mol kg}^{-1}$ , in the control and high CO<sub>2</sub> treatment, respectively. Total alkalinity (A<sub>T</sub>) was not significantly different between treatments; with an average 2,086 ( $\pm$  101)  $\mu\text{Eq kg}^{-1}$  and 2,115 ( $\pm$  95)  $\mu\text{Eq kg}^{-1}$ , in the control and high CO<sub>2</sub> treatment, respectively (see Chapter 2 for exploration of the variability, control and reproducibility of the carbonate parameter measurements). There was a slight increase in the pH towards the end of the experiment (~day 84) in both control and high CO<sub>2</sub> (figure 3.1), as a result of an increase in salinity at this time. The CO<sub>2</sub> concentration was maintained at a mean ( $\pm$  95 % C.I.) 346 ( $\pm$  27) ppm and 922 ( $\pm$  72) ppm in the control and high CO<sub>2</sub> treatment, respectively. The high CO<sub>2</sub> treatment was

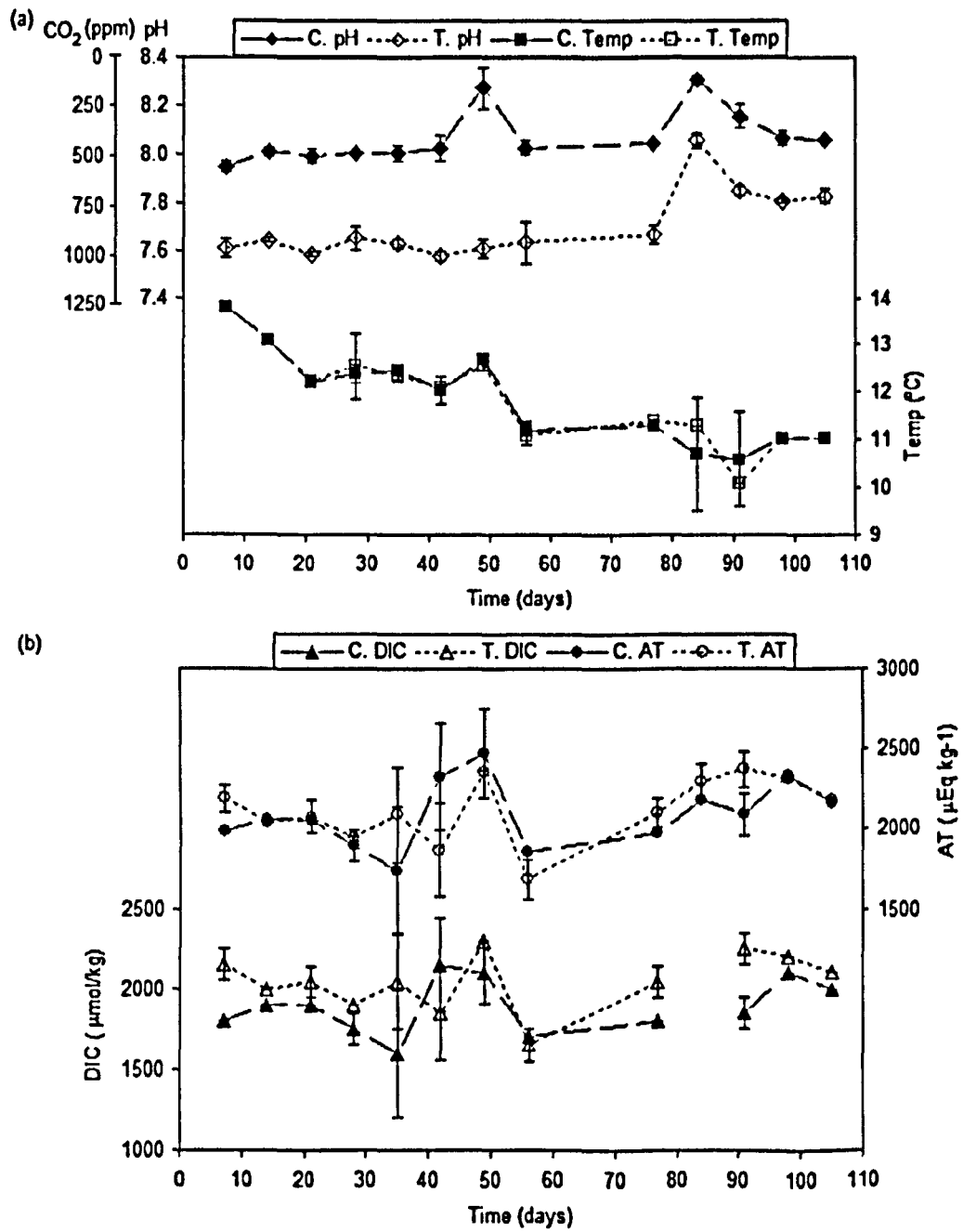


Figure 3.1: (a)  $pH_{NBS}$  (diamonds) and temperature (squares) in the control microcosms (C; filled symbols) and high  $CO_2$  (T; open symbols) microcosms in the over the experimental period and (b) Dissolved inorganic carbon (DIC; triangles) and total alkalinity ( $A_T$ ; circles) in the control microcosms (C. filled symbols) and high  $CO_2$  microcosms (T. open symbols) over the experimental period. Error bars represent 95 % C.I..



undersaturated with respect to aragonite ( $\Omega < 1$ ) and calcite was near saturation ( $\Omega = 1$ ) throughout. Water temperature was an average of 11.9 °C in both treatments, but was set to track local sea surface temperature and hence decreased from 13 °C in November to 10 °C in February (figure 3.1a).

### 3.3.2. Adult survival

Adult survival was significantly lower ( $p = 0.017$ ,  $df = 1$ ) in the high CO<sub>2</sub> treatment than in the control (mean ( $\pm$  95 % CI) 69 ( $\pm$  4.28) % survival compared to mean ( $\pm$  95 % CI) 47 ( $\pm$  4.62) % survival) after 104 days (figure 3.2). There were no significant microcosm effects in any of the end point measures (survival, mineralogy or development).

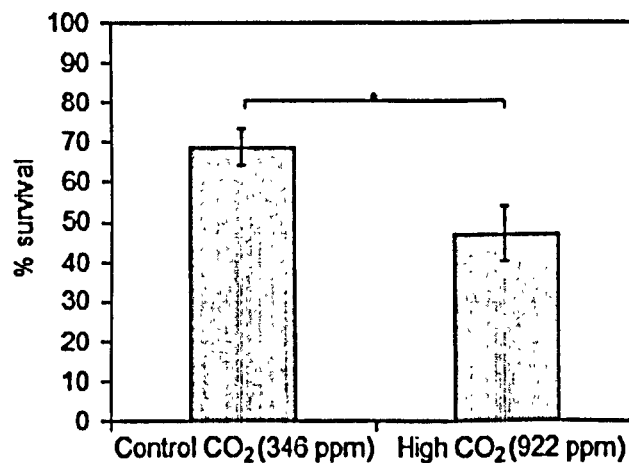


Figure 3.2: Mean percentage of adult *Semibalanus balanoides* barnacles surviving in the control microcosms (CO<sub>2</sub> 346 ppm) and high CO<sub>2</sub> microcosms (922 ppm). Bar with asterisk indicates a significant difference ( $p = 0.017$ ,  $df = 1$ ,  $n = 2$ ). Error bars show the 95 % C.I..

### 3.3.4. Adult shell mineralogy

**Calcium:** The proportion of calcium increased from the control to the high CO<sub>2</sub> treatment in the case of live barnacles but decreased from the control to the high CO<sub>2</sub> in the dead barnacles (figure 3.3a). This indicated that in live barnacles there may have been some dissolution that was compensated for by calcification. However, there was no significant

difference in the proportion of calcium between the treatments or between live or dead barnacles.

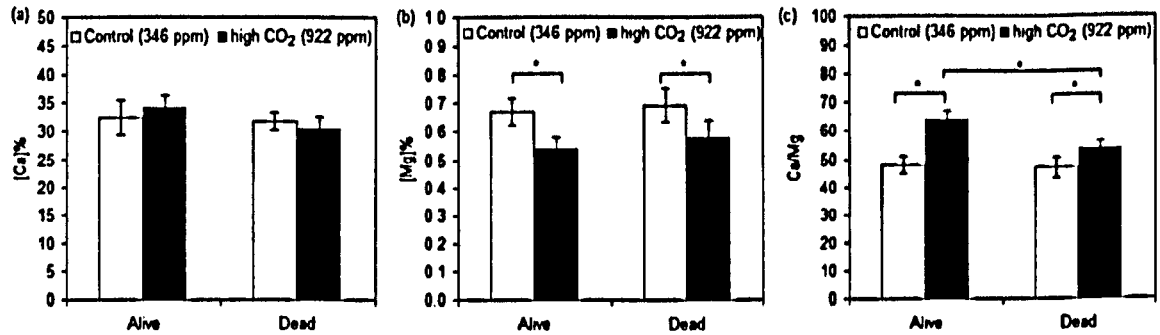


Figure 3.3: Concentrations of (a) calcium and (b) magnesium and (c) the calcium/magnesium ratio in shells of *Semibalanus balanoides* barnacles, in the control ( $\text{CO}_2$  346 ppm) microcosm (white bars) and high  $\text{CO}_2$  (922 ppm) microcosm (dark bars), as a percentage of total shell material in barnacles that were alive for the whole experiment and barnacles that were dead at the end of the experiment. Bar with asterisk indicates a significant difference ( $p < 0.05$ ). Error bars show 95 % C.I..

**Magnesium:** There was a significant difference in the proportion of magnesium between the control and high  $\text{CO}_2$  treatment ( $p < 0.000$ ,  $df = 1$ ) and a small significant difference in magnesium between the live and dead barnacles ( $p = 0.048$ ,  $df = 1$ ), the significance was small because of large variability in the data. There was no significant interaction between the treatment and whether the barnacle was dead or alive. Magnesium proportion decreased from the control to the high  $\text{CO}_2$  treatment in both live and dead barnacles (figure 3.3b).

**Ca/Mg:** There was a significant difference in the Ca/Mg ratio between  $\text{CO}_2$  treatments ( $p = 0.000$ ,  $df = 1$ ) and between live and dead barnacles ( $p < 0.000$ ,  $df = 1$ ), with a significant interaction ( $p = 0.032$ ,  $df = 1$ ). The Ca/Mg ratio increased from the control to the high  $\text{CO}_2$

treatment in both live and dead barnacles but this increase was greatest in living barnacles (figure 3.3c).

### 3.3.5. Embryo development

In excess of 50 % of the eggs in egg masses were fertilised at the start of the experiment (day 0) and all the eggs from fertilised animals had reached stage I by day 7. The ANOVA test (Table 3.1) indicated effects of both time and CO<sub>2</sub> concentration on the development of embryos through each stage (figure 3.4a-e) however this difference resulting from elevated CO<sub>2</sub> occurred most significantly at stages III, IV and IVh. On day 104 around 50 % of the embryos had hatched from the control compared to around 20 % in the high CO<sub>2</sub> treatment (figure 3.4e).

The estimated rate of development (figure 3.4f) was significantly greater (see Table 3.2) in the control (0.24 stages d<sup>-0.5</sup>) than in the high CO<sub>2</sub> treatment (0.22 stages d<sup>-0.5</sup>) for stages I to IV. In the high CO<sub>2</sub> treatment the time to hatching (stage IVh) was delayed by 18.95 days. The control was not significantly slower than the time to hatching observed by Crisp (1959) at Brixham (0.26 stages d<sup>-0.5</sup>), whereas hatching in the high CO<sub>2</sub> was significantly slower than at Brixham (regression analysis:  $t[3.60]$ ,  $t[0.05, 2.447]$ , 2-tailed,  $df = 6$ ). The control development rate was significantly greater than Crisp's (1959) data from Bangor (0.20 stages d<sup>-0.5</sup>) but at the high CO<sub>2</sub> concentration the rate was not significantly greater (regression analysis difference between slopes of control vs Bangor data,  $t[2.69]$ ,  $t[0.05, 2.447]$ , 2-tailed,  $df = 6$ ; regression analysis difference between slopes of low pH vs Bangor data,  $t[1.48]$ ,  $t[0.05, 2.447]$ , 2-tailed,  $df = 6$ ). For the Bangor population Crisp (1959) observed that hatching took 54.5 days longer than the Brixham population.

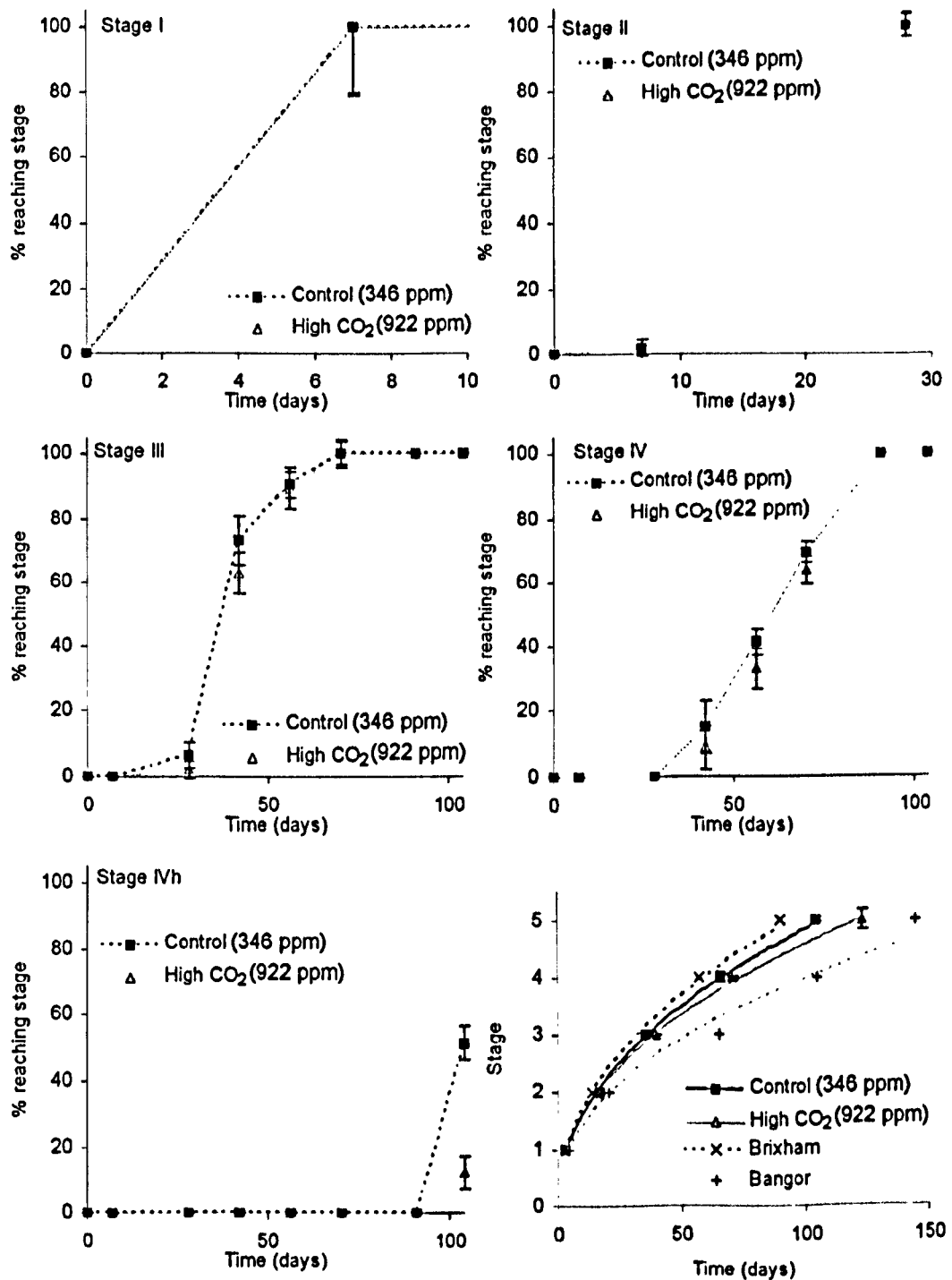


Figure 3.4: Mean percentage of eggs from 20 *Semibalanus balanoides* barnacles reaching each stage (stage I – IVh (a) to (e)) at particular time periods (days after start of experiment) in the control (CO<sub>2</sub> 346 ppm) microcosms (squares) and the high CO<sub>2</sub> (928 ppm) microcosms (triangles). Error bars represent the 95 % C.I.. (f) Time for 50 % of samples to reach each stage in the control (black squares), high CO<sub>2</sub> (grey triangles) and Crisp (1959) Brixham data (x) and Bangor data (+), also displaying the lines of best fit (power equation, day<sup>0.5</sup>).

Table 3.1: Nested (microcosm) repeated-measures ANOVA for the proportion of embryos at each stage (I, II, III, IV and IVh) over time. Significance ( $p > 0.05$ ) is indicated by \*

	DF	Seq SS	Adj SS	Adj MS	F	P	Sig
Stage I							
pH	1	0.0001	0.0001	0.0001	0.44	0.574	
Microcosm(pH)	2	0.0007	0.0007	0.0003	0	0.996	
Day	5	63.4435	63.4435	12.6887	139.8	0	*
pH*day	5	0.0007	0.0007	0.0001	0	1	
Stage II							
pH	1	0.0001	0.0001	0.0001	0.44	0.574	
Microcosm(pH)	2	0.0007	0.0007	0.0003	0	0.996	
Day	5	63.4435	63.4435	12.6887	139.8	0	*
pH*day	5	0.0007	0.0007	0.0001	0	1	
Stage III							
pH	1	1.2349	1.2349	1.2349	1932.45	0.001	*
Microcosm(pH)	2	0.0013	0.0013	0.0006	0.03	0.97	
Day	5	51.6918	51.6918	10.3384	494.65	<0.001	*
pH*day	5	2.0738	2.0738	0.4148	19.84	<0.001	*
Stage IV							
pH	1	1.49	1.49	1.49	3293.02	<0.001	*
Microcosm(pH)	2	0.001	0.001	0	0.03	0.972	
Day	5	178.969	178.969	35.794	2257.78	<0.001	*
pH*day	5	1.806	1.806	0.361	22.78	<0.001	*
Stage IVh							
pH	1	1.0462	1.0462	1.0462	222.37	0.004	*
Microcosm(pH)	2	0.0094	0.0094	0.0047	0.59	0.555	
Day	5	19.6909	19.6909	3.9382	493.85	<0.001	*
pH*day	5	5.2312	5.2312	1.0462	131.2	<0.001	*

Table 3.2: PERMANOVA table of results for nested (microcosms) regression (stages) at each pH condition, where pH condition 1 = control (346 ppm), pH condition 2 = high CO<sub>2</sub> (922 ppm), pH condition 3 = Crisp (1959) Brixham data, and pH condition 4 = Crisp (1959) Bangor data.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Stage	3	15801	5267	39751	0.001	998
pH	3	1141.9	380.64	2872.7	0.016	45
Microcosm(pH)	2	0.265	0.1325	1	0.6328	53
StagexpH	9	922.81	102.53	773.85	0.001	999
Res	6	0.795	0.1325			
Total	23	18608				

### 3.4. DISCUSSION

At atmospheric CO<sub>2</sub> concentrations predicted for the year 2100 under the IPCC's IS92a scenario the probability of adult *Semibalanus balanoides* barnacles surviving the winter period was 22 % lower than under present (2007/2008) winter sea surface temperature and pH conditions. Adult barnacles appeared to maintain their calcium carbonate mineral structure in the face of increased CO<sub>2</sub> despite changes in the Ca/Mg ratio suggesting that their shell structure should disintegrate as a result of dissolution in corrosive (undersaturated) seawater. The relatively large decline in survival rate possibly comes as a consequence of the metabolic cost of maintaining the shell. Embryos developing within the adults developed more slowly as a result of increased CO<sub>2</sub> levels. Both delayed development and reduced naupliar production, leading to delayed settlement, have the potential to impact local populations (Pechenik et al. 1993; Jarrett 2003).

#### 3.4.1. Adult shell mineralogy and survival

Under low CO<sub>2</sub> (control) conditions, a large change in the mineral structure of the shells in this experiment was not expected because adults do not expend much energy on growth during the winter period and they feed minimally at this time (Barnes et al. 1963). Therefore in the high CO<sub>2</sub> treatment, where enhanced dissolution is expected, I would predict a loss of calcium carbonate structure. There was, however, no significant difference in calcium concentration between the control and the high CO<sub>2</sub> treatments in living individuals, suggesting that in the high CO<sub>2</sub> treatment more energy was being invested in shell maintenance than there was in the control.

There were also significant differences between the live and dead barnacles: in the high CO<sub>2</sub> treatment there was less Ca in dead adult barnacles, resulting in a lower Ca/Mg ratios in dead compared to live barnacles. This indicates that dissolution of the dead shells was

occurring in the high CO<sub>2</sub> treatment while there was biological precipitation of CaCO<sub>3</sub> in the shell of the living barnacles.

Barnes et al. (1976) investigated the Ca/Mg ratio in several barnacle species. *Chthamalus depressus* was found to have an increased Ca/Mg ratio in extreme hypobiotic individuals (found in caves and other dark locations) compared to those on the open shore. This increased ratio was accompanied by a reduction in the total organic matter content as a result of reduced protein. As calcite is the major form of CaCO<sub>3</sub> in these organisms any magnesium present in the shell is held within the lattice matrix but is not tightly bound and hence dissolution will cause ions such as Mg to be lost before the dissolution of CaCO<sub>3</sub>. Therefore a larger decrease in Mg compared to Ca was both expected and observed in the high CO<sub>2</sub> treatments. The absence of photosynthetic organisms found in environments with hypobiotic individuals could lead to an elevated level of CO<sub>2</sub> in the seawater occurring as a net result of high respiration but low photosynthetic rates. This could result in seawater with carbonate chemistry properties similar to those seen in this study and may explain why both high CO<sub>2</sub> and hypobiotic individuals show similar results.

Wickens (1984) investigated CO<sub>2</sub> impacts on growth and mineralisation in penaeid prawns, showing that with increasing CO<sub>2</sub>-induced acidification there was an increase in Ca and no change in Mg and hence an increase in the Ca/Mg ratio. This agrees with findings here in several respects as there was an increased Ca/Mg ratio and, although not significant at the CO<sub>2</sub> level used here, there was an increase in Ca. Increasing CO<sub>2</sub> is accompanied by an increase in HCO<sub>3</sub><sup>-</sup> which is taken up to be used both as a buffer to rising haemolymph pH and as a substrate for CaCO<sub>3</sub>. The Mg decrease in *S. balanoides* but not in the prawns, suggests there is either a difference in the mechanisms associated with uptake of Mg into the shell structure, with less Mg being incorporated at lower pH in *S. balanoides*, a

difference in relation to external erosion properties or, more likely, a difference in the shell mineralogy.

The lower survival of adults in the high CO<sub>2</sub> treatment could have resulted from either physiological or dissolution effects. Like all other organisms barnacles expend energy on maintenance, repair, reproduction and respiration (Sibly & Calow 1986). They feed minimally during the winter period and hence must rely on food reserves (lipid stores) while undergoing a period of lowered metabolism with minimal growth (Barnes et al. 1963). Despite acidosis in extracellular fluids being considered as a 'normal' feature of intertidal barnacles during periods of emersion (e.g. Fyhn et al. 1972), prolonged acidosis as a result of sustained exposure to low pH seawater, as found for other crustacean species (e.g. Spicer et al. 2007), could lead to disruption of normal physiological processes. Additionally, an increased use of lipid stores for energetic maintenance could result in a loss of protein biosynthesis, which in turn enhances mortality (Barnes et al. 1963). Exposure to corrosive seawater may have led to some dissolution of the shell, as well as creating hypercapnic conditions within the organism further stressing the animals and hence decreasing survival. Further work investigating lipid storage and cirral movement during the winter period under normal and acidified conditions will aid the understanding in this energy balance, although energetic trade-offs have been shown in other species under hypercapnic conditions (Wood et al. 2008; Chapter 5).

### **3.4.2. Embryo development**

Comparing the present study with the investigation of Crisp (1959) it can be seen that the naupliar development rates recorded by Crisp at Brixham, geographically the closest site to the barnacle populations used here, were similar to those estimated for the control. On the basis of this observation it can be assumed that the 1.5 °C increase in temperature over the last 50 years as a result of global warming (MCCIP 2008) has had little impact on



the development rates. The estimated development rate in the high CO<sub>2</sub> treatment was significantly slower than both the control (average winter temp 12 °C) and those of the Brixham population (average winter temp 11 °C), but was similar to the rate from the Bangor population (average winter temp 8 °C). There is no information on the annual variation of development rates at either Bangor or Brixham. Crisp (1959) suggested that the difference between the rate of development at different locations arises from temperature differences (winter average temperature in Brixham is approximately 2 °C warmer than in Bangor) together with the implication that towards the later stages in development embryos are more deprived from oxygen or inhibited by excess CO<sub>2</sub> in the warmer conditions. Both O<sub>2</sub> and CO<sub>2</sub> are known to impact the rate of egg development (Root 1930; Strathmann & Strathmann 1995; Cohen & Strathmann 1996). Root (1930) demonstrated that the rate of O<sub>2</sub> consumption of *Arbacia* eggs decreases rapidly when CO<sub>2</sub> increases (O<sub>2</sub> consumption decreased by 21 % for every ~ 1,315 µatm CO<sub>2</sub> increased), but only begins to decrease below pH 6.25 if the acidity is changed using HCl. This implies that bubbling CO<sub>2</sub> has a much greater effect on egg respiration than resulting from using HCl to lower pH and is in agreement with the results obtained here which showed CO<sub>2</sub> had a small yet significant effect at 922 ppm. Mayor et al. (2007) showed that elevated CO<sub>2</sub> (8,000 ppm) was associated with nearly an 86 % reduction in hatching success of copepods despite apparently normal growth and reproduction of adults. In these experiments there was no impact of elevated CO<sub>2</sub> on hatching success; however there was an estimated 19 day delay in reaching hatching stage.

A change in the rate of embryo development induced by elevated CO<sub>2</sub> may be important when considering that larvae are released into the plankton to coincide with the spring bloom. Adults are triggered to release their larvae by a chemical cue or "hatching substance" (Clare & Walker 1986) produced when the adult begins feeding. There is considerable interannual and geographic variability in release timing (Barnes 1962,

Kendall et al. 1985) therefore effects of delayed development would be greatest in early release years and in areas where spawning tends to be early. In other years, developmentally intact larvae can be held for some time before release takes place. Larval release tends to start at the southern range edge (South West UK and northern Portugal) as early as February, therefore the already short period between fertilisation and spawning could be problematic. Delaying the time to hatching could prevent synchronisation with the spring bloom and lead to high mortality as a result of competition with the holoplankton. Even if the larvae were able to survive, late settlement would leave juveniles susceptible to additional stresses: on average, the later in the year that a larva settles the greater is its chance of encountering high air and rock temperatures and dying from either heat or desiccation. Hence the timing of recruitment is crucial with early and late settlers often having lowest survival (Kendall et al. 1985; Pineda et al. 2006).

Here evidence is provided that relatively small changes in CO<sub>2</sub> and pH, to levels that are predicted to occur globally within the next 100 years (Caldiera & Wickett 2003), can lower survival of adults and decrease the development rate of embryos. These findings further the work of Kurihara & Shiryama (2004), Havenhand et al. (2008) and Dupont et al. (2008) as well as others who have found impacts on early life stages at realistic levels of future CO<sub>2</sub>. However, the CO<sub>2</sub>-induced slowing in development rate still falls within the range of rates seen in natural populations. It is known that temperature limits the development rate of embryos at the southern edge of their range (Crisp 1959), but from the results here it could be inferred that ocean acidification could further compromise barnacle development at this range edge (cf. synergistic effects of temperature and elevated CO<sub>2</sub> on the thermal limits of the edible crab *Cancer productus* – Metzger et al. 2007). Further work is necessary to establish more exactly how temperature and CO<sub>2</sub> will interact over the entire geographic range.

Both the growth and embryo development of an organism that is normally exposed to seasonal and daily fluctuations in its environment can be significantly affected by lowering pH to levels predicted under future ocean acidification scenarios. Nevertheless, in assessing the significance of these findings their limitations must be considered. One consideration for this experiment is that CO<sub>2</sub> levels were increased over a period of days, as opposed to the increase on yearly timescales seen in nature, and thus removed the possibility that the barnacles might adapt or acclimate over longer time periods. The evidence that in high CO<sub>2</sub> conditions some adults would be able to survive and that their larvae are able to hatch may indicate that the species are able to find suitable habitats and survive. At the southern edge of its range the additional temperature stress may make populations more vulnerable to local extinctions.

## **CHAPTER 4. POST-LARVAL DEVELOPMENT**

### **Post-larval development of two intertidal barnacles at elevated CO<sub>2</sub> and temperature**

Aspects of this chapter have been published as:

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#### 4.1. INTRODUCTION

Many studies have already shown that elevated temperatures can have an impact on populations of intertidal species (Kendall et al. 1985; Harley & Helmuth 2003; Mieszkowska et al. 2006), and that the specific habitat occupied, even down to the direction and aspect an organism is orientated on the shore (Denny et al. 2006), can be important in determining the extent to which it is stressed. Seasonal variation in environmental conditions may also be important in determining the probability of an organism surviving. For example, the barnacle *Semibalanus balanoides* releases its larvae to coincide with the plankton spring bloom which provides food for the pelagic phase. *S. balanoides* larvae settle on the shore approx. 4 weeks later but if during this settlement period there are high air temperatures, the number of surviving recruits will be very low (Kendall et al. 1985). Coincidentally, in the temperate and sub-polar regions occupied by *S. balanoides*, the spring and summer phytoplankton blooms cause an increase in seawater pH (Blackford & Gilbert 2007) and carbonate ion saturation state (Findlay et al. 2008), thereby inadvertently providing optimal conditions for growth and development of their calcareous shells.

Some studies have investigated the impacts of ocean acidification on intertidal species; however they have all previously carried out the experiments in subtidal conditions (e.g. Michaelidis et al. 2005; Gazeau et al. 2007; Bibby et al. 2008; Beesley et al. 2008). These early studies suggested that processes such as calcification (Gazeau et al. 2007 at pH < 7.7), growth, acid-base balance and metabolism (Michaelidis et al. 2005 at pH < 7.5) and health (Phagocytotic response, Bibby et al. 2008 and lysosome membrane stability, Beesley et al. 2008) can all be negatively impacted by lowered pH. Moreover, few studies have attempted to put into context ocean acidification as an environmental stressor, particularly in relation to temperature. Consequently, two species of intertidal barnacles were used in intertidal microcosms (Chapter 2) to assess whether organisms usually exposed to a highly

variable environment are less susceptible to changes in pH; and whether they are more, less or equally impacted by pH or by temperature. This chapter investigates how CO<sub>2</sub> and temperature, and their interaction, affects growth, shell development and survival in developing post-larvae during the metamorphic period of their life-history.

The two intertidal barnacle species chosen, *Semibalanus balanoides* and *Elminius modestus*, are found in similar habitats around the United Kingdom, indeed in many locations they co-occur. However, these barnacles differ in some key aspects of their biology; for example *E. modestus* is often more successful in estuarine environments, whereas *S. balanoides* prefers more exposed sites (Rainbow 1984). *S. balanoides* is a boreo-arctic species and shows a marked seasonal response in feeding rate (Ritz & Crisp 1970), moulting (Crisp & Patel 1960), and reproduction and development (Crisp 1956, 1962), all of which peak during the spring and summer. Cyprid larvae settle onto the shore in the spring (late February in the south (Southward 1958) to June in NE England (Kendall et al. 1985)) in the UK, and metamorphose into juveniles when they first lay down a calcified shell (Bourget & Crisp 1975). The average metamorphosis time occurs in 1.5 days (Connell 1961). *E. modestus* by contrast is an invasive, warm-water species, which was first introduced into the UK from New Zealand around 1945 (Bishop 1947). Its European distribution extends from Faro (southern Portugal) to southern Denmark (Harms & Anger 1989; O'Riordan & Ramsay 1999). In contrast to *S. balanoides*, *E. modestus* releases larvae to the plankton throughout the year (Crisp & Davies 1955).

#### 4.2. MATERIALS AND METHODS

*Semibalanus balanoides* were collected on settlement panels (10 x 10 cm ceramic tiles) deployed for one week (beginning 30<sup>th</sup> April 2007 - settlement panels were placed on the shore and checked weekly for the onset of settlement) on north-facing rocks in the mid-shore at Looe, southwest England (50°20'N, 004°27'W). On collection, the panels

contained a mixed age population of barnacles ranging from newly settled cyprids to week-old post-larvae. *Elminius modestus* were collected on identical settlement panels placed for one week (beginning 13<sup>th</sup> August 2007) on south-facing rocks in the mid-shore at Mount Edgecombe, southwest England (50°20'N, 004°10'W).

For each experiment (one experiment on *S. balanoides* and one experiment on *E. modestus*), at least two settlement panels were placed into one of eight microcosms (30 x 15 x 20 cm) so that each microcosm contained in excess of 200 individual barnacles. The microcosms were identical to those described in Chapter 2 and were kept in a controlled temperature facility. Four microcosms were set at 14 °C to simulate 2008 summer conditions and the remainder were set to 18 °C to represent average year 2100 summer sea surface temperature. The temperatures chosen were based on the IPCC 2007 A2 scenario of 4 °C warming.

At each temperature, two microcosms were set at ambient CO<sub>2</sub> conditions (pH 8.07, CO<sub>2</sub> = ~380 ppm) and two were allocated to a high CO<sub>2</sub> treatment (pH 7.70, CO<sub>2</sub> = ~1,000 ppm). The CO<sub>2</sub> concentration, and hence pH level, and temperature was maintained in each microcosm using the CO<sub>2</sub> mixing system described in Chapter 2. pH (NBS scale, Mettler-Toledo pH meter), CO<sub>2</sub> (Licor LI-6262 CO<sub>2</sub> analyser), temperature and salinity (WTW LF197 combination temperature and salinity probe) were recorded weekly. Total alkalinity (A<sub>T</sub>), dissolved inorganic carbon (DIC), bicarbonate (HCO<sub>3</sub><sup>-</sup>), carbonate (CO<sub>3</sub><sup>2-</sup>), and the saturation states for aragonite and calcite were all calculated from pH and pCO<sub>2</sub> values using CO2sys (Pierrot et al. 2006) with dissociation constants from Mehrbach et al. (1973) refit by Dickson & Millero (1987) and KSO<sub>4</sub> using Dickson (1990).

The tidal regime of the microcosms was programmed weekly to coincide with the local Plymouth tide times. Salinity was 35 and seawater was delivered to the microcosms during

the flood tide period at a rate of 10 ml min<sup>-1</sup>. Day length (Polylux XL 58 W) was maintained to within 15 min of the sunrise/ sunset times for London, UK, roughly equating to a 14 h on/ 10 h off cycle throughout the experiment. Natural, filtered (10 µm), seawater was used and was replenished twice weekly to avoid salinity increases through evaporation. The experiment ran for 30 days and barnacles were fed every two days with a mixed diatom-flagellate diet at 15,000 cells ml<sup>-1</sup> (Shellfish Diet 1800®, Reed Mariculture).

Changes in barnacle abundance on each settlement panel were recorded every 2-3 days using a digital camera (FujiFilm A510 FinePix) which was maintained in consistent alignment using a stand. The photographic images were analysed (Image-Pro Plus v.4.5, Media Cybernetics) to estimate both growth and survival. Growth was estimated by measuring the diameter of the operculum of each barnacle on each panel at each time point. Growth rate was calculated as an average over the 30 day experimental period of all the barnacles in each microcosm. Barnacle survival was estimated from the images taken at the beginning and the end of the experiment by counting living and dead individuals. Prior to photography individuals were gently touched to check whether they were able to close their operculum and were classed as dead when the operculum either remained open or the shell was empty. Survival, recorded as a proportion, was square root arcsine transformed before analysis so that data were normally distributed.

The calcium carbonate composition of the shell was estimated by analysing the calcium (Ca) concentration as a proxy for changes in calcification or dissolution (Chapter 5). The shells of five individuals were haphazardly selected from each microcosm at the end of the experiment and the Ca concentration was measured using methods described in Spicer & Eriksson (2003); briefly this involved dissolving the shells in 10 % HNO<sub>3</sub> after drying and weighing, then using an atomic absorption spectrophotometer (Varian SpectrAA 50) to measure the Ca concentration. The proportion of Ca in the shell was calculated from the



mass of the shell and volume of acid used in the digest (Ca [mg] / total shell mass [mg]). The proportion of Ca in the total shell was then square root arcsine transformed before statistical analysis so that data were normally distributed.

The growth rate, transformed-survival and transformed- Ca data were tested for normality using a Kolmogorov-Smirnov test and for homogeneity of variances using Bartlett's test. Once these assumptions were met a two-way nested ANOVA was used to determine the effects of temperature, CO<sub>2</sub> or any interaction between them. Microcosms were nested, as a random factor, within CO<sub>2</sub> treatment (n = 2). All statistical analysis was performed using Minitab® 15.1.0.0 (© 2006, Minitab Inc.).

### 4.3. RESULTS

Environmental data remained stable at the pre-set levels of temperature, salinity, CO<sub>2</sub> and pH over the experimental periods (Table 4.1). Table 4.1 also shows the difference in carbonate ion concentration and aragonite and calcite saturation states between the control and the high CO<sub>2</sub> treatments and the calculated saturation states for calcite, which did not become undersaturated at any point in either experiment.

*Elminius modestus* exhibited a significantly slower growth rate with increasing CO<sub>2</sub> at the higher temperature (ANOVA,  $F_{df=1} = 19.15$ ,  $p = 0.012$ ). No other significant impacts of temperature and / or pH manipulation on rate of growth were observed in either barnacle species (figure 4.1). The mean growth rate of *Semibalanus balanoides* in the cold control CO<sub>2</sub> treatment was greater than in all other treatments ( $14 \mu\text{m d}^{-1}$ ) but was highly variable (SE = 5.96). This large variability in growth rates (Table 4.2) suggests that there may be some error in measuring the exact growth rates over this short period of time or that there is large natural variability within a population. A PERMANOVA test for difference between treatments over time also revealed no significant affects of CO<sub>2</sub> or temperature on

growth (PERMANOVA pH\*time  $F_{18,72} = 1.104$ ,  $p = 0.355$ ; PERMANOVA temperature\*time  $F_{18,72} = 1.074$ ,  $p = 0.395$ ).

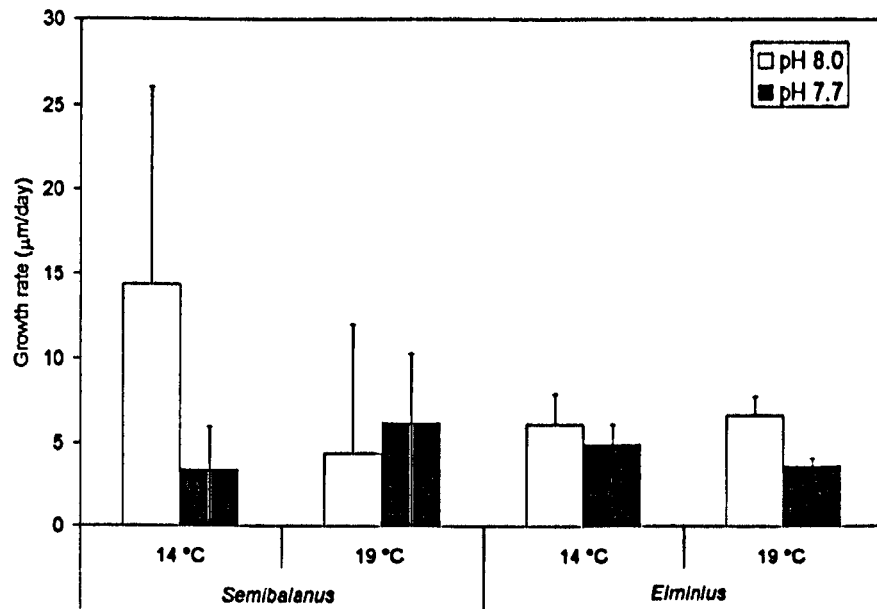


Figure 4.1: Mean growth rate of *Semibalanus balanoides* and *Elminius modestus* under different temperature (14 °C and 19 °C) and pH conditions (pH 8.0 and pH 7.7). Error bars represent the 95 % C.I..

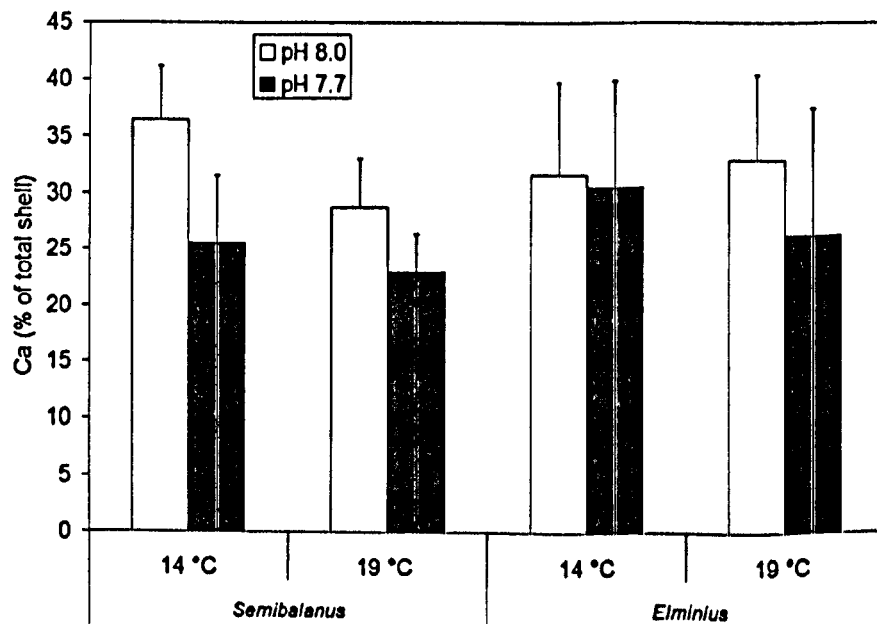


Figure 4.2: Mean calcium content as a percentage of total shell of *Semibalanus balanoides* and *Elminius modestus* under different temperature (14 °C and 19 °C) and pH conditions (pH 8.0 and pH 7.7). Error bars represent the 95 % C.I..

The mean calcium content in shells of both *S. balanoides* and *E. modestus* decreased with increasing CO<sub>2</sub> (figure 4.2); while these reductions seemed much greater in *S. balanoides*, neither were actually significant (ANOVA, *S. balanoides*  $F_{df=1} = 2.53$ ,  $p = 0.253$ , *E. modestus*  $F_{df=1} = 1.13$ ,  $p = 0.295$ ). The calcium content in *S. balanoides* decreased with increasing temperature and was significantly lower in the high temperature control CO<sub>2</sub> than in the low temperature control CO<sub>2</sub> treatment (ANOVA,  $F_{df=1} = 12.48$ ,  $p = 0.001$ ). Temperature had no significant effect on the calcium content of shells from *E. modestus*. No clear relationship between calcium content and mass of the shell within any of the *S. balanoides* or *E. modestus* treatments could be demonstrated in view of the different size ranges of the shells (Fig. 3).

*Table 4.1: Environmental conditions in the treatments for the Semibalanus balanoides experiment and for the Elminius modestus experiment. Mean ± 95 % C.I. are given for salinity, temperature (°C), pH, CO<sub>2</sub> (µatm), calcite saturation state (Ω<sub>cal</sub>) in each treatment. Additionally the difference in carbonate ion concentration (ΔCO<sub>3</sub><sup>2-</sup> µmol kg<sup>-1</sup>), calcite (ΔΩ<sub>cal</sub>) and aragonite saturation (ΔΩ<sub>arg</sub>) states between the high CO<sub>2</sub> treatment and the low CO<sub>2</sub> treatments are also provided.*

		<i>Semibalanus balanoides</i>		<i>Elminius modestus</i>	
		Low Temp	High Temp	Low Temp	High Temp
Low CO <sub>2</sub>	Salinity	35.74 ± 0.30	35.79 ± 0.37	34.5 ± 0.33	34.6 ± 0.34
	Temp (°C)	14.39 ± 0.25	19.77 ± 0.25	14.65 ± 0.23	19.65 ± 0.70
	pH <sub>(NBS)</sub>	8.05 ± 0.027	8.07 ± 0.030	7.96 ± 0.022	7.98 ± 0.044
	CO <sub>2</sub> (µatm)	409 ± 17	423 ± 23	413 ± 4	412 ± 9
	Ω <sub>cal</sub>	2.4 ± 0.26	2.9 ± 0.31	1.9 ± 0.15	2.4 ± 0.51
High CO <sub>2</sub>	Salinity	35.63 ± 0.31	35.74 ± 0.32	34.5 ± 0.30	34.7 ± 0.32
	Temp (°C)	14.70 ± 0.27	19.73 ± 0.26	14.95 ± 0.20	20.03 ± 1.05
	pH <sub>(NBS)</sub>	7.73 ± 0.022	7.71 ± 0.025	7.73 ± 0.051	7.73 ± 0.036
	CO <sub>2</sub> (µatm)	1132 ± 43	1109 ± 43	1076 ± 34	1075 ± 40
	Ω <sub>cal</sub>	1.5 ± 0.14	1.5 ± 0.13	1.4 ± 0.34	1.5 ± 0.22
Difference From control	ΔCO <sub>3</sub> <sup>2-</sup> (µmol kg <sup>-1</sup> )	- 37 ± 14	- 37 ± 13	- 18 ± 16	- 14 ± 10
	ΔΩ <sub>cal</sub>	- 0.89 ± 0.34	- 0.87 ± 0.31	- 0.44 ± 0.37	- 0.34 ± 0.25
	ΔΩ <sub>arg</sub>	- 0.56 ± 0.22	- 0.55 ± 0.20	- 0.28 ± 0.24	- 0.2 ± 0.16

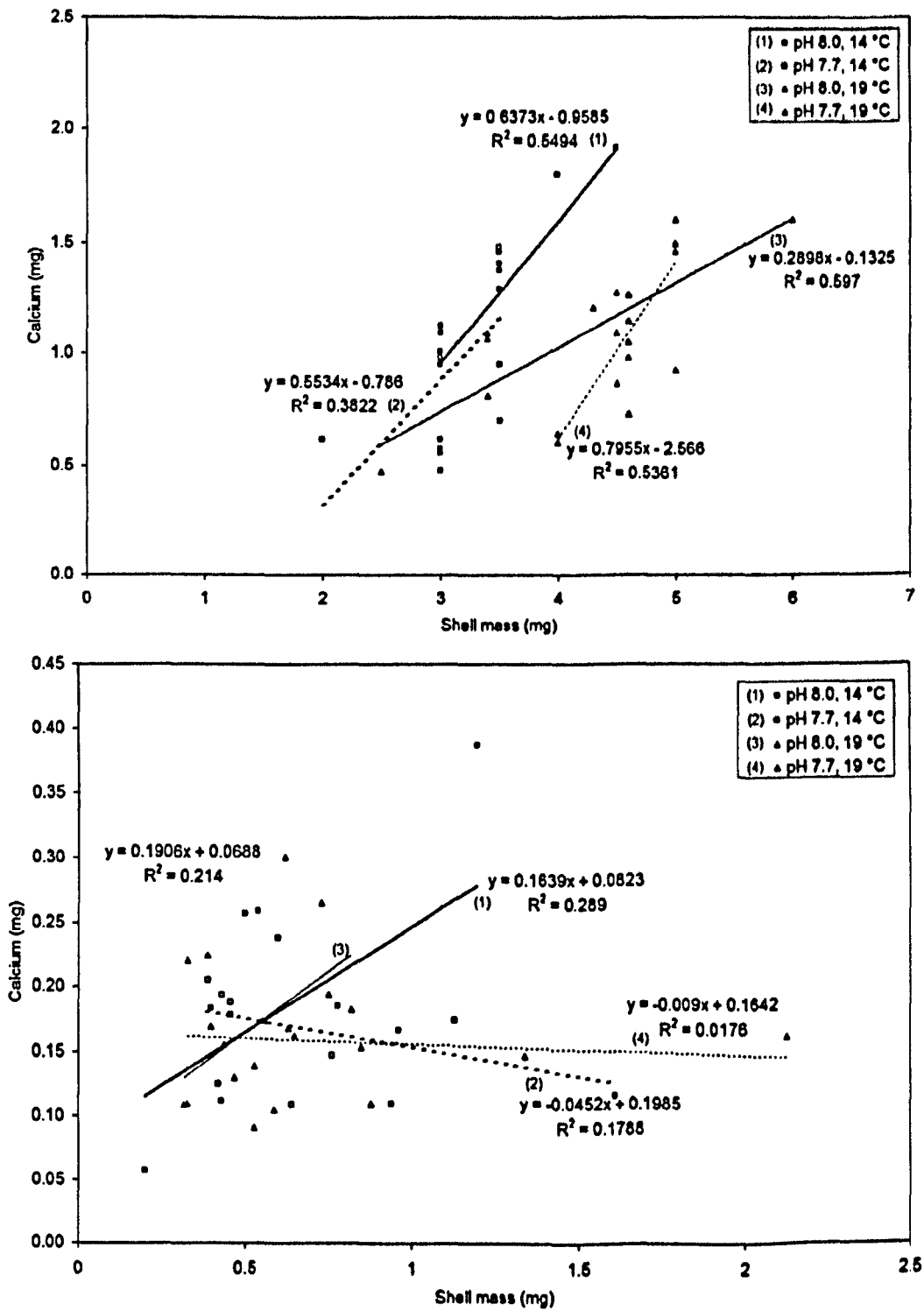


Figure 4.3: Mass of calcium present in the shell (mg) in relationship to total shell mass (mg) for (a) *Semibalanus balanoides* and (b) *Elminius modestus* for each treatment (1) pH = 8.0, temperature = 14 °C, (2) pH = 7.7, temperature = 14 °C, (3) pH = 8.0, temperature = 19 °C, and (4) pH = 7.7, temperature = 19 °C. Linear regression lines are shown for each treatment.

Table 4.2: Abundance, size, growth rate and crowding data for each species (*Elminius modestus* and *Semibalanus balanoides*) in each treatment (pH 8.0 and pH 7.7, temperature 14 °C and 19 °C).

Treatment	Species	Time point	Count	Mean Size (mm)	Size ( $\sigma^2$ )	Growth rate ( $\mu\text{m d}^{-1}$ )	Crowding (ind. $\text{cm}^{-2}$ )	
pH 8.0, 14 °C	<i>E. modestus</i>	Initial	210	0.46	0.315	6.14	0.70	
		Final	162	0.65	0.675		0.54	
	<i>S. balanoides</i>	Initial	355	0.59	0.116		14.35	1.78
		Final	316	0.95	0.095			1.68
pH 7.7, 14 °C	<i>E. modestus</i>	Initial	298	0.50	0.063	4.92	0.99	
		Final	240	0.65	0.284		0.80	
	<i>S. balanoides</i>	Initial	190	0.61	0.116		2.94	2.00
		Final	150	0.68	0.114			1.58
pH 8.0, 19 °C	<i>E. modestus</i>	Initial	252	0.49	0.437	6.68	0.84	
		Final	187	0.69	0.716		0.62	
	<i>S. balanoides</i>	Initial	594	0.85	0.133		4.34	3.96
		Final	320	0.96	0.004			3.43
pH 7.7, 19 °C	<i>E. modestus</i>	Initial	293	0.48	0.088	3.64	0.98	
		Final	202	0.59	0.088		0.67	
	<i>S. balanoides</i>	Initial	317	0.86	0.117		6.23	1.92
		Final	85	1.02	0.052			1.70

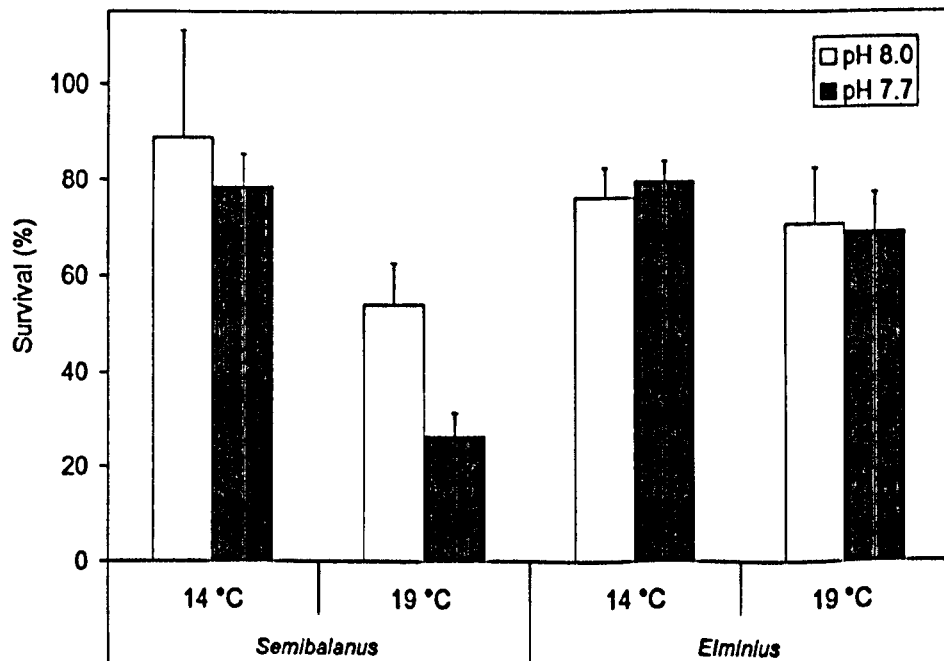


Figure 4.4: Mean percentage survival of *Semibalanus balanoides* and *Elminius modestus* under different temperature (14 °C and 19 °C) and pH conditions (pH 8.0 and pH 7.7). Error bars represent the 95 % C.I..

Table 4.3: Results from t-test regressions analysis difference between slopes (calcium content vs. shell mass) for *Elminius modestus* experiment. T values are given for each pair of treatments,  $df = 16$ , significant values ( $p < 0.05$ ) are shown in bold.

t value	pH 7.7, 14 °C	pH 8.0, 19 °C	pH 7.7, 19 °C
pH 8.0, 14 °C	<b>2.241</b>	0.251	1.866
pH 7.7, 14 °C		<b>3.072</b>	0.645
pH 8.0, 19 °C			<b>2.627</b>

Increasing temperature decreased the survival of both *S. balanoides* (ANOVA,  $F_{df=1} = 102.69$ ,  $p = 0.010$ ) and *E. modestus* (ANOVA,  $F_{df=1} = 7.99$ ,  $p = 0.047$ ) (figure 4.4). There was no significant effect (or interaction with temperature) of  $CO_2$  on survival of either species.

#### 4.4. DISCUSSION

This study investigated the impacts of both elevated temperature and  $CO_2$  (at levels predicted for 2100 based on the IPCC A2 scenario) on the developing post-larvae of two intertidal barnacle species that, as adults, are thought to be relatively tolerant to changes in abiotic conditions (Newell 1979). Temperature appears to be the overriding factor determining survival with no additional impact from elevated  $CO_2$ . In common with many recent investigations (e.g. Ellis et al. 2009; Arnold et al. 2009; Hauton et al. 2009; McDonald et al. 2009), the impacts of  $CO_2$  were more subtle and sub-lethal. Both elevated temperature and  $CO_2$  affected growth and shell development, although the responses were not consistent between the species. Below the relative impacts of temperature and  $CO_2$  on *Semibalanus balanoides* and *Elminius modestus* are discussed; comparisons are made with previous studies of environmental stressors, including ocean acidification, which enable the results presented here to be placed in the context of seasonal and life-history variability.

#### 4.4.1. Response to elevated temperature and CO<sub>2</sub>

The newly settled *Semibalanus balanoides* post-larvae investigated in this study had significantly less calcium in their shells when kept under elevated temperature than when kept under ambient conditions. This lower calcium content could have resulted either indirectly from physiological changes or directly from temperature control of the mineralogy (Freitas et al. 2008). However, temperature had no impact on the calcium content of *Elminius modestus* shells suggesting that the nature or extent by which shell mineralogy is biologically controlled differs between the two barnacle species. Raising CO<sub>2</sub> levels results in the lowering of saturation states for calcium carbonate minerals, such as calcite, in seawater and this causes increased corrosion of calcitic shells. However, at no point in this experiment did calcite become undersaturated, although it was lowered to  $\Omega_{\text{cal}} = \sim 1.5$ . It seems more likely that any impacts on shell mineralogy from elevated CO<sub>2</sub> came from indirectly affecting rates of associated physiological processes; a similar response was found in larval lobsters under high CO<sub>2</sub> conditions (Arnold et al. 2009).

*Semibalanus balanoides* post-larval growth rates under control conditions were similar to rates found in the field (10 – 20  $\mu\text{m d}^{-1}$ ; Jarrett 2003) but lower than other laboratory experiments (30 – 40  $\mu\text{m d}^{-1}$ ; Jarrett 2003). There are several differences between the laboratory experiment of Jarrett (2003) that might explain the relatively slow growth found in this study: 1) the juveniles used in Jarrett (2003) were continuously submerged, whereas here they were grown in an intertidal regime. An intertidal regime means that the barnacles feed for less than 12 hours a day which immediately explains a 50 % difference in growth rates if feeding under continuously submerged conditions is not restricted; 2) The field observations of Jarrett (2003) were made on intertidal juveniles and are more directly comparable to the tidal regime in this present study; 3) In the laboratory experiment Jarrett (2003) used over three times more algal cells  $\text{m}^{-3}$  to feed the juveniles than used in the study. If there is a constant relationship between food concentration and growth rate then

an increase in food to match the concentration used in Jarrett (2003) would raise growth rates in this study to about  $46 \mu\text{m d}^{-1}$ . Furthermore, Bourget (1977) examined growth checks to show that barnacles do not grow over the low tide period. The differences between the growth rates recorded in this experiment and those of other laboratory studies appear to be related to keeping the barnacles in a tidal regime that simulates natural conditions.

In the experiments described here, *Elminius modestus* also had low growth rates compared to previous studies. Crisp & Patel (1961) showed that under intertidal field conditions *E. modestus* can reach about 4.2 mm in 56 days ( $\sim 70 \mu\text{m d}^{-1}$  growth rate) at temperatures between 15 - 21 °C, although the rate of development varies with crowding and again with tidal height (Crisp & Patel 1961, Table 4.2). The lower growth rates observed in this study are most likely caused by a low food supply, which although the same as for *S. balanoides*, was inadequate for the faster developing *E. modestus*.

As a consequence of the high variability in the *S. balanoides* growth rates there were no significant differences in growth rate between the various experimental treatments. This variability may have arisen from difficulties of accurately measuring small increments in growth over a short time period or may be indicative of high variation naturally occurring within populations (Wethey 1983). *E. modestus* post-larvae did appear to have some reduced growth under elevated CO<sub>2</sub> conditions but were less impacted by elevated temperature. Such findings support those of Harms (1986) who showed that *E. modestus* larvae reared in salinity extremes had reduced growth rates but were less impacted by temperature between 9 °C and 19 °C.

Metamorphosing cyprids may not have the ability to compensate for environmental stressors in the same way that adults do (Chapter 3; Wood et al. 2008), as there may be



mechanistic differences between metamorphic shell production and long-term shell production. In particular, metamorphosing cyprids are likely to have different metabolic priorities, as they are in immediate danger of desiccation and hence must lay down their shell rapidly (Foster 1971). Adults will spend more energy on maintenance but high metabolic priority is also given to reproduction (Wu & Levings 1978). Larval metamorphosis is an energetically expensive process during which cyprids may consume up to 30% of their own body organic carbon, primarily through breakdown of lipids but also proteins (Lucas et al. 1979). The extent of a cyprid's energy reserve has been shown to correlate with its survival to the juvenile stage (Thiyagarajan et al. 2005). In the present experiment *S. balanoides* did not appear to have altered growth rates but did have lower levels of calcium in their shells under elevated temperature and CO<sub>2</sub>. Such changes may have diverted energy from metamorphosis and resulted in reduced survival. *E. modestus* growth was lowered under elevated CO<sub>2</sub> but the levels of calcium in their shells appeared to be less impacted although there was a significant difference in the relationship between calcium content and shell mass between elevated CO<sub>2</sub> and normal CO<sub>2</sub> treatments. In this species it could be speculated that the energy available was not adequate for growth and essentially the individual reduced its metabolic processes causing little change in shell development but survival remained relatively high. Additional measures of feeding rates would show whether the individuals were able to elevate their energy intake.

The differences in observed responses between *Semibalanus balanoides* and *Elminius modestus* may well reflect the contrasting biogeography/niche requirements of the two species. Although *S. balanoides* and *E. modestus* may overlap in their distribution, their environmental preferences are significantly different; *S. balanoides* is a cold-water species while *E. modestus* is from warmer waters. In the populations used in the present study, *S. balanoides* is already nearing its southern geographic limit, which is likely set by high temperature (Barnes 1958). In the summer SST in southwest UK, at the southern range

edge, is about 15 °C on average but ranges from 12 °C to 19 °C. Hence these animals may be more susceptible to warming than animals from populations in the middle of this species' range. Indeed, *S. balanoides* appeared to be more affected in the present experiment than *E. modestus*, particularly under elevated temperature. As a warm-water species, *E. modestus* are commonly found in areas that experience sea temperatures greater than 19 °C. Harms (1986) recorded that New Zealand sea temperatures range between 15 °C and 21 °C and hence it might be expected that the experimental temperatures are within the range to which *E. modestus* as a species is adapted.

Harms (1986) discovered that highest mortality of *E. modestus* larvae occurred at salinity extremes (>40 and <20), while temperature impacts were most evident at the salinity range of 30-40. In a similar manner, the *E. modestus* survival response in this study was most impacted by temperature while at nominal values of CO<sub>2</sub> / pH. The temperature effect on survival of *S. balanoides* seen in the present study, however, was more similar to the survival response observed by Harms (1986) for *E. modestus* when held under temperature extremes (at temperatures <9 °C or >20 °C). Once again this indicates potential differences in the susceptibility of organism living at the edge of their natural tolerance range and those living towards the centre. Such observations highlight the difficulties in making population or species comparisons (Spicer & Gaston 1999) as much research is still needed to understand the variability of responses within species.

#### 4.4.2. Seasonal and life-history variability

Connell (1961) reviewed the causes of mortality for settled *S. balanoides* cyprids and post-larvae between and within different locations (height up shore), shores, and years. He concluded that if cyprids were exposed to hot or sunny days they were less likely to survive; but once the cyprids had metamorphosed, the highest mortalities occurred when the individuals were submerged, largely as a result of abrasion during storm events.

Weaker shells, through reduced calcification or increased corrosion in lower pH, or indeed higher temperature, seawater may increase mortality resulting from abrasion and predation.

During the summer period it is well known that either extreme events or an accumulation of hot days will cause mortality (Kendall et al. 1985) because post-larvae are susceptible to desiccation in just a few hours. For example, at 18 °C the mean lethal time is 6 hours for *S. balanoides* and 7 hours for *E. modestus* (Foster 1971). In the present experiments, the microcosms recreated relatively calm conditions with high humidity (> 80 % relative humidity) and so a different explanation must be considered for increased mortality, such as changes in metabolism and resource allocation.

The different growth response of *E. modestus* compared to the *S. balanoides* supports the hypothesis that differences in life history strategies influence the ability to populate space on the shore. *E. modestus* develops rapidly and reproduces within months of settlement, requiring a continuous supply of food throughout the year. *S. balanoides* is pre-adapted to conditions of discontinuous food supply and hence has slower growth and reproduces just once each year (Rainbow 1984). In the experiments here, a combination of low food supply, high CO<sub>2</sub> and elevated temperature prevented *E. modestus* from having rapid growth.

Growth of barnacles varies with crowding of both post larvae and adults and with both the quality and the quantity of their food supply. Neither barnacle in this study was impacted by crowding, as densities were < 2 individuals cm<sup>-2</sup>. However it appears that food supply may have prevented *E. modestus* from attaining normal field growth rates. Sanford et al. (1994) show that flow, food-supply and temperature affect the feeding rate of *S. balanoides*. Sanford et al. (1994)'s low flow regimes appears representative of the microcosm laboratory conditions found in this present study and show that temperature

reduces feeding in both adults and juveniles significantly from 15 °C to 20 °C (the range used in this experiment). These findings are consistent with the results presented here that growth rate may be impacted by temperatures above 15 °C.

The barnacles used in these experiments were subjected to a tidal regime which simulates an overcast, windless, low wave exposure day on the mid-rocky intertidal. Understanding the environment from which the organisms are taken is of fundamental importance when interpreting results and attempting to generalise relationships. *E. modestus* and *S. balanoides* were placed in exactly the same conditions (representative of the field) and have shown different responses to elevated temperature and CO<sub>2</sub>. Nevertheless I would emphasise that extrapolating these results to reach conclusions on populations or community dynamics must be carried out with caution because of the extremely variable physical nature of the area of the intertidal zone that is occupied by the two barnacle species.

Ocean acidification studies on early life stages thus far have primarily been carried out on planktonic larvae prior to settlement and have shown that the development of calcareous skeletons, growth and survival may be adversely affected (e.g. Kurihara & Shirayama 2004; Kurihara et al. 2007; Dupont et al. 2008a, 2008b). Such a combination of impacts has led to the conclusion that larvae may be more susceptible to ocean acidification than adults (Dupont & Thorndyke 2009). One previous study investigated benthic larval stages (Ellis et al. 2009) and this showed subtle impacts on shell morphology and development, which could have more serious consequences for later life. McDonald et al. (2009) showed that ocean acidification had no impact on the early life stages of the barnacle *Amphibalanus amphitrite*, a tropical species, although calcification increased in the post-larvae and adults. If the results from the present study are considered in terms of future ocean conditions, they suggest that a decrease by 0.4 pH<sub>(NBS)</sub> units alone (year 2100 IPCC

(2007) A2 emissions scenario) would not be sufficient to have a direct impact on the survival of barnacles during the first few days of their intertidal life. However, if such changes in pH occur in conjunction with changes in other environmental parameters, such as a 4-5 °C increase in temperature, it is highly probable that significant changes to the biology of these organisms will ensue. These changes could be sufficient to affect the abundance of the adult population, even though larger, older animals are far better adapted to living in a fluctuating environment.

In interpreting these results it must also be considered that this study investigated larvae and post-larvae that came from populations unaffected by either elevated temperature or elevated CO<sub>2</sub>. Different responses may be found if larvae came from similarly exposed parents, particularly with relation to the amount of energy invested in reproduction. However, only one study so far has investigated exposure of high CO<sub>2</sub> on more than one generation (Kurihara & Ishimatsu 2008), demonstrating that copepods appeared tolerant to high CO<sub>2</sub> through two generations.

In conclusion, post-larvae of the barnacles *Semibalanus balanoides* and *Elminius modestus* showed changes in shell structure and growth, respectively, under conditions of elevated temperature and CO<sub>2</sub>. *S. balanoides* were able to continue growth but were not able to maintain the mineral structure of their calcified shells under elevated temperature and CO<sub>2</sub> conditions, whilst *E. modestus* showed an opposite response by maintaining the mineral structure of their calcified shells but with limited growth rates. This contrast could result from either different physiological strategies in the two species or possibly different levels of tolerance associated with populations from different parts of their geographic distribution. In general, these post larval intertidal barnacles had lowered survival in elevated temperature and CO<sub>2</sub> conditions, which likely results from changes in energy consumption at a period in their life-history when they are non-feeding. These subtle

changes may be evidence that rates of physiological processes have altered corresponding with a shift above their optimal rates in the case of *S. balanoides* or more towards their optimum in the case of *E. modestus*.

## **CHAPTER 5. CALCIFICATION**

### **Comparing the impact of high CO<sub>2</sub> on calcium carbonate structures in different marine organisms**

Aspects of this chapter have been submitted as:

Findlay HS, Wood HL, Kendall MA, Spicer JJ, Widdicombe S (in review) Comparing the impact of high CO<sub>2</sub> on calcium carbonate structures in different marine organisms. *Journal of Experimental Marine Biology and Ecology*

## 5.1. INTRODUCTION

Calcifying marine organisms (molluscs & foraminifera, crustaceans, echinoderms and corals, coccolithophores) are predicted to be most vulnerable to decreasing oceanic pH (ocean acidification) because calcification rates may decrease as a result of reduced carbonate ion availability (Fabry et al. 2008). However, the possibility for increased or maintained calcification under high carbon dioxide ( $\text{CO}_2$ ) conditions originates from evidence that calcifying organisms are not reliant on carbonate ions to calcify. Iglesias-Rodriguez et al. (2008) first showed data of increased calcification with elevated  $\text{CO}_2$  levels in coccolithophores. The study here will focus primarily on invertebrates however. Investigations principally of molluscs (Wilbur 1964; Erez 2003) but also of corals (Al-Horani et al. 2003; Erez 2003), barnacles (Bubel 1975) and echinoderms (Decker & Lennarz 1988) show that bicarbonate ( $\text{HCO}_3^-$ ) or  $\text{CO}_{2(\text{aq})}$  and not carbonate ( $\text{CO}_3^{2-}$ ) is the origin of the carbon used in calcification. When either  $\text{HCO}_3^-$  or  $\text{CO}_2$  is the substrate for biogenic  $\text{CaCO}_3$ , the formation of  $\text{CaCO}_3$  structures (calcification) should not be inhibited directly by decreasing  $\text{CO}_3^{2-}$  concentrations (*via* ocean acidification). Although not new, this information often seems to be overlooked when explaining observed decreases in net calcification. Furthermore, many of these organisms produce calcium carbonate ( $\text{CaCO}_3$ ) at a crystallisation site isolated from the surrounding seawater (Wilbur 1964; Hart & Podolsky 2004; de Nooijer et al. 2008).

Molluscan shell calcification takes place away from the surrounding ambient seawater, at a crystallisation site in the extrapallial space (Wilbur & Yonge 1964). Detailed investigations of shell-forming cells indicated that calcium transport and secretion may in part be dependent on metabolic energy derived from the generation of ATP. This has been shown also for corals (review by Cohan & McConnaughey 2003). Additionally an increasing amount of glycogen has been found to be present in these shell-forming cells and this may provide a source of  $\text{CO}_2$ , which can be converted to  $\text{CO}_3^{2-}$  by the enzyme carbonic



anhydrase (Wilbur & Jodrey 1955) and used to form  $\text{CaCO}_3$ . In barnacles, calcification takes place in the mantle cavity and, again, examination of the structure of shell-secreting cells reveals a large presence of glycogen and mitochondria (Bubel 1975). Ophiuroids possess a mesodermal skeleton, yet the epithelium is very thin and the internal barrier separating coelomic fluid from the test is not well developed (Hyman 1955). This structure can therefore be exposed to changing seawater chemistry. The skeletal structure of echinoderms is made of magnesium calcite and is therefore highly susceptible to dissolution at lowered pH. Current understanding of the calcification process in echinoderms is based mainly on echinoid studies, with little known of the process in ophiuroids (Hart & Podolsky 2005).

Here biogenic calcification is defined as the formation of calcium carbonate by marine organisms, which is a process independent of dissolution of  $\text{CaCO}_3$ . Most current techniques for investigating changes in biogenic calcification are proxies for a change in the calcium carbonate concentration of calcified structures. Methods such as the alkalinity anomaly technique, quantifying calcium concentration in the calcified material (either by radioactive labelled calcium ( $\text{Ca}^{45}$ ) or by spectrophotometer measurements), or measuring changes in morphological parameters of a calcified structure (e.g. shell length and mass) all indicate a net change in calcium carbonate, i.e. the overall product of calcification and dissolution. This is often correctly termed net calcification but is sometimes wrongly interpreted as the individual's ability to produce calcium carbonate. There are no studies measuring *in vivo* dissolution, as far as I am aware, as there have been no successful methods designed to isolate the dissolution process without impacting the animal itself. Hence impacts from ocean acidification on shell growth, mineralogy or water chemistry cannot be assigned solely to a decrease in calcification but may result from expected increases in dissolution or changes in the innately-linked physiological processes. All physiological processes are closely interlinked and all of which are equally relevant for

organism survival. In calcifying organisms calcification is integral in the control of other processes such as growth, metabolism and regulation of internal body pH (Pörtner 2008). Five different calcifying organisms were used to assess the impacts of ocean acidification on aspects of whole animal physiology and calcification in this study: three mollusc species, a gastropod limpet (*Patella vulgata*), a gastropod snail (*Littorina littorea*), and a bivalve mussel (*Mytilus edulis*); one crustacean, a cirripede (*Semibalanus balanoides*); and one echinoderm, a brittlestar (*Amphiura filiformis*). Either the calcium (Ca) concentration in the calcified structures or shell morphological parameters was measured as a proxy for a net change in calcium carbonate in live individuals exposed to lowered pH. In order to quantify the rates at which some of these organisms' calcium carbonate structures dissolve, the Ca concentration in isolated shells and arms exposed to lowered pH was also measured. This measurement allowed observation of the change in calcium carbonate when biogenic calcification was absent, which enabled determination of a species' ability to calcify compared to dissolution across decreasing levels of pH and thus also across calcite and aragonite saturation states.

## 5.2. MATERIALS AND METHODS

The *Amphiura filiformis*, *Mytilus edulis*, *Littorina littorea* and *Semibalanus balanoides* experiments were initially carried out as part of a number of different studies with different aims to this investigation, focusing on other physiological, histological, and ecological impacts of ocean acidification, and hence the experiments were not all conducted at identical pH levels. The calcium and metabolism data for *A. filiformis* were previously published in Wood et al. (2008), some morphometric measurement and metabolism data for *L. littorea* have been published in Bibby et al. (2007) and preliminary data from *S. balanoides* are presented in Chapter 3. However, the data presented on *M. edulis* and *P. vulgata* are novel to this study and the data from Bibby et al. (2007), Wood et al. (2008) and Chapter 3 have been reanalysed. This study also bring together information on other

physiological impacts, examples from the studies mentioned above and other literature, as well as paleoecological examples to gain a greater understanding of the processes impacting the whole organisms.

### 5.2.1. Experimental setups

The *Amphiura filiformis*, *Patella vulgata*, *Mytilus edulis* and *Littorina littorea* experiments were carried out using acidified seawater by means of pH adjustment through bubbling of CO<sub>2</sub> into header tanks, and drawing water from these header tanks into the experimental containers as described in Widdicombe & Needham (2007). For details of the *A. filiformis* experiment see Wood et al. (2008); the *P. vulgata* experiment was run alongside the *A. filiformis* experiment. Ten *P. vulgata* individuals were placed in replicate 5 l containers at each pH condition; briefly the pH levels for these two experiments were 8.0, 7.7, 7.3 and 6.8. The *M. edulis* experiment is detailed in Beesley et al. (2008) with pH levels set at 8.0, 7.8, 7.6 and 6.8. The *L. littorea* experiment is detailed in Bibby et al. (2007), where only two pH conditions were examined: pH 8.0 and 6.45. The *Semibalanus balanoides* experiment was carried out in tidal microcosm systems (Chapter 2) containing high CO<sub>2</sub> - air detailed in Chapter 3, with two pH conditions: pH 8.0 and 7.7. Table 5.1 presents relevant information on exposure conditions and state of the organisms, while Table 5.2 presents information on the seawater conditions and carbonate system. In all experiments pH (NBS scale, Mettler-Toledo pH meter), dissolved inorganic carbon (DIC) (Ciba-Coming 965D Total CO<sub>2</sub> Analyser, Olympic Analytical Service), temperature and salinity (WTW LF197 combination temperature and salinity probe) were recorded throughout the experimental periods. Total alkalinity, bicarbonate (HCO<sub>3</sub><sup>-</sup>), carbonate (CO<sub>3</sub><sup>2-</sup>), and the saturation states for aragonite and calcite were all calculated from pH and DIC using CO<sub>2</sub>sys (Pierrot et al. 2006) with dissociation constants from Mehrbach et al. (1973) refit by Dickson & Millero (1987) and KSO<sub>4</sub> using Dickson (1990).

### 5.2.2. Measurement of calcium content

The calcium carbonate composition of the shells (*P. vulgata*, *M. edulis* and *S. balanoides*) or arms (*A. filiformis*) was estimated by analysing the calcium (Ca) concentrations as a proxy for any changes in calcification or dissolution. Live individuals produce calcium carbonate (i.e. calcify) during shell growth, however there may also be some dissolution of the shell or indeed some abiotic influence of shell formation. The dissolution factors, as discussed in section 5.1, may be enhanced in high CO<sub>2</sub> conditions. Ca is abundant in seawater and hence are not limiting. Formation of CaCO<sub>3</sub> involves combining inorganic carbon with Ca. Therefore any observed changes in Ca should indicate how the shell structure changes over time through calcification and dissolution processes. This principle is the same whether measuring radioactive labelled <sup>45</sup>Ca incorporation into shells (Comeau et al. 2009) or Ca content, *via* spectrophotometric techniques. Comparing the concentration of Ca in shells/arms of non-living calcified structures (shells and arms) with the concentrations in the structures from live animals provides an estimate of a organism's ability to calcify relative to any dissolution or abiotic effects because biogenic calcification will not be taking place in dead individuals.

All shells and arms were taken at the end of each experiment and frozen at -20 °C for further analysis. Concentration of Ca was measured using methods described in Spicer & Eriksson (2003); briefly this involved dissolving the shells and arms in 10 % nitric acid after rinsing in distilled water, drying and weighing and the total Ca concentration determined using atomic absorption spectrophotometry (Varian SpectrAA 50). The proportion of calcium (%Ca) in the shell or arm (mg Ca / mg shell) was calculated from the known total mass of the shell or arm (mg) and the volume of acid used in the digest (l).

*Table 5.1: Experimental information for each species, detailing when the experiments were carried out, where the animals were collected from, what reproductive state the animals were in, how long the exposures lasted for and the mean pH of each exposure treatment.*

Species	Date of experiment	Collections location	Adult reproductive state	Exposure period	Treatments (pH)	Feeding
<i>Mytilus edulis</i>	Sep. – Nov.	Trebarwith Sand, Cornwall, UK	Spawned prior to day 30	60 days	8.08, 7.72, 7.54, 6.41	Mixed algal diet
<i>Patella vulgata</i>	Dec. – Jan.	Wembury Bay, Devon, UK	Dormant	40 days	7.88, 7.70, 7.36, 6.60	Preconditioned biofilm slides
<i>Littorina littorea</i>	Nov. – Dec.	Wembury Bay, Devon, UK	Dormant	15 days	7.97, 6.63	3 algal spp.
<i>Amphiura filiformis</i>	Dec. – Jan.	Plymouth Sound, Devon, UK	Dormant	40 days	7.87, 7.69, 7.36, 6.80	Deposit feeding from cores
<i>Semibalanus balanoides</i>	Nov. – Feb.	Looe Bay, Cornwall, UK	Dormant	104 days	8.07, 7.70	Mixed algal diet

### 5.3. RESULTS

All five species showed a response to acidified conditions with perhaps the most surprising result being that four of these five had increased concentrations of calcium in low pH conditions (figure 5.1).

Over the respective experimental exposures, the %Ca of shells of live *Patella vulgata* and *Semibalanus balanoides* and the arms of live *Amphiura filiformis* either remained constant or increased significantly (ANOVA,  $F_{2,59} = 16.58$ ,  $p < 0.001$ ) compared to the control as the pH treatments decreased (figure 5.1a). The %Ca in the shells of live *Mytilus edulis* (figure 5.1a) did not differ significantly compared to the controls as pH decreased. These changes occurred despite the seawater in the low pH treatments having lower calcite and aragonite saturation states (Table 5.2) due to a reduction in carbonate ions. In some cases, treatments were completely undersaturated for  $\text{CaCO}_3$ , with calcite becoming undersaturated at ~ pH 7.3 and aragonite becoming undersaturated at ~ pH 7.6.

The %Ca in isolated shells of *P. vulgata*, *M. edulis* and *S. balanoides*, and arms of *A. filiformis*, decreased over the exposure period (7 d) compared to the controls (figure 5.1b). The percent change in %Ca  $\text{d}^{-1}$  (overall increase or decrease) relative to the control showed that %Ca  $\text{d}^{-1}$  in isolated *M. edulis* shells decreased by up to 1.5 %Ca  $\text{d}^{-1}$  while live shells did not differ from the control (figure 5.2). A similar pattern was exhibited by *P. vulgata*, *S. balanoides* and *A. filiformis* (figure 5.2). The decrease in %Ca observed in the isolated shells and arms of all four species correlates strongly with a decrease in carbonate ion concentration (figure 5.2b), yet this decline is not observed in the live individuals in any of the species.

Table 5.2: System data (mean  $\pm$  95 % C.I.) for the treatment (control, pH-1, pH-2, pH-3) used in each of the five experiments. For all experiments salinity, temperature, pH and DIC data were measured, all other data ( $A_T$  = total alkalinity;  $CO_3^{2-}$  = carbonate ion concentration;  $\Omega_{calcite}$  = calcite saturation state;  $\Omega_{aragonite}$  = aragonite saturation state) were calculated from pH and DIC using CO2sys with the solubility constant of Mehrbach et al, (1973) refit by Dickson & Millero (1989).

		<i>Mytilus edulis</i>	<i>Patella vulgata</i>	<i>Littorina littorea</i>	<i>Amphiura filiformis</i>	<i>Semibalanus balanoides</i>
	Temp	17.74 ( $\pm$ 0.24)	14.83 ( $\pm$ 0.39)	15	14.83 ( $\pm$ 0.39)	11.88 ( $\pm$ 0.06)
	Sal	35.13 ( $\pm$ 0.07)	36	35	36	35.60 ( $\pm$ 0.11)
Control	pH	8.08 ( $\pm$ 0.09)	7.88 ( $\pm$ 0.04)	7.96 ( $\pm$ 0.04)	7.89 ( $\pm$ 0.05)	8.07 ( $\pm$ 0.03)
pH-1		7.72 ( $\pm$ 0.12)	7.70 ( $\pm$ 0.03)		7.69 ( $\pm$ 0.03)	7.70 ( $\pm$ 0.03)
pH-2		7.54 ( $\pm$ 0.09)	7.36 ( $\pm$ 0.07)		7.37 ( $\pm$ 0.10)	
pH-3		6.41 ( $\pm$ 0.22)	6.60 ( $\pm$ 0.06)	6.64 ( $\pm$ 0.06)	6.60 ( $\pm$ 0.06)	
Control	DIC (mmol kg <sup>-1</sup> )	1.88 ( $\pm$ 0.65)	1.92 ( $\pm$ 0.11)	1.24 ( $\pm$ 0.22)	1.94 ( $\pm$ 0.11)	1.88 ( $\pm$ 0.89)
pH-1		1.95 ( $\pm$ 0.62)	2.05 ( $\pm$ 0.14)		2.04 ( $\pm$ 0.14)	2.05 ( $\pm$ 0.83)
pH-2		1.97 ( $\pm$ 0.70)	2.04 ( $\pm$ 0.15)		2.08 ( $\pm$ 0.15)	
pH-3		2.39 ( $\pm$ 0.24)	1.83 ( $\pm$ 0.23)	2.54 ( $\pm$ 0.24)	2.39 ( $\pm$ 0.24)	
Control	$\Omega_{calcite}$	4.37 ( $\pm$ 1.34)	2.56 ( $\pm$ 0.38)	1.76 ( $\pm$ 0.12)	2.79 ( $\pm$ 0.38)	3.22 ( $\pm$ 0.34)
pH-1		2.12 ( $\pm$ 0.63)	1.81 ( $\pm$ 0.22)		1.86 ( $\pm$ 0.22)	1.59 ( $\pm$ 0.13)
pH-2		1.36 ( $\pm$ 0.45)	0.81 ( $\pm$ 0.32)		0.95 ( $\pm$ 0.32)	
pH-3		0.10 ( $\pm$ 0.08)	0.14 ( $\pm$ 0.04)	0.15 ( $\pm$ 0.02)	0.16 ( $\pm$ 0.04)	
Control	$\Omega_{aragonite}$	2.83 ( $\pm$ 0.87)	1.66 ( $\pm$ 0.25)	1.13 ( $\pm$ 0.07)	1.80 ( $\pm$ 0.25)	2.07 ( $\pm$ 0.22)
pH-1		1.37 ( $\pm$ 0.41)	1.17 ( $\pm$ 0.14)		1.19 ( $\pm$ 0.14)	1.02 ( $\pm$ 0.09)
pH-2		0.88 ( $\pm$ 0.29)	0.52 ( $\pm$ 0.21)		0.61 ( $\pm$ 0.21)	
pH-3		0.06 ( $\pm$ 0.05)	0.09 ( $\pm$ 0.02)	0.10 ( $\pm$ 0.01)	0.10 ( $\pm$ 0.02)	
Control	$A_T$ ( $\mu$ Eq kg <sup>-1</sup> )	2160 ( $\pm$ 697)	2094 ( $\pm$ 120)	1363 ( $\pm$ 213)	2099 ( $\pm$ 120)	2086 ( $\pm$ 101)
pH-1		2069 ( $\pm$ 635)	2175 ( $\pm$ 153)		2125 ( $\pm$ 153)	2115 ( $\pm$ 95)
pH-2		2019 ( $\pm$ 707)	2134 ( $\pm$ 156)		2067 ( $\pm$ 156)	
pH-3		2057 ( $\pm$ 620)	1943 ( $\pm$ 232)	2116 ( $\pm$ 155)	1981 ( $\pm$ 232)	
Control	$CO_3^{2-}$ ( $\mu$ mol kg <sup>-1</sup> )	189.0 ( $\pm$ 57.98)	107.4 ( $\pm$ 16.07)	76.0 ( $\pm$ 5.10)	117.1 ( $\pm$ 16.07)	144.3 ( $\pm$ 20.02)
pH-1		91.8 ( $\pm$ 27.40)	75.8 ( $\pm$ 9.29)		77.9 ( $\pm$ 9.29)	71.6 ( $\pm$ 12.9)
pH-2		58.9 ( $\pm$ 19.54)	33.7 ( $\pm$ 13.77)		39.9 ( $\pm$ 13.77)	
pH-3		4.2 ( $\pm$ 3.47)	5.8 ( $\pm$ 1.65)	6.7 ( $\pm$ 0.90)	6.4 ( $\pm$ 1.65)	

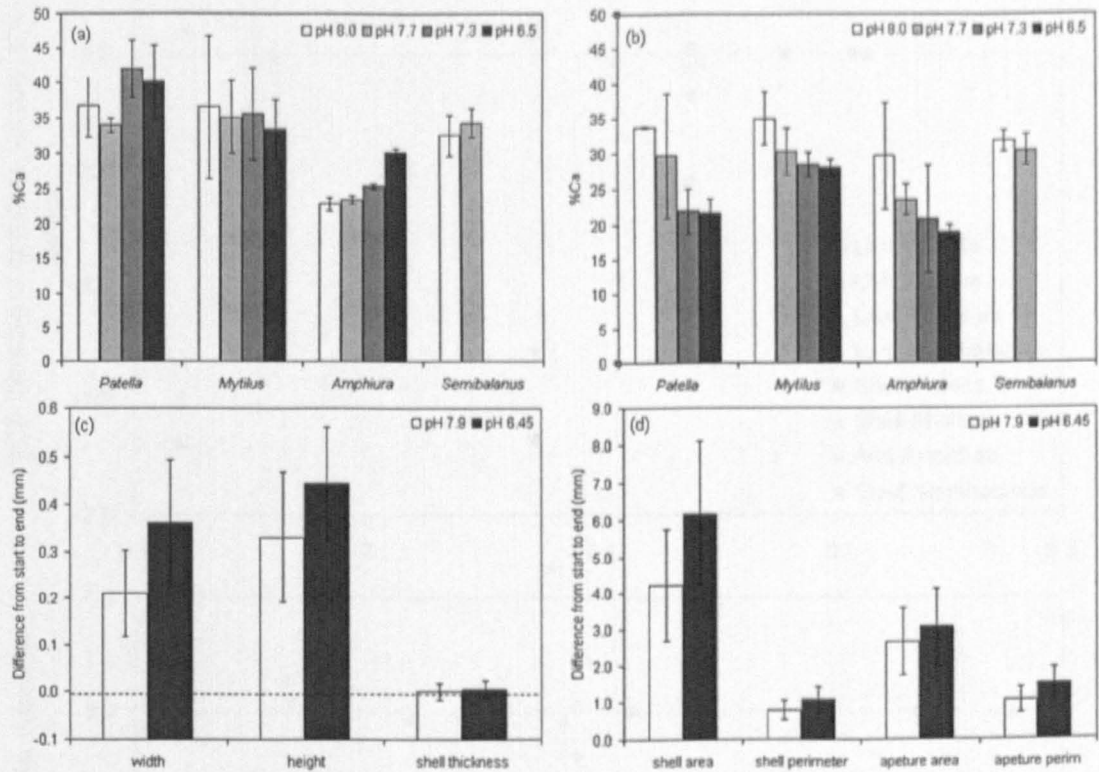


Figure 5.1: (a) Calcium ion concentration (percentage of total structure) in the shells of live *Patella vulgata*, *Mytilus edulis*, *Semibalanus balanoides* and arms of *Amphiura filiformis* (from Wood et al., 2008) in control pH 8 (white bars), pH 7.7 (light grey bars), pH 7.3 (dark grey bars), pH 6.8; (b) Calcium ion concentration (percentage of total structure) in the shells of dead *Patella vulgata*, *Mytilus edulis*, *Semibalanus balanoides* and arms of *Amphiura filiformis* in control pH 8 (white bars), pH 7.7 (light grey bars), pH 7.3 (dark grey bars), pH 6.8 (black bars); (c) Mean difference (measurement at end – measurement at start of experiment) in shell parameters of *Littorina littorea* (shell width, height, thickness) and (d) *Littorina littorea* (shell area, shell perimeter, aperture area and aperture perimeter) in the control pH 7.9 (white bars) and the treatment pH 6.45 (black bars), where values above zero represent an increase (mm). Error bars represent 95 % C.I..



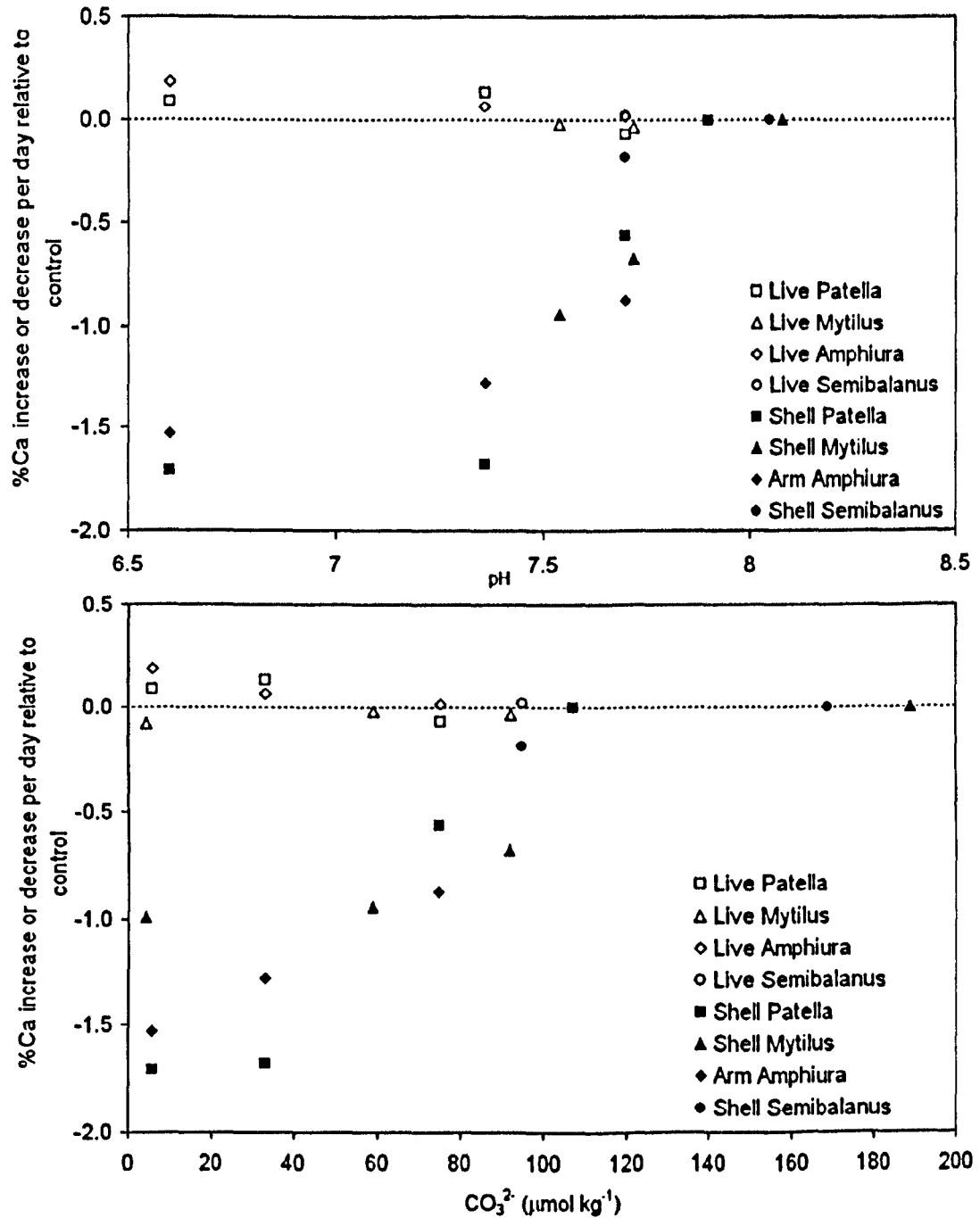


Figure 5.2: The increase or decrease in calcium ion concentration (percentage total structure) in each treatment (a) shows actual pH measured in the exposure tanks (b) shows calculated carbonate ion concentration in the exposure tanks, of live (open squares) and dead *Patella vulgata* shells (closed squares), live (open diamonds) and dead *Amphiura filiformis* arms (closed diamonds), live (open triangles) and dead *Mytilus edulis* shells (closed triangles) and live (open circles) and dead *Semibalanus balanoides* (closed circles). The means are standardised to an increase or decrease per day, assuming that there was a linear change over the experimental time period.

All the morphological shell parameters in *L. littorea* (width, height, thickness, area, perimeter, aperture area, and aperture perimeter) increased in low pH treatments compared to the control (figure 5.1c & 5.1d): there was ~67 % more growth in shell height, ~30 % more growth in shell width and ~40 % more growth in shell thickness under low pH conditions compared to the control. This increased growth implies that acidification was not preventing the animals from producing their shells and hence formation of CaCO<sub>3</sub> was possible at lowered pH. No measures of the mineral structure of the shell were made and therefore cannot ascertain if there was any impact on shell structure however both calcite and aragonite were undersaturated in the low pH treatment, indicating that dissolution is likely to have been occurring in the low pH treatment.

#### 5.4. DISCUSSION

There was a large amount of dissolution taking place on isolated shells and arms while the presence of a live animal within its calcium carbonate structure offset this dissolution; although the dissolution rate observed here may have been greater than might be expected to occur *in situ*. *In situ* dissolution remains an expected response to lowered pH primarily due to both the continued external exposure of CaCO<sub>3</sub> structures to the lowered pH water in the shell bearing species (*Mytilus edulis*, *Littorina littorea*, *Patella vulgata*, and *Semibalanus balanoides*) and the poor internal regulatory capacity of both *Amphiura filiformis* and the aforementioned species. Continued exposure and poor internal regulatory capacity results in the internal fluids having similar chemical composition to the surrounding seawater, therefore the endoskeleton and inner shell surface respectively are also bathed in lowered pH fluid. The results here showing continued presence and in some cases growth of calcified structures, demonstrates that the animals are still able to produce CaCO<sub>3</sub>, i.e. calcify, thus replacing the CaCO<sub>3</sub> lost through dissolution. This supports the hypothesis that calcification in molluscs, crustaceans and echinoderms relies on either HCO<sub>3</sub><sup>-</sup> or CO<sub>2</sub> and is not dependent on the CO<sub>3</sub><sup>2-</sup> concentration or calcite/aragonite

saturation states but may be related to metabolism (Lewis & Cerrato 1997). Perhaps more importantly it demonstrates that there is a great degree of biological control on calcification with complex links to other physiological processes (e.g. Pörtner 2008). In some instances organisms were able to completely overcome dissolution to increase their levels of calcium carbonate, while in other organisms levels were simply maintained (e.g. *Mytilus*).

Understanding how biological processes such as calcification influence the oceans' natural feedback mechanisms is fundamental when attempting to predict how the oceans' carbonate system will change in the future. Models indicate that under ocean acidification  $\text{CaCO}_3$  saturation states will become undersaturated (Caldeira & Wickett 2003) leading to increased  $\text{CaCO}_3$  dissolution. These results have shown, however, that biogenic calcium carbonate formation may increase or remain constant despite falling carbonate saturation levels and associated increasing dissolution (Andersson et al. 2006). Future net calcium carbonate production will represent a trade off between the antagonistic processes of calcification and dissolution. Dissolution may exert a cost, physically or energetically on organisms and additional impacts of hypercapnia and acidosis on metabolism and physiology may also interfere with an animal's homeostatic function (Pörtner 2008).

Recent experiments focusing on a single physiological process, such as growth of calcifying organisms under hypercapnia, potentially overlook the possibility that increased calcification may have counteracted some, or all, shell dissolution that was occurring at the same time as the animals were growing (e.g. Michaelidis et al. 2005; Gazeau et al. 2007; Cooper et al. 2008). Shell growth or net calcification may appear to be slower or reduced under hypercapnic conditions compared to the control, yet this may be a result of increased dissolution rates or impairment to other physiological processes, not necessarily a reduction in the animals' ability to calcify. Increasing evidence is appearing in the

literature which agrees with the results of this study: McDonald et al. (2009) show another barnacle species (*Amphibalanus amphitrite*) to continue, and possible even increase, calcification in conditions with pH 7.4; Arnold et al. (2009) demonstrate larval lobsters (*Homarus gammarus*) are able to lay down calcium carbonate structure in pH conditions 0.3 units below the control levels; Checkley et al. (2009) show 'young' fish have enhanced aragonite otolith growth when grown under elevated CO<sub>2</sub>; Maier et al. (2009) showed that although there was a decrease in calcification in cold-water corals, overall they showed a positive net calcification at aragonite saturation states below 1, and longer-term experiments suggest that they may actually maintain or even increase calcification over longer timescales at low pH (pers comm. U. Riebesell).

While the five species presented in this study are all benthic calcifiers, they vary greatly in life history. Therefore it needs to be considered whether the abiotic environments differ in their natural pH conditions. The most notably different species is the brittlestar *Amphiura filiformis* which lives within the sediment which is naturally lower in pH (Widdicombe unpublished data). However, it has been shown (Zhu et al. 2006) that burrow irrigation results in porewater pH reflecting the overlying water rather than that of the sediment; it can be assumed this is the case for *A. filiformis* which continually ventilates its burrow. The remaining species investigated in this study were all intertidal, and studied under immersed conditions (except *S. balanoides*, which was studied under tidal conditions), thus the altered seawater pH reflects the conditions these species experienced. Under natural conditions these species typically, with the exception of *Littorina littorea*, close up during emersion. Therefore their internal pH may decrease for short term periods due to the build up of respiratory CO<sub>2</sub>, however this does not occur in these experiments due to the short term nature of the natural episodes, and because these current experiments result in the total immersion, both internally and externally, of the animal in lowered pH seawater.

The present findings also have implications for the understanding of past episodes of CO<sub>2</sub> rise, ocean acidification and biodiversity crisis, and find support in recent paleoecological studies. The fossil record is an archive of global-level experimental data on the response of the biosphere to climatic and environmental change, and understanding past changes allows us to place the present-day crisis in its historical and scientific context. The geochemical and paleontological proxies that are used to estimate past levels of atmospheric CO<sub>2</sub>, such as the stomatal index of fossil leaves (McElwain et al. 1999) and the carbon isotope signature of ancient soil carbonates (Cerling 1991), demonstrate that CO<sub>2</sub> has fluctuated over the Phanerozoic and at times in the past has greatly exceeded present-day levels and the maximum predictions for the coming century (Royer et al. 2004), albeit on very different timescales to the present-day crisis. All of the major mass extinction events of the past 500 million years show evidence of associated climate change, including CO<sub>2</sub> rise and global warming (Twitchett 2006). The Late Triassic mass extinction event, for example, occurred during a relatively fast 400 % rise in atmospheric CO<sub>2</sub> levels from ca. 600 to 2,400 ppm (e.g. McElwain et al. 1999; Beerling & Berner 2002) and increased dissolution may have had a leading role to play in the extinctions of marine invertebrates (Hautmann 2004). Measurements of bivalve size and shell thickness through this event demonstrated a temporary reduction in size but increase in shell thickness (Mander et al. 2008), which would be a predicted response to increasing acidification based on laboratory studies. The timescale of present day climate change is faster than the events recorded in the fossil record, where changes are more likely to result from evolutionary adaptation. However such evidence does support the survival and continued calcification potential of benthic invertebrates in a high CO<sub>2</sub> world. In addition, the metabolic change seen in paleoecological data (Hautmann 2006) is consistent with the results of some recent ocean acidification studies highlighted here (e.g. Wood et al. 2008; Bibby et al. 2007) which also found increased calcification and metabolism in species today under ocean acidification conditions.

At ocean acidification levels predicted to occur within the next 100 – 300 years, a pH decrease by 0.30 – 0.77 units (IS92a carbon dioxide emissions scenario, IPCC 2007), there is evidence that increasing calcification comes at a cost. Investigations of whole-animal physiology and behavioural measures, such as general health (using lysosomal leakage as a proxy), reproduction (assessment of gonad state), muscle mass, metabolism and predation response have shown that several are impacted as a consequence of the up-regulation of calcification and metabolism: for example, there was increased muscle degradation in *Amphiura filiformis* (Wood et al. 2008), a lowered predation avoidance response in *Littorina littorea* (Bibby et al. 2007), and reduced health in *Mytilus edulis* (Beesley et al. 2008). Other investigations, with similarly small changes in pH, show that acid-base balance cannot be maintained in other mollusc and echinoderm species under acidified conditions (Michaeladis et al. 2005; Miles et al. 2007). Indeed Table 5.1 illustrates that all species that showed increased %Ca were in a dormant reproductive state, while *M. edulis*, which spawned during the exposure, only had maintained and slightly lower %Ca. This again hints at differences in energy allocation and metabolic demand resulting in different effects. A longer term (6 month) sea urchin acidification experiment (Shirayama & Thornton 2005) appears to provide evidence that some species are not able to maintain a high rate of calcification in order to overcome an increased rate of dissolution. The decrease seen in test thickness (Shirayama & Thornton 2005) did not account for total mass loss of *Hemicentrotus pulcherrimus* and *Echinometra mathaei* indicating a loss of soft tissue, as seen in *A. filiformis* (Wood et al. 2008). Ocean acidification therefore may not directly result in a reduced ability to calcify, but it does appear to cause negative impacts on all tested organisms. This highlights the importance of bringing together the present literature to gain a holistic insight when evaluating parameters such as calcification but also the need to investigate other processes in both calcifying and non-calcifying species.

Paleoecological studies of past episodes of CO<sub>2</sub> rise provide some data concerning longer term changes in species life-history. One characteristic of extinction episodes, especially those associated with CO<sub>2</sub> rise such as the Late Permian and Late Triassic events, is a dramatic decline in the size of marine organisms (the Lilliput effect) (Hautmann 2006; Twitchett 2007; Mander et al. 2008). The costs associated with the need for increased calcification may have a role to play in this phenomenon. Changes in shell mineralogy, from aragonite to calcite, have also been observed in Triassic-Jurassic bivalves and interpreted as reflecting a need to conserve energy as metabolic rates increased (Hautmann 2006). This change in mineral structure, which may also be an adaptation to ocean acidification by benthic calcifiers today, reduces metabolic costs of calcification indirectly because calcite is less prone to dissolution and hence the rate at which the structure needs to be replenished in low pH conditions is reduced. There are apparent differences in the deep-sea coral ecosystems between the North Atlantic and the North Pacific, the latter of which has much a shallower aragonite saturation horizon (ASH). In the North Pacific six of the seven stylasterid species of coral (Cairns & Macintyre 1992), used calcite to form their spicules and skeletons, yet only 10 % of all known stylasterid species produce calcite instead of the more soluble aragonite. Cold-water corals have also been found living close to the ASH, suggesting they have mechanisms to cope with high rates of dissolution, yet they do not flourish or form large structure, as in the North Atlantic (Guinotte et al. 2006). At marine volcanic CO<sub>2</sub> vent sites, although the abundance of calcifying animals' decreases with increasing pH, these organisms are nonetheless found under acidified seawater conditions (Hall-Spencer et al. 2008).

Results from both the laboratory and from paleoecological records suggest that animals are capable of altering their biology to be able to cope with a decrease in pH. As research now homes in on realistic scenarios, investigations may find that within the predicted pH ranges, at least in terms of producing calcium carbonate, animals are able to compensate. Other

physiological processes are more likely to be impacted as a cost to increased energy expenditure of producing calcified material in a more acid ocean, therefore organisms may grow less (i.e. become smaller on average, as is evident from experimental and paleoecological data) and/or over longer timescales they may change their mineralogical structures. While work to date (see Fabry et al. (2008) for review) has made some progress in determining physiological responses to high levels of CO<sub>2</sub>, research should focus on whole animal physiology in both non-calcifying and calcifying organisms as well as investigate the possibility of mineral and size changes over longer time-scales.



## CHAPTER 6. POPULATIONS & MODELS

**Can ocean acidification affect *Semibalanus balanoides* population dynamics at its southern range edge?**

Aspects of this chapter have been submitted as:

Findlay HS, Burrows MT, Kendall MA, Spicer JI, Widdicombe S (in review) Can ocean acidification affect *Semibalanus balanoides* population dynamics at its southern range edge.

*Ecology*

## 6.1. INTRODUCTION

Models are useful tools for exploring the relationships between environmental factors (biotic and abiotic) and population dynamics (Lauzon-Guay et al. 2006). In the study of the marine environment, models have been used to understand processes such as local and regional scale population dynamics (Lima et al. 2007; Wethey & Woodin 2008), dispersal (Wieters et al. 2008), and interspecific interactions (Poloczanska et al. 2008). In the majority of these models temperature is assumed to be the dominant controlling factor. Certainly as climate change research progresses it is becoming increasingly important to understand what role temperature plays on population processes, how this changes across both temporal and spatial scales, and whether scientists can make useful predictions. Climate change is not, however, the only consequence of elevated atmospheric CO<sub>2</sub>. The oceans are a carbon sink and have absorbed *ca.* 48 % of all the anthropogenic carbon released into the atmosphere since the industrial revolution (Sabine et al. 2004). This increasing concentration of CO<sub>2</sub> is altering the ocean chemistry predominantly causing an increase in hydrogen ions (and hence a decrease in pH) and a decrease in carbonate ions (Orr et al. 2005). Ocean acidification, as it has been termed, has yet to be included in biological models. Only one paper to date has investigated ecological dynamics and their correlation to pH dynamics over the same time period (Wootton et al. 2008).

Wootton et al. (2008) provided observational and modelling analysis of rocky shore community dynamics as well as pH and associated physical factors over a number of years (2000 – 2008). Their analysis suggests that there has been a decrease in ocean pH over this period of 0.045 unit yr<sup>-1</sup>, which is significantly faster than seen in other long-term time series station data, such as the Hawaiian Ocean Time-series (Brix et al. 2004), the European Station for Time-series in the Ocean, Canary Islands (Santana-Casiano et al. 2007) and the Bermuda Atlantic Time-Series (Bates & Peters 2007). Associated with this declining pH is a shift in ecosystem structure from a mussel to an algal-barnacle dominated

community (Wootton et al. 2008). Ocean acidification can alter processes such as calcification (e.g. Gazeau et al. 2007), growth (e.g. Michaelidis et al. 2005), immune function (Bibby et al. 2008), and behaviour (Bibby et al. 2007). However, understanding how these individual impacts considered together alter behaviour and interactions between individuals, populations and communities is more difficult.

Many factors influence the population dynamics of intertidal animals, with some of the most well studied being the barnacles (e.g. Barnes 1999). It has long been debated whether pre-settlement or post-settlement factors contribute more to controlling recruitment of barnacles (Hatton & Fischer-Piette 1932; Hatton 1938). Field observations and dispersal models suggest that pre-settlement conditions play an important role, for example wind strength and pattern during the larval phase (Kendall et al. 1982; Delafontaine & Flemming 1989); however survival of settled post-larvae and juveniles has been shown to be highly influenced by temperature over the summer months (Kendall et al. 1985) as well as by predation and abrasion (Connel 1961; Foster 1971). Thermal models have been developed to show that the temperature on a rocky shore can exceed barnacles' thermal limits and their survival depends on small-scale habitat conditions such as position on the shore (Wethey et al. 2002). Few of these modelling studies attempt to include multiple factors for forcing the population, yet scientists know that a multitude of changes are occurring in the oceans which have the potential to alter ecosystem dynamics (Helmuth et al. 2006). There is also a need to take empirical evidence of impacts on single organisms, even at the level of cellular and physiological processes, and place this in a wider context of how these impacts might affect a population or even a community.

The purpose of this study is twofold: First to attempt to incorporate the impacts of ocean acidification into a population model forced through environmental parameters. Secondly to scale up from experimental data to predict how populations, at a very basic level, might

be impacted by ocean acidification and climate change. This is the first study, to my knowledge that attempts to place ocean acidification experimental data into a population model for a benthic sessile organism. This study adapts a model used to predict climate change impacts on two barnacle species (Poloczanska et al. 2008). The model is generated utilising experimental information on survival of post-larval *Semibalanus balanoides* under different ocean acidification and climate change scenarios, and compare this with data from the southwest coast of England. Then the model is used to (a) forecast the population abundance at the southern edge of the *S. balanoides* geographic distribution, and (b) to understand and therefore predict the sensitivity of this species to ocean acidification and climate change.

## **6.2. MATERIALS AND METHODS**

### **6.2.1. Data sets**

#### ***6.2.1.1. Population data***

*Semibalanus balanoides* abundance data were extracted from Southward (1991, Table 6.1) as the mean adult abundance (individuals cm<sup>-2</sup>) at all tidal heights from autumn 1951 to 1990. The counts were made at Cellar Beach, on the south coast of England. Autumn counts are used here because they give a good indication of the population after the spring/summer recruitment period and survival over the summer.

#### ***6.2.1.2. Temperature data***

Temperature data were obtained from station E1 (data available from the PML L4 website [http://www.westernchannelobservatory.org.uk/e1\\_ctdf/](http://www.westernchannelobservatory.org.uk/e1_ctdf/)) from 1903 to present (2008). The data for June sea surface temperature (SST) was used following Poloczanska et al. (2008) where June SST from the previous year was used as a predictor for the model (figure 6.1a). The variation around the data was calculated ( $\sigma^2 = 1.17$ ), as was the linear trend (June SST = 0.0142.year – 13.799, mean square deviation (MSD) = 0.9786).

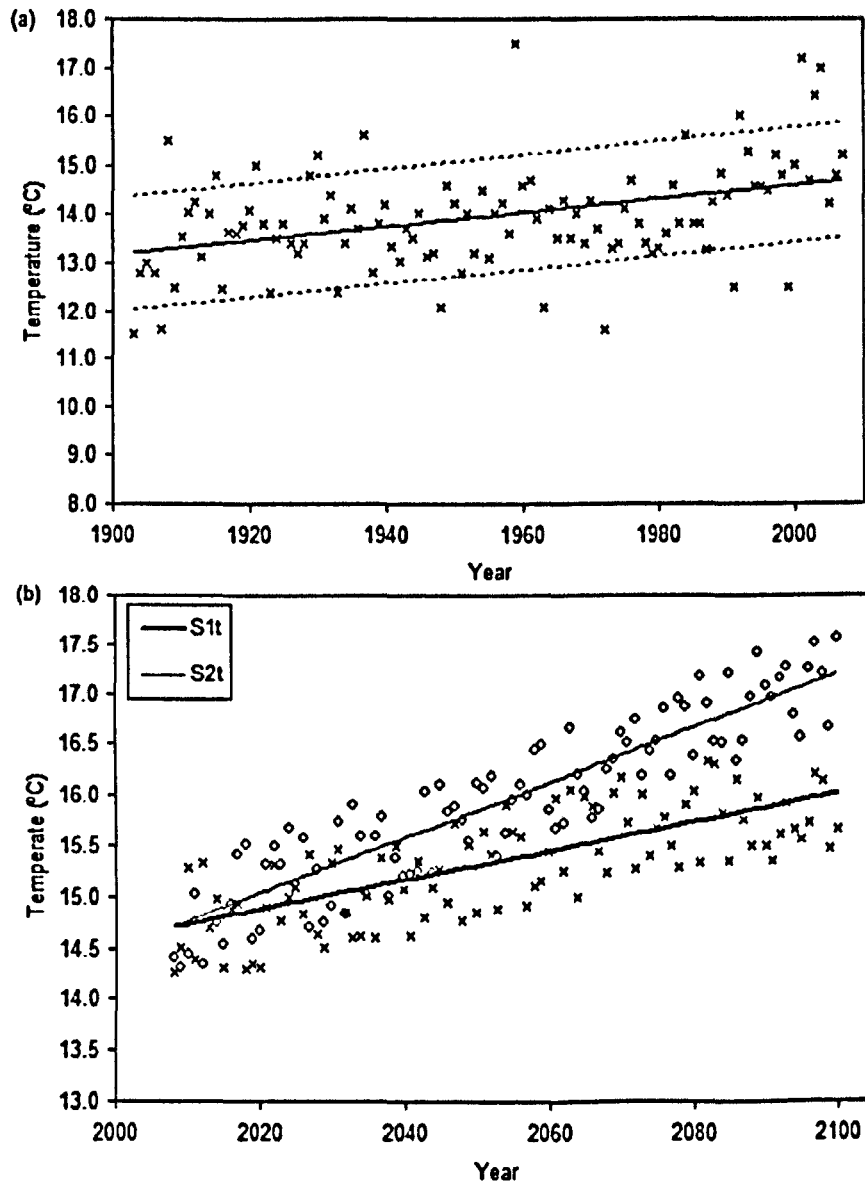


Figure 6.1: (a) Temperature dataset for period 1903 – 2008, mean pH (continuous line) =  $0.0142 \cdot \text{yr} - 13.799$ , dashed lines are equal to 1 standard deviation ( $\sigma^2 = 1.17$ ), crosses show the El temperature dataset. (b) Future projections: S1t, mean increase  $0.0142 \text{ yr}^{-1}$  (thick line) with associated example of stochastic dataset (crosses) and S2t, mean increase  $0.027 \text{ yr}^{-1}$  (thin line) with associated example of stochastic dataset (circles).

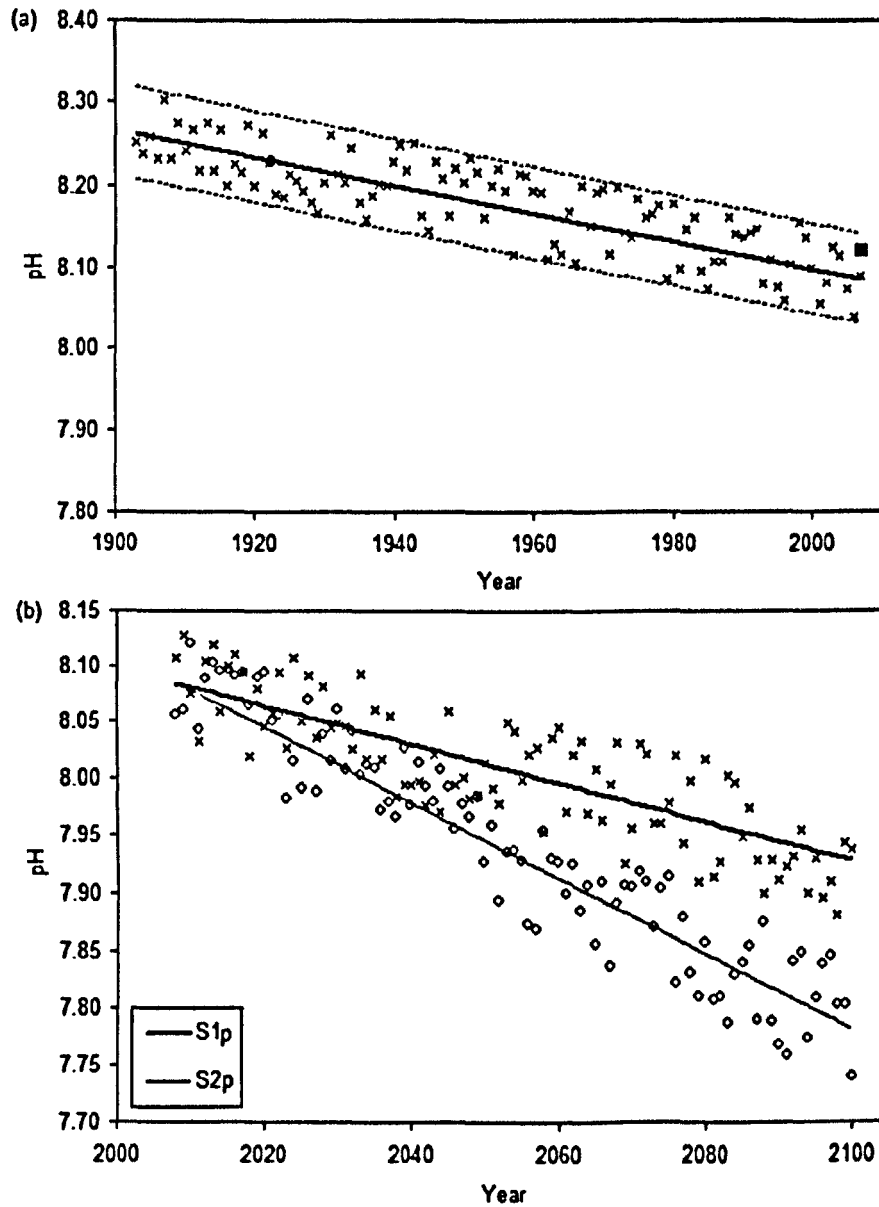
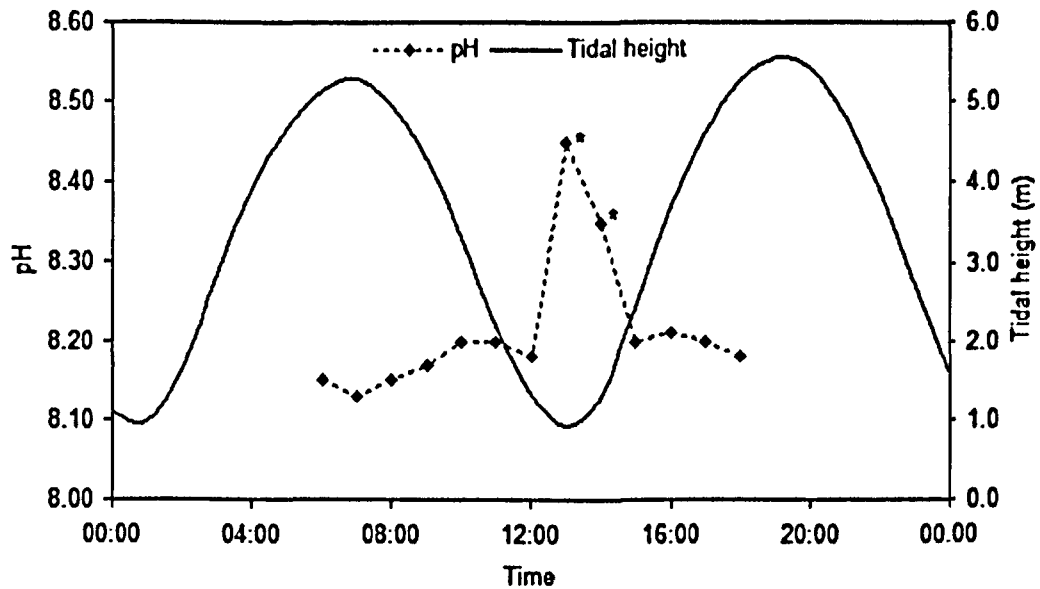


Figure 6.2: (a) Modelled pH dataset for period 1903 – 2008, mean pH (continuous line) =  $-0.0017 \cdot \text{yr} + 11.498$ , dashed line are equal to 1 standard deviation ( $\sigma^2 = 0.119$ ), crosses give an example of the random distribution of pH values selected for one dataset, solid circle represents data from Atkins (1923) and the solid square represents data from Cellar Beach, June 2008. (b) Modelled future projections: S1p, mean decrease  $0.0017 \text{ yr}^{-1}$  (thick line) with associated example of stochastic dataset (crosses) and S2p, mean decrease  $0.0033 \text{ yr}^{-1}$  (thin line) with associated example of stochastic dataset (circles).



*Figure 6.3: Tidal height (solid line) and pH (dashed line) measured at the intertidal rocky shore of Cellar Beach over a tidal cycle throughout the one day (24/06/09). The asterisks denote measurements of pH taken over sand instead of rock (see section 6.2.1.3).*

*Future temperature projections were based on two scenarios:*

- (1) *S1t*: Extension of the linear trend observed in the E1 SST data from 1903 to 2008 (+0.0142 °C yr<sup>-1</sup>)
- (2) *S2t*: 2.5 °C expected warming by 2100 (+0.027 °C yr<sup>-1</sup>) (MCCIP 2007)

Stochastic variation was added to each mean linear trend (figure 6.1b) to match the variance observed in the E1 data ( $\sigma^2 = 1.17$ ).

### **6.2.1.3. pH data**

pH data were available for this location but only for one time point. One pH dataset was created in 1922 (Atkin 1923) at several locations off Plymouth Sound, including at station E1 (June pH = 8.23). The nearest long-term time series of pH is the European Station Time-series of the Ocean, Canaries (ESTOC), data from which has been used to show that pH is decreasing by 0.0017 unit yr<sup>-1</sup> (Santano-Casiano et al. 2007). From this information

pH was modelled for the same period as available temperature data (1903 – 2008). Stochastic variation was added to the mean trend of pH decline, by calculating the variation found in the available Waterbase database (<http://www.waterbase.nl/index.cfm>) June pH data (Applezak 30 km station (Note, E1 is approx. 34 km from shore), 1975 - 1982),  $\sigma^2 = 0.119$  (figure 6.2a).

Additional pH data were collected from the rocky intertidal over a tidal cycle (over 13 hours) at Cellar Beach in June, 2009 (figure 6.2a and figure 6.3); measurements were made at the sea surface 50 cm out from the waterline, for most of the tidal cycle this was above the rocky shore; however, at low tide this was overlying sand.

*Future pH projections were based on two scenarios:*

(1) *S1p*: extension of the linear trend described in the ESTOC data (-0.0017 pH unit yr<sup>-1</sup>)

(2) *S2p*: 0.3 pH unit decrease by 2100 (-0.0033 pH unit yr<sup>-1</sup>) (IPCC 2007)

Stochastic variation was again added to the mean linear trend (figure 6.2b) to match the variance in the pH data set ( $\sigma^2 = 0.119$ ).

### 6.2.2. Model description

The model was based on that of Poloczanska et al. (2008) but was modified to model only the *Semibalanus balanoides* six age classes. The basic assumptions were (1) the population is open and recruitment is proportional to the free space available, (2) mortality is age-specific and density-independent and (3) that there *Chthamalus-Semibalanus* interactions were not important at this site, as the two species are not in full competitions for space since there is always some free space for settlement (Southward 1991). The model has two time steps per year (June and December) with *S. balanoides* recruiting to the adult population in June. The number of individuals entering each age class of six months and above is:



$$n_{x,i+1,t+1} = P_{x,i} n_{x,i,t} \quad (6.1)$$

where  $P_{x,i}$  is the probability of surviving the six months from age class  $i$  to age  $i+1$  for species  $x$ . The last age class was assumed to be additive. The input parameters: survival size, growth rates and maximum recruitment values, are taken from Poloczanska et al. (2008) (Table 6.1).

Recruitment,  $R$ , is defined as the number of settlers alive at the end of the settlement season, taken to be June for *S. balanoides* as in Poloczanska et al. (2008). The equations are provided in Table 6.2.

Recruitment rate,  $S$ , is a temperature and pH dependent function (figure 6.4):

$$S = S_{max} \cdot f(t, T_{crit}, c) \cdot A_{t,pH} \quad (6.2)$$

where  $S_{max}$  is the maximum recruitment rate per unit free space at cool temperatures, set to a constant 30 recruits per square centimetre,  $f$  is a cumulative Gaussian function,  $t$  is June SST,  $T_{crit}$  is the SST at which recruitment is at 50 % maximum, and  $c$  represents the rate of decline of recruitment per unit free space with increasing SST.  $A_{t,pH}$  represents a matrix of probabilities of cyprids surviving to become settlers, and hence potential recruits, as a function of temperature and pH. The probability of surviving at each temperature and pH was provided from experimental data (Chapter 4) and linearly interpolated between the data points.

*Table 6.1: Abbreviations are: Operculum = average operculum length, Basal Area = average basal area, p(survival) = probability of surviving to the next age class. Size at age data and survival probabilities were taken from the work of Burrows (1988) on intertidal barnacles in southwest England and Poloczanska et al. (2008). Amended from Poloczanska et al. (2008)*

Input	Age Class (Months)					
	0 – 6	6 – 12	12 – 18	18 – 24	24 – 30	30+
Operculum (mm)	1	2	2.1	2.5	2.6	3
Basal Area (mm <sup>2</sup> )	2.5	8.1	8.8	11.9	12.7	16.2
p(survival)	0.5	0.5	0.6	0.6	0.6	0.6

Table 6.2: Variable and parameter descriptions with values and units used in the model; where variable values are given, these are the initial values.

	Description	Value	Unit
<b>Variables</b>			
$F$	Free Space	100	mm <sup>2</sup>
$J_{pop}$	Total adult population in June	2	Ind. cm <sup>-2</sup>
$R$	Recruitment rate per unit free space: $R = S \times F \times G$		recruits cm <sup>-2</sup>
$G$	Gregariousness function: $G = \frac{P_{SB}}{b + P_{SB}}$		
$P_{SB}$	Proportion of total area occupied by adult <i>S. balanoides</i>		mm <sup>2</sup>
$S$	Recruitment rate: $S = S_{max} \cdot f(t, T_{crit}, c) \cdot A_{t,pH}$		recruits cm <sup>-2</sup>
<b>Parameters</b>			
$S_{max}$	Maximum recruitment rate	30	Ind. cm <sup>-2</sup>
$b$	Gregariousness half-saturation constant	0.0009	
$t$	June SST (E1)		°C
$T_{crit}$	Temperature at 50 % maximum recruitment	13.11	°C
$c$	Rate of recruitment decline with increasing SST	0.62	

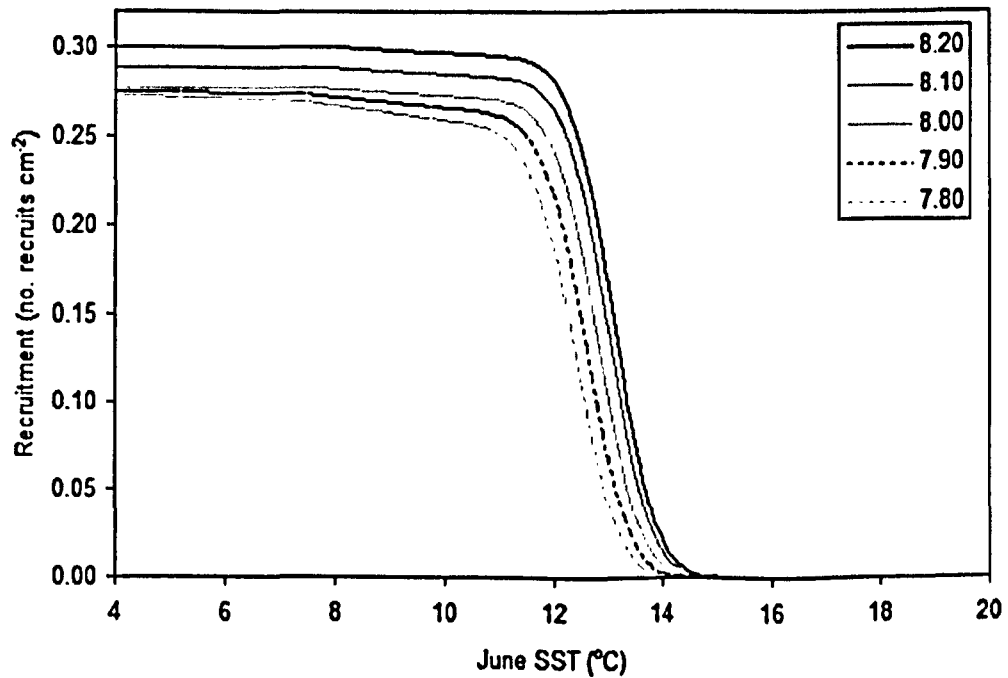


Figure 6.4: Fitted function for *Semibalanus balanoides* recruitment rate into free space with June sea surface temperature (SST, °C) and at different pH levels (pH 8.20 thick line, pH 8.10 dashed black line, pH 8.00 thin black line, pH 7.90 dashed grey line, pH 7.80 thin grey line).

### 6.2.3. Statistical analysis

The model runs were compared with the Southward (1991) data (from 1950 – 1990) by calculating the sum of squared deviation ( $SS^2$ ), the Pearson's correlation coefficient ( $r$ ) and the Akaike's Information Criterion (AIC). An  $SS^2 = 0$  or  $AIC = 0$  shows a model that does not deviate at all from the data (i.e. perfect fit and position) while an  $r = 1$  would demonstrate complete correlation between model and data (i.e. perfect phasing and cycling).

To assess the differences between the data and the model hindcast runs (1950 – 1990), the  $SS^2$  and  $r$  were compared with the average (mean of 100 runs)  $SS^2$  and  $r$  for the time-series with decreasing pH ( $-0.0017 \text{ unit yr}^{-1}$ ) by converting both to z-values. It was assumed that the 100 runs came from a normal distribution and that the z-value standardises the normal.

### 6.2.4. Experimental analyses

Empirical evidence is used to infer whether, over the whole life cycle of *Semibalanus balanoides*, it is possible to achieve the levels of mortality in lowered pH conditions which will cause a significant impact on the population. For this, experimental data on survival of embryos (Chapter 3), nauplius larvae and post-larvae (Chapter 4) were used. The nauplius larval experiment was carried out during March and April 2008. Three replicate 500 ml culture bottles were filled with filtered seawater (100 and 1  $\mu\text{m}$  filters), which had been either equilibrated with air (380 ppm) or  $\text{CO}_2$ -air mix ( $\sim 1,000$  ppm). Barnacles were collected from the rocky shore and taken back to the laboratory on rock chips, without dislodging. Once in the laboratory 20 barnacles were removed from the rocks and examined for the presence of egg masses. Gravid barnacles were placed into individual Petri-dishes (10 cm diameter) containing natural filtered seawater and after a few hours any nauplii larvae that had been released from the adults were collected. In excess of 200 nauplius larvae were then transferred into each of the culture bottles. On days 1, 6, 12, 15,

20, 26 and 33 a subsample of 20 ml was taken from each culture bottle making sure that the seawater was well mixed. The samples were preserved in 4 % formalin and then photographed under low power (x 10). The length and width of each nauplius was then measured using Image Analysis. Survival was expressed as the counts of nauplii in each subsample that appeared complete and had no abnormalities.

### 6.3. RESULTS

#### 6.3.1. Model comparison to *Semibalanus balanoides* abundance data 1950 – 1990

*Table 6.3: Statistical analysis of the hindcasting (1950 – 1990) produced from the different pH levels (No pH function/ pH constant at 8.2, pH constant at 8.1, pH constant at 8.0, pH constant at 7.9, pH constant at 7.8 and pH decreasing with stochastic variation at  $-0.0017 \text{ yr}^{-1}$ ) compared to the average time-series (mean of 100 runs) produced with decreasing pH ( $-0.0017 \text{ unit yr}^{-1}$ ) with stochastic variation.  $SS^2$  Deviation = sum of squares deviation,  $r$  = Pearson's correlation coefficient, AIC = Akaike's Information Criterion.*

	No pH function/ pH 8.2	pH 8.1	pH 8.0	pH 7.9	pH 7.8	pH $-0.0017 \text{ yr}^{-1}$
$SS^2$ Dev	55.66	50.90	53.94	60.93	70.53	54.99
z-score	0.55	-3.36	-0.87	4.88	12.76	
$r$	0.43	0.39	0.33	0.27	0.19	0.42
z-score	0.97	-2.11	-5.88	-10.07	-15.67	
AIC	23.87	20.39	22.65	27.40	33.11	23.40

The model is able to reproduce the Cellar Beach mean population data (figure 6.5a,  $SS^2 = 55.66$ ,  $r = 0.43$ , AIC = 23.87). When the pH function was introduced as a constant pH = 8.2 this had no effect on the model (figure 6.5b). However, with the exception of pH = 8.1, as the constant pH was decreased, the correlation between the data and the model also decreased (Table 6.3, figure 6.5b). pH = 8.1 had a better  $SS^2$  deviation and AIC but a lower Pearson's correlation ( $SS^2 = 50.90$ ,  $r = 0.39$ , AIC = 20.39), which suggests that at this pH the model was better related to the data but did not accurately describe the cycling of the

data. When the pH decreasing with time ( $-0.0017 \text{ unit yr}^{-1}$ ) model was used, the time-series produced the best Pearson's correlation and AIC value with a relatively low  $SS^2$  deviation in relation to the Southward (1991) data ( $SS^2 = 54.99$ ,  $r = 0.42$ ,  $AIC = 23.40$ ).

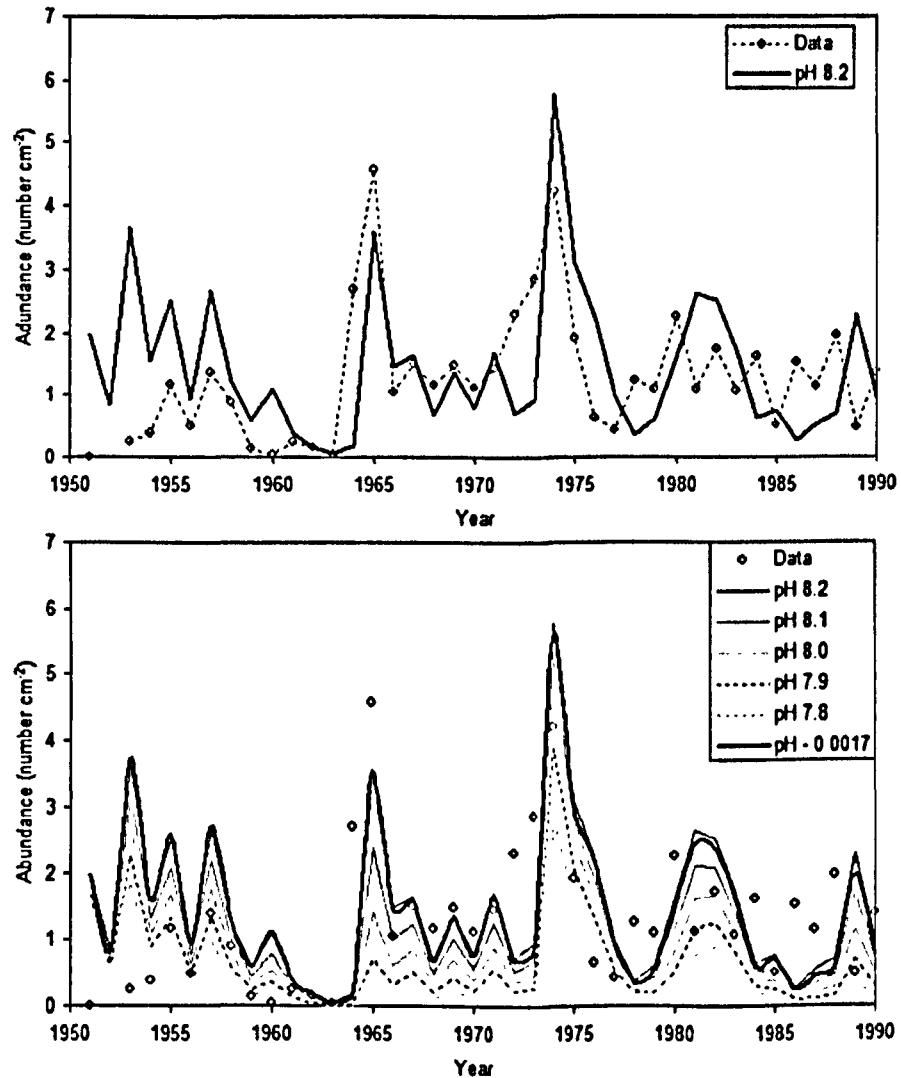


Figure 6.5: (a) Model (thick line) and data (dashed line & diamonds) for population density at Cellar beach, UK (Southward, 1991), between 1950 and 1990 with June sea surface temperature forcing the model population. (b) Modelled population with constant pH = 8.2 (thin black line), pH = 8.1 (thin dark grey line), pH = 8.0 (thin light grey line), pH = 7.9 (dashed black line), pH = 7.8 (dashed grey line), pH decreasing  $-0.0017 \text{ unit yr}^{-1}$  (thick black line). Open circles are the data from Southward (1991).

### 6.3.2. Model projections for 1990 – 2008

The E1 temperature data and decreasing pH ( $-0.017$  unit  $\text{yr}^{-1}$ ) produced a time-series where the abundance of *Semibalanus balanoides* was overall decreasing, except where it increased in 1992 when there was a below average cold year the previous year ( $12.5$  °C). The population increased again in 2000, after the cold year in 1999 ( $12.5$  °C) although this was a much smaller increase because there was a very small population ( $<0.01$  individual  $\text{cm}^{-2}$ ) the previous year. After this the population decreased to near zero (figure 6.6). There was very little difference between the time-series predicted by temperature only and the time-series predicted by temperature and pH ( $\text{SS}^2$  deviation from temperature only model = 0.23). However, at constant pH levels below 8.0, the population remained very low even after the cold years of 1991 and 1999 because recruitment levels were much lower.

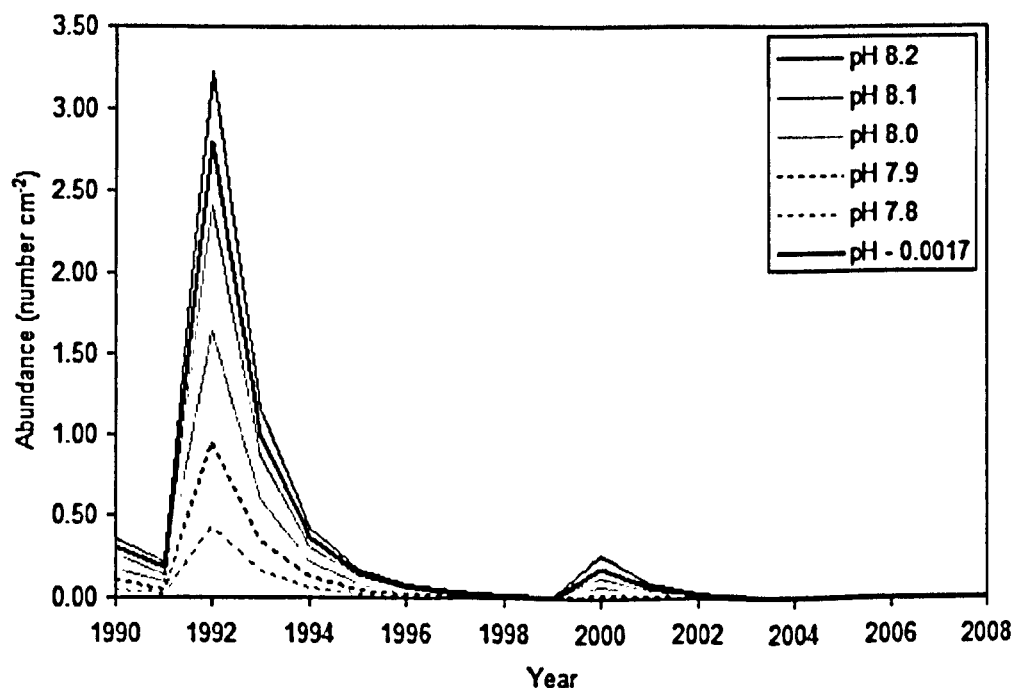


Figure 6.6: Modelled population from year 1990 to 2008 with constant pH = 8.2 (thin black line), pH = 8.1 (thin dark grey line), pH = 8.0 (thin light grey line), pH = 7.9 (dashed black line), pH = 7.8 (dashed grey line), pH decreasing  $-0.0017$  unit  $\text{yr}^{-1}$  (thick black line).

### 6.3.3. Model projections for 2008 – 2100

After the year 2008 there was no significant population; model values were  $< 0.01$  individual  $\text{cm}^{-2}$  (figure 6.7). A small increase in population could occur by introducing a low temperature year, however because the population is so small ( $< 1 \times 10^{-5}$  individuals  $\text{cm}^{-2}$ ) even a maximum recruitment (30 recruits  $\text{cm}^{-2}$ ) only increased the population to just over 0.0001 individuals  $\text{cm}^{-2}$ , and hence a recruitment of  $>1,000$  individuals  $\text{cm}^{-2}$  would be needed to recover the population to “real” observable levels. After the year 2020 the model population died out completely under all the scenarios. The decreasing pH scenarios  $S1p$  and  $S2p$  both caused the population to die out slightly quicker than when there was no pH function included. There was negligible difference between the different scenarios of either temperature ( $S1t$  and  $S2t$ ) or pH ( $S1p$  and  $S2p$ ).

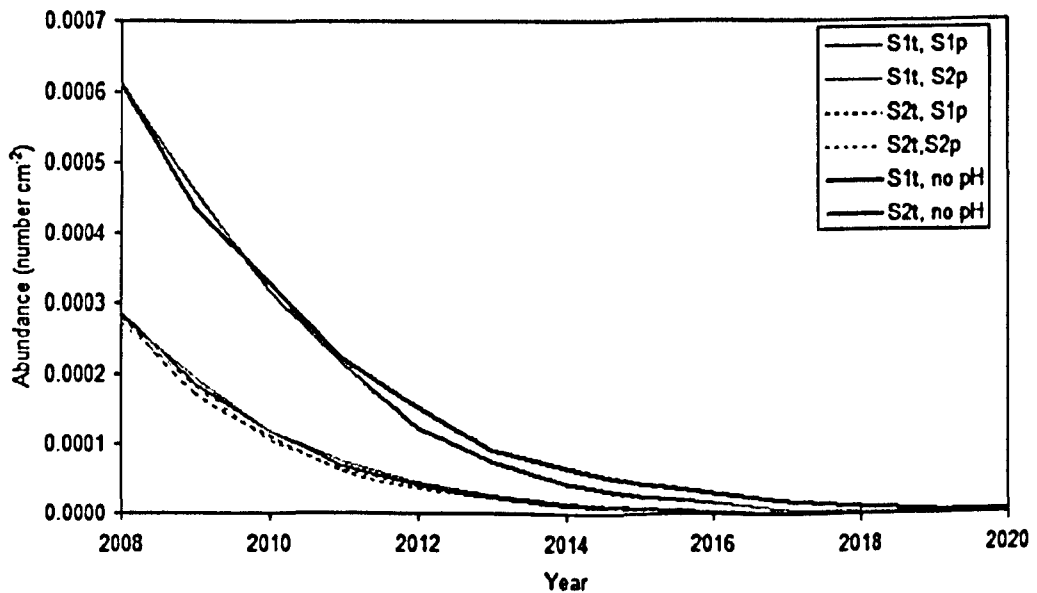


Figure 6.7: Modelled population from year 2008 to 2020 with different scenarios of temperature increase and pH decrease. Abbreviations are:  $S1p = -0.0017 \text{ unit yr}^{-1}$ ,  $S2p = -0.0033 \text{ unit yr}^{-1}$ ,  $S1t = 0.014 \text{ }^\circ\text{C yr}^{-1}$ ,  $S2t = 0.027 \text{ }^\circ\text{C yr}^{-1}$ , no pH  $f = \text{no pH function}$ .

#### 6.3.4. Experimental survival rates

*Semibalanus balanoides* embryos showed no mortality (chapter 3), whereas nauplius larvae began to show increased mortality in lower pH conditions after 12 days. This increased further through days 15, 20 and 26 such that survival was 15 % lower than the control after 26 days. The post-larval mortality varied with temperature and pH, such that at low temperatures (4 – 8 °C) there was negligible impact on post-larval survival (2 – 3 % lower than the control), whereas at higher temperature (14 – 19 °C) and low pH there was more impact on survival (10 – 27 % lower than the control). Cumulatively the lowered pH decreases survival of early-life stages in the laboratory from ~79 % (control) to ~58 % at present (year 2008) summer SST (14 °C). The modelled equilibrium population at 14 °C (no pH affect included) is 0.803 (individuals cm<sup>-2</sup>). The equilibrium population decreases to 0.645 individuals cm<sup>-2</sup> and 0.479 individuals cm<sup>-2</sup> when a lowered survival of 79 % and 58 % are incorporated into the model respectively.

#### 6.4. DISCUSSION

This study integrates results from an ocean acidification and climate change experiment with a population model forced by temperature and pH. It predicts how climate change and ocean acidification together might impact a population of the barnacle *Semibalanus balanoides* at the southern edge of its geographic range. This study highlights that given that temperature is a main driver in the population dynamics at this site, small decreases in pH can have a significant impact on population level by further lowering recruitment. Nevertheless the projected decline in modelled pH over the past fifty years does not appear to significantly impact the population dynamics compared to the relative increase in temperature over this period.

There is good agreement between the temperature-only population model and the population data from Southward (1991). As with Poloczanska et al. (2008) June sea



surface temperature in this model is a proxy for a suite of environmental influences on the success of early life stages. Adding an additional factor, such as pH, may not result in a large change in the population dynamics as the impacts resulting from this factor may already be captured by the June SST function. The model sensitivity analysis (figure 6.8) suggests that in response to a relative increase or decrease in pH or SST (by  $\pm 2.5\%$ ) the *S. balanoides* abundance is more sensitive to changes in pH than changes in SST up until the critical temperature of about  $13\text{ }^{\circ}\text{C}$  (although these changes are non-linear). This implies that changes in pH have more of an impact on the population in cold water, whereas when in warmer water the population responds more strongly to changes in temperature.

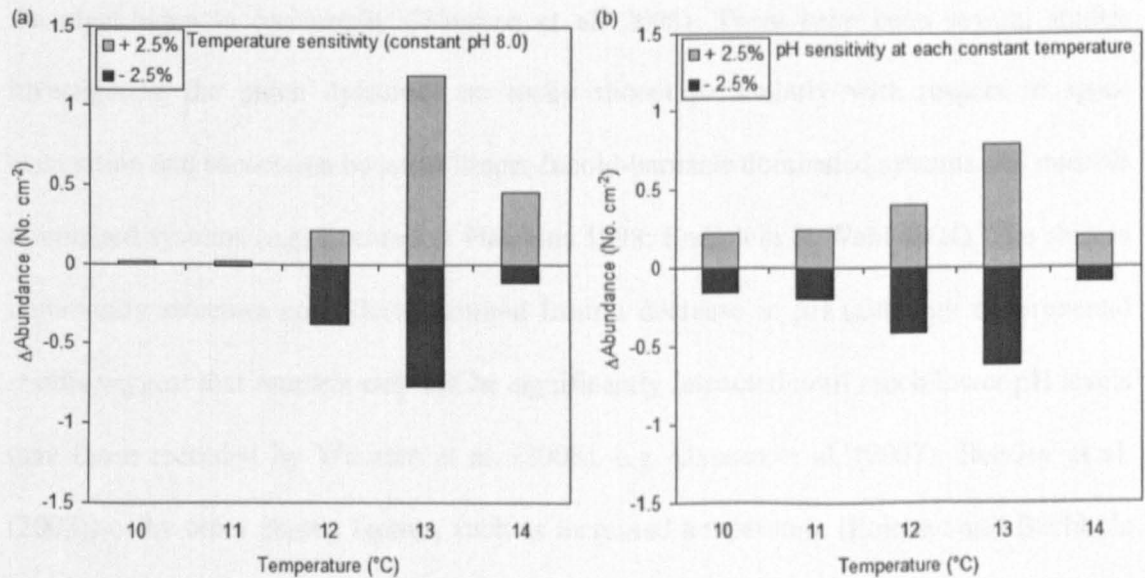


Figure 6.8: Sensitivity of the model (change in population abundance) to a 2.5 % increase and 2.5 % decrease in (a) sea surface temperature (with constant pH 8.0) and (b) pH (at each constant temperature) after the model has equilibrated to pH 8.0 and each of the temperatures ( $10\text{ }^{\circ}\text{C} - 14\text{ }^{\circ}\text{C}$ ).

At the southern edge of *Semibalanus balanoides*' range increasing temperature undoubtedly will result in local population extinctions as is already observed in some locations (Southward et al. 1995; Mieskowska et al. 2006). Decreasing pH could potentially bring forward the time when this occurs and may also enhance extinctions in

some areas, e.g. local upwelling, where pH is particularly low. At present (year 2008) summer sea temperatures (14 °C) the impacts of experimentally lowered pH on survival across all the early-life stages of *S. balanoides* (embryo, nauplii and cyprids) can result in a significant reduction in population abundance.

In contrast with the predictions provided here, a community modelling study by Wootton et al. (2008) suggests that acorn barnacle abundance increased with decreasing pH over an eight year period. This appears to primarily be a response to alterations in the ecosystem dynamics as opposed to a direct response to changing environmental conditions. The mussel (*Mytilus*) community appears to have decreased which gave rise to an increase in the algal-barnacle community (Wootton et al. 2008). There have been several studies investigating the patch dynamics on rocky shores particularly with respect to space occupation and succession between limpet-fucoid-barnacle dominated systems and mussels dominated systems (e.g. Burrows & Hawkins 1998; Enderlein & Wahl 2004). The shift in community structure could have resulted from a decrease in pH (although experimental results suggest that mussels may not be significantly impacted until much lower pH levels than those recorded by Wootton et al. (2008), e.g. Gazeau et al. (2007); Beesley et al. (2008)) or by other abiotic factors, such as increased temperature (Reichert and Buchholz 2006). Equally the changes could have resulted from biological factors such as changes in predator abundance (Enderlein & Wahl 2004; Navarrete & Manzur 2008). Temperature appears to have increased slightly over the observation period (Wootton et al. 2008, figure S1) but there are no records for abundance of mobile predators such as dogwhelks, starfish or crustaceans. The projected proportion of cover of acorn barnacles (*Semibalanus cariosus* and *Balanus glandula*) does not appear to directly follow a declining pH trend (Wootton et al. 2008, figure 3a), as the proportional cover is actually lowest in the mid-pH range (8.29 – 8.37). The Wootton et al. (2008) study highlights the importance of

investigating community dynamics as well as individual or population responses to environmental change, which this present investigation does not take into account.

Wootton et al. (2008) temperature data shows that summer temperatures are, on average, below about 13 °C during their eight year monitoring period; although in the last three years temperatures reached nearly 16 °C. For *S. balanoides* these lower temperatures would mean that a change in pH could have an impact on the population dynamics. However, as highlighted by Chapter 4, different species of acorn barnacle appear to respond to elevated CO<sub>2</sub> and temperature in different ways, and hence the species presented in Wootton et al. (2008) might also respond differently. At the pH levels reported in Wootton et al. (2008), recruitment of barnacles is not likely to be significantly impacted (Chapter 3; Chapter 4; McDonald et al. 2009) and hence observed changes in community structure are more likely due to a response to biological interactions.

As emphasised by Poloczanska et al. (2008) changes in population abundance of competing species can significantly impact on the abundance of their competitors. *Chthamalus* species have yet to be examined in response to elevated CO<sub>2</sub>, although the disappearance of *S. balanoides* will create free space for *Chthamalus* and other species. *Elminius modestus*, for example, appears to tolerate elevated CO<sub>2</sub> at similar temperatures to those described here, showing only subtle impacts on growth rate but no impact on survival or shell development (Chapter 4). *E. modestus* is a spatial competitor to *S. balanoides* and may therefore benefit to some extent from future changes in temperature and ocean chemistry.

At mid- to northern- locations within *S. balanoides* range, ocean acidification is likely to have different consequences for local populations. From model results showing that lower temperatures will allow pH to have a more prevalent impact than increasing temperature,

the model predicts that the abundance of *S. balanoides* in populations which are not restricted by temperature could become reduced. Although the model presented in this study is a simple representation of one species population abundance, it is a first link between experimental data on individuals and predicting population effects. Future modelling studies could become more detailed, including other species or investigating differences between habitats on a local scale.

The outcomes of these models are only valid using the assumption that no adaptation or acclimation takes place in these organisms. It seems unlikely that, at the southern edge of their geographic range, *Semibalanus balanoides* will be able to tolerate increasing temperatures (range shifts); however, in mid and northern parts of the range, where pH has more impact than temperature, this may be a possibility. Transplant field experiments showed that local adaptation in thermal tolerance can occur across small spatial scales (Bertness & Gaines 1993). There is also evidence of organisms adapting to lowered pH conditions by either changing their shell mineral content (e.g. cold-water corals living close to the aragonite saturation horizon in the Pacific ocean, Guinotte et al. 2006) or by forming protective outer membranes (e.g. mussels found on the edge of hydrothermal vents, Tunnicliffe et al. 2009).

Furthermore, the model here assumes that the population does not shift its life history, temporally, as an adaptive response to increasing temperature with time. If the population were able to bring forward its development such that the critical window before recruitment was maintained at the optimal temperature, then it would be expected that the population would persist at this location. Evidence from previous warming and cooling events suggests that such adaptation has not previously been observed (Southward, 1991).

One other important assumption that warrants discussion here is the assumed linear increase in temperature and linear decrease in pH over time. It is clear that environmental variations are not linear; however in the interest of producing an initial assessment of population susceptibility to environmental fluctuations, and the relative impact of temperature and ocean acidification, the linear trends provide simple, useful models. Further studies should include analysis using perhaps best-fit curves or five-year averaging to capture the shorter time-scale variations. However it appears that the overall long-term predictions for *S. balanoides* populations would remain the same in this location and using the model functions defined in this chapter, independent of the exact function (linear/non-linear) used to for a temperature increase and pH decrease over the next 100 years.

## **CHAPTER 7. BROAD-SCALE ECOLOGY**

**Relative influences of ocean acidification and temperature on intertidal barnacle post-larvae at the northern edge of their geographic distribution.**

Aspects of this chapter are in press in:

Findlay HS, Kendall MA, Spicer JI, Widdicombe S (in press) Relative influences of ocean acidification and temperature on intertidal barnacle post-larvae at the northern edge of their geographic distribution. *Estuaries, Coastal and Shelf Science*

## 7.1. INTRODUCTION

The Arctic Ocean appears to be particularly vulnerable to change. Increasing temperatures are causing ocean warming and sea ice melting (IPCC 2007), while the colder seawater temperatures facilitates greater CO<sub>2</sub> absorption thereby increasing the rate of ocean acidification and carbonate ion decline (Steinacher et al. 2009). The Arctic Ocean is predicted to first become undersaturated with respect to aragonite as early as 2040 (Steinacher et al. 2009), with undersaturation of calcite occurring a couple of decades later.

Intertidal species may be particularly sensitive to environmental fluctuations because of the steep gradients of abiotic factors that occur in intertidal habitats (Southward et al. 1995; Hawkins et al. 2003). Furthermore, the effect of climatic variables should be greatest where a range edge is set mainly by physical factors (Pearson et al. 2009). For example, the northern limit for the intertidal barnacle *Semibalanus balanoides* is closely paralleled by the summer limits of pack ice (Barnes 1957); hence populations can be found on the coasts of northern Norway and Svalbard in the high Arctic (Barnes 1999).

Unfortunately, perhaps due to logistic constraints, intertidal studies to date have concentrated mainly on temperate, with little attention paid to polar regions. The Arctic intertidal zone has received little attention with respect to understanding species distribution in relation to climate change, despite shifts in ecological dynamics being observed in the pelagic realm (Greene et al. 2008). A study on the intertidal macroorganism distribution and biomass of Svalbard (Weslawski et al. 1993) suggests that *Fucus-Semibalanus* (previously *Balanus*) communities were the richest in terms of diversity and biomass of all the Arctic intertidal communities that they investigated. This assemblage occurred on 8 % of the investigated coastline (South Spitsbergen National Park, South East Svalbard National Reserve and Isfjorden), but it is also known to occur further

north along the west coast of Spitsbergen, where warm Atlantic water maintains ice-free conditions in summer (Kukliński & Barnes 2008).

As the Arctic warms, a greater area of rocky shore will become available for colonisation by boreal and temperate species extending their ranges northwards. The west coast of Spitsbergen provides an ideal study site to investigate how a key space occupier responds to warming and the additional stress of ocean acidification. For example, areas that were previously inaccessible to species like the barnacle *Semibalanus balanoides* might, in the future, become available if larvae are able to disperse to these new locations and post-larvae are able to survive to recruit to form new populations.

This study investigated the potential impacts of ocean acidification and climate change on populations of an intertidal species, in this case the barnacle *Semibalanus balanoides*, at the northern edge of its range. This was carried out by assessing the survival, growth and development, as well as shell mineralogy, of post-larval barnacles in the Arctic. Post-larval mortality plays an important role in population abundance (Rainbow 1984); and poor development in the early stages can have impacts later on in the life history of barnacles (Jarrett 2003). The results from this study are also compared to results from a similar study on barnacles at their southerly range edge (Chapter 4), as well as other studies investigating the impact of ocean acidification on barnacles (e.g. MacDonald et al. 2009).

## **7.2. MATERIALS AND METHODS**

### **7.2.1. Collection site**

Kongsfjorden, on the west coast of Spitsbergen (79 °N, figure 7.1), is a fjord system into which several glaciers feed. It is open to the North Atlantic on its western edge with the largest glacier flowing into the fjord at its eastern edge (see Svendsen et al. 2002 for details of physical environment). The shoreline is typically scoured by floating ice during the



summer months and is overlain by fast ice during winter (Hop et al. 2002; Kukliński & Barnes 2008). Seasonal temperatures range from -1.8 °C to 4 °C at the sea surface (SST) and from -15 °C to 5 °C in the air (still air) from winter to summer respectively (Svendsen et al. 2002). In August 2008 mean SST was 4.5 °C and mean salinity was 34.5. No previous carbonate system measures have been made from water in this fjord so it is assumed that the control seawater used through the experiment system was near natural fjord levels, although seawater was pumped in from 80 m depth (see details of the set up below) and is therefore likely to contain slightly lower levels of anthropogenic CO<sub>2</sub>.

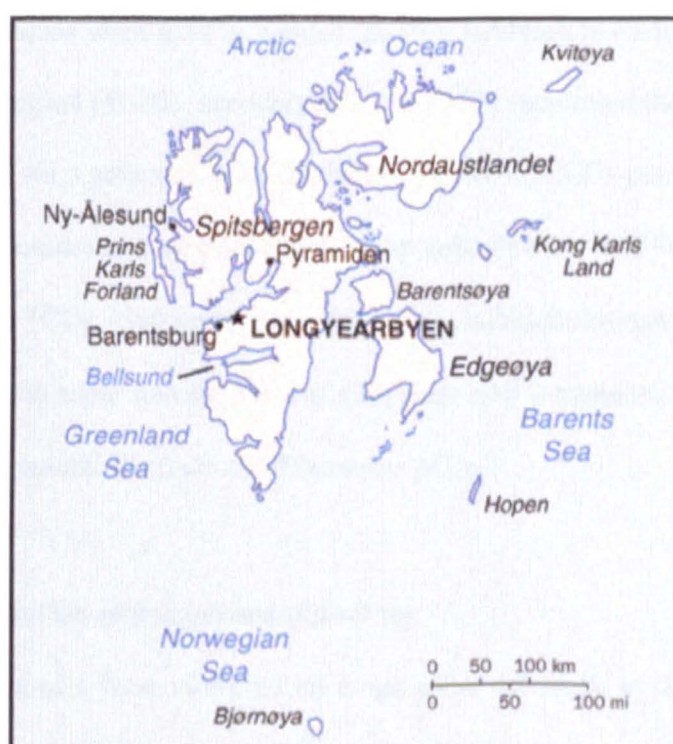


Figure 7.1: Map of Spitsbergen showing Kongsfjorden and location of Ny Alesund (Red dot). Insert shows all islands of Svalbard in relation to northern Norway.

### 7.2.2. CO<sub>2</sub> system set up

The experimental system was constructed using six header tanks (vol. = 200 l) housed in a large experimental room. Each of the six header tanks was used for one of each of the pH and temperature levels. The nominal pH levels were pH 8.1 (control, ambient CO<sub>2</sub> ~380

ppm), pH 7.7 (mid CO<sub>2</sub> ~1,000 ppm, year 2100 IS92a scenario) and pH 7.3 (high CO<sub>2</sub> ~3,000 ppm, year 2300 scenario) (Table 7.1).

Seawater was pumped, *via* a pair of filters (100 and then 20 µm), into two large reservoir tanks direct from the fjord (from 80 m depth), which then fed into each of the header tanks. In the reservoir tanks the temperature of the seawater was adjusted to maintain two constant temperatures throughout the experiment. The nominal temperatures were 4.5 °C and 8.5 °C.

The nominal pH values were used to control the CO<sub>2</sub> bubbling in each header tank. A pH controller (Aqua Digital pH-201, accuracy ± 0.1 % +0.02) monitored the pH and controlled the CO<sub>2</sub> bubbling, *via* a solenoid valve feedback system, to a CO<sub>2</sub> gas cylinder (CP grade 99.95 % carbon dioxide) in a near-identical set up described by Widdicombe & Needham (2007). Natural air (CO<sub>2</sub> ~380 ppm) was additionally bubbled through each of the header tanks to maintain the same starting point of CO<sub>2</sub> across all treatments, therefore buffering the system against natural fluctuations of seawater pCO<sub>2</sub>.

### **7.2.3. Animal collection and experimental set up**

*Semibalanus balanoides* were collected on chips taken off rocks in the mid-shore at Ny Ålesund, Svalbard (78°55'N, 011°56'E), on the 4<sup>th</sup> Aug 2008. Ny Ålesund is located roughly 10 km from the entrance of the fjord along the southern shore of Kongsfjorden (figure 7.1). On collection, the rock chips contained a mixed age population of juvenile barnacles ranging from newly settled cyprids to week-old post-larvae but there were no adults.

Three rock chips were placed into one of twelve microcosms (30 x 15 x 20 cm) so that each microcosm contained in excess of 200 individual barnacles. The microcosms allow

the mimicking of diurnal tidal exposure while controlling temperature and CO<sub>2</sub> and were identical to those described in Chapter 2. All microcosms were kept in a controlled temperature experimental room. The tidal regime of the microcosms was programmed weekly to coincide with the local tide times. Seawater (S = 35) was supplied to the microcosms from the header tanks during the flood tide at a rate of 10 ml min<sup>-1</sup> and ebbing seawater ran to waste. Light was maintained continuously to replicate the 24 h daylight over this period. The experiment ran for 20 days and barnacles were fed every two days with a mixed diatom-flagellate diet at 15,000 cells ml<sup>-1</sup> (Shellfish Diet 1800®, Reed Mariculture).

Changes in barnacle abundance on each rock chip were recorded every five days using a digital camera (Canon EOS 400D) which was maintained in consistent alignment using a stand. The photographic images were analysed (Image-Pro Plus v.4.5, Media Cybernetics) to estimate both growth and survival. Growth was estimated by measuring the diameter of the operculum of each barnacle on each panel at each time point. Growth rate was calculated as an average over the 20 day experimental period of all the barnacles in each microcosm. Barnacle survival was estimated from the images taken at the beginning and the end of the experiment by counting living and dead individuals. Prior to photography individuals were gently touched to check whether they were able to close their operculum and were classed as dead when the operculum either remained open or the shell was empty. Survival, recorded as a proportion of the initial number of individuals, was square root arcsine transformed before analysis so that data were normally distributed.

The mineral content of the shell was calculated by analysing the calcium (Ca) and magnesium (Mg) concentrations. The shells of ten individuals were haphazardly selected from each microcosm at the end of the experiment and the concentrations of both divalent ions were measured using methods described in Spicer & Eriksson (2003); briefly this

involved dissolving the shells in 10 % nitric acid after drying and weighing, then using Inductively Coupled Plasma (ICP) optical emissions spectrometer (Varian 725-ES) to measure Ca and Mg simultaneously. The proportion of each ion in the shell was calculated from the mass of the shell and volume of acid used in the digest (ion [mg] / total shell mass [mg]). The proportion of each ion in the total shell was then square root arcsine transformed before statistical analysis so that data were normally distributed.

Growth rate, transformed-survival and transformed-ion data were tested for normality using a Kolmogorov-Smirnov test and for homogeneity of variances using Bartlett's test. Once these assumptions were confirmed a two-way nested ANOVA was used to determine the effects of temperature, CO<sub>2</sub> or any interaction between them. Microcosms were nested, as a random factor, within CO<sub>2</sub> treatment (n = 2). Statistical analysis was performed using Minitab® 15.1.0.0 (© 2006, Minitab Inc.). PERMANOVA (Primer-E) (Anderson 2001) with a nested (replicate microcosms) regression design was used to test for difference in post-larval size increase over time in the different pH and temperature treatments.

### **7.3. RESULTS**

The temperatures in the microcosms were maintained slightly above the nominal temperatures ( $5.2 \pm 0.64$  and  $9.5 \pm 0.35$  °C in the low and high temperatures respectively) because of a small increase in temperature occurring while seawater passed through the tubing. Salinity remained between 33 and 34 in all microcosms. The carbonate system parameters are provided in Table 7.1.

The percentage of post-larvae and juveniles surviving the 20 day exposure period ranged between 85 to 90 % and was not affected by either temperature or pH level (figure 7.2).

*Table 7.1: Environmental conditions in the microcosms (mean  $\pm$  standard deviation). Measured values are given for salinity, temperature ( $^{\circ}\text{C}$ ), pH and DIC,  $p\text{CO}_2$  ( $\mu\text{atm}$ ), total alkalinity (TA), bicarbonate, carbonate, and the saturation states for calcite ( $\Omega_{\text{cal}}$ ) and aragonite ( $\Omega_{\text{arg}}$ ) were calculated from pH and DIC using CO2sys with the solubility constant of Mehrbach et al, (1973) refit by Dickson & Millero (1989).*

	Low Temperature			High temperature		
	8.1	7.7	7.3	8.1	7.7	7.3
Temp ( $^{\circ}\text{C}$ )	5.68 $\pm 0.82$	5.17 $\pm 0.43$	4.92 $\pm 0.44$	9.8 $\pm 0.39$	9.5 $\pm 0.26$	9.4 $\pm 0.32$
Salinity	33.7 $\pm 0.08$	33.7 $\pm 0.05$	33.5 $\pm 0.18$	33.7 $\pm 0.04$	33.7 $\pm 0.04$	33.6 $\pm 0.28$
pH	8.12 $\pm 0.032$	7.68 $\pm 0.031$	7.35 $\pm 0.054$	8.14 $\pm 0.022$	7.71 $\pm 0.050$	7.36 $\pm 0.043$
$p\text{CO}_2$	352 $\pm 27.6$	1086 $\pm 94.9$	2429 $\pm 336$	343 $\pm 13$	1060 $\pm 133$	2448 $\pm 277$
DIC ( $\mu\text{mol kg}^{-1}$ )	1800	1983 $\pm 75$	2116 $\pm 75$	1783 $\pm 41$	1975 $\pm 61$	2092 $\pm 49$
TA ( $\mu\text{mol kg}^{-1}$ )	1924 $\pm 12$	1982 $\pm 73$	2016 $\pm 59$	1937 $\pm 48$	1997 $\pm 57.1$	2014 $\pm 39$
$\text{HCO}_3^-$ ( $\mu\text{mol kg}^{-1}$ )	1693 $\pm 5.4$	1891 $\pm 71.6$	1972 $\pm 62$	1661 $\pm 36.0$	1883 $\pm 58.5$	1961 $\pm 41.0$
$\text{CO}_3^{2-}$ ( $\mu\text{mol kg}^{-1}$ )	89.3 $\pm 6.8$	35.2 $\pm 2.2$	17.0 $\pm 1.9$	107 $\pm 6.7$	44.5 $\pm 4.8$	20.6 $\pm 1.7$
$\Omega_{\text{cal}}$	2.14 $\pm 0.16$	0.85 $\pm 0.05$	0.41 $\pm 0.04$	2.57 $\pm 0.16$	1.07 $\pm 0.11$	0.50 $\pm 0.04$
$\Omega_{\text{arg}}$	1.35 $\pm 0.10$	0.53 $\pm 0.03$	0.26 $\pm 0.03$	1.63 $\pm 0.10$	0.68 $\pm 0.07$	0.31 $\pm 0.03$

All the cyprids had metamorphosed to post-larvae by the start of the experiment. Development of a number of individual post-larvae was followed throughout the experimental period (figure 7.3) as well as measuring overall population growth, which included larger (older) juveniles (but no adults). The post-larvae in the control pH (high and low temperatures) increased in size relatively uniformly over the 20 day exposure period. At pH 7.70 initial growth rate of post-larvae was not significantly different from control pH. However after day 10 the growth rate decreased and became significantly slower than the low temperature control after 15 days. At pH 7.30, within the first five days there appeared to be reduced growth and the rate decreased further after 10 days. The

individually monitored post-larvae showed significant differences between the average increase in length as a result of pH and temperature effects (figure 7.3; PERMANOVA,  $F_{2,29} = 83.404$ ,  $p = 0.001$  and PERMANOVA,  $F_{1,29} = 20.065$ ,  $p = 0.002$  for pH and temperature respectively). The mean growth rate of all the post-larvae in each treatment decreased significantly with decreasing pH (figure 7.4, ANOVA  $F_{2,29} = 4.75$ ,  $p = 0.018$ ) and appeared to decrease with increasing temperature, but this was not significant (figure 7.4, ANOVA  $F_{1,29} = 0.82$ ,  $p = 0.375$ ); there was no significant interaction between the temperature and pH.

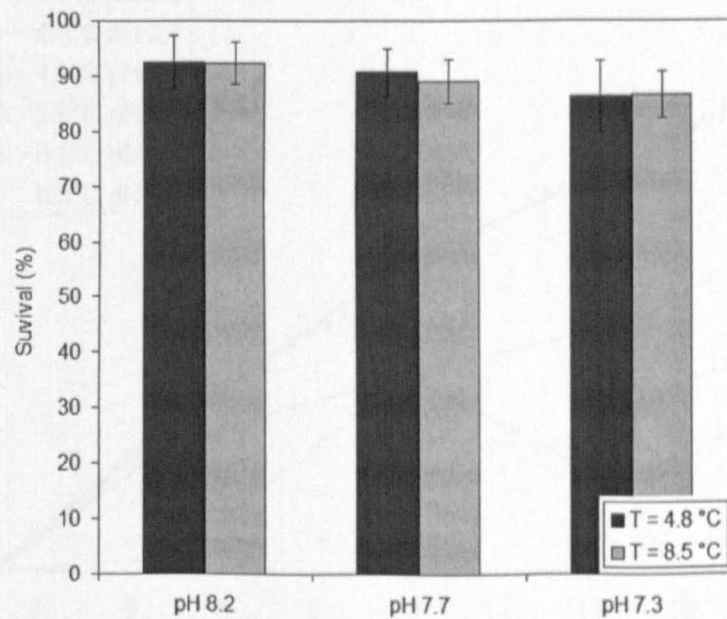


Figure 7.2: Survival (mean percentage) of post-larvae at each pH (pH 8.2, 7.7 and 7.3) and temperature level (4.8 °C, dark bars and 8.5 °C, grey bars). Error bars represent 95 % C.I..

Table 7.2: PERMANOVA table of results for average increase in length of post-larvae over the 20 day exposure period testing for differences between fixed factors of pH, temperature (Temp) and over time, and interactions between them.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
pH	2	2.64	1.32	83.404	0.001	999
Temp	1	0.31755	0.31755	20.065	0.002	996
Time	4	4.0836	1.0209	64.506	0.001	999
pHxTemp	2	0.13107	6.55E-02	4.1409	0.066	998
pHxTime	8	1.1826	0.14783	9.3406	0.002	998
TempxTime	4	0.17714	4.43E-02	2.7982	0.117	999
Res	8	0.12661	1.58E-02			
Total	29	8.6585				

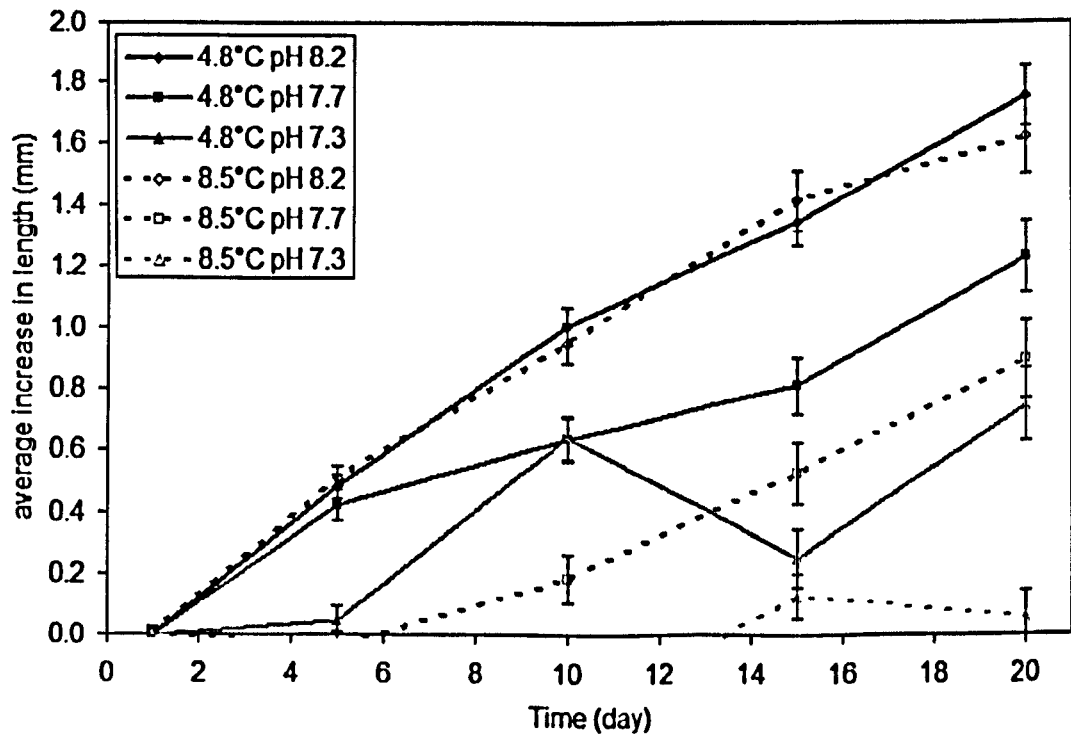


Figure 7.3: Mean increase in length (mm) of barnacles over the experimental time period under different conditions of temperature (4.8 °C, lines and 8.5 °C, dashed lines) and pH (pH 8.2, diamonds, pH 7.7, squares, and pH 7.3, triangles). Error bars are 95 % C.I..

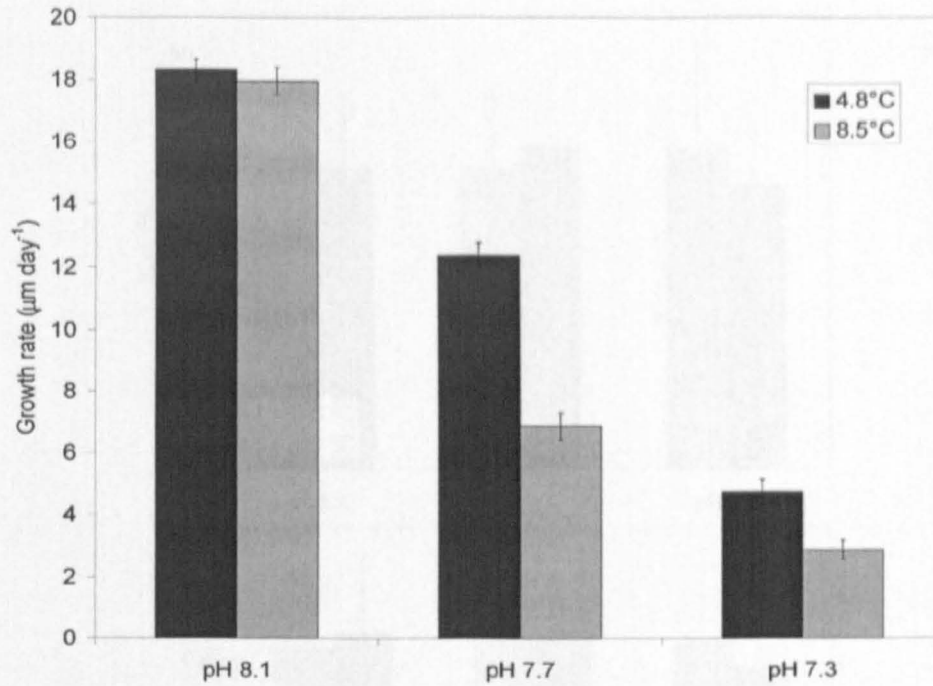


Figure 7.4: Mean growth rate ( $\mu\text{m d}^{-1}$ ) over the 20 day experiment period for each pH (pH 8.2, pH 7.7, and pH 7.3) and temperature (4.8 °C, black bars and 8.5 °C, grey bars) treatment. Error bars are 95 % C.I..

Table 7.3: ANOVA table of results for average growth rate of post-larvae over the experimental period, testing for differences between fixed factors of pH and temperature (Temp), and the interactions between them.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	2	913.74	913.74	456.87	4.75	0.018
Temp	1	78.69	78.69	78.69	0.82	0.375
pHxTemp	2	34.1	34.1	17.05	0.18	0.839
Error	24	2307.25	2307.25	96.14		
Total	29	3333.78				

The calcium and magnesium contents of the shells did not change with either increased temperature or decreased pH (figure 7.5). Calcium content ranged between 35 – 40 % of the shell, while magnesium made up 0.4 – 0.45 % of the shell, irrespective of treatment. There was a relatively low concentration of magnesium in the shells resulting in a relatively high Ca:Mg ratio, between 1:80 and 1:90.



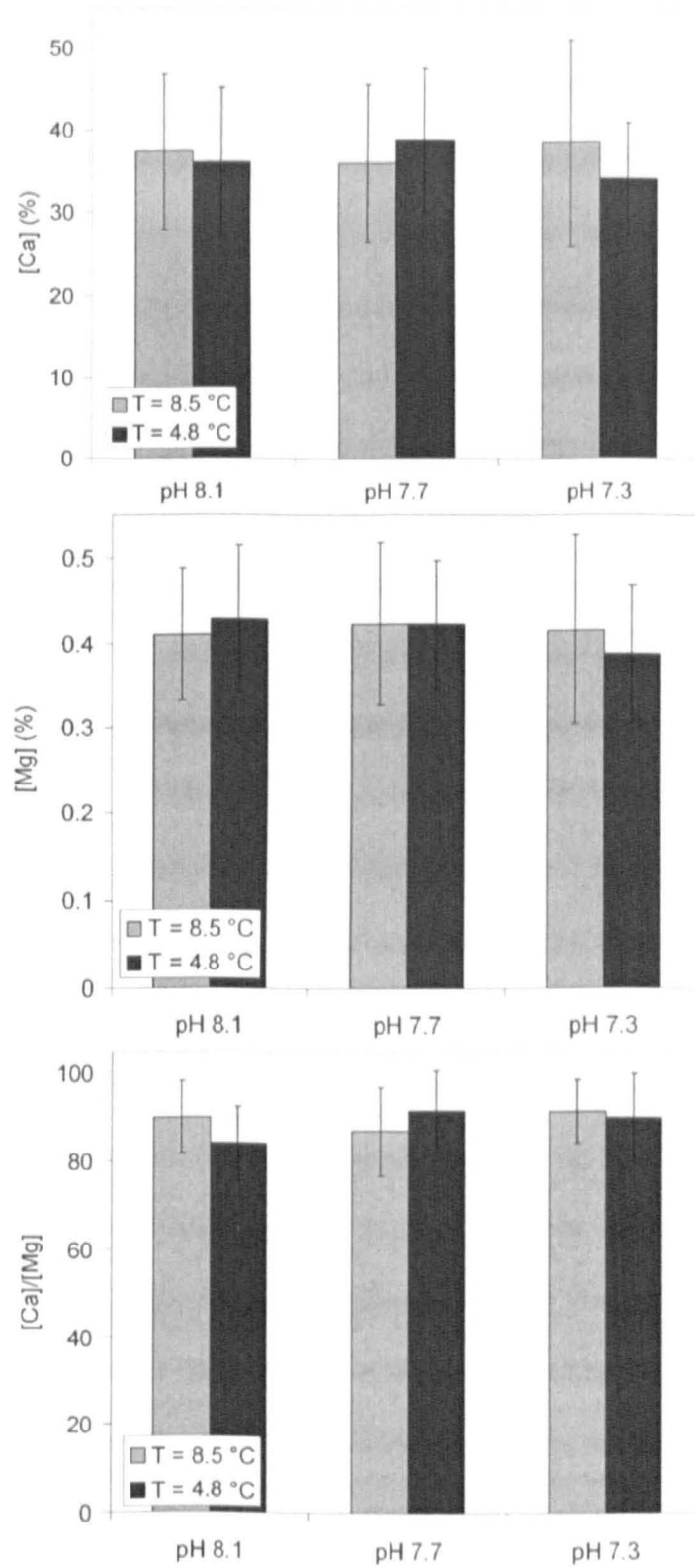


Figure 7.5: Concentrations of (a) calcium and (b) magnesium and (c) the calcium/magnesium ratio in shells of *Semibalanus balanoides* barnacles, in each pH treatment (pH 8.2, pH 7.7 and pH 7.3) at 4.5 °C (black bars) and 8.5 °C (grey bars), as a percentage of total shell material. Error bars are 95 % C.I...

#### 7.4. DISCUSSION

*Semibalanus balanoides* is a boreal species found on rocky shores across much of northern Europe and North America (Barnes & Barnes, 1976). Therefore it is not a true polar species in that it is not restricted (and so presumably is not adapted) specifically to cold waters. Early investigations of *S. balanoides* suggest that a maximum temperature between 10 – 12 °C is required for successful gonad and brood development (Crisp & Clegg 1960) while embryo and larval development rates are optimal at about 10 °C (Crisp 1959; Barnes & Barnes, 1976). It was therefore not expected to find a marked effect of increasing temperature from 4 °C to 8 °C on cyprid survival or development, although it was predicted that growth rates would increase with increasing temperature. Surprisingly temperature did not have any significant impacts on the rate of post-larval growth.

Post-larval growth and development was significantly impacted by lowered pH. All levels of lowered pH caused a significant reduction in growth rate, although neither survival nor shell mineral content was significantly different over the 20 day period. Reduced growth rates as a response to lowered pH have been observed in studies on other species (e.g. Harris et al. 1999; Michaelidis et al. 2005; Gazeau et al. 2007), particularly when shell growth has been measured. The stability in mineral content of the shell noted here indicates that post-larvae were still capable of forming their shells and growing even in conditions of lowered calcite saturation state. The combination of reduced growth and maintained mineral content means that there may have been a change in the energetic balance of the exposed individuals. In undersaturated conditions more mineral will dissolve from the shell and hence more energy is required to maintain the mineral integrity. Therefore, energy that would normally be invested into growth could be reallocated and hence organisms growing in lowered pH grow more slowly and end up smaller than individuals grown in higher pH conditions. If true, it could mean that it is more important that these organisms maintain shell integrity to protect against desiccation, predation and

abrasion than that they grow to a larger size. Sessile invertebrates such as intertidal barnacles are unable to avoid mortality from predation or environmental impacts by moving into shelter. The ability to form a hard shell which is thick and strong can deter predators from drilling through the shell and will additionally protect against abrasion by ice or rocks (Barnes 1999). Although this could be construed as an unnecessary response by *S. balanoides* which are found in the Arctic and have few predators, it is certainly a critical factor for surviving early life on more temperate rocky shores, where desiccation and predation are two main causes of post-settlement mortality (Connell 1961; Foster 1971). Hence it is possible that this may be a feature of *S. balanoides* evolution that has been maintained across their geographical distribution.

Counterintuitively the impact of multiple factors acting on *S. balanoides* post-larvae concomitantly does appear to further reduce growth rate. Previous studies investigating both temperature and pH have shown that either (a) an increase in temperature results in an increase in growth rate which is offset by a decrease in growth resulting from lowered pH (Chapter 3; Reynauds et al. 2003; Jones et al. in review) or (b) both cause an increase in growth rate (Gooding et al. 2009). There are two possible explanations which could account for the further reduction in growth rate seen here. First, the cyprids and post-larvae are likely to be cold-acclimated, and hence experience a short period of heat adjustment and acclimation (Pörtner & Knust 2007) which impacts on growth in the early period of the experiment (as can be seen in figure 7.1). Second (and possibly related to the previous point), the greater reduction in growth could be the result of a change in energy balance as suggested above. There is a short period during and after metamorphosis when the post-larvae is non-feeding and reliant on energy reserves (Rainbow & Walker 1977). This period is thought to be particularly energetically costly (Lucas et al. 1979). Elevated metabolism accompanying increased temperature (Newell & Northcroft 1965) may be detrimental to the post-larvae at this time because it draws essential energy from a limited

pool. The larval phase is already a particularly extended period in the Arctic (Petersen 1966) and hence reserves may not be sufficient to buffer long-term changes in metabolism. Neither of these hypotheses are tested by this present study. They require additional studies on cyprid and post-larval biochemistry as well as time of onset of feeding and feeding rates. However, changes to the energy balance may well have long term effects on the fitness of these barnacles.

Towards the southern edge of its biogeographical range, temperature appears to have a much more significant impact on post-larval *Semibalanus balanoides*, particularly with respect to overall survival (Chapter 4). At its southern range edge, *S. balanoides* is already high temperature limited, and hence the effects of increased temperature will be sufficiently abrupt so as to mask the impacts of other stressors. However, at the northern limits, as discussed above, a temperature increase would not be expected to be limiting for this species and hence other environmental parameters increase in importance (see figure 7.6). Conversely, there are differences in the Ca/Mg ratio of the shells between barnacles of southern and northern populations. The Ca/Mg ratio is  $1:91.4 \pm 8.10$  (mean  $\pm$  s.d.), which is very similar to values from northern Scotland (1:92, Barnes et al. 1976) but much greater than values from barnacles taken from a southern population (Chapter 4), which have a ratio  $1:51.5 \pm 8.58$ . The reduction in the ratio from north to south corresponds with an increase in the Mg content. Although barnacles use calcite as the primary form of calcium carbonate in their shells, the decrease in shell Mg indicates a shift from a higher Mg content (making calcite more soluble) in warmer waters to a lower Mg content (less soluble calcite) in colder waters (Andersson et al. 2008). Shell composition is known to vary with environmental conditions, such as temperature (Gussone et al. 2005). There may also be some level of biological control as it is more energetically costly to maintain a more soluble shell in waters where there is a lower level

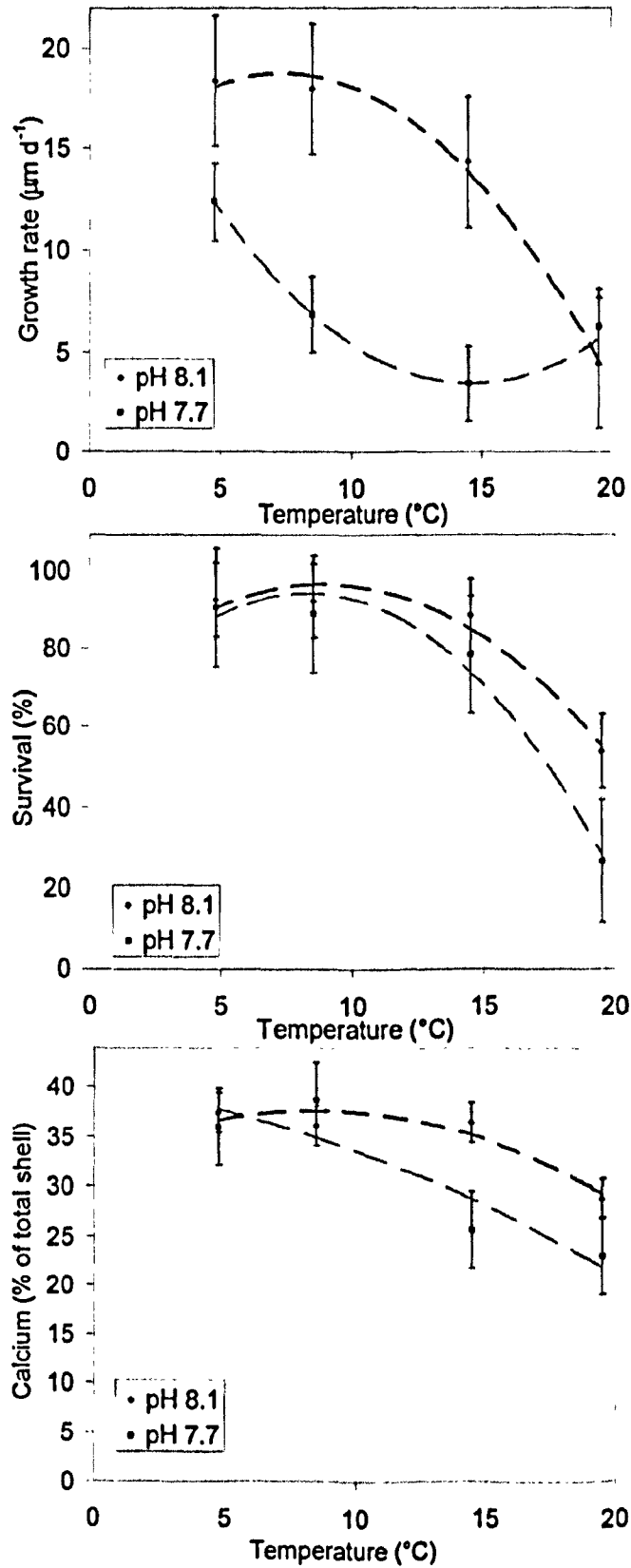


Figure 7.6: Impacts of pH (black circles pH 8.0, grey squares pH 7.7) across a range of temperatures on (a) growth rate ( $\mu\text{m d}^{-1}$ ), (b) survival (%) and (c) calcium content (% of total shell). Error bars are 95 % C.I.. Dashed lines show a polynomial fit to the data points.

of saturation of carbonate minerals (Freitas et al. 2008) and hence the Mg fraction may be lower under these conditions (Andersson et al. 2008).

From what has gone before a simple conceptual model of energy allocation for post-larvae can be derived from northern and southern populations (figure 7.7). This model can be used to predict juvenile morphology. This is presented in figure 7.7 (c.f. figure 7.6) showing that (a) the low energy demands in cold temperatures allows continuous growth and calcium incorporation and results in a relatively large barnacle with a well developed shell; (b) increased energy demand from changes in metabolism and shell formation cause lowered growth rates but maintained calcium incorporation, which results in a relatively small barnacle with a well developed shell; (c) small increase in energy demand from changes in metabolism cause slightly lower growth rates but maintained calcium incorporation, which results in a smaller barnacle but with well developed shell; and (d) increased energy demand from changes in metabolism and shell formation cause lowered growth rates and lower calcium incorporation, which results in a small barnacle with a poorly developed shell. This highlights the need to evaluate single processes in a context of both a whole organism but also across an ecological scale. It is also suggested that predictions of the fate of a species under a changing climate cannot easily be inferred without accounting for inter-population variation.

Within a relatively short time period much more of the Arctic coastline is likely to become available for settlement of intertidal species. *Semibalanus balanoides* is able to disperse to these areas because of its extended planktonic larval development period, and hence is a prime candidate for colonising any newly uncovered, 'free' space. In particular, if elevated temperatures were to occur alone, *S. balanoides* would likely expand its range into these new areas. Along with the settlement of *S. balanoides* there is also likely to be settlement of other organisms and possibly the production of a diverse ecosystem (Weslawski et al.

1993). However, with the addition of ocean acidification there may be a much slower extension of this species.

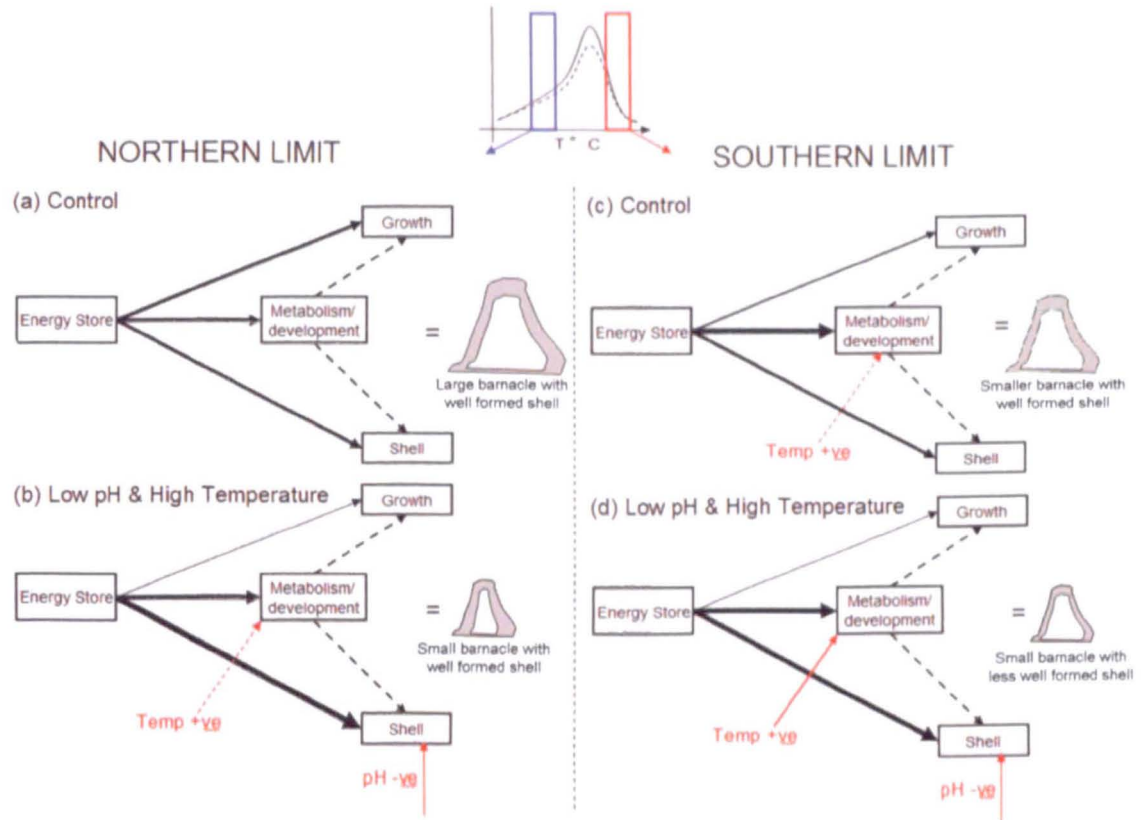


Figure 7.7: A simple conceptual model of energy allocation in post-larvae from northern and southern populations under control (“normal” conditions of temperature and pH) and under lowered pH and elevated temperature conditions. Black arrows indicate possible routes of energy allocation from a finite energy reserve (lipid store in the case of metamorphosing cyprids) to growth, metabolism and development into adult form, and shell formation. Red arrows indicate potential impacts from pH and temperature. Temperature is likely to increase metabolism, requiring more energy, however pH will have negative impacts on the shell (either through direct dissolution) or indirectly through metabolism. See text for further explanation.

## **CHAPTER 8. OVERALL DISCUSSION AND CONCLUSIONS**



## 8.1. SUMMARY OF EXPERIMENTS

The aim of this thesis was to investigate how ocean acidification and climate change could impact populations of rocky shore species. Experiments were carried out in a controlled laboratory environment which simulated (a) realistic patterns of tidal emersion and (b) present and future intertidal conditions, with respect to CO<sub>2</sub> and temperature (Chapter 2). The intertidal barnacle, *Semibalanus balanoides*, was used as a model intertidal organism; its biology and ecology are well studied, they are easily accessible on the rocky shore, and they occur over a wide biogeographic range. Importantly, previous studies (e.g. Burrows & Hawkins 1998; Hyder et al. 1998; Svensson et al. 2004; Wethey & Woodin 2008; Poloczanska et al. 2008) have attempted to model, and so predict, the population dynamics of this species. The existence of such models provides the opportunity to place the results from laboratory experiments into a framework that can be manipulated to make predictions of barnacle population dynamics in a future, high CO<sub>2</sub> world; the results of modelling are presented and discussed in Chapter 6. Furthermore, like many marine invertebrate species, barnacles have a bi-phasic life cycle (Roughgarden et al. 1988), a planktonic larval phase and a sessile adult phase. The population dynamics can in principle be affected by a changing environment experienced during either phase. Factors that affect the larval stage are commonly referred to as pre-settlement processes, while factors that affect the adult phase are referred to as post-settlement processes. Previous ocean acidification literature (e.g. review by Kurihara 2008) suggests that the early life stages will be more vulnerable than the adults to a changing environment and therefore that existing predictions, which have been made on the basis of the susceptibility of adults, may not be appropriate. The hypothesis of greater juvenile susceptibility was assessed by carrying out experiments investigating the relative impacts of CO<sub>2</sub> and temperature on each of the pre- and post-settlement phases: the embryonic stage (Chapter 3), the naupliar stage (Chapter 6), the post-larvae stage (Chapter 4) and the overwintering adults (Chapter 3).

Much of the early ocean acidification literature focused on the ability of calcifying organisms, such as barnacles, to produce and maintain their calcium carbonate structures in a more acidic ocean. In this thesis the view is taken that shell formation is an energetically costly process and hence it is imperative that calcification was also investigated alongside other physiological processes to inform the understanding of the allocation of energy budgets of these organisms. Chapter 5 describes the impact of ocean acidification on the calcification process in barnacles, along with four other species, in relation to other physiological processes.

The urgent need for predictions, primarily driven by policymakers, has meant that general statements about the response of whole ecosystems are often formulated from results of experimental work on just a few species (e.g. Guinotte & Fabry 2008; Fabry et al. 2008). This approach has a number of shortcomings; for example species responses are often predicted on the basis of similar species with shared habitats or life styles. Equally, within a species there has been a tendency to assume that all populations, irrespective of where they occur in their geographical range, are likely to present a similar response to environmental change. This thesis critically assesses how applicable a generalist approach is by (a) comparing another species of barnacle, *Elminius modestus*, which often co-occurs with *S. balanoides* (Chapter 4) and (b) by investigating two populations of *S. balanoides*, one from near the southern limits of their geographic range and one from the northern limit of their geographic range (Chapter 7).

The remainder of this discussion chapter aims to summarise the major findings from the experiments carried out on the various life stages of *Semibalanus balanoides*, before bringing together those results to assess the ability of *S. balanoides* populations to survive in a future ocean. The conditions to which *S. balanoides* will be exposed in the future are considered (sections 8.3.1 & 8.3.2.). Models are then developed to (a) assess the relative

contribution of temperature and ocean acidification on impacting each life stage (section 8.3.3) and (b) make predictions on the future distribution of *S. balanoides* (section 8.3.4). Section 8.4 addresses the shortcomings and caveats associated with making these predictions as well as examining areas for development of future experiments and models. Finally in section 8.5 some overall conclusions are drawn from the experimental and modelling analysis carried out in this thesis, which address some issues raised here in this section:

- The hypothesis that early life stages are more vulnerable than adult stages;
- The hypothesis that the process of calcification is more vulnerable to ocean acidification than other processes;
- The relative impacts of temperature and CO<sub>2</sub> on different population within the same species, species plasticity and different species sharing similar habitats;
- The ability to scale up experimental results to the real world using models.

## **8.2. SUMMARY OF *SEMIBALANUS BALANOIDES* RESULTS**

This section will briefly summarise the main findings from each experimental chapter and begin to make predictions about the relative impacts of temperature and CO<sub>2</sub> on each life-history stage.

### **8.2.1. Embryonic development**

Embryonic development rate was significantly slower under elevated CO<sub>2</sub> conditions compared to the control, but still resembled 'field' rates recorded in populations found in similar environmental conditions. There was an estimated delay in development of 19 days under elevated CO<sub>2</sub> conditions, which resulted in a 60 % reduction in the number of nauplii reaching hatching stage at the time when over 50 % of the control nauplii were able to hatch. However, larvae were still able to hatch, indicating that there is no or little impact on embryonic survival. Furthermore there were no obvious morphological differences in

these hatchlings. These results agree with those from a recent study on a tropical barnacle species, *Amphibalanus amphitrite*, which showed no impacts of elevated CO<sub>2</sub> levels on egg production (McDonald et al. 2009).

Although the impacts of increased temperature on embryo development were not studied as part of this thesis, there have been several studies on this topic (e.g. Crisp 1959; Barnes & Barnes 1976; Lucas & Crisp 1987), as well as effects of embryo size (Barnes 1965). Early studies found a latitudinal gradient in embryo development rates and size, suggestive of a correlation with temperature. *S. balanoides* embryo development was faster under warmer conditions (Crisp 1959) which resulted in smaller embryos and hence smaller nauplii being released (Barnes 1965). This pattern occurred both over the broad geographic scale (from southern Europe to northern Norway) and on a local scale where levels of exposure and position on the shore alter the microhabitat and hence the temperature conditions actually experienced by individuals (Helmuth & Hofmann 2001).

Elevated temperature and CO<sub>2</sub> together may therefore, at the very least, offset each other (Chapter 3) but the level of the impact will be determined by the starting temperature and the magnitude of change. These predictions will be discussed in more detail later in this chapter (section 8.3.3). However the counteractive effects of CO<sub>2</sub> and temperature are supported by work carried out on early life stages of other organisms such as urchins (Kurihara & Shirayama 2004), brittlestars (Dupont et al. 2008) and serpulid polychaetes (Jones et al. in press).

### **8.2.2. Naupliar development**

Initial results on *Semibalanus balanoides* nauplii indicated that, for a constant temperature, mortality was greater under elevated CO<sub>2</sub> conditions than under control conditions. No difference in either growth rate or naupliar size was found between control and high CO<sub>2</sub>

conditions. Other studies investigating nauplius development and cyprid attachment agree that there are limited impacts of elevated CO<sub>2</sub> on these early life stages (McDonald et al. 2009).

Studies investigating the impacts of elevated temperature on barnacle nauplius development have shown that naupliar size (Barnes 1965), development rate (Barnes & Barnes 1958) and hence time spent in the plankton (Crisp 1977), were all correlated with temperature, although both food availability and larval quality have also been shown to be important (Barnes & Barnes 1958). Warmer conditions result in quicker development, smaller nauplii and a shorter planktonic stage. Unless the elevated rates of development in warmer conditions are able to offset the lowered survival seen in high CO<sub>2</sub> treatments, it would be expected that in combination elevated temperature and CO<sub>2</sub> would cause an overall decrease in survival of the planktonic larvae. Again, these predictions will be discussed in more detail below (section 8.3.3).

### **8.2.3. Post-larval development**

Elevated CO<sub>2</sub> had a significant impact on the metamorphosis and growth of post-larvae at all temperatures and on their shell mineralogy at the higher temperatures but did not appear to alter the survival of post-larvae. The ability to alter the rates of processes in response to the changing environment, such as growth, metabolism, shell development and reproduction, comes from the ability to reallocate resources within an individual. During the period of metamorphosis from cyprid larva to post larva, the individual is reliant on stored energy reserves to differentiate, grow and form a calcium carbonate shell. Once it has developed to form a post-larva it can begin feeding. The experiments described in Chapter 7 indicate that the mechanism of resource allocation under different environmental regimes can influence the adult form. Increasing temperature, under a constant energy regime, will result in a decrease in the energy available for growth and reproduction

(Sebens 1982) as a result of higher metabolic rate. CO<sub>2</sub> can also alter adult morphology and fitness during the early stages of development (Chapter 4). At low temperatures, metabolic rate is relatively low and hence more stored energy within the post-larvae is available for growth and development resulting in post-larvae that initially grow relatively fast. When under conditions of elevated CO<sub>2</sub>, shell formation continues but using energy that would otherwise have been allocated for growth; the individual therefore has lowered growth rates but maintains its shell mineral structure. When under conditions of both elevated CO<sub>2</sub> and temperature, maintaining the shell requires additional energy, consequently growth declines further until eventually there is minimal resources left for maintenance and mortality rates begin to increase.

In *S. balanoides*, reduced growth rates of post-larvae could have two possible consequences for life-history strategy. Either juveniles remain small and mature at a smaller size or they take longer to reach a mature size; each strategy has its own benefits and trade-offs (Sibly & Calow 1986). Smaller adults might have lowered risk from post-settlement mortality factors such as predation (Connell 1961; Barnett 1979) but may be more susceptible to desiccation and exposure because of their smaller volume to surface area (Hunt & Scheibling 1997). Smaller adults will produce smaller egg masses (Barnes & Barnes 1968) but are likely to occur in higher densities and hence there will be a higher number of propagules produced per unit area. Larger adults with longer lives and later maturity will still be able to produce large egg masses but may have increased risk of mortality before reaching maturity (Barnes & Barnes 1968).

#### **8.2.4. Adult survival over winter**

Under experimental conditions the adults maintained their shell mineralogy but overall survival was 22 % lower under elevated CO<sub>2</sub> conditions than in the control at the end of experimental period. Demand for resources from maintenance requirements would have

increased under elevated CO<sub>2</sub>, because of the high levels of energy required to form calcite shells under enhanced dissolution. Limited resources would have been used up more quickly and hence overall fitness and survival of these barnacles was reduced. This change in resource allocation is similar to the post-larval response seen under low temperature and elevated CO<sub>2</sub>. Although the impacts of elevated temperature on the adults was not investigated here, it seems unlikely that temperature would directly impact the adult physiology as they have high thermal tolerance (Foster 1969) but would act more significantly on gonad and embryo development (Barnes 1957; Lewis 1976).

#### 8.2.5. Summary

The experiments, combining elevated temperature and CO<sub>2</sub> concentration, have revealed a complex set of responses across the different life-stages. The early-life stages were negatively impacted by ocean acidification in some instances however there were also important changes occurring within the adults. The adults and developing post-larvae reallocated their resources from growth to shell maintenance, which potentially alters their fitness and could lead to increased mortality. Calcification is impacted in both the developing post-larvae and the adults, but the individuals affected tend to switch energy from processes such as growth in order to maintain their shells. In several of the experiments individuals responded to an increase in temperature in such a way that their response offsets the impacts from ocean acidification. Many of the responses involved a change in process rates (such as growth rate and development rate) as opposed to complete inhibition of a process. The question remains as to whether these small, often sub-lethal impacts occurring at various life stages actually matter at the level of the population and hence impact on community and ecosystem dynamics.

### 8.3. REAL WORLD PREDICTIONS

This section seeks to scale up the results from the experiments to understand how populations might be impacted in the real world. The approach that will be taken is to place the experimental results into a context of existing predictions that are based on the way in which the marine environment in general, and the rocky shore environment in particular, will be affected by a warmer more acidic ocean. From this starting point, further conclusions will be developed by expanding ecological principles and developing conceptual models.

#### 8.3.1. How is the ocean changing across the biogeographic range of the experimental species?

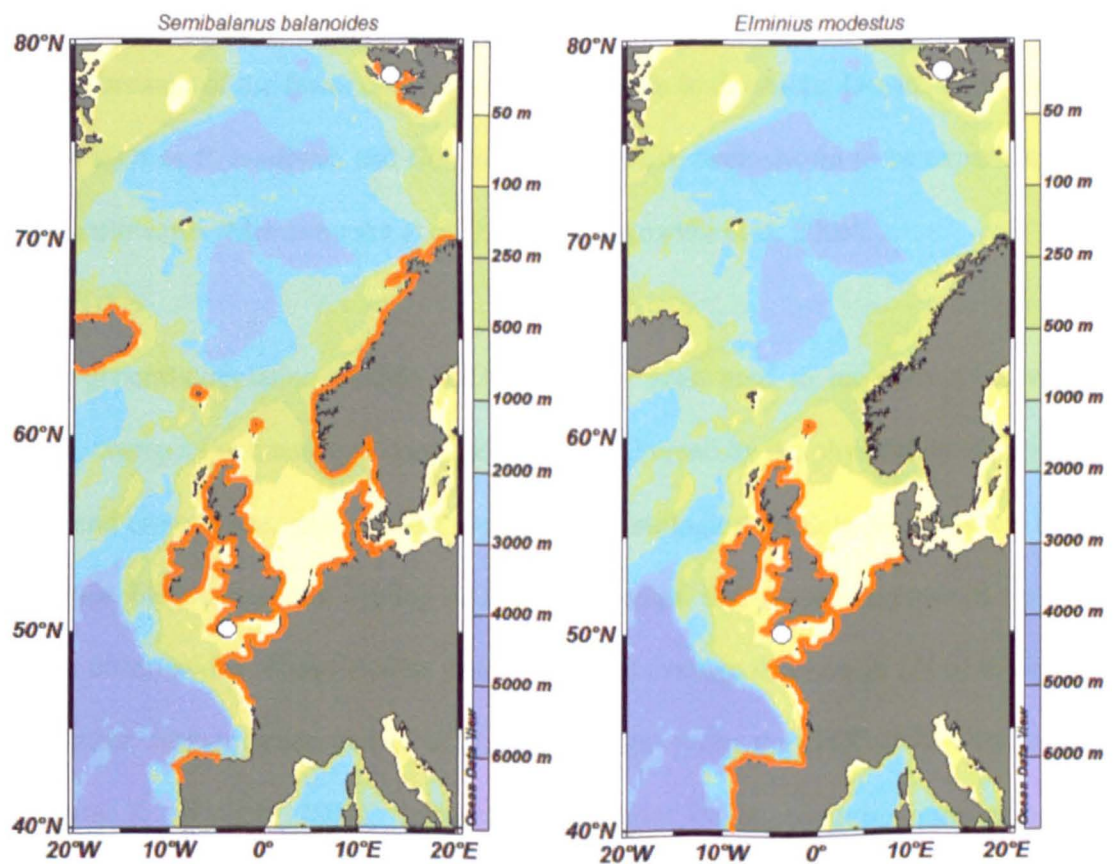


Figure 8.1: Geographic distribution of a) *Semibalanus balanoides* and b) *Elminius modestus* along the European coastline. The map also outlines land (grey) and bottom topography (depth, m). White circles mark Plymouth in the south and Ny Ålesund in the north.



Physical, ecological, evolutionary and physiological factors acting on the processes of reproduction, birth, dispersal, recruitment and mortality are all involved in shaping species ranges (c.f. geographic ranges for *Semibalanus balanoides* and *Elminius modestus* in figure 8.1). Both the southern edge of a cold-adapted species and the northern edge of a warm-adapted species are predominantly controlled by temperature acting on a particular aspect of a species life-history (Hutchins 1947). The observational evidence suggests that regional climate warming since the mid 1980s has caused cold-adapted species such as *S. balanoides* to retract within their range, i.e. the southern edge is moving north (Mieszkowska et al. 2006). Limited observations have been made at the northern edges of species range. One such report was of the mussel *Mytilus edulis*, which was discovered for the first time in Svalbard (Berge et al. 2005). However, there is little evidence to suggest further changes in the populations of cold-adapted species at the northern range edge, perhaps because of the fewer observations being made in the Arctic Ocean. Warm-adapted species such as *E. modestus* and *Chthamalus* spp. have been shown to be expanding their range northwards (Mieszkowska et al. 2005; Mieszkowska et al. 2006).

Ocean general-circulation models (OGCMs) have been used to reconstruct, as well as predict, changes in climate and ocean acidification. Forced by the physical dynamics of the ocean and atmosphere, and coupled together with biological models, OGCMs are able to reproduce biogeochemical cycling within the oceans that closely represents past and present observations. These models predict a global average decrease in pH of 0.4 by year 2100 in the surface ocean and of 0.77 by year 2300 under the IPCC IS92 CO<sub>2</sub> scenario (Caldeira & Wickett 2003). Introducing changes in temperature, weathering and sedimentation into these simulations only reduced this maximum decline in pH by 10 % (Caldeira & Wickett 2003). More detailed predictions of both carbonate ion and CO<sub>2</sub> concentration for different oceans regions and across latitudinal gradients strongly imply that the polar and sub-polar oceans are particularly vulnerable to ocean acidification (Orr et

al. 2005). The carbonate ion concentration is already much lower in these regions so they are particularly susceptible to a reduction in pH (Bellerby et al. 2005) such that it is predicted that Arctic waters will become undersaturated with respect to aragonite by 2030 (Steinacher et al. 2009). Predictions of ocean acidification in the Arctic Ocean are made using coupled ocean models that incorporate future scenarios of global warming, sea ice melt, salinity changes due to ice melt and some changes in primary production. Indeed, the model used by Steinacher et al. (2009) shows that climate change amplifies the decrease in average annual mean Arctic surface aragonite saturation state and pH by 22 % and 27 %, respectively. The changes that will occur in the Arctic Ocean as a result of climate change are complex and require further research to unravel how they will impact populations.

From a consideration of the observational data it appears that species ranges are shifting as predicted from temperature changes alone. It is important then to ask what impact CO<sub>2</sub> has already had, if any, and what impact it might have on future range shifts. Furthermore, it is necessary to consider whether species employ physiological plasticity to acclimate to a changing environment. The latter has, in many respects, already been answered for *S. balanoides*. The natural, observable variation in growth rates, morphology, egg mass, and other life-history traits, clearly indicates that this species is highly plastic in its response to temperature and many other stresses previously investigated in the literature (e.g. salinity, Foster 1970). The experiments in this thesis show that individuals are able to alter their resource allocation over a relatively short time period in response to a different CO<sub>2</sub> regime. The longer-term implications of these changes in life-history trait on populations are more difficult to ascertain. Survival of recruits is important in the short term but population persistence over generations is more important for long-term survival of the population. For example, populations at the range edges tend to be regulated at persistent levels of 1-5 individuals cm<sup>-2</sup>. If recruitment is reduced by 20 % every year, either through lowered

survival of nauplii (i.e. less settling) or post-larvae, this will, overtime, reduce the population.

One method for attempting to answer the question of what impact CO<sub>2</sub> will have on the distribution of a species as the range shifts with temperature is to formulate conceptual models of the relative impacts of ocean acidification and temperature on the processes that most influence population dynamics. Firstly though, it is useful to assess what future conditions these intertidal organisms may actually experience.

### **8.3.2. What will intertidal organisms actually experience through their life history and does variability matter?**

Chapter 1 describes present (year 2008) intertidal conditions at the locations where the organisms used in the experiments were collected. The changes in temperature and CO<sub>2</sub> used throughout these experiments, and many studies described in the literature are nominal mean values of change, such as the Intergovernmental Panel on Climate Change (IPCC) global average CO<sub>2</sub> and sea surface temperature predictions. However, there is much information, particularly with reference to intertidal temperature variability, detailing the regional and local differences in environmental conditions, which have profound implications on population dynamics (e.g. Helmuth & Hofmann 2001). While experimental conditions and many predictions of the future distribution of species are based on changes in average temperature, it is clear that, at least in the intertidal, extreme events are often more important than average conditions (e.g. Harley & Helmuth 2003; Denny et al. 2006). High temperatures during and immediately after settlement can contribute significantly to post-larval and juvenile mortality (Kendall et al. 1985) through desiccation (Foster 1971) and heat death (Wetthey 2008). For intertidal sessile organisms, high body temperatures are caused by a combination of high air temperatures, direct sunlight, low wind speeds and long periods of emersion associated with low tides (Wetthey

2002). Barnacle body temperature is related most significantly with the rock temperature and the ability of the substratum to store heat (Thomas 1987; Bertness 1989). Additionally storm events can lead to loss of high density of barnacles that have formed hummocks (Barnes & Powell 1950) as well as from abrasion (Hunt & Scheibling 1997).

With respect to ocean acidification, the decline in pH is broadly similar across most regions of the world's oceans (Orr et al. 2005), although somewhat faster in the polar oceans. In the long term (interannual and decadal timescales) continued uptake of CO<sub>2</sub> by the oceans is predicted to cause some coastal areas to be completely outside their natural range of pH (Blackford & Gilbert 2007). Hence, for the whole lifespan of an organism the global average pH value often used in ocean acidification studies gives a reasonable representation of the conditions which an organism will experience. On much shorter timescales (seasonal and daily) the variability of CO<sub>2</sub> (and pH) becomes more important for the physiology of the individual, yet such variability is seldom taken into account by those making predictions concerning vulnerability.

For example, benthic organisms are subjected to higher CO<sub>2</sub> levels than those in surface/water column (Blackford & Gilbert 2007). Similarly, the CO<sub>2</sub> level, pH and saturation state of calcium carbonate minerals can change substantially over a seasonal cycle in both temperate and polar regions (Blackford & Gilbert 2007; Findlay et al. 2008). As release of *S. balanoides* larvae always occurs with the spring bloom (Barnes 1962), the nauplii will develop in higher (and presumably more suitable) levels of pH and calcium carbonate saturation states because of the consumption of CO<sub>2</sub> from the surface waters by phytoplankton. On the other hand overwintering adults will be subjected to much lower levels of pH and saturation states.

It is difficult to quantify how important variability in temperature and CO<sub>2</sub> are. One view, particularly in relation to temperature, is that extreme and acute events cause the highest levels of mortality. Alternatively, it is clear that over longer time-scales the average conditions capture the general dynamics of populations (Chapter 6). Organisms such as these intertidal barnacles, which experience, and tolerate, a naturally fluctuating environment have been shown to be impacted by chronic changes in ambient conditions. Hence, it is most important to choose the appropriate conditions for both experiments and models that best represent those experienced by the life-stage and life-history of interest.

To date, there are limited observations of present changes in ocean biology as a result of ocean acidification. This may be in part a result of (a) a lack of chemical data with which observations can be correlated and (b) a need for more observations in this emerging area. However it may also be an artefact of organisms' ability to cope with short term variability in pH. Impacts may not become apparent until they are subjected to longer periods of lower pH or the whole pH range that they experience is reduced. Observed changes, for example in species distribution which have been attributed to changes in climate, pollution, ecosystem deterioration, may have masked the role of ocean acidification. Hence, understanding the variability and the responses to that variability is important particularly when making predictions about acclimation and the possibility of adaptation.

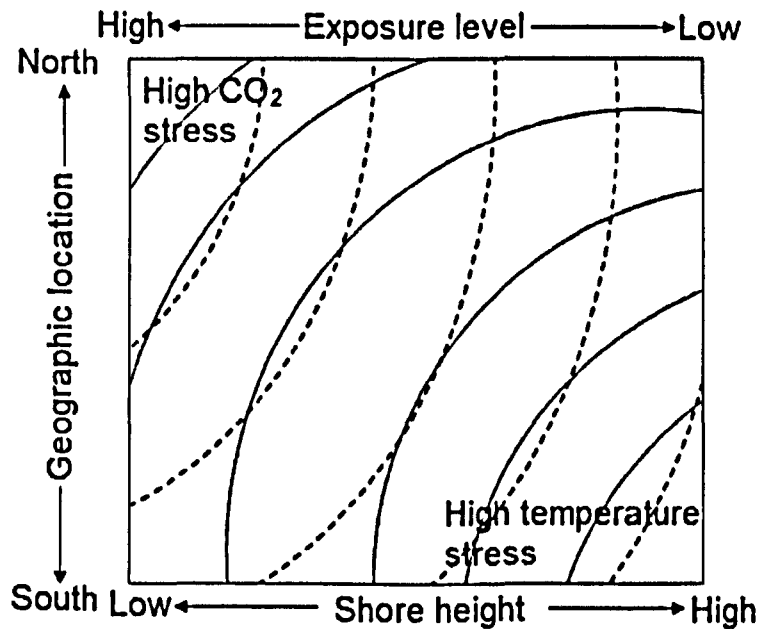
### **8.3.3. South vs. north, closed vs. open, and everything in between!**

The experimental results from this thesis will now be placed into context of the present ecological thinking in order to attempt to understand how populations may respond in the future. The advance in ecological theory over the last century can provide a framework for understanding and predicting population dynamics. Unfortunately, the highly variable nature of intertidal populations means that there is a complexity underlying the overarching theories which has caused much debate. Taking into consideration these debates, it is most

suitable to first consider the experiments under two separate themes: the location of a population across stress gradients (large scale: geographic range, small scale: exposure level); and pre- and post-settlement processes, before attempting to formulate a more overarching model.

#### ***8.3.3.1. Relative impacts of CO<sub>2</sub> and temperature across environmental gradients***

Across a geographic range, temperature and desiccation stress increase with decreasing latitude, while seawater CO<sub>2</sub> stress (ocean acidification) will to some extent decrease with decreasing latitude (Steinacher et al. 2009). Thus, there are opposing forces governing the impacts on individuals and populations across a large scale gradient (figure 8.2). This pattern in temperature (and desiccation) and CO<sub>2</sub> stress is mirrored at the local scale too, shore height and degree of exposure (figure 8.2). Increasing height up the shore and decreasing exposure will increase a barnacle's stress from temperature and desiccation but will decrease seawater CO<sub>2</sub> stress. Individuals on the low shore or on exposed sites will be exposed to longer periods of ocean acidification but will have some relief from high temperatures. However, in areas where there is lower temperature stress there are increased biotic interactions (Lewis 1964). A number of authors have indicated that temperature sensitivity within a species can be variable across a single shore, with high shore individuals being more tolerant to temperature than low shore (Ware & Hartnoll 1996; Somero 2002). But how do the various life-history stages respond to a combination of ocean acidification and temperature across these gradients?



*Figure 8.2: A hypothetical model of relative impacts on temperature and CO<sub>2</sub> across large-scale geographic location and small-scale position on shore and exposure level. Solid lines indicate temperature stress, with highest stress occurring high in the shore, sheltered sites and at the southern limits of the geographic distribution. Strength of CO<sub>2</sub> (pH) stress is indicated by dashed lines with highest stress occurring in the low shore, more exposed sites and at the northern limits of the geographic distribution.*

The results from experiments on post-larval development which were carried out at different temperature and CO<sub>2</sub> conditions across the biogeographic range are most easily applied to prediction. Overall survival of post-larvae was reduced by both increasing temperature and by elevated CO<sub>2</sub> conditions with little interaction between the two variables (Chapter 7). Such findings lead to the prediction that temperature is more important in driving post-settlement development in warmer conditions, whereas elevated CO<sub>2</sub> becomes more dominant in colder conditions (Chapter 6).

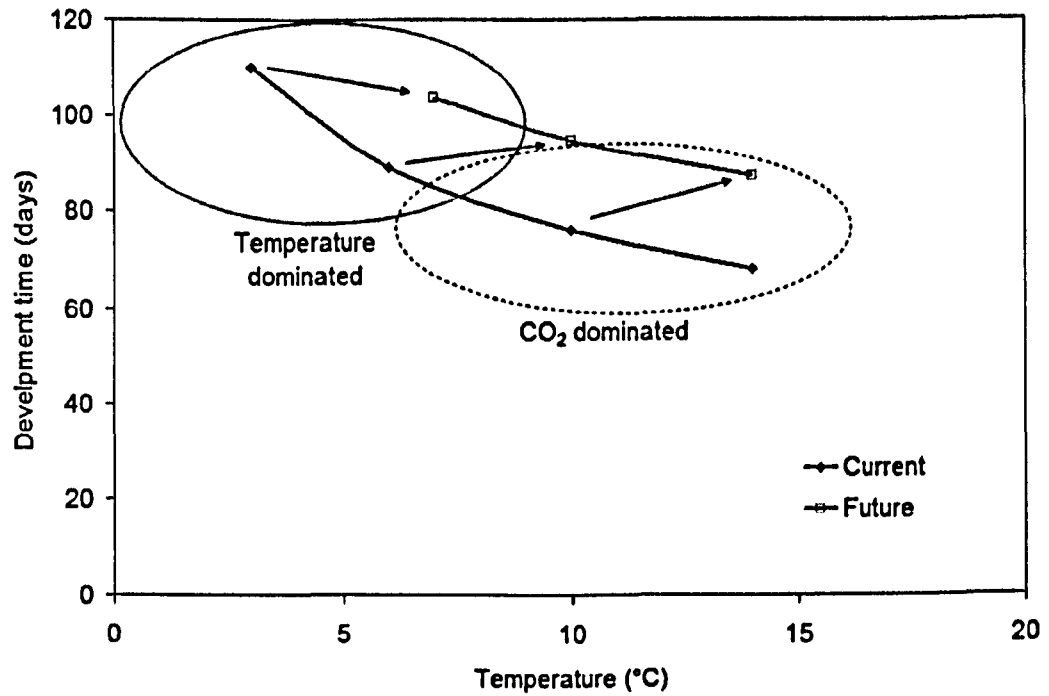


Figure 8.3: Example model of embryo development time (days) at different temperatures under low CO<sub>2</sub> conditions (~350 ppm) using data taken from Crisp (1959) forming the relationship  $y = ax^b + c$ . Arrows indicate the shift in development times under future CO<sub>2</sub> conditions (~920 ppm). The solid oval indicates there is a decline in development time caused by a dominance of increased temperature; the dashed oval indicates that the delay in development time caused by elevated CO<sub>2</sub> is not overcome by the temperature change.

The combined impacts of elevated temperature and CO<sub>2</sub> on embryo and naupliar development are somewhat more difficult to assess. Crisp (1959) provided experimental data on the development time of embryos under different temperatures and constant emersion. These data can be used to create a simple model to predict development time at a specific temperature, e.g.:

$$t_d = aT^{-b}, \quad (8.1)$$

where  $t_d$  = development time in days and  $T$  = temperature °C,  $a$  and  $b$  are constants).

With respect to elevated CO<sub>2</sub>, the experiments on embryo development in this thesis suggested that there was a 19 day delay in development under elevated CO<sub>2</sub> at constant



temperature. Assuming that there is no interaction between temperature and CO<sub>2</sub> (this assumption will be discussed further in section 8.4.1) then the model can be amended to predict for elevated CO<sub>2</sub> conditions, i.e.

$$t_d = aT^{-b} + c, \quad (8.2)$$

where  $c =$  is a constant related to the delayed development caused by CO<sub>2</sub> at constant temperature.

Using this improved model it can be shown that under IPCC year 2100 predictions of elevated temperature and CO<sub>2</sub>, compared to control conditions, development time would be lower (rates increase) in cold-water locations, but development time would faster (rates decrease) in warm-water locations (figure 8.3). This simple model shows that temperature is dominant over CO<sub>2</sub> in determining embryo development rates in colder locations but CO<sub>2</sub> becomes dominant at warmer locations. Furthermore, there is an upper thermal tolerance limit (> 14 °C) after which no development will occur (Crisp 1959), this limit will prevent the persistence of more southerly populations.

The experiments on naupliar development were only partially successful; development rate was determined for naupliar stages I to III, as was survival to stage III. Development rate was apparently unaffected by CO<sub>2</sub> but survival decreased by about 20 %. This would suggest that, again assuming there are no interactions between temperature and CO<sub>2</sub> (see section 8.4.1), across a temperature gradient, the development rate would not be influenced by CO<sub>2</sub>, and is therefore determined only by temperature. Survival on the other hand, does not appear to be temperature-sensitive within the range that *S. balanoides* nauplii experience in the field (up to 18 °C, Barnes 1958) and hence CO<sub>2</sub> would reduce survival equally across a temperature gradient up until this thermal limit. These findings lead to the proposition that CO<sub>2</sub> and temperature affect naupliar development relatively equally. However, temperature induced changes will be most important in presently warm locations

while ocean acidification driven changes will be largest in colder locations. Therefore, it will be predicted that temperature is the dominant factor on nauplii development in warm conditions while CO<sub>2</sub> is the dominant factor in cold conditions (figure 8.4).

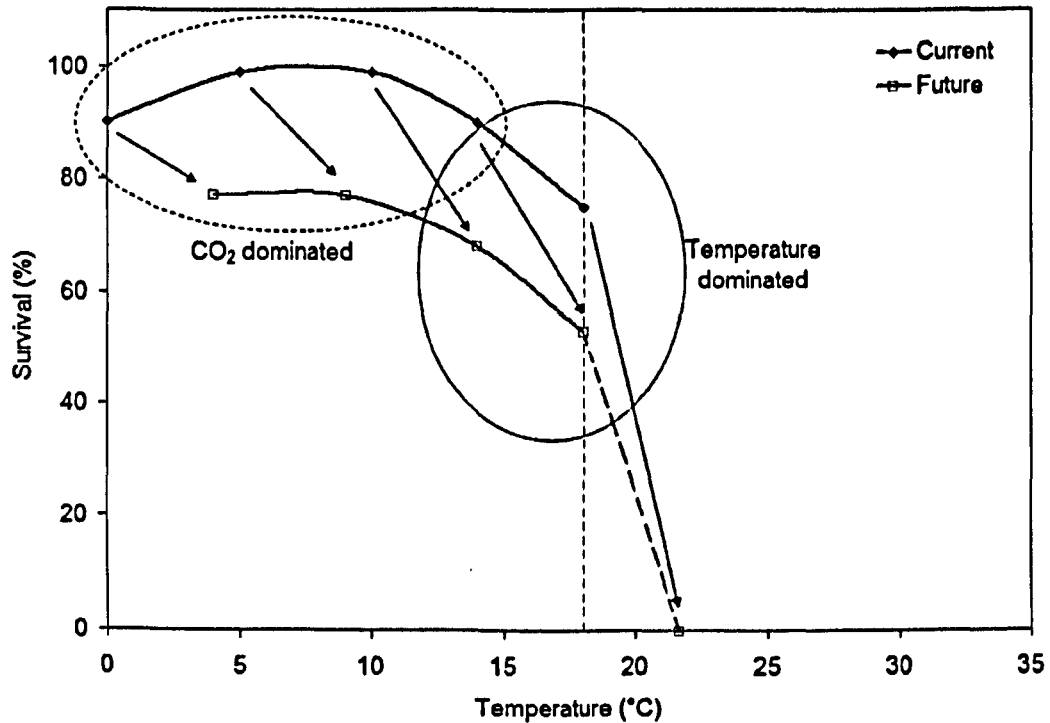


Figure 8.4: Example model of naupliar survival at different temperatures under low CO<sub>2</sub> conditions (~350 ppm) using information from Barnes (1959) that survival is not affected by temperature until 18 °C. Arrows indicate the shift in survival under future CO<sub>2</sub> conditions (~920 ppm). The solid oval indicates there is a decline in survival caused by reaching the temperature threshold of 18 °C, the dashed oval indicates that survival is lowered by elevated CO<sub>2</sub>.

### 8.3.3.2. Relative impacts of CO<sub>2</sub> and temperature on pre- and post-settlement phases

There has been great debate over the last fifty years as to whether it is pre-settlement processes or post-settlement processes that have most influence on the population dynamics of intertidal species (e.g. Thorson 1950; Young 1990; Grosberg & Levitan 1992; Menge 2000). Studies have included field observations (e.g. Barnes & Powell 1950; Gaines & Roughgarden 1985; Hunt & Scheibling 1997; Jenkins 2005), manipulation experiments (e.g. Barnett 1979; Hawkins 1983; Olivier et al. 2000; Menge 2000; Hills &

Thomason 2003) and modelling (e.g. Roughgarden et al. 1988; Delafontaine & Flemming 1989; Alexander & Roughgarden 1996; Crimaldi et al. 2002; Shanks et al. 2003; Svensson et al. 2004; Pfeiffer-Hyot & McManus 2005). Initially, attention was paid to the more tractable adult populations and interactions associated with predation, competition and the physical environment such as heat stress and exposure. Underwood & Denley (1984) criticised marine ecologists for not considering the influence of variable recruitment on population dynamics. The focus of research then switched to extend the “recruit-adult hypothesis” which suggests that density of recruits had a large influence on the adult barnacle abundance (Gaines & Roughgarden 1985; Roughgarden et al. 1988). Since then, a wealth of evidence has been found to support both the “supply-side” and the post-recruitment arguments. Todd (1998) succinctly summarised the evidence to show that both sets of processes can be equally important to general dynamics. Simply put, when supply is variable and limiting to a population (e.g. the population is ‘open’) then the population dynamics will be more significantly influenced by the density of recruits, and hence by pre-settlement factors, rather than by post-settlement factors. Conversely, when supply is not limited (i.e. the population is ‘closed’) then the dynamics are driven primarily by post-settlement factors because there will always be a high density of recruits. By this understanding, it is possible to assess the relative impacts of ocean acidification and temperature on different populations.

The first step in placing the experimental results from this thesis into categories of pre- or post-settlement effect is initially relatively easy. The direct impacts of temperature and CO<sub>2</sub> on naupliar stages can be classed as pre-settlement, while the direct impacts of temperature and CO<sub>2</sub> on post-larvae, adults and embryo development can be classed as post-settlement. It could be argued that embryo development should be classed as pre-settlement; however an ‘open’ population will not be influenced by its own embryo development while a ‘closed’ population will be impacted by its own embryo development.

However, embryo development occurs within the adult mantle cavity which is under the influence of post-settlement processes. By combining the ideas of stress gradients and population types, a conceptual model can be constructed (figure 8.5) to describe the relative impacts of CO<sub>2</sub> and temperature on the most important processes that influence population dynamics according to the population type (closed or open) and location (large-scale and local-scale).

The conceptual model presented in figure 8.5 shows that at the northern edge of the range and/or in conditions of lowered wave exposure, a closed population will most strongly be influenced by ocean acidification acting negatively on post-larval development. In an open population at the north range edge, the population dynamics will be primarily influenced by ocean acidification impacts acting on larval development and survival. At the southern range edge however, closed and open populations will be more significantly influenced by temperature acting on post-larval and naupliar larvae, respectively.

Geographic range (Large-scale)	Closed population	Mixed population	Open population	Exposure level (Local-scale)
North	Post-settlement factors Embryo develop CO <sub>2</sub> < T °C Post-larvae CO <sub>2</sub> >> T °C	↔	Pre-settlement factors Naupli survival CO <sub>2</sub> > T °C	Low
Mid	↕	⊗	↕	Mid
South	Post-settlement factors Embryo develop CO <sub>2</sub> > T °C Post-larvae CO <sub>2</sub> << T °C	↔	Pre-settlement factors Naupli survival CO <sub>2</sub> < T °C	High

Figure 8.5: Conceptual model of the changes in dominant factors acting on the process that most influences the population dynamics at specific locations

It is much more difficult to assess how the indirect affects of temperature and CO<sub>2</sub> will alter population dynamics. These include factors such as oceanographic and hydrographic

conditions, competition, predation, and disturbance, and will be briefly summarised immediately below in relation to temperature and ocean acidification.

*Oceanographic and hydrographic influences:* Oceanographic and hydrographic factors predominantly influence dispersal and settlement rate (e.g. Barnes 1970; Kendall et al. 1978; Jackson & Strathman 1981; Alexander & Roughgarden 1996; Crimaldi et al. 2002; Shanks et al. 2003). Ocean acidification and climate change may alter physical oceanography and dispersal either directly or indirectly. Direct impacts from changes in temperature could modify the physical dynamics by increasing the water column stratification and/or changing flow of currents (IPCC 2007). Although not easy to assess, it should be made clear that at certain locations, oceanographic and hydrographic factors may be responsible for recruitment failure of whole year classes (Todd 1998), thereby overriding any direct biological impacts from temperature and ocean acidification.

Increases in development rate caused by elevated temperature will influence the length of time spent in the plankton. As a consequence there will be a reduction in the length of the planktonic stage which would be manifested in reduced dispersion (Crisp 1977) and the probability of an increased supply of larvae settling on the shore. Ocean acidification could directly influence dispersal by affecting the rate of development and hence extend the planktonic stage, which is in the opposite direction to temperature affects. Consequently, in some locations (e.g. mid-range), the interaction of the two factors may well prevent any direct changes becoming apparent. In others, however, it seems likely that either temperature effects will dominate (e.g. northern-range edge, figure 8.3) and increase the development rate or ocean acidification effects will dominate (e.g. southern-range edge, figure 8.3) and development rate will decrease.

***Intra-specific competition:*** *Semibalanus balanoides* is a gregarious species that relies on the availability of free-space surrounding conspecifics on the rocky shore for settlement (Knight-Jones & Crisp 1953; Crisp 1961). Conversely, during periods of heavy settlement, mortality of post-larvae becomes highly density-dependent (Barnes & Powell 1950; Crisp 1961; Lewis 1964; Todd 1998). The reduction in growth rates of post-larvae seen under elevated temperature and CO<sub>2</sub> could have two different consequences on competition. If reduced growth rates reduced the overall size of juveniles then there will be reduced spatial competition. Heavy settlement will not cause as many individuals to grow in such crowded conditions, thereby relieving post-settlement mortality caused by hummocking. Conversely, if reduced growth rates lead to increased longevity instead of reduced size then competition will be delayed but will be inevitable. Very heavy settlement will still result in self-eliminations.

***Inter-specific competition:*** Other barnacle species such as *Chthamalus* spp. have yet to be examined with regard to their response to elevated CO<sub>2</sub>, although where ranges overlap the disappearance of *S. balanoides* would create free space for *Chthamalus* and other species such as *Elminius modestus* (Southward & Crisp 1956). The ability of such species to exploit the loss of *S. balanoides* of course relates to their own response to temperature and CO<sub>2</sub>. *E. modestus*, for example, appears to be more tolerant of elevated CO<sub>2</sub> than *S. balanoides* when found at similar temperatures (Chapter 4). Elevated CO<sub>2</sub> caused only subtle impacts on growth rate in this species and had no impact on its survival or shell development. In some locations, principally in areas of disturbance or lowered salinity, *E. modestus* is a spatial competitor to *S. balanoides* (Crisp 1958; Barnett et al. 1979) and hence in some places it may benefit from future changes in temperature and ocean chemistry. If *E. modestus* were to replace *S. balanoides* it could arguably have no impact on ecosystem function. They are both sessile filter-feeders and are of relatively similar size therefore are likely to have similar functions as well as secondary production levels (for

one population of *E. modestus* organic production was estimated at 40 – 145 g<sub>AFDW</sub> m<sup>-2</sup> yr<sup>-1</sup>, while total CO<sub>2</sub> production was estimated at 7 – 27 mol CO<sub>2</sub> m<sup>-2</sup> yr<sup>-1</sup>, Gollety et al. 2008).

Few climate change and ocean acidification experiments have been carried out on macro-algae, although one study on macro-algae and seagrasses showed that there may be an increase in production as a result of increased CO<sub>2</sub> (Beer & Koch 1996). Hence they may not be detrimentally impacted by ocean acidification, however their relative success is likely to influence future community dynamics. An increase in macro-algae on the shore will prevent *S. balanoides* cyprids from settling or establishing and hence further restrict recruitment potential (Hawkins 1983). Conversely, field observations suggest that climate warming might lead to a reduction in cold-adapted species of brown macro-algae around its southern limits (Southward et al. 1995), which could potentially reduce spatial competition, reduce cover for limpets and reduce mortality of cyprids being swept away by algal fronds.

There will also be interaction affects with limpets, such as *Patella* spp., which browse on macro-algae and hence increase settlement by preventing algal growth (Hawkins 1983). Initial experiments on *Patella vulgata* show that they may be able to compensate for changes in ocean acidification (Chapter 5), especially if algae, their food source, also increase in abundance. Interestingly, *Patella vulgata* is mid-range in the UK and hence perhaps it would be expected that temperature and ocean acidification are not the main factors influencing the local populations of this species.

***Biotic interactions (e.g. predation):*** Biotic interactions become more important lower down on the shore and similarly at the mid-range of a species geographic distribution (Lewis 1964; Menge 2000). It is apparent from the earlier discussion of resource allocation that the formation of calcified shells will become more energetically costly as ocean

acidification increases, altering the energetic balance of the barnacles and possibly resulting in smaller adults. The predatory gastropod, *Nucella lapillus*, preferentially preys on larger barnacles (Connell 1961; Barnett 1979) so initially it appears that predation risk may be reduced. Assessing the predation risk is complicated by two factors: 1) the direct impacts on ocean acidification and elevated temperature on the predators themselves; and 2) the implications that ocean acidification will lead to smaller barnacles that may also have weaker or thinner shells. If the broad assumption was made that a predatory gastropod responded to ocean acidification in a similar way to the gastropod *Littorina littorea* (Chapter 5), energy demand for those individuals would increase. Under sub-optimal conditions many predators become less specific with their prey selection (Burrows & Hughes 1991) and the foraging strategy of the predator may be shifted. The prey-selection strategy will depend on the higher energy rewards of consuming larger prey being offset by the frequency at which they will come across large prey (search time). When the second factor of thinner or weaker shells is also taken into account, the balance is tipped again in the favour of predators. A predator will gain by consuming smaller barnacles, which have lower energy rewards, as long as they are common and easy to consume (Burrows & Hughes 1991).

**Physical disturbance:** The reduced calcium levels and smaller individuals that occur at the southern range edge in elevated temperature and CO<sub>2</sub> are more likely to be affected by physical disturbance, such as wave action, than populations in the north of the range, which are able to grow larger and maintain strong shells. Lower shore individuals and individuals in more exposed locations may be eliminated more frequently because not only will they experience more wave action, but they are also exposed to acidified conditions for longer periods and hence will experience more shell dissolution.



**Summary:** There are a multitude of factors that potentially impact population dynamics, yet understanding all the various responses to ocean acidification and climate change is a complex and difficult processes. The conceptual model in figure 8.5 describes the direct impacts on a population and can be useful to make reliable predictions. As stated by ecologist Robert T. Paine (1994) “excessive detail can obscure dynamically based patterns”, so accordingly it is important to understand if it is necessary to include the complexity of these other responses, and what the predictive limitations are, if they are not included.

#### **8.3.4. How much detail is needed in a model to make it useful?**

A simple population model (such as that used in Chapter 6) can provide insight into the relative impacts of temperature and CO<sub>2</sub> on a southern range edge population of *Semibalanus balanoides* over the last 50 years. In the model outputs the contribution of temperature to controlling abundance is much greater than the contribution of a declining or fluctuating CO<sub>2</sub> (pH) level; nevertheless, during cold years, ocean acidification has a stronger influence on abundance than in warmer years. Although the addition of a pH function to the model did alter the dynamics slightly, the major dynamics could be predicted from sea surface temperature and perhaps the model does not require the pH functions in order to predict the general outcome. This model makes reasonable hindcast and forecast predictions for this specific location but it may be unlikely to do so well at other locations within *S. balanoides* range because of the shift in dominant controlling factors. The model will not be representative for all locations because the dynamics of an open population towards the southern limits of the geographic distribution are driven primarily by pre-settlement factors and temperature will influence these factors more than ocean acidification (figure 8.5), whereas at other locations post-settlement factors may be more important or ocean acidification may be more influential.

Using the conceptual model presented in figure 8.5 it can be shown that, depending on location, a population model could be designed which takes into account the basic assumptions associated with the location of the population and whether the population can be considered closed or open. Such findings support recent studies that suggest that models of organisms with complex life histories must contain the appropriate detail of both the pre-settlement and post-settlement processes (Menge 2000). The conceptual model in figure 8.5 indicates that a reasonable understanding and prediction of *S. balanoides* population dynamics can be made from knowledge of the population type, the level of exposure, shore height and geographic location. Local population type, level of exposure and geographic location can be readily discovered with the present technology and existing networks of observations. Further experimental analysis can then be carried out on the most appropriate life-history stage, according to population type and location, which can then be placed into the model.

Arguably then, other factors, such as oceanographic conditions, predation, competition, can be considered as simple mortality terms within these models when necessary. This allows model complexity to be reduced but still capture the most important variables and dynamics. Moreover, many of these factors are also primarily driven by temperature and will produce a large element of covariance, which would also have to be resolved.

#### **8.3.5. What does the future hold for *Semibalanus balanoides* and similar organisms?**

The conceptual model indicates that for *Semibalanus balanoides*, temperature will negatively influence the population dynamics towards the southern edge of their range, through both pre- and post-settlement processes. At the northern edge of the range, ocean acidification impacts will negatively influence the same processes. In combination this may prevent the northern edge from extending any further north as the ice-edge recedes. Had ocean acidification not been occurring, the geographic range of cold-adapted species

such as *S. balanoides* would likely have shifted northwards and locations that become ice-free as a result of climate warming could have provided new habitat for the species to occupy.

#### **8.4. WHERE SHOULD RESEARCH GO FROM HERE?**

The work carried out in this thesis forms the basis of a novel line of thinking within the field of ocean acidification. It takes the shape of parameterising mathematical and conceptual models with empirical data in order to assess how populations might respond to changing environmental conditions while maintaining some of the necessary complexity of the real world. This approach obviously comes with limitations, both practically and theoretically. These caveats will be discussed below and will be followed by some suggestions for future work and improvements.

##### **8.4.1. Understanding the limitations of experiments and models**

While the modelling approach adopted in this thesis has produced potentially useful information, it has been unable to fill a number of important gaps in predicting how individuals, populations and ecosystems respond to changing environments. These will now be briefly discussed.

One common problem with the present experimental work related to ocean acidification and climate change is the relatively short experimental periods on which conclusions are based. On the one hand these short-term experiments are useful to gain an understanding of possible responses but on the other hand, they do not allow organisms time to acclimatise or, on even longer time-scales, for species to adapt. An optimistic view may be that for organisms with short generation times, micro-evolutionary adaptation could be rapid and that individuals adversely affected by high CO<sub>2</sub> could be replaced by more CO<sub>2</sub>-tolerant individuals (e.g. as can occur in response to temperature, Ware & Hartnoll 1996). A more

pessimistic view is that ocean acidification and climate change are occurring at such unprecedented rates and in combination with other stressors, such as fishing, habitat destruction, hypoxia, eutrophication, etc., that it will be impossible for sensitive groups to compete ecologically, which could result in extinctions.

In the example of *Semibalanus balanoides*, generation times are relatively short and it is therefore plausible that micro-evolutionary processes could occur. Frankham (2005) reviewed adaptation experiments and demonstrated that the pattern of adaptation to a particular force was one of diminishing rate with time until no more adaptation occurred. Laboratory studies have shown adaptation in a closed population can occur within 25 to 100 generations (Frankham 2005). Nevertheless, the oceanographic conditions that affect dispersal, as well as the location of the population, will affect the ability of these organisms to adapt. For example, Bertness & Gaines (1992) showed that bays with low flushing rates, and hence longer residence times, restricted *S. balanoides* larval dispersal, which in turn promoted local adaptation of the populations to the thermal stresses of bay habitats within a few generations. From the conceptual model it might be predicted that closed populations in the south will have more opportunity to adapt to temperature stress, while closed populations in the north and on the east coast of the UK will have more opportunity to adapt to ocean acidification stress. Adaptation of open populations is likely to be much slower.

*Elminius modestus*, however, provides an example of a species that has shown no obvious phenotypic adaptation in a period of 50 years. *Elminius modestus* responded differently to temperature and CO<sub>2</sub> than did *S. balanoides* in the experiments carried out in this thesis (Chapter 4), despite it being found in similar conditions to *S. balanoides* around the colder UK waters for the last 60 years (Bishop 1947). *E. modestus* requires a much larger food supply than *S. balanoides* in order to reproduce within a few months of settling. *E.*

*modestus* produces more offspring than *S. balanoides* and does this throughout the year, hence has a higher generation rate (Crisp & Davies 1955). At times of lower food supply (i.e. winter) however, *E. modestus* offspring would be less likely to survive (Rainbow 1984). In the temperate and sub-polar locations there is a large seasonal cycle in phytoplankton (and hence food supply) yet there has been no change in the life-history strategy of *E. modestus* to this food selection pressure. Perhaps the food selection pressure was not large enough compared to *E. modestus*' ability to rapidly colonise areas when food supply was high; or perhaps the openness of the populations prevents adaptation from occurring. More analysis is required in the future to assess the level of fitness, the associated trade-offs and the amount of selection pressure resulting from a changing climate which might cause adaptation to occur.

*Semibalanus balanoides* reaches its geographic limit in the Arctic Ocean were changes in the physical environment will be complicated by a number of factors; one that has many associated consequences is the pattern of solar radiation. The fact that over winter the Polar Regions receive no sunlight has fundamental consequences for both physics and biology. Present predictions suggest that there will be no summer sea ice cover in the Arctic within the next 30 years (Wang & Overland 2009) and as a consequence the Arctic Ocean will be open to many new species (Greene et al. 2008). The winter ice cover however will continue to form because of the lack of sunlight with the result that there will still be large seasonal dynamics of temperature, ice-cover, nutrients, etc. which will ultimately control the phytoplankton bloom and the effects will propagate up the food chain. Therefore, while the timing of the spring bloom may become earlier in the year in the lower latitudes (Edwards et al. 2001), in the Arctic, the spring bloom will only be able to become earlier to the point where sunlight becomes non-limiting; there will be a strong limit to the period in which the spawning of *S. balanoides* can be advanced. Food-supply is an important factor throughout all stages of an organism's life however predicting how food-supply will

change in the future, although important, is particularly difficult. The models presented in this thesis assume a constant food supply.

The assumption that no interaction takes place between temperature and CO<sub>2</sub> in relation to embryo development rates and naupliar survival is based on interpretation of the experimental data presented here (section 8.3.3). Interestingly, these experimental results reveal that there is no clear interaction between temperature and CO<sub>2</sub>. In some cases it appears that the two stressors are antagonistic (e.g. development rates) but there is no clear pattern and no evidence of synergy. The result of antagonistic responses is counter to some predictions that temperature and CO<sub>2</sub> will act synergistically (e.g. Pörtner et al. 2005) and suggests more multiple stress studies need to be carried out to understand how and where the two stresses interact.

#### **8.4.2. Future experimental and modelling requirements**

Finally, the limitations of the present understanding of ocean acidification and climate change can be used to assess what is required for developing observation networks, experiments and models in the future. Understanding the key life-stage, processes and conditions are the most important requirements for providing a good understanding of how a changing environment will influence populations and hence community structure. To that end, new experiments should not only investigate physiological processes of one individual, but should focus on aspects of population dynamics such as reproduction, dispersal, recruitment and mortality; and on processes rather than individuals. It is now vital that the ocean acidification community attempts to understand adaptation and recovery using experimental and modelling tools. Continual effort should also be made to assess multiple impacts relative to the specific area of interest.

## 8.5. CONCLUSIONS

- Early life stages may not always be the most vulnerable to ocean acidification. Adults show changes in resource allocation, which although may not affect the adult directly, could have implications for long-term persistence of the population. Additionally, conditions vary throughout the year and, in the case of *S. balanoides*, CO<sub>2</sub> and pH conditions are more suitable during the period of nauplii and post-larval phases but less suitable during the adult over-wintering phase. Furthermore, temperature changes can be equally important and antagonistic to ocean acidification responses.
- Calcification is just one physiological process that is coupled to many other physiological processes (particularly metabolism) and should be taken in context of the whole organism, as a change in one process has implications on resource allocation and energy budget of the individual.
- Species that occupy the same habitat show different responses to ocean acidification and temperature, because of their different life-history strategies.
- Intertidal organisms, such as *Semibalanus balanoides*, show large plasticity. However, adaptation will depend on the openness of populations and the timescale of change, as shown by *Elminius modestus*.
- Population responses to ocean acidification and temperature differ. This can be modelled to some level, when there is an understanding of what factors are most important in controlling the population at each location.
- Models can be developed based on relatively simple experiments and these can be used to make reasonable predictions about populations across a geographic range.

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## APPENDIX 1: pH AND BASIC CARBONATE CHEMISTRY

### A1.1. THE pH CONCEPT

pH is a measure of the acidity or basicity of a solution. The concentration of  $H^+$  ( $[H^+]$ ) is expressed as moles/litre (molar, M) and the pH scale expresses the negative log  $[H^+]$ .

Water dissociates ( $H_2O \leftrightarrow H^+ + OH^-$ ) but in pure water  $[H^+]$  and  $[OH^-]$  are equal; with  $[H^+] = 10^{-7}$  or  $pH = 7$ . When an acid is added to water, it donates protons ( $H^+$ ) and has a pH lower than 7. When a base is added to water, it accepts protons and has a pH higher than 7. The  $[H^+]$  of strong acids is roughly equal to the amount added to water, e.g. add 0.01 mol HCl to water then the  $[H^+] = 0.01$  M or  $pH = 2$ . However, weak acids do not always fully dissociate. The extent to which an acid dissociates is expressed by its equilibrium acid dissociation constant,  $K_a$ :

$$K_a = \frac{[H^+][A^-]}{[HA]}, \quad (A.1)$$

which can be rearranged:

$$[H^+] = K_a \frac{[A^-]}{[HA]} \quad (\text{The Henderson equation}) \quad (A.2)$$

### A1.2. THE CARBONATE SYSTEM

$CO_2$  dissolves in the surface ocean as it passes between the air-sea interface. At equilibrium the partial pressure of  $CO_2$  in seawater ( $pCO_2^{sw}$ ) will be equal to the partial pressure of  $CO_2$  in the atmosphere ( $pCO_2^{atm}$ ). The air-sea flux of  $CO_2$  is driven by the partial pressure difference between the two reservoirs (Brostrom, 2000).  $CO_2$  is more soluble in the cold surface waters of high latitudes; hence these waters take up gas from the atmosphere and then sink to form deep waters that circulate slowly through the ocean. Thereby helping to keep the surface waters lower in  $CO_2$  than the deep waters, and promoting an overall flux of the gas from the atmosphere to the ocean (Fasham et al. 2001).

The  $p\text{CO}_2^{\text{atm}}$  is independent of local conditions and the mean value remains fairly constant, spatially, throughout the year.  $p\text{CO}_2^{\text{sw}}$  is dependent on sea surface temperature (SST), salinity, DIC and alkalinity (definitions below).

$\text{CO}_2$  dissolves in seawater and remains in aqueous form ( $\text{CO}_{2(\text{aq})}$ ) but also reacts with seawater (eq. 1) to form a weak acid  $\text{H}_2\text{CO}_3$  (carbonic acid), which rapidly dissociates (eq. A.4) releasing  $\text{H}^+$ :



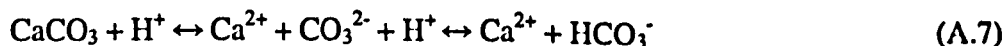
Carbonate ions ( $\text{CO}_3^{2-}$ ) are bases and act as buffers to soak up  $\text{H}^+$  (eq. A.5) and form more bicarbonate ( $\text{HCO}_3^-$ ).

The concentration of DIC can be considered:

$$[\text{DIC}] = [\text{CO}_{2(\text{aq})}] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}] \quad (\text{A.6})$$

In seawater with a pH of 8.1, about 90% of DIC is present as  $\text{HCO}_3^-$ , 9% as  $\text{CO}_3^{2-}$ , and 1% as  $\text{CO}_{2(\text{aq})}$ .

[DIC] is a function of both biological and physical processes and therefore has considerable seasonal and regional variation. Photosynthesis consumes inorganic carbon from seawater, thereby reducing [DIC], while respiration releases inorganic carbon to seawater. Additionally, biological precipitation of calcium carbonate ( $\text{CaCO}_3$ ) by calcifying organisms also substantially impacts on [DIC]. Formation of  $\text{CaCO}_3$  removes inorganic carbon from seawater, while dissolution (breakdown of  $\text{CaCO}_3$ ) increases [DIC] by adding inorganic carbon:



Total alkalinity ( $A_T$ ) is a measure of the excess of bases over acids in the ocean.  $A_T$  is the sum of the carbonate alkalinity

$$[\text{Alk}] = [\text{HCO}^-] + 2[\text{CO}_3^{2-}] \quad (\text{A.8})$$

plus the concentration of other ions, such as borate, nitrate, phosphate, etc.  $A_T$  is predominantly impacted by processes that affect the  $[\text{CO}_3^{2-}]$ , such as calcification, addition from river runoff and aeolian deposition.

The rate of dissolution of the mineral  $\text{CaCO}_3$  is dependent on the solubility product  $K_{sp}^{\text{CaCO}_3}$  and the saturation concentrations:

$$K_{sp}^{\text{CaCO}_3} = [\text{CO}_3^{2-}]_{\text{sat}} [\text{Ca}^{2+}]_{\text{sat}} \quad (\text{A.9})$$

where  $[\text{CO}_3^{2-}]_{\text{sat}}$  and  $[\text{Ca}^{2+}]_{\text{sat}}$  are the concentrations of carbonate and dissolved calcium ions in equilibrium with mineral  $\text{CaCO}_3$ .

The degree of saturation (saturation state,  $\Omega$ ) is the ratio of the product of the solute over the product of the solutes at saturation:

$$\Omega = \frac{[\text{CO}_3^{2-}][\text{Ca}^{2+}]}{[\text{CO}_3^{2-}]_{\text{sat}}[\text{Ca}^{2+}]_{\text{sat}}} = \frac{[\text{CO}_3^{2-}][\text{Ca}^{2+}]}{K_{sp}^{\text{CaCO}_3}} \quad (\text{A.10})$$

As changes in  $\text{Ca}^{2+}$  are relatively small compared to changes in  $\text{CO}_3^{2-}$ , this definition can be simplified to:

$$\Omega \approx \frac{[\text{CO}_3^{2-}]}{[\text{CO}_3^{2-}]_{\text{sat}}} \quad (\text{A.11})$$

## **APPENDIX 2: PUBLISHED PAPERS**





# Novel microcosm system for investigating the effects of elevated carbon dioxide and temperature on intertidal organisms

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**ABSTRACT:** In addition to the predicted rise in temperature, a recognised consequence of increased atmospheric CO<sub>2</sub> is ocean acidification. The response of marine organisms to the stresses associated with acidification is still not understood, and a number of recent experiments have addressed this problem. The starting point for many of these studies has been the development of a system by which seawater pH can be altered and then maintained. The current paper presents details of a temperature- and pH-controlled microcosm system, which enables the establishment of a tidal regime, for the experimental investigation of intertidal organisms. Two climate scenarios were simulated to evaluate the system's precision and accuracy; Year 2008 ('low' [CO<sub>2</sub>]: 380 ppm and 14°C) conditions and Year 2100 ('high' [CO<sub>2</sub>]) conditions (based on the IPCC—Intergovernmental Panel on Climate Change—2007 A2 scenario, 'high' [CO<sub>2</sub>]: 1250 ppm and 2.0 to 5.4°C warming). The temperature and seawater carbonate chemistry were reliably maintained for 30 d during which time newly settled barnacle cyprids were allowed to metamorphose into juveniles, then grow and develop. The pH and [CO<sub>2</sub>] had 95% confidence intervals of ±0.03 units and ±17 ppm, respectively, under low [CO<sub>2</sub>] conditions, and of ±0.02 units and ±43 ppm, respectively, under high [CO<sub>2</sub>] conditions. The tidal regime is fully adjustable, and on this occasion was set to a 6 h cycle. These microcosms have proved ideal for studying benthic organisms from a variety of near-surface environments and at different stages of their life-cycle.

**KEY WORDS:** Ocean acidification · pH · Carbon dioxide · Climate change · Global warming · Microcosm · Intertidal · Larvae

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## INTRODUCTION

Increasing atmospheric [CO<sub>2</sub>] is causing a rapid decrease in seawater pH (ocean acidification) as a result of the imbalance between the rate of CO<sub>2</sub> uptake into the ocean and the ocean's ability to buffer the resulting increase in hydrogen ions (Caldeira & Wickert 2003). Seawater pH has declined by an average of 0.1 units since the industrial revolution (Kleypas et al. 2006). It is estimated that pH will decrease from a current global average of ~8.1 to as low as ~7.7 by 2100 (IPCC 2007). Not only does this decline in pH have the potential to induce hypercapnia and acidosis in some

marine organisms (e.g. Pörtner et al. 2004, Shirayama & Thornton 2005, Miles et al. 2007), but, for organisms that produce calcium carbonate structures (like corals or molluscs), there may be an additional impact on the calcification rate due to declining saturation states of calcium carbonate minerals (e.g. Fabry 1990, Gattuso et al. 1998a, Orr et al. 2005).

Atmospheric and sea-surface temperatures (SSTs) are also increasing (e.g. Levitus et al. 2005, Mackenzie & Sciedek 2007): global SST has increased by 0.76 (ranging between 0.57 and 0.95°C) from between 1850 and 1899 to between 2001 and 2005 (IPCC 2007). While climate change is increasingly recognised as an

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impact to whole marine ecosystems (e.g. coral reefs: Burke & Maidens 2004; Arctic marginal sea ice: Loeng et al. 2005) and the geographic distribution of key pelagic and benthic species (e.g. Southward et al. 1995, Beaugrand & Reid 2003, Hays et al. 2005, IPCC 2007), the concern over the radical changes in the ocean carbonate cycle and how this impacts marine organisms is only just emerging (Pörtner et al. 2005, Raven et al. 2005, Turley et al. 2006, Guinotte et al. 2006). It is therefore not surprising that there are even fewer studies on the relationships between temperature and acidification and how they interact to affect individual animals' physiology and impact ecosystem processes (e.g. Metzger et al. 2007).

Early experiments investigating physiological responses of marine organisms to various gas mixtures were initially carried out using relatively crude techniques of saturating water with the gas (e.g. CO<sub>2</sub>) and then diluting with normal seawater before adding the test animal (e.g. Fox & Johnson 1934). Experiments were aimed primarily at eliciting a physiological response and not at replicating realistic environment conditions; hence, [CO<sub>2</sub>] was often very high, and accurate monitoring of levels was not of primary importance (e.g. Dale et al. 1970). The methods mentioned above and some that followed, involved sealing the experimental containers once the treated water had been added, restricting the size of organisms that could be used because of the build-up of wastes in the containers and changes in the gas mixtures by respiration and by diffusion. Regulated flows of gases, mixed using precision gas mixing pumps prior to bubbling through seawater, were developed to enable more accurate control of the final gas concentrations (Pörtner 1987, Cameron & Iwama 1987). These pumps are expensive and therefore in relatively short supply, which prevented large numbers of treatments and long exposure times from being used.

As [CO<sub>2</sub>] and pH became more topical with respect to environmental change, techniques were developed to regulate seawater pH using pH sensors via feedback systems coupled to a regulated source of CO<sub>2</sub>. This greatly improved the long-term reliability and still remains one of the most implemented techniques to date (Riebesell et al. 1993, Pörtner et al. 1998, Green et al. 2004, Kurihara & Shirayama 2004, Kurihara et al. 2004, Michaelidis et al. 2005, Metzger et al. 2007, Widdicombe & Needham 2007). These systems, however, can be expensive and still rely on pH measurements for control, and not on precise measurements of CO<sub>2</sub>. Adding pulses of CO<sub>2</sub> gives a stable pH, on average, but the pulsing of CO<sub>2</sub> may alter the equilibrium of the carbonate system and cause variability in total dissolved inorganic carbon (DIC). Fluctuations in the carbonate system can be accounted for by additionally

monitoring DIC and/or total alkalinity (A<sub>T</sub>). Use of these systems to investigate longer term seasonal variations is more challenging and has yet to be accurately carried out.

An alternative method developed more recently for investigating realistic environmental scenarios is the 2-phase system (Delille et al. 2005). The tops of these *in situ* mesocosms were isolated from the surrounding atmosphere forming tents that covered >90% of each mesocosm surface area. The atmospheric [CO<sub>2</sub>] in each tent was then controlled by continuous addition of high-[CO<sub>2</sub>] air mixes. The seawater carbonate system was allowed to develop naturally and equilibrate with the atmosphere. This particular set-up involved the use of premixed gases; these types of premixed gases have also been used in experiments to bubble into seawater (e.g. Pane & Barry 2007). Premixed gases are readily available, but are expensive and rapidly used up making long-running experiments costly.

These previous methods have been designed for mimicking subtidal situations. However, the intertidal is a significant component of the shallow-water ocean margin, is important in the global carbon cycle (Gattuso et al. 1998b, Ver et al. 1999, Chen et al. 2003, 2004) and is a key habitat for many calcifying organisms. As yet, there is no published account of experimental apparatus that enables the combined effects of higher seawater temperatures and lower pH on intertidal habitats/organisms to be investigated.

Building on the experience of Widdicombe & Needham (2007) and J. I. Spicer (pers. obs.); we developed a flow-through tidal microcosm system that allows the investigator to simultaneously manipulate temperature and CO<sub>2</sub> to simulate a realistic intertidal scenario. The system was designed primarily with the aim of investigating survival, growth and development of larvae, juveniles and adults of species from the intertidal zone; it is also clearly a suitable experimental tool for tackling a wide range of questions related to the development, settlement and growth of intertidal organisms. The incorporation of a cheap, yet reliable gas mixing system builds on previous gas mixing techniques, allowing the investigator to easily control the gas mix over long periods of time (weeks, months). The small scale of the system allows it to be portable between laboratories and flexible with respect to the variety of organisms that can be studied. The stability, precision and reproducibility of this system have been assessed by investigating the response of benthic post-larva to the experimental conditions created by this system. The target conditions and variability of the system are discussed in comparison to a similar experiment conducted using the mesocosm system of Widdicombe & Needham (2007) and data extracted from the literature.

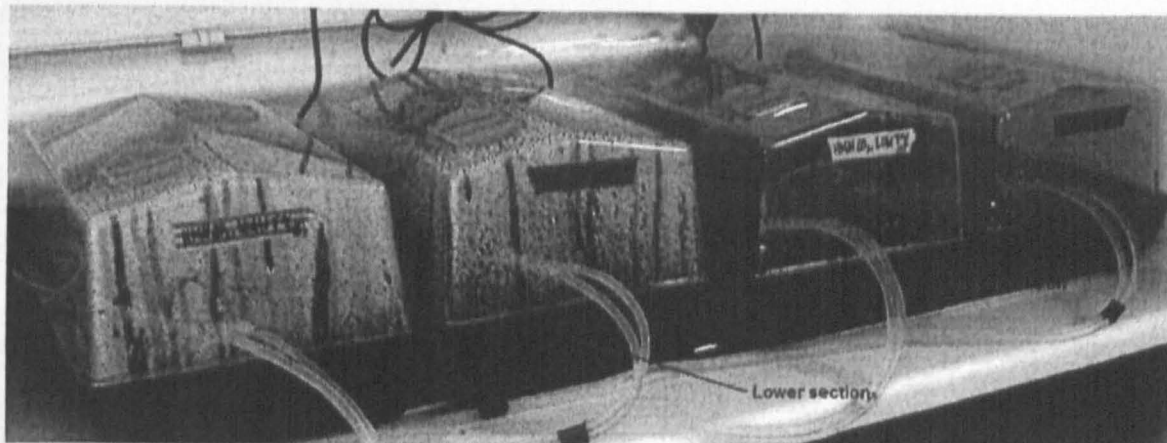


Fig. 1. Four tidal microcosms showing the upper and lower sections and water inflow pipes

## MATERIALS AND METHODS

**The microcosms.** Microcosms were constructed by adapting variable controlled, commercially available (B&Q), electric-heated plant propagators (30 × 15 × 20 cm). Each microcosm consisted of 2 parts: a lower section containing the internal heating elements and an upper section (Fig. 1). The heating elements were connected to an enclosed controllable thermostat, which regulated the temperature, via a thermocouple, to between ambient (in this case 14°C) and 40°C ( $\pm 0.01$ ); the heat was spread evenly throughout the

lower section. The upper section contained 2 adjustable vents, which prevented a build-up of gas that might otherwise have occurred from the constant addition of high-[CO<sub>2</sub>] air. In each microcosm there were water input and outlet pipes and an air/high-[CO<sub>2</sub>] air input pipe. The air input pipe had an aquarium air stone attached to maintain fine air bubbling into the bottom of the lower section.

The ambient system discussed here was set up for current southwest UK seawater temperatures, which range from about 8 to 15°C; however, alternative temperatures can be achieved most simply by setting up the experimental system in a suitable controlled temperature/environment room. The use of chillers and heaters (e.g. an Aqua Medic Minicooler) would also be possible, depending on the size of the containers used as microcosms.

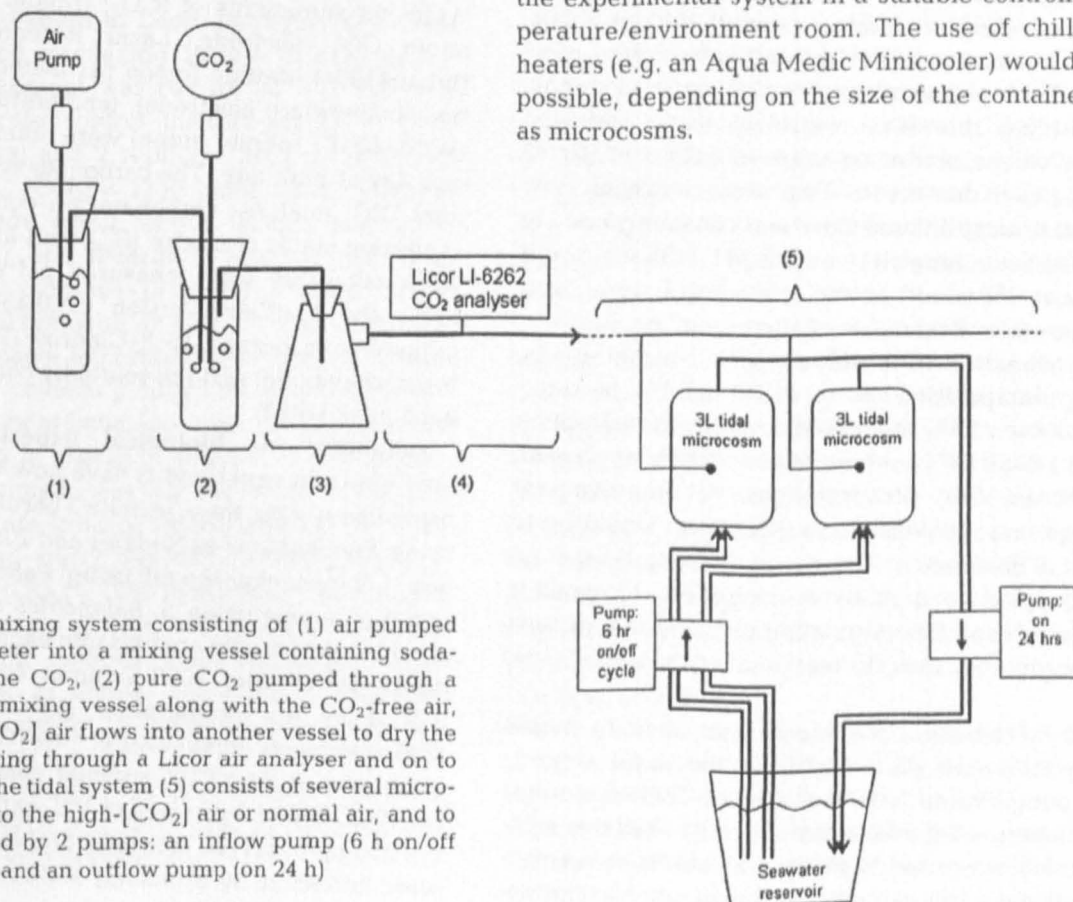


Fig. 2. Air-CO<sub>2</sub> mixing system consisting of (1) air pumped through a flow meter into a mixing vessel containing soda-lime to remove the CO<sub>2</sub>, (2) pure CO<sub>2</sub> pumped through a flow meter into a mixing vessel along with the CO<sub>2</sub>-free air, (3) mixed high-[CO<sub>2</sub>] air flows into another vessel to dry the air before (4) passing through a Licor air analyser and on to the microcosms. The tidal system (5) consists of several microcosms connected to the high-[CO<sub>2</sub>] air or normal air, and to seawater controlled by 2 pumps: an inflow pump (6 h on/off cycle) and an outflow pump (on 24 h)

**CO<sub>2</sub> manipulation.** The concentration of CO<sub>2</sub> flowing into the microcosms was manipulated through an air–CO<sub>2</sub> gas mixing system (Fig. 2). In this system, air was pumped through a flow meter (1 in Fig. 2), into an airtight and pressure-resistant bottle (e.g. a 1 l Dreschel mixing bottle) containing 4 % NaOH, which both cleaned the air and removed the CO<sub>2</sub>. Carbon dioxide (BOC, CP grade carbon dioxide 99.995 %) (2 in Fig. 2) was pumped through a second flow meter into a second airtight and pressure-resistant bottle (in this case a 1 l Buchner mixing flask) containing distilled water. The cleaned air was also passed into this mixing flask, and the use of aquarium air stones, which created fine bubbles of both CO<sub>2</sub> and air, allowed the 2 gases to mix. The mixed high-[CO<sub>2</sub>] air then fed into a final airtight and pressure-resistant bottle (3 in Fig. 2; here a 1 l Buchner flask) before flowing through a Licor LI-6262 CO<sub>2</sub> analyser (4 in Fig. 2) and then out to the microcosms. The flow rates of the air and the CO<sub>2</sub> were adjusted to create the final high-[CO<sub>2</sub>] air mix; for example, to create a [CO<sub>2</sub>] of 1250 ppm, a flow of 6.25 ml min<sup>-1</sup> of CO<sub>2</sub> needs to be mixed with cleaned air flowing at 5 l min<sup>-1</sup>. The composition of the mixture was monitored using the CO<sub>2</sub> analyser. The high-[CO<sub>2</sub>] air was bubbled continuously into the lower section of the microcosms using air stones (see above), allowing the seawater [CO<sub>2</sub>] to reach equilibrium. The control (low [CO<sub>2</sub>]) microcosms were bubbled with ambient air pumped using a Hailea air pump (Model V-20). Standard aquarium connectors and valves were used to connect non-porous silicone tubing to the air stones. The bubbling rate was controlled using standard aquarium valves, producing a flow rate of about 50 cm<sup>3</sup> min<sup>-1</sup> into each microcosm. To produce a greater flow rate overall, an additional valve was constantly open to release the remaining air flow. The pH (NBS scale) and [CO<sub>2</sub>] were measured as the microcosms were filled with water (see 'Evaluation of microcosm' below).

To investigate a wide range of CO<sub>2</sub> concentrations, the CO<sub>2</sub> manipulation set-up would need to be replicated for each CO<sub>2</sub> concentration, with the exception of using 1 Licor CO<sub>2</sub> analyser to periodically analyse all the different CO<sub>2</sub> concentrations. An improvement (although more expensive) to this set-up would be to use digital flow meters (e.g. use of a standard solenoid valve coupled to a millivolt controller), to monitor the pressure and flow regulating the CO<sub>2</sub> and air flow accordingly and, thus, to maintain a precise CO<sub>2</sub>–air mix.

**Tidal mechanism.** The mechanism used to mimic tidal conditions is presented in in Fig. 2 (5). A peristaltic pump (Pump 1; Watson-Marlow 503S 6 h on/off cycle, manipulated with a commercially available electrical timer) was used to pump seawater from a reservoir (volume = 15 l, salinity = 35.7 psu) into each micro-

cosm. A second pump (Pump 2) continually pumped the seawater out of the microcosms back into the seawater reservoir. This produced a tidal cycle of low tide (microcosms are empty), flooding tide (Pump 1 on, Pump 2 on, microcosms take 6 h to fill), high tide (full microcosms) and ebbing tide (Pump 1 off, Pump 2 on, microcosms take 6 h to empty). Peristaltic pump tubing (Gradko International Ltd 116-0536-18) and silicone tubing (Fisher Scientific FB56471) were used to feed seawater through the system.

The outflow pump was set to produce a flow rate of 8.3 ml min<sup>-1</sup> so as to remove 3 l of water in 6 h; however, it remained constantly 'on'; therefore, the inflow pump was set to a flow rate of 16.6 ml min<sup>-1</sup> to fill the 3 l microcosms during the 6 h flood, while allowing a continuous flow-through of water. Alternatively, digital timers can be used to set the exact tidal regime with the inflow pump turning on and the outflow pump turning off at low tide and vice versa at high tide.

**Evaluation of the microcosm.** To evaluate the ability of the system to reproduce a high-temperature, high-[CO<sub>2</sub>] (HTHC) atmosphere and equilibrated seawater conditions, an experiment was conducted in which each microcosm was set up with a specific combination of temperature and [CO<sub>2</sub>] levels. These combinations assessed the system's suitability for mimicking real climate scenarios. The experiments ran for 30 d in a controlled-temperature environment (precision ±1°C). Water measurements of [CO<sub>2</sub>] (Model GS-136CO-1S micro CO<sub>2</sub> electrode, Lazar Research Labs), pH (InLab413SG Mettler-Toledo pH meter and combination temperature electrode), temperature and salinity (WTW LF197 salinity probe) were obtained every second day at high tide. The carbonate system variables, total DIC, total A<sub>T</sub>, carbonate ion concentration and saturation states of calcite (Ω<sub>cal</sub>) and aragonite (Ω<sub>arag</sub>), were calculated from measured pH and [CO<sub>2</sub>] data using the MatLab (Version 6.1.0.451) csys.m programme from Zeebe & Wolf-Gladrow (2001) ([www.awi-bremerhaven.de](http://www.awi-bremerhaven.de)) and the solubility constants of Mehrbach et al. (1973).

**Suitability for biological experiments.** Several medium-term experiments have now been carried out using this system; these include 3 barnacle experiments using *Semibalanus balanoides* and *Elminius modestus* and 1 limpet experiment using *Patella vulgata*. The initial experiments on *S. balanoides* and *E. modestus* involved placing the animals into each of 4 tidal microcosms set at Year 2008 summer conditions characterised by low temperature (14°C) and low-[CO<sub>2</sub>] (380 ppm) (low-temperature, low-[CO<sub>2</sub>] [LTLC] scenario) and at Year 2100 summer conditions, based on the IPCC 2007 A2 scenario of 4°C warming and a [CO<sub>2</sub>] of 1250 ppm (HTHC scenario) (Table 1). *S. balanoides* were collected by attaching settlement panels (10 ×

Table 1. Experimental conditions of temperature ( $T$ ), carbon dioxide concentration ( $[CO_2]$ ) and pH. Data are 30 d mean values  $\pm 95\%$  CI

	$T$ ( $^{\circ}C$ )	Low temperature $[CO_2]$ (ppm)	pH	$T$ ( $^{\circ}C$ )	High temperature $[CO_2]$ (ppm)	pH
Low $[CO_2]$	$14.4 \pm 0.25$	$400 \pm 17$	$8.04 \pm 0.027$	$19.7 \pm 0.25$	$405 \pm 23$	$8.06 \pm 0.030$
High $[CO_2]$	$14.6 \pm 0.27$	$1100 \pm 43$	$7.74 \pm 0.022$	$19.9 \pm 0.26$	$1103 \pm 43$	$7.73 \pm 0.025$

10 cm tiles) to north-facing rocks mid-shore at Looe, England ( $50^{\circ}20'N$ ,  $004^{\circ}27'W$ ), for 1 wk (beginning 30 April 2007) during barnacle settlement. On collection the panels contained a mixed age population of barnacles, ranging from newly settled cyprids to 1 wk old metamorphosed individuals. Three settlement panels were placed in each microcosm. Photographs of each panel were taken at low tide on Days 1 and 30 and every second day in between. A stand was set up so that the camera (FujiFilm A510 FinePix digital camera) and plate were aligned consistently. The photographic images were analysed using image analysis software (Image-Pro Plus v.4.5, Media Cybernetics) to estimate both abundance (number of animals per plate) and survival (measured as numbers surviving from one day to the next). The experiment was repeated for settled *E. modestus*.

The limpet experiment was carried out by collecting adult limpets on small rock chips (6 cm < length < 30 cm) from mid-shore at Looe, England, on 13 November 2007 and by placing 10 ind. in each microcosm. This experiment was run for 36 d in 4 microcosms—2 replicate control microcosms ( $T = 12^{\circ}C$ ,  $[CO_2] = 360$  ppm) and 2 replicate high- $[CO_2]$  microcosms ( $T = 12^{\circ}C$ ,  $[CO_2] = 1250$  ppm). Shell measurements were made (shell length, height and width across the apex), and records of numbers surviving were maintained. Light was provided by 4 lights (Polylux XL 58W, 5200 Lm) with an 8/16 h on/off cycle.

The third barnacle experiment (*Semibalanus balanoides*) involved collecting adults on small rock chips from the mid-shore at Looe, England, on 23 November 2007 and placing in excess of 400 ind. in each microcosm. The experiment was run for 91 d in 4 microcosms—2 replicate control microcosms ( $T = 12^{\circ}C$ ,  $[CO_2] = 370$  ppm) and 2 replicate high- $[CO_2]$  microcosms ( $T = 12^{\circ}C$ ,  $[CO_2] = 1250$  ppm). Survival of barnacles, as an average for each microcosm, was calculated from photographic images analysed using image analysis software, as above. The lights (Polylux XL 58W, 5200 Lm) were set to within 15 min of the sunrise/sunset times of London, UK, on a weekly basis; this ranged from roughly an 8/16 h on/off cycle in December, to a 9/15 h on/off cycle in January, and a 10/14 h on/off cycle in February.

## RESULTS

### Evaluation of the microcosm

#### The tidal cycle

The tidal cycle was maintained for the duration of each experiment (ca. 30 or 90 d).  $[CO_2]$  varied the most over each tidal cycle (Fig. 3, triangles), but not significantly enough to affect pH and DIC (Fig. 3, circles and crosses).

#### Measured values of pH, $CO_2$ and DIC

In the first 2 barnacle experiments, only pH and  $[CO_2]$  were measured. They showed that conditions could be kept relatively stable over 30 d periods with only small fluctuations. The atmospheric  $[CO_2]$  was maintained, on average, at 400 and 1100 ppm, respectively, in the low- and high- $[CO_2]$  scenarios (Table 2). The control was higher than the required 380 ppm as result of a slight build-up of ambient  $[CO_2]$  within the laboratory. There was greater variation in the high- $[CO_2]$  scenarios: 95% CIs were  $\pm 9$  and  $\pm 12$  ppm in the low- $[CO_2]$  scenarios and  $\pm 18$  and  $\pm 14$  ppm in the high- $[CO_2]$  scenarios. The seawater pH was thus maintained on average at  $8.05 \pm 0.028$  and  $8.06 \pm 0.030$ , in the low- $[CO_2]$  scenarios and  $7.72 \pm 0.021$  and  $7.73 \pm 0.016$ , in the high- $[CO_2]$  scenarios. The pressure of the air and the  $[CO_2]$  both needed to be checked and modified throughout the experimental period to prevent any alterations in the mixing ratios. There was a slight decline in the mixing system air pressure over the experimental time period, which caused a change in the pH (increase by 0.03 units over 30 d) and  $[CO_2]$  (decrease by  $\sim 10$  ppm over 30 d) in the low- $[CO_2]$  scenarios, and opposite effects in the high- $[CO_2]$  scenarios (pH decreased by 0.04 over 30 d;  $[CO_2]$  increased by  $\sim 60$  ppm over 30 d).

In the subsequent experiments, the DIC was measured in addition to  $[CO_2]$  and pH. This gave a much more stable value for DIC (Table 2), and, in turn, the calculated values of the carbonate system (see 'Results: calculated values of carbonate system variables' be-

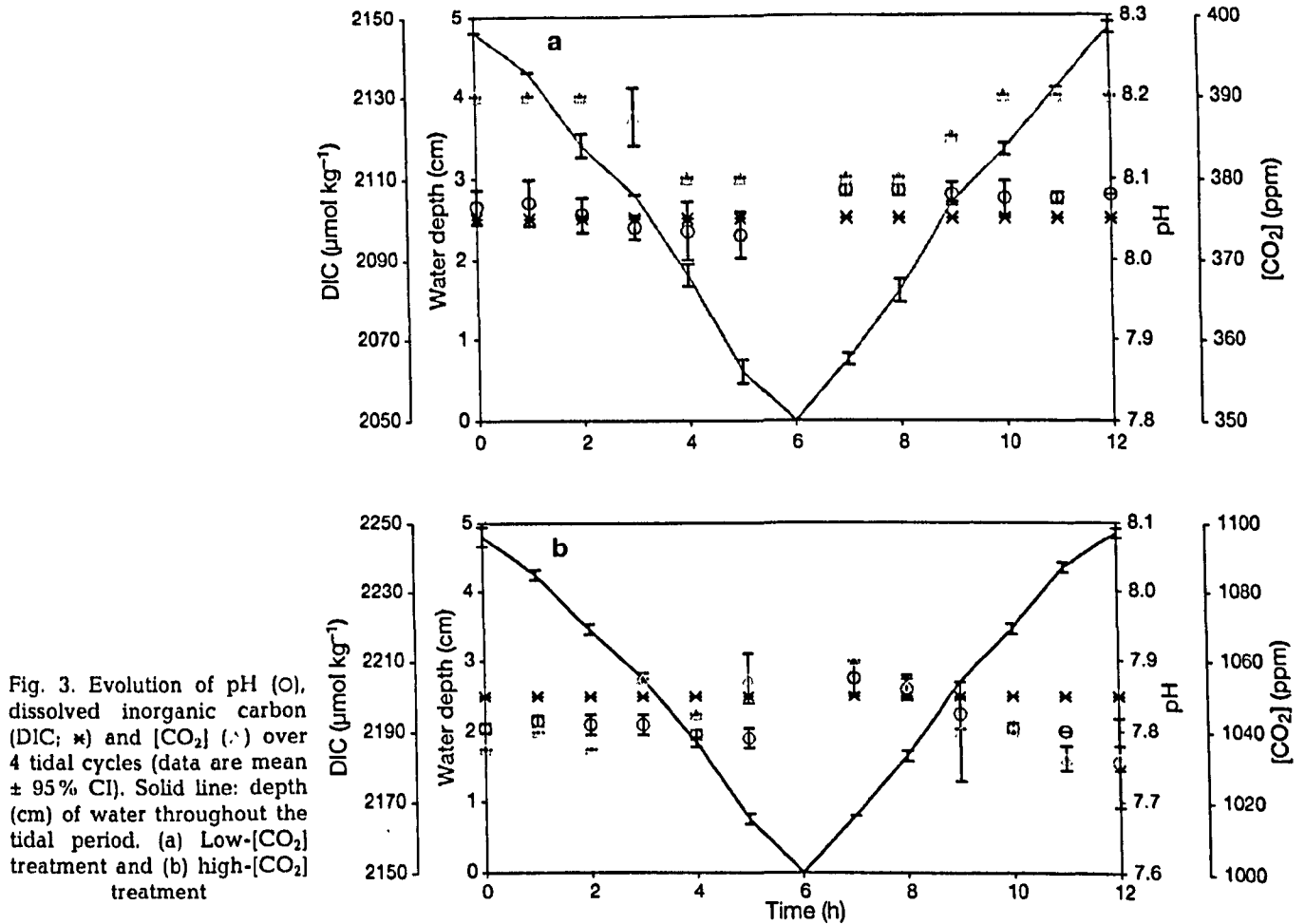


Fig. 3. Evolution of pH (O), dissolved inorganic carbon (DIC; x) and [CO<sub>2</sub>] (·) over 4 tidal cycles (data are mean  $\pm$  95% CI). Solid line: depth (cm) of water throughout the tidal period. (a) Low-[CO<sub>2</sub>] treatment and (b) high-[CO<sub>2</sub>] treatment

Table 2. System data (mean  $\pm$  95% CI) for the 4 experiments—Sb(1): 30 d *Semibalanus balanoides*; Em(2): 30 d *Elminius modestus*; Pv(3): 30 d *Patella vulgata*; Sb(4): 90 d *S. balanoides* for each treatment. For Sb(1) and Em(2), salinity, temperature, pH and [CO<sub>2</sub>] data were measured, all other data (DIC: dissolved inorganic carbon; A<sub>T</sub>: total alkalinity;  $\Omega_{\text{calcite}}$ : calcite saturation state;  $\Omega_{\text{aragonite}}$ : aragonite saturation state) were calculated from pH and [CO<sub>2</sub>] using MatLab csys.m from Zeebe & Wolf-Gladrow (2001) (www.awi-bremerhaven.de) and using the solubility constant of Mehrbach et al. (1973). For Pv(3) and Sb(4), salinity, temperature, pH, [CO<sub>2</sub>] and DIC were measured; all other values were calculated from pH and DIC

Treatment	Salinity (psu)	Temp. (°C)	pH	DIC (μmol kg <sup>-1</sup> )	A <sub>T</sub> (μEq kg <sup>-1</sup> )	[CO <sub>3</sub> <sup>2-</sup> ] (μmol kg <sup>-1</sup> )	$\Omega_{\text{calcite}}$	$\Omega_{\text{aragonite}}$
<b>Low [CO<sub>2</sub>]</b>								
Sb(1)	35.7 $\pm$ 0.30	14.4 $\pm$ 0.25	8.05 $\pm$ 0.028	2185 $\pm$ 110	2417 $\pm$ 133	169 $\pm$ 18.9	3.84 $\pm$ 0.46	2.42 $\pm$ 0.23
Em(2)	34.5 $\pm$ 0.33	14.7 $\pm$ 0.23	7.96 $\pm$ 0.022	2003 $\pm$ 58	2192 $\pm$ 69	137 $\pm$ 9.0	3.22 $\pm$ 0.22	2.06 $\pm$ 0.14
Pv(3)	36.4 $\pm$ 0.97	11.7 $\pm$ 0.36	7.93 $\pm$ 0.030	1995 $\pm$ 106	2144 $\pm$ 114	110 $\pm$ 9.0	2.44 $\pm$ 0.21	1.56 $\pm$ 0.13
Sb(4)	35.5 $\pm$ 1.43	11.8 $\pm$ 0.42	8.07 $\pm$ 0.045	1968 $\pm$ 138	2163 $\pm$ 135	146 $\pm$ 21.0	3.27 $\pm$ 0.37	2.09 $\pm$ 0.23
<b>High [CO<sub>2</sub>]</b>								
Sb(1)	35.6 $\pm$ 0.32	14.8 $\pm$ 0.27	7.72 $\pm$ 0.021	2517 $\pm$ 113	2613 $\pm$ 124	95 $\pm$ 9.1	2.15 $\pm$ 0.20	1.36 $\pm$ 0.13
Em(2)	34.5 $\pm$ 0.30	14.9 $\pm$ 0.20	7.73 $\pm$ 0.051	2652 $\pm$ 301	2753 $\pm$ 329	101.7 $\pm$ 23.7	2.4 $\pm$ 0.69	1.54 $\pm$ 0.35
Pv(3)	36.4 $\pm$ 0.96	11.7 $\pm$ 0.35	7.63 $\pm$ 0.040	2230 $\pm$ 105	2277 $\pm$ 106	63.1 $\pm$ 8.2	1.39 $\pm$ 0.17	0.88 $\pm$ 0.11
Sb(4)	35.6 $\pm$ 1.44	11.9 $\pm$ 0.44	7.689 $\pm$ 0.055	2131 $\pm$ 160	2193 $\pm$ 149	71 $\pm$ 14.0	1.58 $\pm$ 0.25	1.01 $\pm$ 0.157

low). The [CO<sub>2</sub>] was prevented from building up in the laboratory and was thus maintained reliably at the desired levels. In the shorter (30 d) limpet experiment the mean ( $\pm$ 95% CI) [CO<sub>2</sub>] was 362  $\pm$  4.4 and 1258  $\pm$  120 ppm and mean pH levels were 7.94  $\pm$  0.042 and

7.63  $\pm$  0.067, in the low and high [CO<sub>2</sub>] scenarios, respectively. In the longer *Semibalanus balanoides* experiment, [CO<sub>2</sub>] was 377  $\pm$  8.5 and 1270  $\pm$  92 ppm, while the pH was 8.07  $\pm$  0.068 and 7.7  $\pm$  0.075, in the low and high [CO<sub>2</sub>] scenarios, respectively.

### Temperature and salinity

In the first 2 barnacle experiments the controlled-temperature room maintained the ambient air temperature at an average of 14°C. The seawater in the low-temperature scenario incubators reflected the ambient air temperature in the upper sections, and these sections were maintained at  $14.4 \pm 0.25$  and  $14.7 \pm 0.28^\circ\text{C}$  (mean  $\pm$  95% CI). The seawater in the high-temperature treatments was maintained by  $19.8 \pm 0.25$  and  $19.7 \pm 0.27^\circ\text{C}$ , reflecting a  $5^\circ\text{C}$  increase in ambient temperature through heating of the base of each microcosm. The seawater used in the experiment had an average salinity of  $35.7 \pm 0.33$  psu (Table 2).

In the winter experiments, the limpets were maintained at  $11.7 \pm 0.36^\circ\text{C}$  and  $36.4 \pm 0.96$  psu). Over the longer term *Semibalanus balanoides* experiment, the temperature was slowly increased from  $11.1 \pm 0.1$  to  $13.1 \pm 0.1^\circ\text{C}$  over the 90 d period to reflect and increase in seawater temperature during spring. Salinity was maintained at  $35.5 \pm 1.43$  psu) over the whole period.

### Calculated values of carbonate system variables

Calculated  $A_T$  and total DIC showed greater fluctuations over the initial barnacle experiments and displayed greater variation between treatments than measured pH and  $[\text{CO}_2]$  values (Fig. 4a; means:  $A_T = 2435 \pm 98 \mu\text{Eq kg}^{-1}$  and  $\text{DIC} = 2554 \pm 78 \mu\text{mol kg}^{-1}$  in the low- $[\text{CO}_2]$  scenarios and  $A_T = 2554 \pm 76 \mu\text{Eq kg}^{-1}$  and  $\text{DIC} = 2445 \pm 70 \mu\text{mol kg}^{-1}$  in the high- $[\text{CO}_2]$  scenarios).  $[\text{CO}_3^{2-}]$  was greatest in the HTLC treatment ( $215 \mu\text{mol kg}^{-1}$ ) and lowest in the LTHC treatment ( $95 \mu\text{mol kg}^{-1}$ ), confirming that both temperature and  $[\text{CO}_2]$  have an affect on the concentration of carbonate ion in the water. The decrease in  $[\text{CO}_3^{2-}]$  from the year 2008 (LTLC) to the A2 2100 scenario (HTHC) is not as large as might be expected if the concentration had been calculated using only an increased  $[\text{CO}_2]$  level and not an increased temperature level. The calcite saturation state ( $\Omega_{\text{cal}}$ ) does not become undersaturated in any treatment, but still falls to a level that may be marginal for dissolution of calcium carbonate; and saturation state was  $>30\%$  lower in the HTHC scenario compared to the control. The aragonite saturation state ( $\Omega_{\text{arag}}$ ) is again not undersaturated in any of the treatments, although it is again  $30\%$  lower in the HTHC scenario compared to the control (Fig. 4c).

In the limpet and long-term *Semibalanus balanoides* experiment, the measured value of DIC produced more stable values of the carbonate system

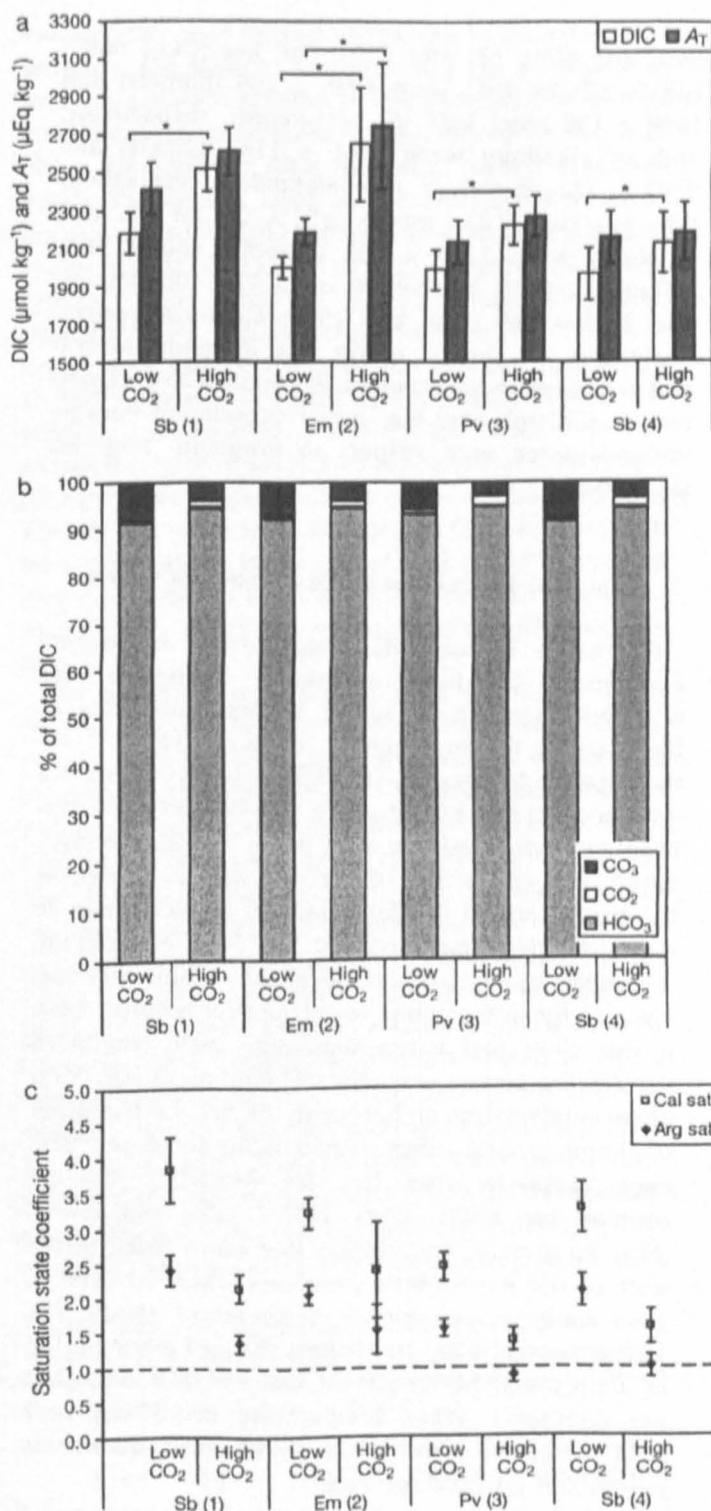


Fig. 4. (a) Mean alkalinity ( $A_T$ ) and total dissolved inorganic carbon (DIC) concentrations for the low- and high- $[\text{CO}_2]$  treatments for each experiment—Sb(1): 30 d *Semibalanus balanoides*; Em(2): 30 d *Elminius modestus*; Pv(3): 30 d *Patella vulgata*; Sb(4): 90 d *S. balanoides*. Error bars: 95% CI, \* $p < 0.05$ . (b) Composition of DIC in each of the treatments in all experiments, given as  $\text{CO}_3^{2-}$ ,  $\text{CO}_2$  and  $\text{HCO}_3^-$  percentage of total dissolved inorganic carbon. (c) Mean calcite (cal) and aragonite (arg) saturation (sat) states for each of the treatments in all experiments. Error bars: 95% CI, dashed line: saturation state of 1—below this line calcite and aragonite will be undersaturated

than just using pH and CO<sub>2</sub>. The low-[CO<sub>2</sub>] mean ( $\pm 95\%$  CI) for DIC were  $1995 \pm 106$  (limpets) and  $1968 \pm 138 \mu\text{mol kg}^{-1}$  (*S. balanoides*), respectively, and for alkalinity were  $2144 \pm 114$  (limpets) and  $2163 \pm 135 \mu\text{mol kg}^{-1}$  (*S. balanoides*), respectively (Fig. 4a). High-[CO<sub>2</sub>] means ( $\pm 95\%$  CI) for DIC and alkalinity were  $2230 \pm 105$  (limpets) and  $2131 \pm 160 \mu\text{mol kg}^{-1}$  (*S. balanoides*) and  $2277 \pm 106$  (limpets) and  $2193 \pm 149 \mu\text{mol kg}^{-1}$  (*S. balanoides*), respectively (Fig. 4a). Again, calcite and aragonite saturation states were reduced in the high-[CO<sub>2</sub>] experiments, although only the limpet experiment became undersaturated with respect to aragonite (Fig. 4c;  $\Omega_{\text{arg}} = 0.88$ ).

#### Biological application of the microcosm system

By chance in the initial *Semibalanus balanoides* experiment, the high-temperature treatments had a greater number of longer settled individuals at the start of the experiment compared to the low-temperature treatments. In addition to estimating the growth of all the individuals, a sub-sample of 10 similar-sized individuals on each panel was also investigated. The probability of survival was calculated as the percentage of the total number of individuals in each treatment that survived the 30 d experiment, multiplied by the percentage of individuals that survived in the sub-sample, to account for relative sizes. It was clear that when simulating 2008 conditions (LTLC), the system was able to maintain almost complete survivorship of barnacles (97%). In the other treatment combinations, survivorship decreased with each treatment when the size variability was accounted for: LTHC 89%, HTLC 73% and HTHC 59%. In general, individuals that were small at the start of the experiment were less likely to survive, particularly in the higher temperature treatments. The increased [CO<sub>2</sub>] treatments showed more mortality than the LTLC treatment, but the difference was not significant. When temperature and [CO<sub>2</sub>] were both increased there was a significant reduction in cyprid and juvenile survival.

The second barnacle *Elminius modestus* study had a more even distribution of new and longer settled individuals. Survival was highest in the LTLC conditions (80%) and was reduced slightly to 76, 70 and 69%, respectively, in the LTHC, HTLC and HTHC treatments. There was 100% survival in all limpet treatments, except in Microcosm 3 (low-[CO<sub>2</sub>] control), where 1 individual died. In the third barnacle experiment (90 d, *Semibalanus balanoides*), 69% survived on average in the controls compared to an average of 48% surviving in the low-pH treatments.

## DISCUSSION

### Evaluation of the system

The system described in the present paper reliably simulates the environmental conditions associated with raised atmospheric [CO<sub>2</sub>] and temperature under a variety of climate change scenarios. We have also demonstrated the ability of this system to incorporate a tidal mechanism enabling experiments to be conducted on intertidal organisms. The success of this system is primarily a result of it being a 2 phase system in which atmospheric [CO<sub>2</sub>] was controlled and seawater [CO<sub>2</sub>] was able to equilibrate with the atmosphere. This provided a good simulation of the natural mechanism of oceanic uptake of CO<sub>2</sub>, but on a much smaller scale and faster timescale. Importantly, the system was able to lower and maintain seawater pH without significantly altering the alkalinity of the water.

Temperature was controlled to replicate the heating of rock surfaces during periods of emersion and cooling with the flood tide, thus allowing some thermal relief over the tidal cycle. Previous acidification systems have not included a heating system, although such systems have been used in global climate warming experiments (McKee et al. 2000, Baulch et al. 2003). Liboriussen et al. (2005) controlled and altered temperature in a shallow lake mesocosm system continuously for 16 mo. Their flow-through heating system was able to maintain, and vary, the temperature according to seasonal settings with little deviation from the target temperature (the deviation ranged between 0.11 and 0.26°C). The seawater temperature in the system described here had larger deviations from the target temperatures of 14 and 19°C by 0.80 ( $\pm 0.17$ ) and 0.84°C ( $\pm 0.61$ ), respectively, compared to Liboriussen et al. (2005). However, as with Liboriussen et al. (2005), the temperature control allows the system to be manipulated on a seasonal basis and is monitored periodically to prevent over or under heating.

The tidal section was created as a flow-through system with a relatively short water residence time (3 h). The tidal system enabled a 6 h semidiurnal tidal cycle to be used, although such a cycle deviates from both the natural general sinusoidal pattern of tidal ebb and flood and from the 6 h 12 min semidiurnal rhythm often seen in nature. The feeding rhythm of many intertidal animals is synchronised with this longer tidal cycle, and so over a long period of time animal feeding patterns may become out of synchrony with the system if they are maintained in a constant 6 h tidal system. The advantage of the tidal flow-through system was to prevent evaporation and salinisation in the microcosms, particularly at higher temperatures. In addition, with the incorporation of the appropriate controllers, such



as a digitally programmable, commercially available electronic timer, a 6 h 12 min cycle, or indeed any rhythm, could be achieved.

In these experiments we did not investigate whether there was a significant build-up of nutrients or toxins in the system. We have not done so to date because of the small size of the organisms used (barnacles and recently settled limpets) compared to the high turnover rate of the water (average  $0.5 \text{ l h}^{-1}$ ). Although the seawater is recirculated through the system, this is done for about 1 wk, and then it is replaced with fresh seawater. This change was primarily done in order to prevent salinity from increasing, although it will also prevent a major build-up of toxins. The reservoir tanks hold nearly twice the amount of seawater necessary to fill the microcosms and are continually mixed by bubbling with air. If larger organisms were being used, the microcosm size would need to be increased to accommodate them. The husbandry of many common species is routine in marine laboratories, and, hence, it is relatively easy to find out the necessary turnover rates needed to prevent the organism from being affected by ammonia production, for example. If sediments are to be incorporated into the experimental set-up, we would suggest that the investigator needs to carefully consider the flow rate and the volume of the container compared to the volume of sediment, as well as taking into account the sediment depth and nutrient flux from the sediment. To prevent toxin build-up or changes in nutrients in larger systems, we would suggest either using a flow-through system in which fresh seawater is used in every tidal cycle, or incorporating filters into the recirculating system.

One major improvement to the initial barnacle experiments described here, or indeed any acidification system, would be to monitor the carbonate system better so that more appropriate parameters of the carbonate system are measured. pH and  $[\text{CO}_2]$  are not

conservative with respect to changes in state (i.e. temperature and salinity) and thus may not be providing such an accurate account of the carbonate system as could be given by combining these measurements with either alkalinity or total inorganic carbon measurements. The latter 2 experiments described here additionally measured DIC and gave more stable carbonate system values (Table 2). A comparison of the carbonate system results from the 2 initial barnacle studies described here, together with data from the literature and a similar study, but using the experimental set-up of Widdicombe & Needham (2007) can elucidate the stability and precision of this set-up. Table 3 demonstrates the relative pH values measured at each target  $[\text{CO}_2]$  in several experiments found in the literature. The pH SDs range from as little as 0.00 to 0.11 units. In the present study, the low- and high- $[\text{CO}_2]$  treatments had a pH standard deviation of 0.06 and 0.04 units, respectively, which are within ranges of other experiments.

The additional experiments were conducted to repeat the one described here, using *Elminius modestus*, *Patella vulgata* and finally *Semibalanus balanoides*, but over a longer time period. The conditions remained stable over all experiments, and the precision of the first experiment was easily repeated in the others (Fig. 4). The gradual decrease in air pressure seen in the first experiment described above did not occur in the latter experiments, as we were aware of the problem and as such constantly monitored the gas pressures via the flow rates and altered the flow rates as necessary. The confidence intervals shown in Fig. 5 demonstrate that there is much better control (more accuracy) when maintaining the systems at set pH and  $[\text{CO}_2]$  levels using this microcosm system than a large pH feedback system, especially when DIC is additionally measured. It is clear that without measuring DIC or alkalinity, the calculated values are greater than

Table 3. pH values ( $\pm$  SD) measured for a nominal  $[\text{CO}_2]$ . Values are obtained from the literature to compare with the initial *Semibalanus balanoides* experiment in the present study. K04: Kurihara et al. (2004) [*H.p.*: *Hemicentrotus pulcherrimus*; *E.m.*: *Echinometra mathaei* (b: before; a: after experiment)]; S05: Shiryama et al. (2005) (1, 2 and 3: 3 replicate tanks; SDs not given in literature); Miles07: Miles et al. (2007); Mich07: Michaelidis et al. (2007)

$[\text{CO}_2]$ ( $\mu$ atm)	Present study	K04 ( <i>H.p.</i> )b	K04 ( <i>H.p.</i> )a	K04 ( <i>E.m.</i> )b	S05 1	S05 2	S05 3	Miles07	Mich07
Control	8.05 $\pm$ 0.06	7.99 $\pm$ 0.10	7.97 $\pm$ 0.16	8.11 $\pm$ 0.00	7.945	7.937	7.936	7.96 $\pm$ 0.07	8.05 $\pm$ 0.02
560					7.899	7.902	7.897		
860		7.74 $\pm$ 0.02	7.73 $\pm$ 0.13	7.80 $\pm$ 0.01					
1250	7.72 $\pm$ 0.04								
1860		7.59 $\pm$ 0.00	7.56 $\pm$ 0.11	7.68 $\pm$ 0.04					
2340								7.46 $\pm$ 0.03	
3860		7.35 $\pm$ 0.04	7.33 $\pm$ 0.08	7.34 $\pm$ 0.02					
5090									7.3 $\pm$ 0.03
8860		7.03 $\pm$ 0.07	7.16 $\pm$ 0.06	7.13 $\pm$ 0.01					
18860		6.83 $\pm$ 0.01	6.95 $\pm$ 0.10	6.78 $\pm$ 0.00					
22470								6.63 $\pm$ 0.11	

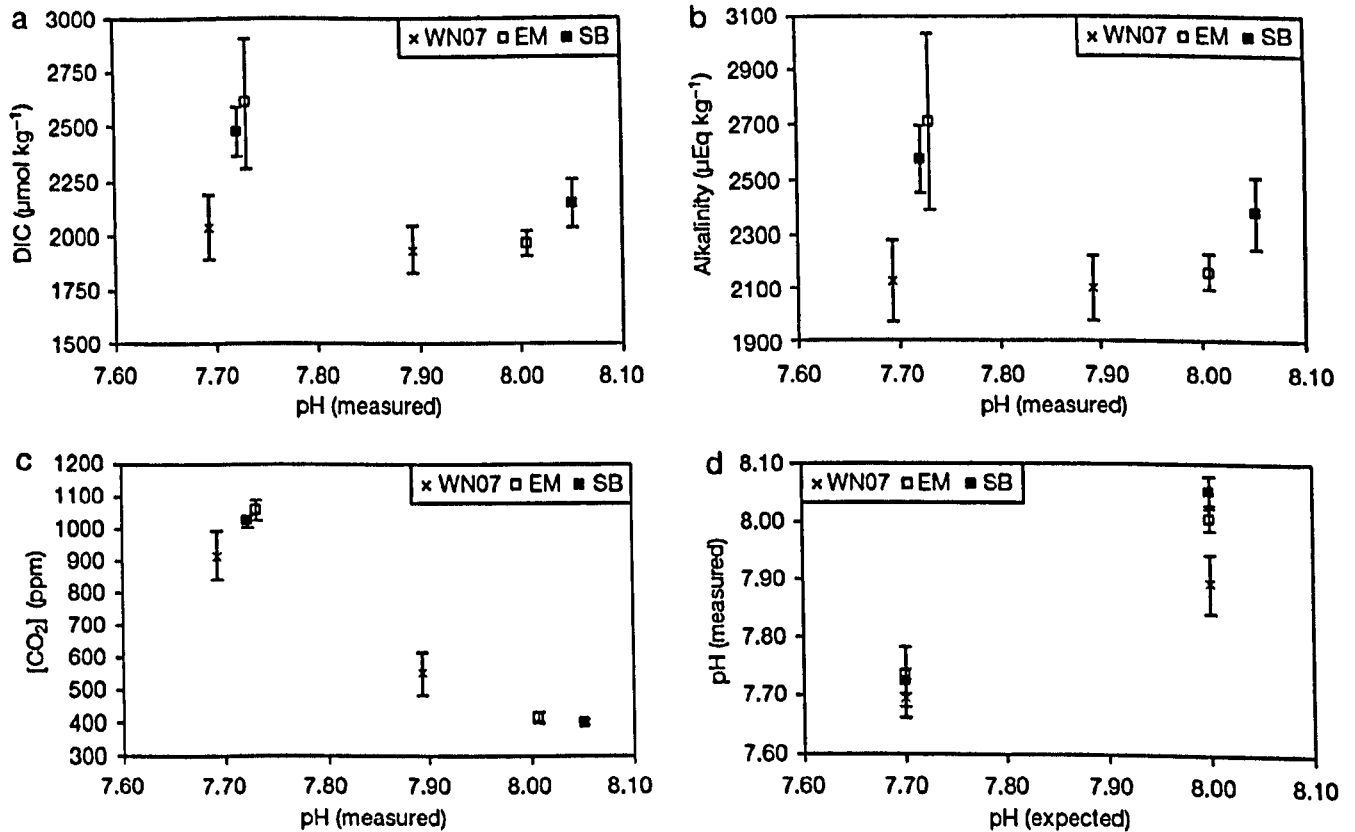


Fig. 5. Carbonate system values from an experiment using the Widdicombe & Needham (2007) set-up (x, WN07), from the initial experiment described here (■, SB) and from a second experiment using the same equipment as SB, but using a different species (□, EM). Values are calculated for the experimental periods, mean  $\pm$  95% CI. (a) Total dissolved inorganic carbon (DIC) plotted against mean measured pH over the experimental period, (b) alkalinity plotted against mean measured pH over the experimental period, (c)  $[\text{CO}_2]$  plotted against mean measured pH over the experimental period, and (d) pH plotted against expected/target pH

expected and have larger uncertainty than the set-up of Widdicombe & Needham (2007).

In any application of the system described here it is important also to consider daily and seasonal variability. We chose to maintain the conditions with minimal variability in order to investigate a specific time period within an organism's life-cycle. However, observations have shown that there is a natural seasonal cycle of pH,  $[\text{CO}_2]$  and temperature. pH in the intertidal zone is thought to vary annually by about 1 unit, reaching highest levels ( $\sim 8.5$ ) during winter and lowest ( $\sim 7.5$ ) in summer, as a result of varying levels of biological production and temperature (Hinga 2002);  $[\text{CO}_2]$  also has seasonal and daily cycles as a result of changes in DIC and alkalinity. In the North Atlantic there is a decrease in sea surface  $[\text{CO}_2]$  in summer and an increase in winter, predominantly as a result of biological productivity (Sarmiento & Gruber 2006). This system can run for a long period, and, with minor alterations, it would be possible to reproduce seasonal variability in atmospheric  $[\text{CO}_2]$  together with a seasonally varying temperature regime (as demonstrated by the longer term *Semibalanus balanoides* experiment). Such a capability enables investigation of longer term climate

change impacts on whole life cycles of short-lived organisms.

This initial proof-of-concept study has demonstrated that the microcosm system is ideal for small-scale studies and thereby justifies further studies with greater replication. Although we acknowledge that size matters (Schindler 1998), these microcosm systems allow us to focus on specific conditions and endpoint measures in a subject area that is lacking in fundamental data. These microcosms are appropriate for studying organisms from a variety of near-surface environments with a variety of substratum types. The system as described here is relatively small and easy to set up, which makes it adaptable to many locations. Here, we used a controlled-temperature environment to maintain ambient conditions and a similar room would be necessary if the *in situ* conditions were highly variable on a daily basis or if the required target conditions were lower than those of the ambient conditions. The ability to move the systems between locations means that the system can reduce long-distance transport of animals and so minimise experimental artefacts resulting from handling and transport.

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# Future high CO<sub>2</sub> in the intertidal may compromise adult barnacle *Semibalanus balanoides* survival and embryonic development rate

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**ABSTRACT:** The effects of CO<sub>2</sub>-induced acidification on survival, shell mineralogy, embryonic development and the timing of larval release were investigated in the intertidal barnacle *Semibalanus balanoides* using an intertidal microcosm system. Compared to that in the control (CO<sub>2</sub> = 344 ppm, pH = 8.07), adult survival was 22% lower in the high-CO<sub>2</sub> treatment (CO<sub>2</sub> = 922 ppm, pH = 7.70) and significant changes in the mineral structure of the adult shell were observed. Embryonic development rate was significantly slower in the high-CO<sub>2</sub> treatment than in the control but still resembled 'natural' rates seen in populations found in similar locations. There was an estimated 19 d delay in development under high-CO<sub>2</sub> conditions, which resulted in a 60% reduction in the number of nauplii reaching hatching stage at the time when over 50% of the control nauplii had hatched. We conclude that ocean acidification could potentially further compromise embryonic development in a species already stressed by temperature, which could in turn impact naupliar development and recruitment. *S. balanoides*, the adults of which live in a highly variable environment, has been shown to be detrimentally impacted by a chronic change in chemical conditions (pH lowered beyond the current range) over a crucial period in their life cycle. Under experimental high-CO<sub>2</sub> conditions, some adults were able to survive and larvae were able to hatch. This may indicate that there is still potential for organisms to find suitable habitats and for populations to develop and survive.

**KEY WORDS:** Ocean acidification · pH · Barnacle · Embryonic development · Carbon dioxide · Climate change · Intertidal · Larvae

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## INTRODUCTION

Ocean acidification, which is the decline in ocean pH occurring due to increasing atmospheric CO<sub>2</sub> concentration, is currently thought to impact most significantly those organisms that produce calcium carbonate structures (e.g. Gattuso et al. 1998, Langdon et al. 2000, Kleypas et al. 2006). In addition to a decrease in pH, the dissolution of more CO<sub>2</sub> in seawater also causes a change in chemistry leading to a decrease in the availability of carbonate ions (CO<sub>3</sub><sup>2-</sup>). This can in turn lead to an increase in the dissolution of calcium carbonate minerals (CaCO<sub>3</sub>). Organisms that use CaCO<sub>3</sub> to form shells may also be susceptible to dissolution.

Coastal shelf seas experience a greater range of pH than open oceans due to terrestrial influences such as

river run-off and nutrient enrichment as well as large temperature and salinity fluctuations (Hinga 2002, Blackford & Gilbert 2007). Intertidal systems can experience even greater pH fluctuations, e.g. in rockpools (Morris & Taylor 1983), but knowledge of the environmental conditions in the overlying water during high tide is virtually non-existent (although see Agnew & Taylor 1986 for an example of diurnal fluctuations in pH of 7.5 to 8.5 and Wootton et al. 2008 for recent data for the coastal zone showing a diurnal range of ~0.7 pH units and a seasonal range of ~1 pH unit). Little is known about how sessile organisms in the coastal zone might respond to chronic changes in pH due to current and future increases in CO<sub>2</sub>. Sessile organisms are important contributors to the intertidal ecosystem, providing habitat, food and nutrient cycling, yet they are

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unable to move away from unfavourable environmental conditions and may be more at risk from extreme events (Newell 1979). Fluctuating environmental conditions may provide some relief from unfavourable conditions for sessile organisms that show plasticity in their behaviour and/or physiology (Newell 1979). Ocean acidification lowers the overall pH range, which could reduce the duration and efficacy of these short-term periods of respite. Under the IPCC IS92 (Intergovernmental Panel on Climate Change, IS92 Emission Scenarios) CO<sub>2</sub> scenario (CO<sub>2</sub> concentration = 1000 ppm), ocean pH is expected to decrease by 0.3 units by 2100 (Caldeira & Wickett 2003, Orr et al. 2005, Blackford & Gilbert 2007). Low-pH water is already encroaching on coastal shelf seas in upwelling areas such as the west coast of North America, where pH can fall below 7.75 on a seasonal basis. These waters are undersaturated with respect to aragonite and near saturation with respect to calcite, aragonite and calcite being 2 common polymorphs of biogenic CaCO<sub>3</sub>. Hence, these waters are corrosive to aragonite-forming marine organisms (Feely et al. 2008) and potentially harmful to calcite-forming organisms such as barnacles.

The barnacle *Semibalanus balanoides* is an important space occupier on rocky shores of northern Europe and America. Its calcareous shell plates provide protection from predators, abrasion, and desiccation (Rainbow 1984). *S. balanoides* is a cross-fertilising hermaphrodite, which develops its egg masses within its shell cavity. Fertilisation in the UK normally occurs by mid-November and the embryos develop over the winter period before hatching between February (near the southern limits of its geographic range, southwest England and northern Portugal) and May (at the northern limits, north UK and Norway) (Barnes 1957, Crisp 1962). Crisp (1959) recorded *S. balanoides* *in vivo* development times at Brixham (south Devon) and Bangor (north Wales), demonstrating no appreciable divergence in timing in early stages but nearly twice as rapid development of embryos after Stage 8 (as defined by Crisp 1954) in Brixham as in Bangor. Laboratory studies have shown that the development rate of these embryos is temperature-dependent, with a maximum development time occurring at ~14°C, which is also impacted by the availability of oxygen within the egg cavity (Crisp 1959, Lucas & Crisp 1987). Adults of this species undergo a period of lowered metabolism and activity in winter (Rainbow 1984), during which they carry out oxygenation within the mantle cavity by flushing it with seawater during periods of immersion (Barnes et al. 1963). *S. balanoides* offers an opportunity to examine the impacts of ocean acidification on an important space occupier, particularly an ability to focus on the development of eggs and larvae, which

have been shown in other species to be highly vulnerable to elevated CO<sub>2</sub>.

Previous studies investigating the impacts of CO<sub>2</sub>-induced acidification on larvae and eggs have shown detrimental impacts on development, growth and survival (Kikkawa et al. 2004, Kurihara & Shirayama 2004, Kurihara et al. 2004, 2007, Dupont et al. 2008, Havenhand et al. 2008). However, there have been no specific investigations on the impacts of high CO<sub>2</sub> on embryonic development in *Semibalanus balanoides* and on survival of the adults through this crucial period in their life cycle, when they have minimal food and predominantly rely on lipid reserves for energy. The aim of the current study was to determine whether *S. balanoides*, a species normally exposed to a fluctuating environment, is likely to be impacted by ocean acidification scenarios realistic for the next 100 yr; and particularly whether the embryos contained within the calcium carbonate shell cavity, which may be subject to increased dissolution under high-CO<sub>2</sub> conditions, would develop normally. The study was conducted using microcosms which simulated immersion and emersion on the shore (Findlay et al. 2008) to investigate the effect of elevated CO<sub>2</sub> on (1) the survival of sexually mature adults during the embryonic production and development period, (2) the calcium and magnesium contents of the adult shells as a measure of changes in their calcium carbonate shells, (3) the timing of the appearance of different developmental stages of the embryos within the adults, and (4) the timing of the subsequent release of free-swimming nauplii.

## MATERIALS AND METHODS

**Experimental setup.** *Semibalanus balanoides* adults on small rock chips were collected from the mid-shore at Looe, England (50° 20' N, 004° 27' W) on 23 November 2007. At least 2 rock chips were placed at random into each of 4 microcosms (30 × 15 × 20 cm) in a constant-temperature room so that each microcosm contained >400 ind. (living and dead). Two microcosms were set at control pH (8.07, CO<sub>2</sub> = 346 ppm) and 2 were set as high-CO<sub>2</sub> treatments (pH 7.70, CO<sub>2</sub> = 922 ppm). The CO<sub>2</sub> concentration, and hence pH level, was maintained in each microcosm using a CO<sub>2</sub> mixing system exactly as described by Findlay et al. (2008), which involved bubbling the microcosm with premixed high-CO<sub>2</sub> air. pH (NBS scale, Mettler-Toledo pH meter), dissolved inorganic carbon (DIC) (Ciba-Corning 965D Total CO<sub>2</sub> Analyser, Olympic Analytical Service), CO<sub>2</sub> (Licor LI-6262 CO<sub>2</sub> analyser), temperature and salinity (WTW LF197 combination temperature and salinity probe) were recorded weekly. Total

alkalinity, bicarbonate (HCO<sub>3</sub><sup>-</sup>), carbonate (CO<sub>3</sub><sup>2-</sup>), and the saturation states ( $\Omega$ ) for aragonite and calcite were all calculated from pH and DIC using CO<sub>2</sub>sys (Pierrot et al. 2006), with dissociation constants from Mehrbach et al. (1973) refit by Dickson & Millero (1987) and KSO<sub>4</sub> using Dickson (1990). The microcosms worked on a tidal system (Findlay et al. 2008) with tide times programmed weekly based on the local Plymouth tide times (salinity = 35, water flow = 10 ml min<sup>-1</sup>). Air temperature in the controlled-temperature room was set so that water temperature followed the Plymouth sea surface temperature. Light conditions (Polylux XL 58 W) were set to within 15 min of the sunrise/sunset times for London, UK, on a weekly basis (roughly 8 h on:16 h off cycle in December, 9 h on:15 h off cycle in January and 10 hr on:14 h off cycle in February). Natural, filtered (10  $\mu$ m) seawater was used in the system and was replenished twice weekly to avoid salinity increases through evaporation. The experiment ran for 104 d.

**Adult survival and shell mineralogy.** Changes in barnacle abundance on each rock chip were recorded using a digital camera (FujiFilm A510 FinePix) which was maintained in consistent alignment using a stand. The photographic images were analysed (Image-Pro Plus v.4.5, Media Cybernetics) to estimate both abundance and survival. Barnacle survival was estimated from the images taken at the beginning and end of the experiment by counting living and dead individuals, accounting for individuals removed for sampling. Prior to photography, individuals were gently touched to check whether they were able to close their operculum, and were counted as dead when the operculum had remained open or the shell was empty.

Adult survival, which was recorded as a proportion, was square root arcsine transformed, tested for normality using the Kolmogorov-Smirnov test, and homogeneity of variances was examined using Levene's test. A 1-way nested ANOVA was used to determine any CO<sub>2</sub> treatment effects, with microcosms being nested within CO<sub>2</sub> treatment ( $n = 2$ ). All statistical analysis was performed using Minitab 15.1.0.0 (Minitab 2006).

The calcium carbonate composition of the shell was estimated by analysing the calcium (Ca) and magnesium (Mg) ion concentrations as a proxy for any changes in calcification or dissolution. Live individuals produce calcium carbonate (calcify) during shell growth, although there may also be some dissolution of the shells; this dissolution, as discussed in the Introduction, may be enhanced in high-CO<sub>2</sub> conditions. Ca and Mg ions are abundant in seawater and hence are not limiting. Formation of CaCO<sub>3</sub> involves combining inorganic carbon with Ca and some Mg is also often incorporated to form Mg-CaCO<sub>3</sub>. Therefore, any observed changes in Ca and Mg should indicate how the shell

structure changes over time through calcification and dissolution. The shells of 10 ind. were haphazardly selected from each microcosm at the end of the experiment. Shells of 10 ind. that were noted as dead at the start of the experiment were also analysed for the concentration of Ca and Mg ions at the end of the experiment. Comparing the concentration in dead animals with that in live animals provides an estimate of a barnacle's ability to calcify relative to any dissolution effects since calcification does not take place in dead individuals. Concentrations of both cations were measured using methods described by Spicer & Eriksson (2003); briefly, this involved dissolving the shells in 10% nitric acid after drying and weighing, and then using an inductively coupled plasma (ICP) optical emissions spectrometer (Varian 725-ES) to measure Ca and Mg simultaneously. The proportion of Ca and Mg in the shell was calculated using the mass of the shell and the volume of acid used in the digestion.

Calcium and magnesium, which were recorded as proportions (cation concentration [mg l<sup>-1</sup>]:total shell concentration [mg l<sup>-1</sup>]), were square root arcsine transformed. The Ca:Mg ratio was calculated (mg Ca l<sup>-1</sup>:mg Mg l<sup>-1</sup>), all 3 datasets were tested for normality using the Kolmogorov-Smirnov test, and homogeneity of variances was examined using Levene's test. A 2-way nested ANOVA ( $n = 10$ ) was then used to test for differences between control and high-CO<sub>2</sub> treatments and between live and dead barnacles, with microcosm being nested within CO<sub>2</sub> treatment.

**Embryonic development.** Embryos were maintained within live adult *Semibalanus balanoides* as naturally fertilised broods. On 8 occasions during the course of the experiment (Days 0 [23 November 2007], 7, 24, 42, 56, 70, 91 and 104), barnacles were removed haphazardly until 20 adults with egg masses were found from each microcosm. After isolating the egg masses in seawater, the embryonic development of 20 eggs from each egg mass at each sampling time was determined under low magnification (40 $\times$ ). Developmental stages were assigned using the classification of Achituv & Barnes (1976):

U: unfertilised, I: early development from being newly laid to having few divisions (equivalent to Stages 1 to 4 in Crisp 1954), II: multicellular (equivalent to Stages 5 to 7 in Crisp 1954), III: limb buds developing (equivalent to Stages 8 to 10 in Crisp 1954), IV: nauplii with eye apparent (equivalent to Stages 11 to 12 in Crisp 1954), and IVh: nauplii hatching (equivalent to Stage 13 in Crisp 1954); following Crisp (1954), the embryos were left for 5 min in seawater on the 2 last sampling points (Days 91 and 104); if any embryos hatched, they were counted as Stage IVh.

Egg development, which was recorded as the proportion of eggs at each stage (I, II, III, IV and IVh) from

20 ind. at each sampling time, was square root arcsine transformed and tested for normality using the Kolmogorov-Smirnov test, and homogeneity of variances was examined using Levene's test. A repeated measures ANOVA ( $n = 20$ ) was then performed to determine the effect of  $\text{CO}_2$  treatment and time (Day 7, 28, 40, 56, 70 and 104), with microcosm being nested within  $\text{CO}_2$  treatment.

Development rate was assessed by first calculating the time at which 50% of the sampled eggs reached each stage, which was in turn calculated by fitting a logistic growth function (as the best model fit) to the embryonic stage data and calculating the time to 50% development. Records of the time taken for each stage to achieve 50% development were analysed using PERMANOVA (Primer-E) (Anderson 2001) with a nested (replicate microcosms) regression design (developmental Stages I to IVh) to test for differences between pH treatments. Time to 50% development for each stage was transformed ( $d^{0.5}$ ) to produce a linear fit (best model fit with maximum  $R^2$ ) whose gradient was taken as the rate of development. Linear regression analysis was then applied to each data set and a 2-tailed *t*-test of the regression was used to assess differences between the slopes of the control and high- $\text{CO}_2$  treatments.

## RESULTS

### Environmental conditions

The pH was maintained at a mean ( $\pm 95\%$  CI) of 8.07 ( $\pm 0.03$ ) and 7.70 ( $\pm 0.03$ ) in the control and high- $\text{CO}_2$  treatments, respectively. Dissolved inorganic carbon (DIC) was on average 1888 ( $\pm 0$ ) and 2045 ( $\pm 83$ )  $\mu\text{mol kg}^{-1}$  in the control and high- $\text{CO}_2$  treatments, respectively. Total alkalinity was not significantly different between treatments, with averages of 2086 ( $\pm 101$ ) and 2115 ( $\pm 95$ )  $\mu\text{Eq kg}^{-1}$  in the control and high- $\text{CO}_2$  treatments, respectively (see Findlay et al. 2008 for exploration of the variability, control and reproducibility of the carbonate parameter measurements). There was a slight increase in pH towards the end of the experiment (~Day 84) in both control and high- $\text{CO}_2$  treatments (Fig. 1) due to an increase in salinity at this time.  $\text{CO}_2$  concentration was maintained at a mean ( $\pm 95\%$  CI) of 346 ( $\pm 27$ ) and 922 ( $\pm 72$ ) ppm in the control and high- $\text{CO}_2$  treatments, respectively. The high- $\text{CO}_2$  treatment was undersaturated with respect to aragonite ( $\Omega < 1$ ) and calcite was near saturation ( $\Omega = 1$ ) throughout. Mean water temperature was 11.9°C in both treatments, but was set to track local sea surface temperature and hence decreased from 13°C in November to 10°C in February (Fig. 1a).

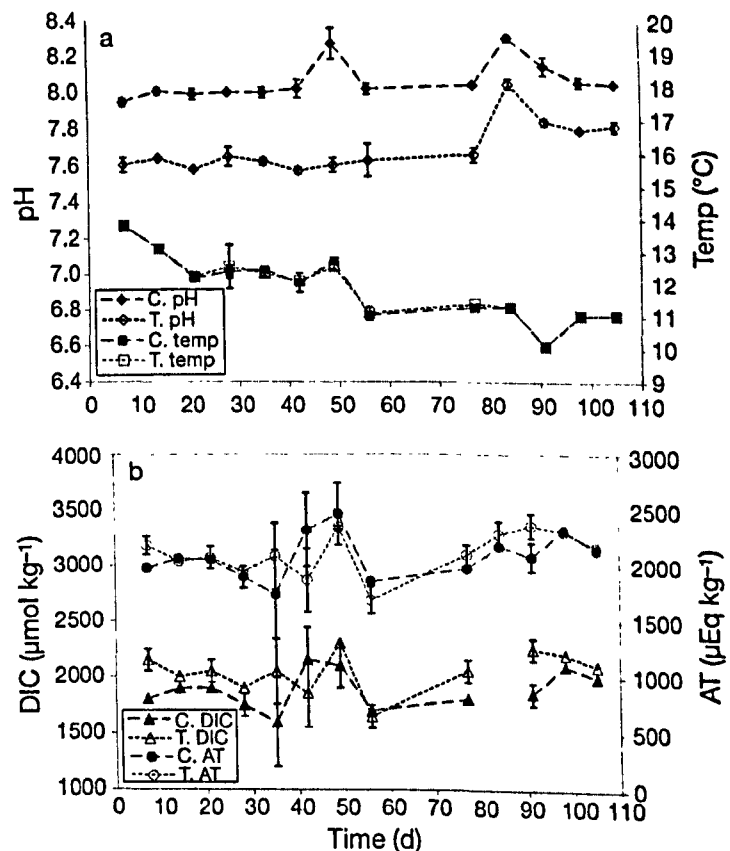


Fig. 1. (a)  $\text{pH}_{\text{NBS}}$  ( $\blacklozenge$ ) and temperature ( $\blacksquare$ ) in control (C; filled symbols) and high- $\text{CO}_2$  (T; open symbols) microcosms over the experimental period, and (b) dissolved inorganic carbon (DIC;  $\blacktriangle$ ) and total alkalinity (AT;  $\bullet$ ) in control (C; filled symbols) and high- $\text{CO}_2$  microcosms (T; open symbols) over the experimental period. Error bars: 95% CIs

### Adult survival

Adult survival was significantly lower ( $p = 0.017$ ,  $df = 1$ ) in the high- $\text{CO}_2$  treatment than in the control (mean ( $\pm 95\%$  CI) of 47 ( $\pm 4.62$ ) vs. 69 ( $\pm 4.28$ )%) after 104 d (Fig. 2). There were no significant microcosm effects in any of the endpoint measures (survival, mineralogy or development).

### Adult shell mineralogy

**Calcium.** The proportion of calcium increased from the control to the high- $\text{CO}_2$  treatment in the live barnacles but decreased from the control to the high- $\text{CO}_2$  treatment in the dead barnacles (Fig. 3a). This indicates that there might have been some dissolution that was compensated for by calcification in live barnacles. However, there was no significant difference in the proportion of calcium between the treatments or between live and dead barnacles.



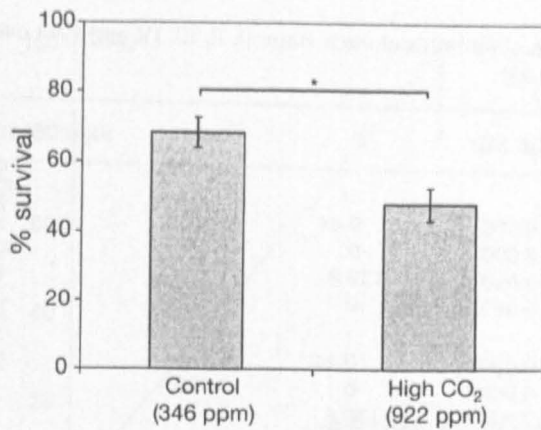


Fig. 2. *Semibalanus balanoides*. Mean percentage of adults surviving in the control (CO<sub>2</sub>: 346 ppm) and high-CO<sub>2</sub> (922 ppm) microcosms. Bar with asterisk: significant difference ( $p = 0.017$ ,  $df = 1$ ,  $n = 2$ ). Error bars: 95% CIs

**Magnesium.** There was a significant difference in the proportion of magnesium between the control and high-CO<sub>2</sub> treatment ( $p = 0.000$ ,  $df = 1$ ) and a small significant difference in magnesium between the live and dead barnacles ( $p = 0.048$ ,  $df = 1$ ); the significance was small due to large variability in the data. There was no significant interaction between the treatment and whether the barnacle was dead or alive. The proportion of magnesium decreased from the control to the high-CO<sub>2</sub> treatment in both live and dead barnacles (Fig. 3b).

**Ca:Mg ratio.** There was a significant difference in the Ca:Mg ratio between CO<sub>2</sub> treatments ( $p = 0.000$ ,  $df = 1$ ) and between live and dead barnacles ( $p = 0.000$ ,  $df = 1$ ), with a significant interaction effect ( $p = 0.032$ ,  $df = 1$ ). The Ca:Mg ratio increased from the control to the high-CO<sub>2</sub> treatment in both live and dead barnacles but this increase was greater in living barnacles (Fig. 3c).

### Embryonic development

More than 50% of the eggs in egg masses were fertilised at the start of the experiment (Day 0) and all the eggs from fertilised animals had reached Stage I by Day 7. ANOVA (Table 1) indicated effects of both time and CO<sub>2</sub> concentration on the development of embryos through each stage (Fig. 4a–e); however, differences resulting from elevated CO<sub>2</sub> occurred most significantly at Stages III, IV and IVh. On Day 104, ~50% of the embryos had hatched in the control compared to only <20% in the high-CO<sub>2</sub> treatment (Fig. 4e).

The estimated rate of development (Fig. 4f) was significantly greater (see Table 2) in the control (0.24 stages  $d^{-0.5}$ ) than in the high-CO<sub>2</sub> treatment (0.22 stages  $d^{-0.5}$ ) for Stages I to IV. In the high-CO<sub>2</sub> treatment, the time to hatching (Stage IVh) was delayed by 18.95 d. The time to hatching in the control was not significantly slower than that observed by Crisp (1959) at Brixham (0.26 stages  $d^{-0.5}$ ), whereas hatching in the high-CO<sub>2</sub> treatment was significantly slower than at Brixham (regression analysis:  $t(3.60)$ ,  $t(0.05, 2.447)$ , 2-tailed,  $df = 6$ ). The development rate in the control was significantly greater than Crisp's (1959) data from Bangor (0.20 stages  $d^{-0.5}$ ), but the rate at the high-CO<sub>2</sub> concentration was not significantly greater (regression analysis between slopes of control vs. Bangor data:  $t(2.69)$ ,  $t(0.05, 2.447)$ , 2-tailed,  $df = 6$ ; regression analysis between slopes of low pH vs. Bangor data:  $t(1.48)$ ,  $t(0.05, 2.447)$ , 2-tailed,  $df = 6$ ). Crisp (1959) observed that hatching took 54.5 d longer for the Bangor than for the Brixham population.

### DISCUSSION

At atmospheric CO<sub>2</sub> concentrations predicted for the year 2100 under the IPCC's IS92a scenario, the probability of adult *Semibalanus balanoides* barnacles sur-

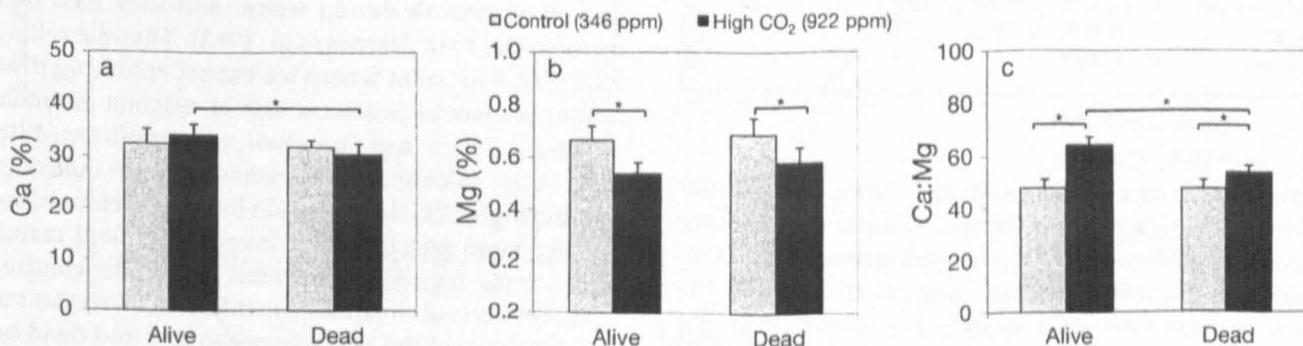


Fig. 3. *Semibalanus balanoides*. Concentrations of (a) calcium and (b) magnesium, and (c) the calcium:magnesium ratio in shells in the control (CO<sub>2</sub>: 346 ppm) (light grey bars) and high-CO<sub>2</sub> (922 ppm) (black bars) microcosms, as a percentage of total shell material in barnacles that were alive for the entire experiment and those that were dead at the end of the experiment. Bars with asterisks: significant difference ( $p < 0.05$ ). Error bars: 95% CIs

Table 1. Nested (microcosm) repeated-measures ANOVA for the proportion of embryos at each stage (I, II, III, IV and IVh) over time. (\*) Significant ( $p < 0.05$ )

Source	df	Seq SS	Adj SS	Adj MS	F	p	Significant
Stage I							
pH	1	0.0001	0.0001	0.0001	0.44	0.574	
Microcosm(pH)	2	0.0007	0.0007	0.0003	0	0.996	
Day	5	63.4435	63.4435	12.6887	139.8	0	*
pH × Day	5	0.0007	0.0007	0.0001	0	1	
Stage II							
pH	1	0.0001	0.0001	0.0001	0.44	0.574	
Microcosm(pH)	2	0.0007	0.0007	0.0003	0	0.996	
Day	5	63.4435	63.4435	12.6887	139.8	0	*
pH × Day	5	0.0007	0.0007	0.0001	0	1	
Stage III							
pH	1	1.2349	1.2349	1.2349	1932.45	0.001	*
Microcosm(pH)	2	0.0013	0.0013	0.0006	0.03	0.97	
Day	5	51.6918	51.6918	10.3384	494.65	<0.001	*
pH × Day	5	2.0738	2.0738	0.4148	19.84	<0.001	*
Stage IV							
pH	1	1.49	1.49	1.49	3293.02	<0.001	*
Microcosm(pH)	2	0.001	0.001	0	0.03	0.972	
Day	5	178.969	178.969	35.794	2257.78	<0.001	*
pH × Day	5	1.806	1.806	0.361	22.78	<0.001	*
Stage IVh							
pH	1	1.0462	1.0462	1.0462	222.37	0.004	*
Microcosm(pH)	2	0.0094	0.0094	0.0047	0.59	0.555	
Day	5	19.6909	19.6909	3.9382	493.85	<0.001	*
pH × Day	5	5.2312	5.2312	1.0462	131.2	<0.001	*

Table 2. PERMANOVA for nested (microcosms) regression (stages) at each pH condition, where pH condition 1: control (346 ppm), pH condition 2: high CO<sub>2</sub> (922 ppm), pH condition 3: Crisp (1959) Brixham data, and pH condition 4: Crisp (1959) Bangor data

Source	df	SS	MS	Pseudo-F	p (perm)	Unique perms
Stage	3	15801	5267	39751	0.001	998
pH	3	1141.9	380.64	2872.7	0.016	45
Microcosm (pH)	2	0.265	0.1325	1	0.6328	53
Stage × pH	9	922.81	102.53	773.85	0.001	999
Res	6	0.795	0.1325			
Total	23	18608				

living the winter period was 22% lower than under current (2007/2008) winter sea surface temperature and pH conditions. Adult barnacles appeared to maintain their calcium carbonate mineral structure in the face of increased CO<sub>2</sub> despite the changes in the Ca:Mg ratio, showing that the isolated shell structure should disintegrate as a result of dissolution in corrosive (undersaturated) seawater. The relatively large decline in survival rate could possibly be due to the metabolic cost of maintaining the shell. Embryos

developing within the adults developed more slowly due to increased CO<sub>2</sub> levels. Both delayed development and reduced naupliar production, which lead to delayed settlement, have the potential to impact local populations (Pechenik et al. 1993, Jarrett 2003).

#### Adult shell mineralogy and survival

Under low-CO<sub>2</sub> (control) conditions, a large change in the mineral structure of the shells was not expected in this experiment because adults do not expend much energy on growth during winter and they feed minimally at this time (Barnes et al. 1963). Therefore, in the high-CO<sub>2</sub> treatment where we expect enhanced dissolution, we would predict a loss of calcium carbonate structure. There was, however, no significant difference in the calcium concentration between the control and the high-CO<sub>2</sub> treatments in living individuals, suggesting more energy being invested in shell maintenance in the high-CO<sub>2</sub> treatment than in the control.

There were also significant differences in the mineral structure of the shells between live and dead barnacles: in the high-CO<sub>2</sub> treatment, there was less Ca in dead adult barnacles, resulting in lower Ca:Mg ratios in dead compared to live barnacles. This indicates that dissolution of the dead shells was occurring in the

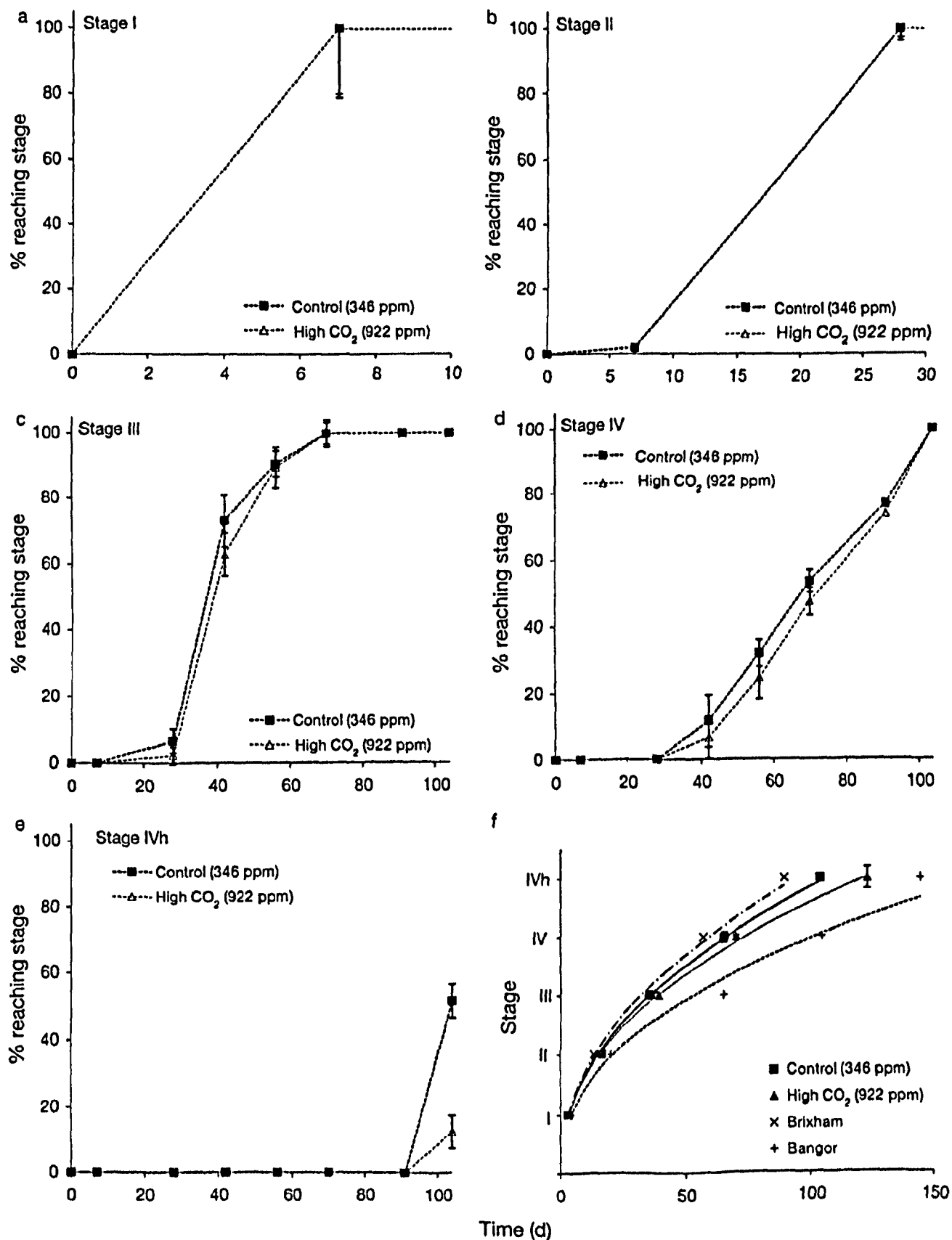


Fig. 4. *Semibalanus balanoides*. Mean percentage of eggs from 20 ind. reaching each stage (stage I – IVh, a to e) at particular time periods (days after start of experiment) in the control (CO<sub>2</sub>: 346 ppm) (■) and the high-CO<sub>2</sub> (928 ppm) (▲) microcosms. Error bars: 95% CIs. (f) Time for 50% of samples to reach each stage in the control (■) and high-CO<sub>2</sub> (▲) microcosms, and in the Crisp (1959) Brixham (×) and Bangor (+) data, also displaying best fit lines (power equation,  $d^{0.5}$ )

high-CO<sub>2</sub> treatment, while there was biological precipitation of CaCO<sub>3</sub> in the living barnacles.

Barnes et al. (1976) investigated the Ca:Mg ratio in several barnacle species. *Chthamalus depressus* was found to have an increased Ca:Mg ratio in extreme hypobiotic individuals (found in caves and other dark locations) compared to those on the open shore. This increased ratio was accompanied by a reduction in the total organic matter content due to reduced protein. As calcite is the major form of CaCO<sub>3</sub> in these organisms, any magnesium present in the shell is held within the lattice matrix but is not tightly bound, hence dissolution will cause ions such as Mg to be lost before the dissolution of CaCO<sub>3</sub>. Therefore, a larger decrease in Mg compared to Ca was both expected and observed in the high-CO<sub>2</sub> treatments. The absence of photosynthetic organisms in hypobiotic environments could lead to an elevated level of CO<sub>2</sub> in the seawater, which occurs as a net result of high respiration but low photosynthetic rates. This could result in seawater with carbonate chemistry properties similar to those seen in this study and may explain why both high-CO<sub>2</sub> and hypobiotic individuals show similar results.

Wickens (1984) investigated CO<sub>2</sub> impacts on growth and mineralisation in penaeid prawns, and showed an increase in Ca, no change in Mg and hence an increase in the Ca:Mg ratio with increasing CO<sub>2</sub>-induced acidification. This agrees with our findings in several respects as we also found an increased Ca:Mg ratio and an increase in Ca, although the latter was not significant at the CO<sub>2</sub> level used here. Increasing CO<sub>2</sub> is accompanied by an increase in HCO<sub>3</sub><sup>-</sup>, which is taken up for use both as a buffer to rising haemolymph pH and as a substrate for CaCO<sub>3</sub>. The decrease in Mg in *Semibalanus balanoides* but not in prawns suggests either a difference in the mechanisms associated with the uptake of Mg into the shell structure (with less Mg being incorporated at lower pH in *S. balanoides*), a difference in relation to external erosion properties or, more likely, a difference in shell mineralogy.

The lower survival of adults in the high-CO<sub>2</sub> treatment could have resulted from either physiological or dissolution effects. Like all other organisms, barnacles expend energy on maintenance, repair, reproduction and respiration (Sibly & Calow 1986). They feed minimally during winter and hence must rely on food reserves (lipid stores) while undergoing a period of lowered metabolism with minimal growth (Barnes et al. 1963). Despite acidosis in extracellular fluids being considered as a 'normal' feature of intertidal barnacles during periods of emersion (e.g. Fyhn et al. 1972), prolonged acidosis resulting from sustained exposure to low-pH seawater, as found for other crustacean species (e.g. Spicer et al. 2007), could lead to disruption of normal physiological processes. Additionally, an in-

creased use of lipid stores for energetic maintenance could result in reduced protein biosynthesis, which in turn could enhance mortality (Barnes et al. 1963). Exposure to corrosive seawater may have led to some dissolution of the shell, as well as created hypercapnic conditions within the organism further stressing the animals and decreasing survival. Further work investigating lipid storage and cirral movement during winter under normal and acidified conditions will aid our understanding of this energy balance, although energetic trade-offs have been shown in other species under hypercapnic conditions (Wood et al. 2008, Findlay et al. 2009).

### Embryonic development

Comparing the current study with the investigation of Crisp (1959), it can be seen that the naupliar development rates recorded by Crisp at Brixham, which is geographically the closest site to the barnacle populations used here, were similar to those estimated for the control. Based on this observation, we assume that the 1.5°C increase in temperature over the last 50 yr as a result of global warming (Baxter et al. 2008) has had little impact on development rates. The estimated development rate in the high-CO<sub>2</sub> treatment was significantly slower than in both the control (average winter temperature of 12°C) and in the Brixham population (average winter temperature of 11°C), but was similar to the rate in the Bangor population (average winter temperature of 8°C). There is no information on annual variation in development rates at either Bangor or Brixham. Crisp (1959) suggested that the difference in the rate of development in different locations arises from temperature differences (average winter temperature in Brixham is ~2°C warmer than in Bangor) together with the implication that towards the later stages of development, embryos are more deprived of oxygen or inhibited by excess CO<sub>2</sub> under warmer conditions. Both O<sub>2</sub> and CO<sub>2</sub> are known to impact the rate of egg development (Root 1930, Strathmann & Strathmann 1995, Cohen & Strathmann 1996). Root (1930) demonstrated that the rate of O<sub>2</sub> consumption of *Arbacia* eggs decreased rapidly when CO<sub>2</sub> increased (O<sub>2</sub> consumption decreased by 21 % for every ~1315 µatm of CO<sub>2</sub> increase), but only began to decrease below pH 6.25 if the acidity was changed using HCl. This implies that bubbling CO<sub>2</sub> has a much greater effect on egg respiration than using HCl to lower pH, and is in agreement with the results obtained here in which CO<sub>2</sub> had a small yet significant effect at 922 ppm. Mayor et al. (2007) showed that elevated CO<sub>2</sub> (8000 ppm) was associated with nearly an 86 % reduction in hatching success of copepods despite

apparently normal growth and reproduction of adults. In our experiments, there was no impact of elevated CO<sub>2</sub> on hatching success; however, there was an estimated 19 d delay in reaching hatching stage.

A change in the rate of embryonic development induced by elevated CO<sub>2</sub> may be important considering that larvae are released into the plankton to coincide with the spring bloom. Adults are triggered to release their larvae by a chemical cue or 'hatching substance' (Clare & Walker 1986) produced when the adult begins feeding. There is considerable interannual and geographic variability in release timing (Barnes 1962, Kendall et al. 1985), therefore effects of delayed development would be greatest in early release years and in areas where spawning tends to be early. In other years, developmentally intact larvae can be held for some time before release takes place. Larval release tends to start at the southern range edge (southwest UK and northern Portugal) as early as February, therefore the already short period between fertilisation and spawning could be problematic. Delaying the time to hatching could prevent synchronisation with the spring bloom and could lead to high mortality resulting from competition with the holoplankton. Even if larvae were able to survive, late settlement would leave juveniles susceptible to additional stresses: on average, the later in the year a larva settles, the greater is its chance of encountering high air and rock temperatures and dying from either heat or desiccation. Hence, the timing of recruitment is crucial, with early and late settlers often having the lowest survival (Kendall et al. 1985, Piñeda et al. 2006).

Here we provide evidence that relatively small changes in CO<sub>2</sub> and pH, to levels that are predicted to occur globally within the next 100 yr (Caldeira & Wickett 2003), can lower the survival of adults and decrease the development rate of embryos. These findings further the work of Kurihara & Shirayama (2004), Dupont et al. (2008), Havenhand et al. (2008) and others who have found impacts on early life stages at realistic levels of future CO<sub>2</sub>. However, the CO<sub>2</sub>-induced slowing in development rate still falls within the range of rates seen in natural populations. It is known that temperature limits the development rate of *Semibalanus balanoides* embryos at the southern edge of their range (Crisp 1959), but it could be inferred from our results that ocean acidification could further compromise barnacle development at this range edge (cf. synergistic effects of temperature and elevated CO<sub>2</sub> on the thermal limits of the edible crab *Cancer productus* in Metzger et al. 2007). Further work is necessary to establish more exactly how temperature and CO<sub>2</sub> interact over the entire geographic range.

We demonstrate that both the growth and embryonic development of an organism that is normally exposed

to seasonal and daily fluctuations in its environment can be significantly affected by lowering pH to levels predicted under future ocean acidification scenarios. Nevertheless, in assessing the significance of these findings, their limitations must be considered. One consideration for this experiment is that we increased CO<sub>2</sub> levels over a period of days, as opposed to increase on yearly timescales seen in nature, which thus removed the possibility that the barnacles might adapt or acclimate over longer time periods. The evidence that some adults were able to survive and their larvae able to hatch in high-CO<sub>2</sub> conditions may indicate that the species is able to find suitable habitats and survive. At the southern edge of its range, the additional temperature stress may make populations more vulnerable to local extinctions.

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## Post-larval development of two intertidal barnacles at elevated CO<sub>2</sub> and temperature

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**Abstract** Ocean acidification and global warming are occurring concomitantly, yet few studies have investigated how organisms will respond to increases in both temperature and CO<sub>2</sub>. Intertidal microcosms were used to examine growth, shell mineralogy and survival of two intertidal barnacle post-larvae, *Semibalanus balanoides* and *Elminius modestus*, at two temperatures (14 and 19°C) and two CO<sub>2</sub> concentrations (380 and 1,000 ppm), fed with a mixed diatom-flagellate diet at 15,000 cells ml<sup>-1</sup> with flow rate of 10 ml<sup>-1</sup> min<sup>-1</sup>. Control growth rates, using operculum diameter, were 14 ± 8 μm day<sup>-1</sup> and 6 ± 2 μm day<sup>-1</sup> for *S. balanoides* and *E. modestus*, respectively. Subtle, but significant decreases in *E. modestus* growth rate were observed in high CO<sub>2</sub> but there were no impacts on shell calcium content and survival by either elevated temperature or CO<sub>2</sub>. *S. balanoides* exhibited no clear alterations in growth rate but did show a large reduction in shell calcium content and survival under elevated temperature and CO<sub>2</sub>. These results suggest that a decrease by 0.4 pH<sub>(NBS)</sub> units alone would not be sufficient to directly impact the survival of barnacles during the first month post-settlement. However, in conjunction with a 4–5°C increase in temperature, it appears that significant changes to the biology of these organisms will ensue.

### Introduction

Increasing atmospheric carbon dioxide (CO<sub>2</sub>) is causing atmospheric and sea surface temperatures to rise (Levitus et al. 2005); additionally, CO<sub>2</sub> is being absorbed into the oceans and reacting with seawater to form a weak acid in a phenomenon termed “ocean acidification” (Caldeira and Wickett 2003). The increasing rate of CO<sub>2</sub> emissions since the industrial revolution has caused global surface temperatures to rise by 0.76°C and global seawater pH to decrease by 0.1 unit (IPCC 2007). It is believed that acting together climate change and ocean acidification will alter many marine ecosystems through changes to species distributions (Widdicombe and Spicer 2008) and survival (Fabry et al. 2008), or changes to nutrient cycling (Wood et al. 2009) and ecosystem functioning (Widdicombe and Spicer 2008).

Intertidal organisms, in comparison with those living sub-tidally, are often relatively tolerant to stressors such as temperature or pH, because they experience larger variations in their abiotic environment (Newell 1979; Somero 2002). This is certainly the case for temperature (body temperature range, in some locations, exceeds 20 or 30°C; e.g., Harley and Helmuth 2003), but our knowledge of the variability in chemical conditions is more limited. There is evidence of diurnal fluctuations in pH (primarily as a result of hypercapnia) from 9.3 to 7.5 in coastal environments (Agnew and Taylor 1986); intertidal rockpool systems may also experience large CO<sub>2</sub>-generated pH fluctuations (e.g., 6.5–9.5 pH units, Morris and Taylor 1983) and pH ranges of 6.7–8.9 have been reported in estuaries (Attrill et al. 1999). Coastal shelf seas experience a greater range of pH than open oceans as a result of terrestrial influences such as river run-off and nutrient enrichment, as well as large temperature and salinity fluctuations (Hinga 2002;

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Blackford and Gilbert 2007). Conversely, recent observations suggest that coastal waters may be more vulnerable to sub-optimal pH conditions because many rivers are already more acidic relative to the ocean (e.g., Salisbury et al. 2008). Furthermore, upwelling regions seasonally bring corrosive water onto shelf seas (Feely et al. 2008). There are reports of increased shell dissolution of organisms exposed to low salinity in estuarine environments, corresponding to low pH conditions (Marshall et al. 2008). Additionally, intertidal organisms may be impacted sooner than animals of the same family (or class) living subtidally, because of the multiple impacts of increasing temperature, eutrophication and pollution occurring first in the surface waters (Helmuth et al. 2006).

Many studies have already shown that elevated temperatures can have an impact on populations of intertidal species (Kendall et al. 1985; Harley and Helmuth 2003; Mieszkowska et al. 2006) and that the specific habitat occupied, even down to the direction and aspect in which an organism is orientated on the shore (Denny et al. 2006), can be important in determining the extent to which it is stressed. Seasonal variation in environmental conditions may also be important in determining the probability of an organism surviving. For example, the barnacle *Semibalanus balanoides* releases its larvae to coincide with the plankton spring bloom that provides food for the pelagic phase. *S. balanoides* larvae settle on the shore approx. 4 weeks later but if during this settlement period there are high air temperatures, the number of surviving recruits will be very low (Kendall et al. 1985). Coincidentally, in the temperate and sub-polar regions occupied by *S. balanoides*, the spring and summer phytoplankton blooms cause an increase in seawater pH (Blackford and Gilbert 2007) and carbonate ion saturation state (Findlay et al. 2008a), thereby inadvertently providing optimal conditions for growth and development of their calcareous shells.

Some studies have investigated the impacts of ocean acidification on intertidal species; however, they have all previously carried out the experiments in sub-tidal conditions (e.g., Michaelidis et al. 2005; Gazeau et al. 2007; Bibby et al. 2008; Beesley et al. 2008). These early studies suggested that processes such as calcification (Gazeau et al. 2007 at pH < 7.7), growth, acid-base balance and metabolism (Michaelidis et al. 2005 at pH < 7.5) and health (Phagocytotic response, Bibby et al. 2008 and lysosome membrane stability, Beesley et al. 2008) can all be negatively impacted by lowered pH. Moreover, few studies have attempted to put into context ocean acidification as an environmental stressor, particularly in relation to temperature. Consequently, we have used two species of intertidal barnacles in intertidal microcosms (Findlay et al. 2008b) to assess whether organisms usually exposed to a highly variable environment are less susceptible to changes in pH;

and whether they are more, less or equally impacted by pH or by temperature. We investigate how CO<sub>2</sub> and temperature, and their interaction, affects growth, shell development and survival in developing post-larvae during the metamorphic period of their life history.

The two intertidal barnacle species chosen, *Semibalanus balanoides* and *Elminius modestus*, are found in similar habitats around the United Kingdom, indeed in many locations they coexist. However, these barnacles differ in some key aspects of their biology; for example, *E. modestus* is often more successful in estuarine environments, whereas *S. balanoides* prefers more-exposed sites (Rainbow 1984). *S. balanoides* is a boreo-arctic species and shows a marked seasonal response in feeding rate (Ritz and Crisp 1970), moulting (Crisp and Patel 1960), and reproduction and development (Crisp 1956, 1962), all of which peak during the spring and summer. Cyprid larvae settle onto the shore in the spring (late February in the south (Southward 1958) to June in NE England (Kendall et al. 1985)) in the UK and metamorphose into juveniles when they first lay down a calcified shell (Bourget and Crisp 1975). The average metamorphosis time occurs in 1.5 days (Connell 1961). *E. modestus* by contrast is an invasive, warm-water species, which was first introduced into the UK from New Zealand around 1945 (Bishop 1947). Its European distribution extends from Faro (southern Portugal) to southern Denmark (Harms and Anger 1989; O'Riordan and Ramsay 1999). In contrast to *S. balanoides*, *E. modestus* releases larvae to the plankton throughout the year (Crisp and Davis 1955).

## Methods

*Semibalanus balanoides* were collected on settlement panels (10 cm × 10 cm ceramic tiles) deployed for 1 week (beginning 30th April 2007—settlement panels were placed on the shore and checked weekly for the onset of settlement) on north-facing rocks in the mid-shore at Looe, southwest England (50°20'N, 004°27'W). On collection, the panels contained a mixed age population of barnacles ranging from newly settled cyprids to week-old post-larvae. *Elminius modestus* were collected on identical settlement panels placed for 1 week (beginning 13th August 2007) on south-facing rocks in the mid-shore at Mount Edgcombe, southwest England (50°20'N, 004°10'W).

For each experiment (one experiment on *S. balanoides* and one experiment on *E. modestus*), at least two settlement panels were placed into one of eight microcosms (30 cm × 15 cm × 20 cm) so that each microcosm contained in excess of 200 individual barnacles. The microcosms were identical to those described by Findlay et al. (2008b) and were kept in a controlled temperature facility.



Four microcosms were set at 14°C to simulate 2008 summer conditions and the remainder were set to 18°C to represent average year 2100 summer sea surface temperature. The temperatures chosen were based on the IPCC (2007) A2 scenario of 4°C warming.

At each temperature, two microcosms were set at ambient CO<sub>2</sub> conditions (pH 8.07, CO<sub>2</sub> = ~380 ppm) and two were allocated to a high CO<sub>2</sub> treatment (pH 7.70, CO<sub>2</sub> = ~1,000 ppm). The CO<sub>2</sub> concentration, and hence pH level, and temperature was maintained in each microcosm using the CO<sub>2</sub>-mixing system described in Findlay et al. (2008b). pH (NBS scale, Mettler-Toledo pH metre), CO<sub>2</sub> (Licor LI-6262 CO<sub>2</sub> analyser), temperature and salinity (WTW LF197 combination temperature and salinity probe) were recorded weekly. Total alkalinity, dissolved inorganic carbon (DIC), bicarbonate (HCO<sub>3</sub><sup>-</sup>), carbonate (CO<sub>3</sub><sup>2-</sup>) and the saturation states for aragonite and calcite were all calculated from pH and pCO<sub>2</sub> values using CO2sys (Pierrot et al. 2006) with dissociation constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987) and KSO<sub>4</sub> using Dickson (1990).

The tidal regime of the microcosms was programmed weekly to coincide with the local Plymouth tide times. Salinity was 35 psu and seawater was delivered to the microcosms during the flood tide period at a rate of 10 ml min<sup>-1</sup>. Day length (Polylux XL 58 W) was maintained to within 15 min of the sunrise/sunset times for London, UK, roughly equating to a 14 h on/10 h off cycle throughout the experiment. Natural, filtered (10 µm), seawater was used and was replenished twice weekly to avoid salinity increases through evaporation. The experiment ran for 30 days and barnacles were fed every 2 days with a mixed diatom-flagellate diet at 15,000 cells ml<sup>-1</sup> (Shellfish Diet 1800<sup>®</sup>, Reed Mariculture).

Changes in barnacle abundance on each settlement panel were recorded every 2–3 days using a digital camera (FujiFilm A510 FinePix), which was maintained in consistent alignment using a stand. The photographic images were analysed (Image-Pro Plus v.4.5, Media Cybernetics) to estimate both growth and survival. Growth was estimated by measuring the diameter of the operculum of each barnacle on each panel at each time point. Growth rate was calculated as an average over the 30-day experimental period of all the barnacles in each microcosm. Barnacle survival was estimated from the images taken at the beginning and at the end of the experiment by counting living and dead individuals. Prior to photography, individuals were gently touched to check whether they were able to close their operculum and were classed as dead when the operculum either remained open or the shell was empty. Survival, recorded as a proportion, was square root arcsine transformed before analysis so that data were normally distributed.

The calcium carbonate composition of the shell was estimated by analysing the calcium (Ca<sup>2+</sup>) concentration as a proxy for changes in calcification or dissolution (Findlay et al. 2009a). The shells of five individuals were haphazardly selected from each microcosm at the end of the experiment, and the Ca<sup>2+</sup> concentration was measured using methods described in Spicer and Eriksson (2003); briefly, this involved dissolving the shells in 10% HNO<sub>3</sub> after drying and weighing, then using an atomic absorption spectrophotometer (Varian SpectrAA 50) to measure the Ca<sup>2+</sup> concentration. Ca<sup>2+</sup> content was converted to moles by dividing mass (g) by molecular mass (Ca = 40.08 g/mole) and report the units as µmol per mg of total shell sample. The proportion of Ca in the total shell was then square root arcsine transformed before statistical analysis, so that data were normally distributed.

The growth rate, transformed-survival and transformed-Ca data were tested for normality using a Kolmogorov–Smirnov test and for homogeneity of variances using Bartlett's test. Once these assumptions were confirmed, a two-way nested ANOVA was used to determine the effects of temperature, CO<sub>2</sub> or any interaction between them. Microcosms were nested, as a random factor, within CO<sub>2</sub> treatment ( $n = 2$ ). All statistical analyses were performed using Minitab<sup>®</sup> 15.1.0.0 (© 2006, Minitab Inc.).

## Results

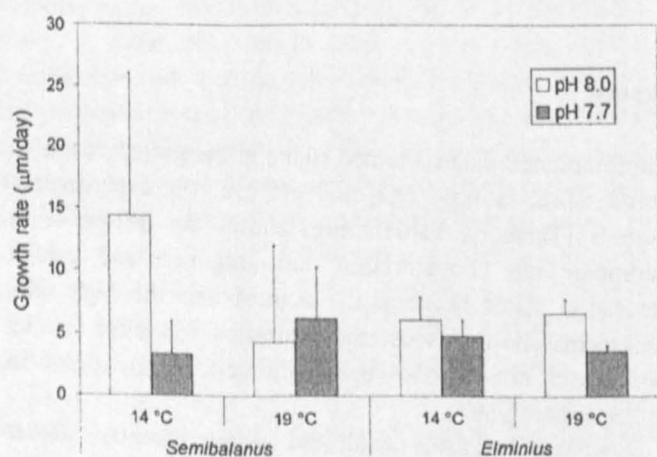
Environmental data remained stable at the pre-set levels of temperature, salinity, CO<sub>2</sub> and pH over the experimental periods (Table 1). Table 1 also shows the difference in carbonate ion concentration and aragonite and calcite saturation states between the control and the high CO<sub>2</sub> treatments and the calculated saturation states for calcite, which did not become undersaturated at any point in either experiment.

*Elminius modestus* exhibited a significantly slower growth rate with increasing CO<sub>2</sub> at the higher temperature (ANOVA,  $F_{df=1} = 19.15$ ,  $P = 0.012$ ). No other significant impacts of temperature and/or pH manipulation on rate of growth were observed in either barnacle species (Fig. 1). The mean growth rate of *Semibalanus balanoides* in the cold control CO<sub>2</sub> treatment was greater than in all other treatments (14 µm day<sup>-1</sup>) but was highly variable (SE = 5.96). This large variability in growth rates (Table 2) suggests that there may be some error in measuring the exact growth rates over this short period of time or that there is large natural variability within a population. A PERMANOVA test for difference between treatments over time also revealed no significant effects of CO<sub>2</sub> or temperature on growth (PERMANOVA pH × time  $F_{18,72} = 1.104$ ,  $P = 0.355$ ;

**Table 1** Environmental conditions in the treatments for the *Semibalanus balanoides* experiment and for the *Elminius modestus* experiment

	<i>Semibalanus balanoides</i>		<i>Elminius modestus</i>	
	Low temp.	High temp.	Low temp.	High temp.
Low CO <sub>2</sub>				
Salinity (psu)	35.74 ± 0.30	35.79 ± 0.37	34.5 ± 0.33	34.6 ± 0.34
Temp. (°C)	14.39 ± 0.25	19.77 ± 0.25	14.65 ± 0.23	19.65 ± 0.70
pH <sub>(NBS)</sub>	8.05 ± 0.027	8.07 ± 0.030	7.96 ± 0.022	7.98 ± 0.044
CO <sub>2</sub> (μatm)	409 ± 17	423 ± 23	413 ± 4	412 ± 9
Ω <sub>cal</sub>	2.4 ± 0.26	2.9 ± 0.31	1.9 ± 0.15	2.4 ± 0.51
High CO <sub>2</sub>				
Salinity (psu)	35.63 ± 0.31	35.74 ± 0.32	34.5 ± 0.30	34.7 ± 0.32
Temp. (°C)	14.70 ± 0.27	19.73 ± 0.26	14.95 ± 0.20	20.03 ± 1.05
pH <sub>(NBS)</sub>	7.73 ± 0.022	7.71 ± 0.025	7.73 ± 0.051	7.73 ± 0.036
CO <sub>2</sub> (μatm)	1,132 ± 43	1,109 ± 43	1,076 ± 34	1,075 ± 40
Ω <sub>cal</sub>	1.5 ± 0.14	1.5 ± 0.13	1.4 ± 0.34	1.5 ± 0.22
Difference from control				
ΔCO <sub>3</sub> <sup>2-</sup> (μmol kg <sup>-1</sup> )	-37 ± 14	-37 ± 13	-18 ± 16	-14 ± 10
ΔΩ <sub>cal</sub>	-0.89 ± 0.34	-0.87 ± 0.31	-0.44 ± 0.37	-0.34 ± 0.25
ΔΩ <sub>arg</sub>	-0.56 ± 0.22	-0.55 ± 0.20	-0.28 ± 0.24	-0.2 ± 0.16

Mean ± 95% confidence intervals are given for salinity, temperature (°C), pH, CO<sub>2</sub> and (μatm) calcite saturation state (Ω<sub>cal</sub>) in each treatment. Additionally, the difference in carbonate ion concentration (ΔCO<sub>3</sub><sup>2-</sup> μmol kg<sup>-1</sup>), calcite (ΔΩ<sub>cal</sub>) and aragonite saturation (ΔΩ<sub>arg</sub>) states between the high CO<sub>2</sub> treatment and the low CO<sub>2</sub> treatments are also provided



**Fig. 1** Growth rate of *Semibalanus balanoides* ( $n = 871$ – $1,456$ ) and *Elminius modestus* ( $n = 791$ – $1,053$ ) under different temperature (14 and 19°C) and pH conditions (pH 8.0 and pH 7.7). Error bars represent the 95% confidence interval

PERMANOVA temperature × time  $F_{18,72} = 1.074$ ,  $P = 0.395$ ).

The mean calcium content in shells of both *S. balanoides* and *E. modestus* decreased with increasing CO<sub>2</sub> (Fig. 2); while these reductions seemed much greater in *S. balanoides*, neither were actually significant (ANOVA, *S. balanoides*  $F_{df=1} = 2.53$ ,  $P = 0.253$ , *E. modestus*  $F_{df=1} = 1.13$ ,  $P = 0.295$ ). The calcium content in *S. balanoides* decreased with increasing temperature and was significantly lower in the high temperature control CO<sub>2</sub>

than in the low temperature control CO<sub>2</sub> treatment (ANOVA,  $F_{df=1} = 12.48$ ,  $P = 0.001$ ). Temperature had no significant effect on the calcium content of shells from *E. modestus*. No clear relationship between calcium content and mass of shell within any of the *S. balanoides* or *E. modestus* treatments could be demonstrated in view of the different size ranges of shell (Fig. 3). The net calcification rate (product of calcification and dissolution) of these barnacles can be estimated, as we know that all individuals were newly metamorphosed within a few days of the start of the experiment. The average net calcification rate (assuming an age between 30 and 35 days) is provided in Table 2, but *Elminius modestus* on average has a higher net calcification rate than *Semibalanus balanoides*.

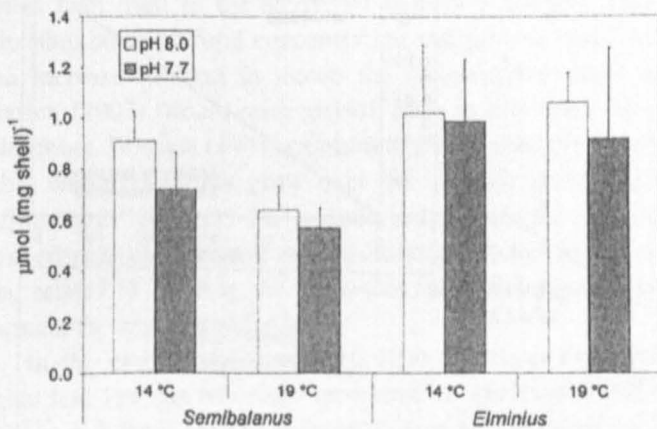
Increasing temperature decreased the survival of both *S. balanoides* (ANOVA,  $F_{df=1} = 102.69$ ,  $P = 0.010$ ) and *E. modestus* (ANOVA,  $F_{df=1} = 7.99$ ,  $P = 0.047$ ) (Fig. 4). There was no significant effect (or interaction with temperature) of CO<sub>2</sub> on survival of either species.

## Discussion

This study investigated the impacts of both elevated temperature and CO<sub>2</sub> (at levels predicted for 2100 based on the IPCC A2 scenario) on the developing post-larvae of two intertidal barnacle species that, as adults, are thought to be

**Table 2** Abundance, size, growth rate and net calcification rate (mean  $\pm$  standard deviation) and crowding data for each species (*Elminius modestus* (*E.m.*) and *Semibalanus balanoides* (*S.b.*)) in each treatment (pH 8.0 and pH 7.7, temperature 14 and 19°C)

Treatment	Species	Time point	Count	Mean size (mm)	Size ( $\sigma^2$ )	Mean growth rate $\pm$ sd ( $\mu\text{m day}^{-1}$ )	Mean NC ( $\mu\text{mol (mg shell)}^{-1} \text{ day}^{-1}$ )	Crowding (ind. $\text{cm}^{-2}$ )
pH 8.0, 14°C	<i>E.m.</i>	Initial	210	0.46	0.315			0.70
		Final	162	0.65	0.675	6.14 $\pm$ 1.62	0.032 $\pm$ 0.0035	0.54
	<i>S.b.</i>	Initial	355	0.59	0.116			1.78
		Final	316	0.95	0.095	14.35 $\pm$ 8.43	0.028 $\pm$ 0.0031	1.68
pH 7.7, 14°C	<i>E.m.</i>	Initial	298	0.50	0.063			0.99
		Final	240	0.65	0.284	4.92 $\pm$ 1.07	0.031 $\pm$ 0.0033	0.80
	<i>S.b.</i>	Initial	190	0.61	0.116			2.00
		Final	150	0.68	0.114	2.94 $\pm$ 1.95	0.022 $\pm$ 0.0024	1.58
pH 8.0, 19°C	<i>E.m.</i>	Initial	252	0.49	0.437			0.84
		Final	187	0.69	0.716	6.68 $\pm$ 1.01	0.035 $\pm$ 0.0036	0.62
	<i>S.b.</i>	Initial	594	0.85	0.133			3.96
		Final	320	0.96	0.004	4.39 $\pm$ 5.51	0.019 $\pm$ 0.0021	3.43
pH 7.7, 19°C	<i>E.m.</i>	Initial	293	0.48	0.088			0.98
		Final	202	0.59	0.088	3.64 $\pm$ 0.41	0.031 $\pm$ 0.0031	0.67
	<i>S.b.</i>	Initial	317	0.86	0.117			1.92
		Final	85	1.02	0.052	6.23 $\pm$ 2.85	0.018 $\pm$ 0.0019	1.70

**Fig. 2** Calcium as micromoles per mg of total shell sample of *Semibalanus balanoides* ( $n = 40$ ) and *Elminius modestus* ( $n = 40$ ) under different temperature (14 and 19°C) and pH conditions (pH 8.0 and pH 7.7). Error bars represent the 95% confidence interval

relatively tolerant to changes in abiotic conditions (Newell 1979). Temperature appears to be the overriding factor determining survival with no additional impact from elevated  $\text{CO}_2$ . In common with many recent investigations (e.g., Ellis et al. 2009; Arnold et al. 2009; Hauton et al. 2009; McDonald et al. 2009), the impacts of  $\text{CO}_2$  were more subtle and sub-lethal. Both elevated temperature and  $\text{CO}_2$  affected growth and shell development, although the responses were not consistent between the species. In the following, we discuss the relative impacts of temperature and  $\text{CO}_2$  on *Semibalanus balanoides* and *Elminius modestus*; comparisons with previous studies of environmental stressors, including ocean acidification, enable the results

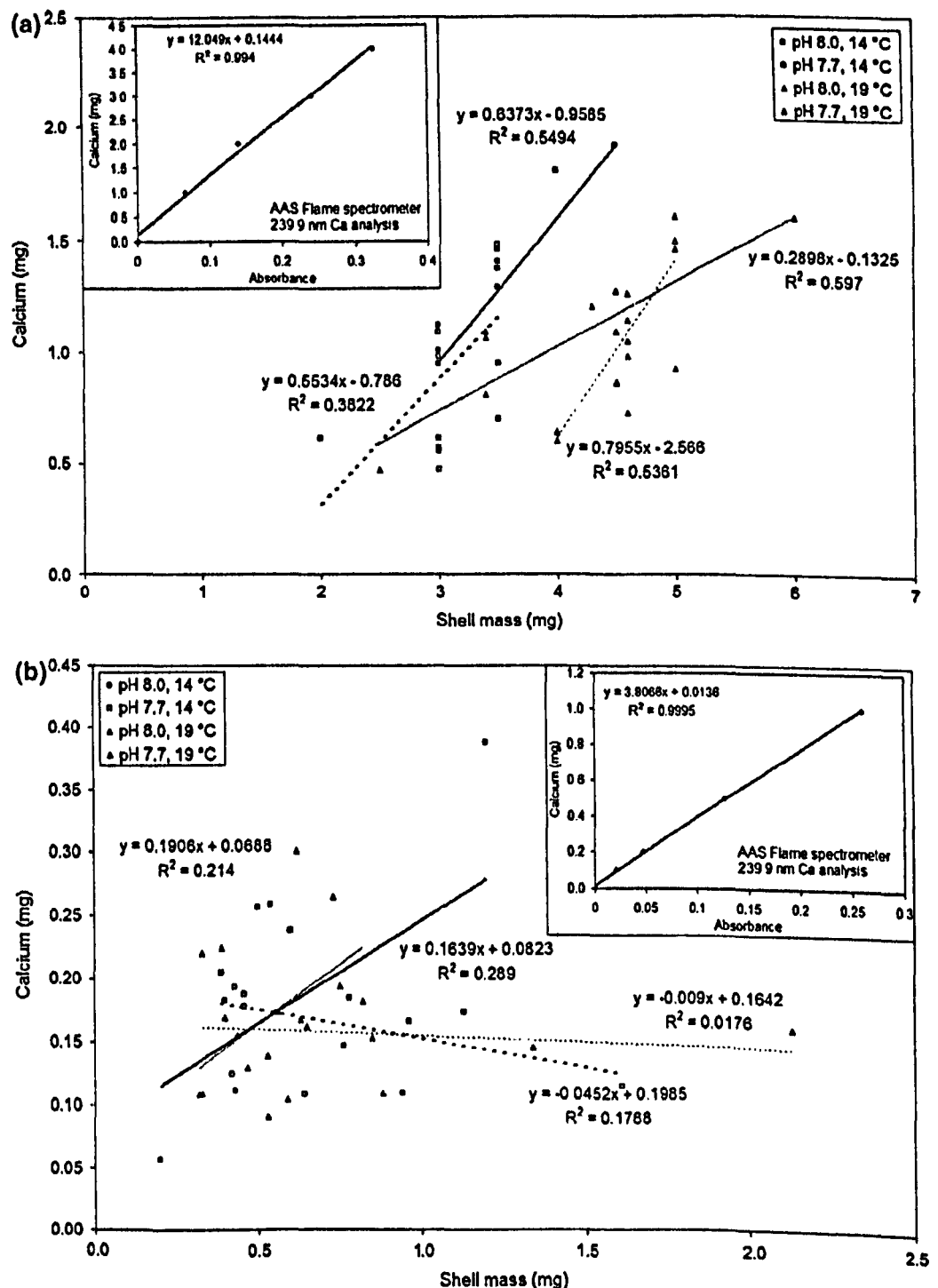
presented here to be placed in the context of life-history variability.

#### Response to elevated temperature and $\text{CO}_2$

The newly settled *Semibalanus balanoides* post-larvae investigated in this study had significantly less calcium in their shells when kept under elevated temperature than when kept under ambient conditions. This lower calcium content could have resulted either indirectly from physiological changes or directly from temperature control of the mineralogy (Freitas et al. 2008). However, temperature had no impact on the calcium content of *Elminius modestus* shells suggesting that the nature or extent by which shell mineralogy is biologically controlled differs between the two barnacle species. Raising  $\text{CO}_2$  levels results in the lowering of saturation states for calcium carbonate minerals, such as calcite, in seawater, and this causes increased corrosion of calcitic shells. However, at no point in this experiment did calcite become undersaturated, although it was lowered to  $\Omega_{\text{cal}} = \sim 1.5$ . It seems more likely that any impacts on shell mineralogy from elevated  $\text{CO}_2$  came from indirectly affecting rates of associated physiological processes; a similar response was found in larval lobsters under high  $\text{CO}_2$  conditions (Arnold et al. 2009).

Studies on barnacle shell structure and mineralogy suggests that <3% of the total shell (by mass) is made of the organic matrix, while the remaining material is formed of minerals, predominantly calcite (Barnes et al. 1976; Bourget 1987). Here, we can estimate calcium carbonate

**Fig. 3** Mass of calcium present in the shell (mg) in relationship to total shell mass (mg) for **a** *Semibalanus balanoides* and **b** *Elminius modestus* for each treatment (1) pH = 8.0, temperature = 14°C, (2) pH = 7.7, temperature = 14°C, (3) pH = 8.0, temperature = 19°C and (4) pH = 7.7, temperature = 19°C. Linear regression lines are shown for each treatment. Inserts in each figure show the calibration curves for each set of samples



that makes up about 93% of the total shell (by mass) in control *Semibalanus balanoides* and 95% in control *Elminius modestus*. These values should not be taken as exact because of the large variability surrounding the calcium measurements, most likely caused by the small mass of shell available for analysis.

*Semibalanus balanoides* post-larval growth rates under control conditions were similar to rates found in the field (10–20  $\mu\text{m day}^{-1}$ ; Jarrett 2003) but lower than other laboratory experiments (30–40  $\mu\text{m day}^{-1}$ ; Jarrett 2003).

There are several differences between the laboratory experiment of Jarrett (2003) that might explain the relatively slow growth found in this study: (1) the juveniles used in Jarrett (2003) were continuously submerged, whereas here, they were grown in an intertidal regime. An intertidal regime means that the barnacles feed for less than 12 h a day, which immediately explains a 50% difference in growth rates if feeding under continuously submerged conditions is not restricted; (2) the field observations of Jarrett (2003) were made on intertidal juveniles and are

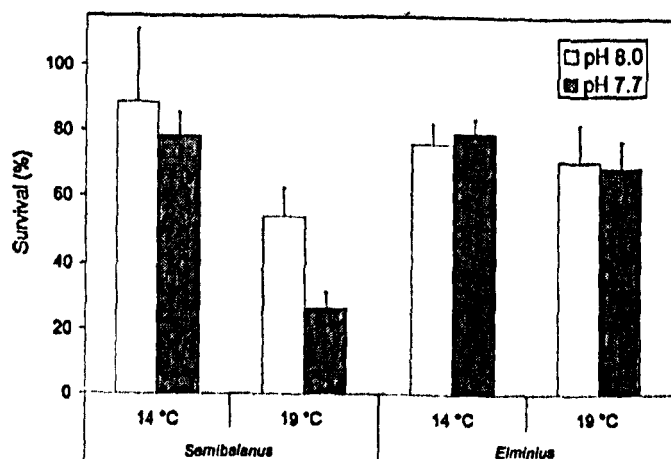


Fig. 4 Percentage survival of *Semibalanus balanoides* and *Elminius modestus* under different temperature (14 and 19°C) and pH conditions (pH 8.0 and pH 7.7). Error bars represent the 95% confidence interval

more directly comparable to the tidal regime in this present study; (3) in the laboratory experiment, Jarrett (2003) used over three times more algal cells per ml to feed the juveniles than used in the study. If there is a constant relationship between food concentration and growth rate, then an increase in food to match the concentration used in Jarrett (2003) should raise growth rates in this study. Furthermore, Bourget (1977) examined growth checks to show that barnacles do not grow over the low tide period. The differences between the growth rates recorded in this experiment and those of other laboratory studies appear to be related to keeping the barnacles in a tidal regime that simulates natural conditions.

In the experiments described here, *Elminius modestus* also had low growth rates compared to previous studies. Crisp and Patel (1961) showed that under intertidal field conditions, *E. modestus* can reach about 4.2 mm in 56 days ( $\sim 70 \mu\text{m day}^{-1}$  growth rate) at temperatures between 15 and 21°C, although the rate of development varies with crowding and again with tidal height (Crisp and Patel 1961, Table 2). The lower growth rates observed in this study are most likely caused by a low food supply, which although the same as for *S. balanoides*, that was possibly inadequate for the faster developing *E. modestus*.

As a consequence of the high variability in the *S. balanoides* growth rates, there were no significant differences in growth rate between the various experimental treatments. This variability may have arisen from difficulties of accurately measuring small increments in growth over a short time period or may be indicative of high variation naturally occurring within populations (Wethey 1983). *E. modestus* post-larvae did appear to have some reduced growth under elevated  $\text{CO}_2$  conditions but were less impacted by elevated temperature. Such findings support

those of Harms (1986) who showed that *E. modestus* larvae reared in salinity extremes had reduced growth rates but were less impacted by temperature between 9 and 19°C.

Metamorphosing cyprids may not have the ability to compensate for environmental stressors in the same way that adults do (Findlay et al. 2009b; Wood et al. 2008), as there may be mechanistic differences between metamorphic shell production and long-term shell production. In particular, metamorphosing cyprids are likely to have different metabolic priorities, as they are in immediate danger of desiccation and hence must lay down their shell rapidly (Foster 1971). Adults will spend more energy on maintenance but high metabolic priority is also given to reproduction (Wu and Levings 1978). Larval metamorphosis is an energetically expensive process during which cyprids may consume up to 30% of their own body organic carbon, primarily through breakdown of lipids but also proteins (Lucas et al. 1979). The extent of a cyprid's energy reserve has been shown to correlate with its survival to the juvenile stage (Thiyagarajan et al. 2005). In our experiment, *S. balanoides* did not appear to have altered growth rates but did have lower net calcification rates under elevated temperature and  $\text{CO}_2$ . Such changes may have diverted energy from metamorphosis and resulted in reduced survival. *E. modestus* growth was lowered under elevated  $\text{CO}_2$  but the net calcification rate appeared to be less impacted. In this species, it could be speculated that the energy available was not adequate for growth, and essentially, the individuals reduced its metabolic processes causing little change in shell development but survival remained relatively high.

The differences in observed responses between *Semibalanus balanoides* and *Elminius modestus* may well reflect the contrasting biogeography/niche requirements of the two species. Although *S. balanoides* and *E. modestus* may overlap in their distribution, their environmental preferences are significantly different; *S. balanoides* is a cold-water species, while *E. modestus* is from warmer waters. In the populations used in the current study, *S. balanoides* is already nearing its southern geographic limit, which is likely set by high temperature (Barnes 1958). In the summer, SST in southwest UK, at the southern range edge, is about 15°C on average but ranges from 12 to 19°C. Hence, these animals may be more susceptible to warming than animals from populations in the middle of this species' range. Indeed, *S. balanoides* appeared to be more affected in the current experiment than *E. modestus*, particularly under elevated temperature. As a warm-water species, *E. modestus* are commonly found in areas that experience sea temperatures greater than 19°C. Harms (1986) recorded that New Zealand sea temperatures range between 15 and 21°C, and hence, it might be expected that the experimental temperatures are within the range to which *E. modestus* as a species is adapted.

Harms (1986) discovered that highest mortality of *E. modestus* larvae occurred at salinity extremes ( $>40$  and  $<20$  psu), while temperature impacts were most evident at the salinity range of 30–40 psu. In a similar manner, the *E. modestus* survival response in this study was most impacted by temperature while at nominal values of  $\text{CO}_2/\text{pH}$ . The temperature effect on survival of *S. balanoides* seen in the current study, however, was more similar to the survival response observed by Harms (1986) for *E. modestus* when held under temperature extremes (at temperatures  $<9$  or  $>20^\circ\text{C}$ ). Once again, this indicates potential differences in the susceptibility of organism living at the edge of their natural tolerance range and those living towards the centre. Such observations highlight the difficulties in making population or species comparisons (Spicer and Gaston 1999) as much research is still needed to understand the variability of responses within species.

#### Life-history variability

Connell (1961) reviewed the causes of mortality for settled *S. balanoides* cyprids and post-larvae between and within different locations (height up shore), shores and years. He concluded that if cyprids were exposed to hot or sunny days, they were less likely to survive; but once the cyprids had metamorphosed, the highest mortalities occurred when the individuals were submerged, largely as a result of abrasion during storm events. Weaker shells, through reduced calcification or increased corrosion in lower pH, or indeed higher temperature, seawater may increase mortality resulting from abrasion and predation.

During the summer period, it is well known that either extreme events or an accumulation of hot days will cause mortality (Kendall et al. 1985) because post-larvae are susceptible to desiccation in just a few hours, e.g., at  $18^\circ\text{C}$ , the mean lethal time is 6 h for *S. balanoides* and 7 h for *E. modestus* (Foster 1971). In our experiments, the microcosms recreated relatively calm conditions with high humidity ( $>80\%$  relative humidity), and so we must consider a different explanation for increased mortality, such as changes in metabolism and resource allocation.

The different growth response of *E. modestus* compared to the *S. balanoides* supports the hypothesis that differences in life-history strategies influence the ability to populate space on the shore. *E. modestus* develops rapidly and reproduces within months of settlement, requiring a continuous supply of food throughout the year. *S. balanoides* is pre-adapted to conditions of discontinuous food supply and hence has slower growth and reproduces just once each year (Rainbow 1984). In our experiments, a combination of low food supply, high  $\text{CO}_2$  and elevated temperature prevented *E. modestus* from having rapid growth.

Growth of barnacles varies with crowding of both post-larvae and adults and with both the quality and the quantity of their food supply. Neither barnacle in this study was impacted by crowding, as densities were  $<2$  individuals  $\text{cm}^{-2}$ . However, food supply may have prevented *E. modestus* from attaining higher growth rates, although long-term records of phytoplankton abundance near Plymouth, UK suggest that total concentrations of phytoplankton peak at about  $12,000$  cells  $\text{ml}^{-1}$  (phytoplankton data form part of the Western Channel Observatory funded under the NERC Oceans 2025 program Theme 10 (sustained observations), data analysed by C. Widdicombe), which is a much lower concentration per barnacle, than used in these experiment. Sanford et al. (1994) show that flow, food-supply and temperature affect the feeding rate of *S. balanoides*. Sanford et al. (1994)'s low flow regimes appears representative of the microcosm laboratory conditions found in this present study and show that temperature reduces feeding in both adults and juveniles significantly from  $15$  to  $20^\circ\text{C}$  (the range used in this experiment). These findings are consistent with the results presented here that growth rate may be impacted by temperatures above  $15^\circ\text{C}$ .

The barnacles used in these experiments were subjected to a tidal regime that simulates an overcast, windless, low wave exposure day on the mid-rocky intertidal. Understanding the environment from which the organisms are taken is of fundamental importance when interpreting results and attempting to generalise relationships. *E. modestus* and *S. balanoides* were placed in exactly the same conditions (representative of the field) and have shown different responses to elevated temperature and  $\text{CO}_2$ . Nevertheless, we would emphasise that extrapolating these results to reach conclusions on populations or community dynamics must be carried out with caution because of the extremely variable physical nature of the area of the intertidal zone that is occupied by the two barnacle species.

Ocean acidification studies on early life stages thus far have primarily been carried out on planktonic larvae prior to settlement and have shown that the development of calcareous skeletons, growth and survival may be adversely affected (e.g., Kurihara and Shirayama 2004; Kurihara et al. 2007; Dupont et al. 2008). Such a combination of impacts has led to the conclusion that larvae may be more susceptible to ocean acidification than adults (Dupont and Thorndyke 2009). One previous study investigated benthic larval stages (Ellis et al. 2009), and this showed subtle impacts on shell morphology and development, which could have more serious consequences for later life. McDonald et al. (2009) showed that ocean acidification had no impact on the early life stages of the barnacle *Amphibalanus amphitrite*, a tropical species,

although calcification increased in the post-larvae and adults. If the results from the current study are considered in terms of future ocean conditions, they suggest that a decrease by 0.4  $\text{pH}_{(\text{nb})}$  units alone (year 2100 IPCC (2007) A2 emissions scenario) would not be sufficient to have a direct impact on the survival of barnacles during the first few days of their intertidal life. However, if such changes in pH occur in conjunction with changes in other environmental parameters, such as a 4–5°C increase in temperature, it is highly probable that significant changes to the biology of these organisms will ensue. These changes could be sufficient to affect the abundance of the adult population, even though larger, older animals are far better adapted to living in a fluctuating environment.

In interpreting the results of this study, it must also be considered that we investigated larvae and post-larvae that came from populations unaffected by either elevated temperature or elevated  $\text{CO}_2$ . Different responses may be found if larvae came from similarly exposed parents, particularly with relation to the amount of energy invested in reproduction. However, only one study so far has investigated exposure of high  $\text{CO}_2$  on more than one generation (Kurihara and Ishimatsu 2008), demonstrating that copepods appeared tolerant to high  $\text{CO}_2$  through two generations.

In conclusion, post-larvae of the barnacles *Semibalanus balanoides* and *Elminius modestus* showed changes in shell structure and growth, respectively, under conditions of elevated temperature and  $\text{CO}_2$ . *S. balanoides* were able to continue growth but were not able to maintain the mineral structure of their calcified shells under elevated temperature and  $\text{CO}_2$  conditions, while *E. modestus* showed an opposite response by maintaining the mineral structure of their calcified shells but with limited growth rates. This contrast could result from either different physiological strategies in the two species or possibly different levels of tolerance associated with populations from different parts of their geographic distribution. In general, these post-larval intertidal barnacles had lowered survival in elevated temperature and  $\text{CO}_2$  conditions, which likely results from changes in energy consumption at a period in their life history when they are non-feeding. These subtle changes may be evidence that rates of physiological processes have altered corresponding with a shift above their optimal rates in the case of *S. balanoides* or more towards their optimum in the case of *E. modestus*.

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## Relative influences of ocean acidification and temperature on intertidal barnacle post-larvae at the northern edge of their geographic distribution

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## ABSTRACT

The Arctic Ocean and its associated ecosystems face numerous challenges over the coming century. Increasing atmospheric CO<sub>2</sub> is causing increasing warming and ice melting as well as a concomitant change in ocean chemistry ("ocean acidification"). As temperature increases it is expected that many temperate species will expand their geographic distribution northwards to follow this thermal shift; however with the addition of ocean acidification this transition may not be so straightforward. Here we investigate the potential impacts of ocean acidification and climate change on populations of an intertidal species, in this case the barnacle *Semibalanus balanoides*, at the northern edge of its range. Growth and development of metamorphosing post-larvae were negatively impacted at lower pH (pH 7.7) compared to the control (pH 8.1) but were not affected by elevated temperature (+4 °C). The mineral composition of the shells did not alter under any of the treatments. The combination of reduced growth and maintained mineral content suggests that there may have been a change in the energetic balance of the exposed animals. In undersaturated conditions more mineral is expected to dissolve from the shell and hence more energy would be required to maintain the mineral integrity. Any energy that would normally be invested into growth could be reallocated and hence organisms growing in lowered pH grow slower and end up smaller than individuals grown in higher pH conditions. The idea of reallocation of resources under different conditions of pH requires further investigation. However, there could be long-term implications on the fitness of these barnacles, which in turn may prevent them from successfully colonising new areas.

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## 1. Introduction

Increasing atmospheric CO<sub>2</sub> concentrations are causing a concomitant change in global temperatures and ocean chemistry (Levitus et al., 2005; Orr et al., 2005; Mackenzie and Schiedek, 2007). Both these changes are now well documented in ocean time-series such as the Hawaiian Ocean Time-series (HOT, Brix et al., 2004), the European Station for Time-series in the Ocean, Canary Islands (ESTOC, Santana-Casiano et al., 2007) and the Bermuda Atlantic Time-Series (BATS, Bates and Peters, 2007). Observations of decreasing pH have also been found in upwelling (Feely et al., 2008) and coastal waters (Wootton et al., 2008) in the NE Pacific and in the Iceland Sea (Olafsson et al., 2009). Global sea surface temperature has increased by 0.76 °C over the last century (IPCC, 2007) and is projected to increase by up to 7 °C under the most extreme CO<sub>2</sub> emissions scenarios (Pope et al., 2008); while the pH of the

oceans has decreased by 0.1 unit since the industrial revolution (Kleypas et al., 2006) and is expected to decrease by 0.4–0.5 units by the year 2100 under similar CO<sub>2</sub> emissions scenarios (IPCC, 2007). The Arctic Ocean appears to be particularly vulnerable to change. Increasing temperatures are causing ocean warming and sea ice melting (IPCC, 2007), while the colder seawater temperatures facilitate greater CO<sub>2</sub> absorption thereby increasing the rate of ocean acidification and carbonate ion decline (Steinacher et al., 2009). The Arctic Ocean is predicted to first become undersaturated with respect to aragonite as early as 2040, with undersaturation of calcite occurring a couple of decades later (Steinacher et al., 2009).

Intertidal species may not be particularly sensitive to environmental fluctuations because of the steep gradients of abiotic factors that occur in intertidal habitats (Southward et al., 1995). However, the effect of climatic variables should be greatest where a range edge is set mainly by physical factors (Pearson et al., 2009). For example, the northern limit for the intertidal barnacle *Semibalanus balanoides* is closely paralleled by the summer limits of pack ice (Barnes, 1957); hence populations can be found on the coasts of northern Norway and Svalbard in the high Arctic (Barnes, 1999).

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Unfortunately, perhaps due to logistic constraints, intertidal studies to date have concentrated mainly on temperate areas, with little attention paid to polar regions. The Arctic intertidal zone has received little attention with respect to understanding species distribution in relation to climate change, despite shifts in ecological dynamics being observed in the pelagic realm (Greene et al., 2008). A study on the intertidal macroorganism distribution and biomass of Svalbard (Weslawski et al., 1993) suggests that *Fucus-Semibalanus* (previously *Balanus*) communities were the richest in terms of diversity and biomass of all the Arctic intertidal communities that they investigated. This assemblage occurred on 8% of the investigated coastline (South Spitsbergen National Park, South East Svalbard National Reserve and Isfjorden), but it is also known to occur further north along the west coast of Spitsbergen, where warm Atlantic water maintains ice-free conditions in summer (Kukliński and Barnes, 2008).

As the Arctic warms, a greater area of rocky shore will become available for colonisation by boreal and temperate species extending their ranges northwards. The west coast of Spitsbergen provides an ideal study site to investigate how a key space occupier responds to warming and the additional stress of ocean acidification. For example, areas that were previously inaccessible to species like the barnacle *Semibalanus balanoides* might, in the future, become available if larvae are able to disperse to these new locations and post-larvae are able to survive to recruit to form new populations.

This study investigated the potential impacts of ocean acidification and temperature on populations of an intertidal species, in this case the barnacle *Semibalanus balanoides*, at the northern edge of its range. This was carried out by assessing the survival, growth and development, as well as shell mineralogy, of post-larval barnacles in the Arctic. Post-larval mortality plays an important role in population abundance (Rainbow, 1984); and poor development in the early stages can have impacts later on in the life history of barnacles (Jarrett, 2003). The results from this study are also compared to results from a similar study on barnacles at their southerly range edge (Findlay et al., 2009), as well as the only other study investigating the impact of ocean acidification on barnacles (McDonald et al., 2009).

*Semibalanus balanoides* is a boreo-arctic species and shows a marked seasonal response in feeding rate (Ritz and Crisp, 1970), moulting (Crisp and Patel, 1960), and reproduction and development (Crisp, 1956, 1962), all of which peak during the spring and summer. Cyprid larvae settle onto the shore in the spring and summer (mid- to late-summer in north of the species range, Barnes, 1957) and metamorphose into post-larvae when they first lay down a calcified shell (Bourget and Crisp, 1975). The average metamorphosis time occurs in 1.5 days (Connell, 1961). These organisms only begin feeding after the shell has been formed and full metamorphosis has

taken place, this takes between 4 and 6 days (Lucas et al., 1979). Fully feeding juveniles then grow and develop, maturing the following year and living up to 5–6 years (Rainbow, 1984). In the arctic there are few spatial competitors with *S. balanoides* (Weslawski et al., 1993). Persistence of populations at the northern limit are believed to be dependent on the time period available for naupliar development, cyprid settlement and spat growth between the melting of winter ice associated with the diatom bloom and the reformation of the ice in autumn (Barnes, 1957).

## 2. Methods

### 2.1. Collection site

Kongsfjorden, on the west coast of Spitsbergen (79° 55' N, 11° 56' E), is a fjord system into which several glaciers feed. It is open to the North Atlantic on its western edge with the largest glacier flowing into the fjord at its eastern edge (see Svendsen et al., 2002 for details of physical environment). The shoreline is typically scoured by floating ice during the summer months and is overlain by fast ice during winter (Hop et al., 2002; Kukliński and Barnes, 2008). Seasonal temperatures range from  $-1.8$  °C to  $4$  °C at the sea surface (SST) and from  $-15$  °C to  $5$  °C in the air (still air) from winter to summer respectively (Svendsen et al., 2002). In August 2008 mean SST was  $4.5$  °C and mean salinity was 34.5. Only one set of carbonate system measures have been taken from water in this fjord and these show measured pH = 8.12 and calculated  $p\text{CO}_2 = 320$   $\mu\text{atm}$  during May 2008 (Comeau et al., 2009). Temperature in the fjord during August 2008 was  $4.5$  °C. We therefore assume that the control seawater used through the experiment system was near natural fjord levels, although seawater was pumped in from 80 m depth (see details of the set up below) and may therefore contain different levels of anthropogenic  $\text{CO}_2$ .

### 2.2. $\text{CO}_2$ system set up

The experimental system was constructed using six header tanks (vol. = 200 l) housed in a large experimental room. Each of the six header tanks was used for one of each of the pH and temperature levels. The nominal pH levels ( $\text{pH}_{\text{NBS}}$ ) were pH 8.1 (control, ambient  $\text{CO}_2 \sim 380$  ppm), pH 7.7 (mid  $\text{CO}_2 \sim 1000$  ppm, year 2100 prediction) and pH 7.3 (high  $\text{CO}_2 \sim 3000$  ppm, year 2300 prediction by Caldeira and Wickett (2003)) (Table 1). Seawater was pumped, via a pair of filters (100 and then 20  $\mu\text{m}$ ), into two large reservoir tanks direct from the fjord (from 80 m depth), which then fed into each of the header tanks. In the reservoir tanks the temperature of the seawater was adjusted to maintain two constant temperatures throughout the experiment. The nominal temperatures were  $4.8$  °C and  $8.5$  °C.

**Table 1**

Environmental conditions in the mesocosms (mean  $\pm$  standard deviation), salinity, temperature (°C), pH and DIC were measured every 2–3 days ( $n = 7$ ).  $p\text{CO}_2$  ( $\mu\text{atm}$ ), total alkalinity (TA), bicarbonate, carbonate, and the saturation states for calcite ( $\Omega_{\text{cal}}$ ) and aragonite ( $\Omega_{\text{arg}}$ ) were calculated from pH and DIC using CO2sys with the solubility constant of Mehrbach et al. (1973) refit by Dickson and Millero (1987).

	Low Temperature			High temperature		
	pH 8.1	pH 7.7	pH 7.3	pH 8.1	pH 7.7	pH 7.3
Temp (°C)	5.68 $\pm$ 0.82	5.17 $\pm$ 0.43	4.92 $\pm$ 0.44	9.82 $\pm$ 0.39	9.55 $\pm$ 0.26	9.40 $\pm$ 0.32
Salinity	33.7 $\pm$ 0.08	33.7 $\pm$ 0.05	33.5 $\pm$ 0.18	33.7 $\pm$ 0.04	33.7 $\pm$ 0.04	33.6 $\pm$ 0.28
pH	8.12 $\pm$ 0.032	7.68 $\pm$ 0.031	7.35 $\pm$ 0.054	8.14 $\pm$ 0.022	7.71 $\pm$ 0.050	7.36 $\pm$ 0.043
$p\text{CO}_2$	352 $\pm$ 27.6	1086 $\pm$ 94.9	2429 $\pm$ 336	343 $\pm$ 13	1060 $\pm$ 133	2448 $\pm$ 277
DIC ( $\mu\text{mol kg}^{-1}$ )	1800 $\pm$ 81	1983 $\pm$ 75	2116 $\pm$ 75	1783 $\pm$ 41	1975 $\pm$ 61	2092 $\pm$ 49
TA ( $\mu\text{mol kg}^{-1}$ )	1924 $\pm$ 12	1982 $\pm$ 73	2016 $\pm$ 59	1937 $\pm$ 48	1997 $\pm$ 57.1	2014 $\pm$ 39
$\text{HCO}_3^-$ ( $\mu\text{mol kg}^{-1}$ )	1693 $\pm$ 5.4	1891 $\pm$ 71.6	1972 $\pm$ 62	1661 $\pm$ 36.0	1883 $\pm$ 58.5	1961 $\pm$ 41.0
$\text{CO}_3^{2-}$ ( $\mu\text{mol kg}^{-1}$ )	89.3 $\pm$ 6.8	35.2 $\pm$ 2.2	17.0 $\pm$ 1.9	107.2 $\pm$ 6.7	44.5 $\pm$ 4.8	20.6 $\pm$ 1.7
$\Omega_{\text{cal}}$	2.14 $\pm$ 0.16	0.85 $\pm$ 0.05	0.41 $\pm$ 0.04	2.57 $\pm$ 0.16	1.07 $\pm$ 0.11	0.50 $\pm$ 0.04
$\Omega_{\text{arg}}$	1.35 $\pm$ 0.10	0.53 $\pm$ 0.03	0.26 $\pm$ 0.03	1.63 $\pm$ 0.10	0.68 $\pm$ 0.07	0.31 $\pm$ 0.03

The nominal pH values were used to control the CO<sub>2</sub> bubbling in each header tank. A pH controller (Aqua Digital pH-201, accuracy  $\pm 0.1\% + 0.02$ ) monitored the pH and controlled the CO<sub>2</sub> bubbling, via a solenoid valve feedback system, to a CO<sub>2</sub> gas cylinder (CP grade 99.95% carbon dioxide) in a near-identical set up described by Widdicombe and Needham (2007). Natural air (CO<sub>2</sub> ~380 ppm) was additionally bubbled through each of the header tanks to maintain the same starting point of CO<sub>2</sub> across all treatments, therefore buffering the system against natural fluctuations of seawater pCO<sub>2</sub>. pH (NBS scale, Mettler-Toledo pH meter), dissolved inorganic carbon (DIC) (Ciba-Corning 965D Total CO<sub>2</sub> Analyser, Olympic Analytical Service), CO<sub>2</sub> (Licor LI-6262 CO<sub>2</sub> analyser), temperature and salinity (WTW LF197 combination temperature and salinity probe) were recorded every 2–3 days. Total alkalinity, bicarbonate (HCO<sub>3</sub><sup>-</sup>), carbonate (CO<sub>3</sub><sup>2-</sup>), and the saturation states for aragonite and calcite were all calculated from pH and DIC using CO<sub>2</sub>sys (Pierrot et al., 2006) with dissociation constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987) and KSO<sub>4</sub> using Dickson (1990).

### 2.3. Animal collection and experimental set up

*Semibalanus balanoides* were collected on chips taken off rocks in the mid-shore at Ny Ålesund, Svalbard (78°55'N, 11°56'E), on the 4th Aug 2008. Ny Ålesund is located roughly 10 km from the entrance of the fjord along the southern shore of Kongsfjorden. On collection, the rock chips contained a mixed age population of juvenile barnacles ranging from newly settled cyprids to week-old post-larvae but there were no adults. Here we use the term post-larvae to distinguish from older juveniles, as we want to focus on the early life stage from metamorphosing cyprid through to commencement of feeding. We class post-larvae as metamorphosed cyprids and early juveniles with an operculum length up to 1.5 mm.

Three rock chips were placed into one of twelve microcosms (30 × 15 × 20 cm) so that each microcosm contained in excess of 200 individual barnacles. The microcosms allow the mimicking of diurnal tidal exposure while controlling temperature and CO<sub>2</sub> and were identical to those described by Findlay et al. (2008). All microcosms were kept in a controlled temperature experimental room. The tidal regime of the microcosms was programmed weekly to coincide with the local tide times. Seawater (salinity = 35) was supplied to the microcosms from the header tanks during the flood tide at a rate of 10 ml min<sup>-1</sup> and ebbing seawater ran to waste. Light was maintained continuously to replicate the 24 h daylight over this period. The experiment ran for 20 days and barnacles were fed every two days with a mixed diatom-flagellate diet at 15,000 cells ml<sup>-1</sup> (Shellfish Diet 1800®, Reed Mariculture).

Changes in barnacle abundance on each rock chip were recorded every five days using a digital camera (Canon EOS 400D) which was maintained in consistent alignment using a stand. The photographic images were analysed (Image-Pro Plus v.4.5, Media Cybernetics) to estimate both growth and survival. Growth was

estimated by measuring the diameter of the operculum (following Wetthey (1983)) of each barnacle on each panel at each time point. Growth rate was calculated as an average over the 20 day experimental period of all the barnacles in each microcosm. Barnacle survival was estimated from the images taken at the beginning and the end of the experiment by counting living and dead individuals. Prior to photography individuals were gently touched to check whether they were able to close their operculum and were classed as dead when the operculum either remained open or the shell was empty. Survival, recorded as a proportion of the initial number of individuals, was square root arcsine transformed before analysis so that data were normally distributed.

The mineral content of the shell was calculated by analysing the calcium (Ca<sup>2+</sup>) and magnesium (Mg<sup>2+</sup>) concentrations. The shells of ten individuals of similar size (total shell mass of individuals ranged between 3.355 and 3.365 mg) were haphazardly selected from each microcosm at the end of the experiment and the concentrations of both divalent ions were measured using methods described in Spicer and Eriksson (2003); briefly this involved dissolving the shells in 10% nitric acid after drying and weighing, then using Inductively Coupled Plasma (ICP) optical emissions spectrometer (Varian 725-ES) to measure Ca<sup>2+</sup> and Mg<sup>2+</sup> simultaneously. The proportion of each ion in the shell was calculated from the mass of the shell and volume of acid used in the digest (ion [mg]/total shell mass [mg]). The proportion of each ion in the total shell was then square root arcsine transformed before statistical analysis so that data were normally distributed. Ca<sup>2+</sup> content was also converted to moles by dividing mass (g) by molecular mass (Ca = 40.08 g/mol) and report the units as μmol per mg of total shell sample, which could then be used to calculate net calcification rate (μmol (g shell)<sup>-1</sup> d<sup>-1</sup>).

The average growth rate, transformed-survival and transformed-ion data were tested for normality using a Kolmogorov-Smirnov test and for homogeneity of variances using Levene's test. Once these assumptions were confirmed a two-way nested ANOVA was used to determine the effects of temperature, CO<sub>2</sub> or any interaction between them. Microcosms were nested, as a random factor, within CO<sub>2</sub> treatment ( $n = 2$ ). Statistical analysis was performed using Minitab® 15.1.0.0 (© 2006, Minitab Inc.). PERMANOVA (Primer-E) (Anderson, 2001) with a nested (replicate microcosms) regression design was used to test for difference in post-larval size increase over time in the different pH and temperature treatments.

These data on growth rate, survival and calcium component of the shell are compared to data produced from experiments carried out on post-larvae from the south coast UK, at the southern edge of *Semibalanus balanoides* range, using exactly the same sampling method and experimental design as in this study, (Findlay et al., 2009). The mean ( $\pm 95\%$  confidence intervals) treatment levels in this comparison study were pH 8.05 ( $\pm 0.027$ ), 14.39 ( $\pm 0.25$ ) °C and pH 7.73 ( $\pm 0.022$ ), 14.70 ( $\pm 0.27$ ) °C for the low temperature treatments and pH 8.07 ( $\pm 0.030$ ), 19.77 ( $\pm 0.25$ ) °C and pH 7.71 ( $\pm 0.025$ ), 19.73 ( $\pm 0.26$ ) °C for the high temperature treatments (Findlay et al., 2009).

**Table 2**

Results for initial size of barnacles, survival (%), shell calcium (μmol (mg of shell)<sup>-1</sup>) and magnesium (μmol (g of shell)<sup>-1</sup>), calcium to magnesium ratio and the net calcification rate (NC) (μmol (g shell)<sup>-1</sup> d<sup>-1</sup>). Values are means  $\pm 95\%$  confidence intervals.

Temperature pH	4.8 °C			8.5 °C		
	8.1	7.7	7.3	8.1	7.7	7.3
Initial size (mm)	0.949 $\pm$ 0.0176	0.953 $\pm$ 0.0271	0.969 $\pm$ 0.0219	0.988 $\pm$ 0.0221	0.987 $\pm$ 0.0285	0.995 $\pm$ 0.0215
Survival (%)	92.5 $\pm$ 4.70	90.6 $\pm$ 4.26	86.4 $\pm$ 6.35	92.3 $\pm$ 3.89	88.9 $\pm$ 3.90	86.6 $\pm$ 4.17
Ca (μmol (mg shell) <sup>-1</sup> )	0.90 $\pm$ 0.103	0.96 $\pm$ 0.099	0.88 $\pm$ 0.091	0.94 $\pm$ 0.111	0.94 $\pm$ 0.118	0.96 $\pm$ 0.141
Mg (μmol (g shell) <sup>-1</sup> )	17.7 $\pm$ 1.81	17.4 $\pm$ 1.60	16.0 $\pm$ 1.42	17.0 $\pm$ 1.58	17.4 $\pm$ 1.74	17.1 $\pm$ 2.02
Ca/Mg (mg/mg)	84.1 $\pm$ 8.45	91.6 $\pm$ 9.24	90.1 $\pm$ 9.90	90.2 $\pm$ 8.26	86.9 $\pm$ 10.17	91.3 $\pm$ 7.31
NC (μmol (g shell) <sup>-1</sup> d <sup>-1</sup> )	40.6 $\pm$ 4.63	43.5 $\pm$ 4.47	39.4 $\pm$ 4.09	42.2 $\pm$ 5.00	42.1 $\pm$ 5.33	43.2 $\pm$ 6.32

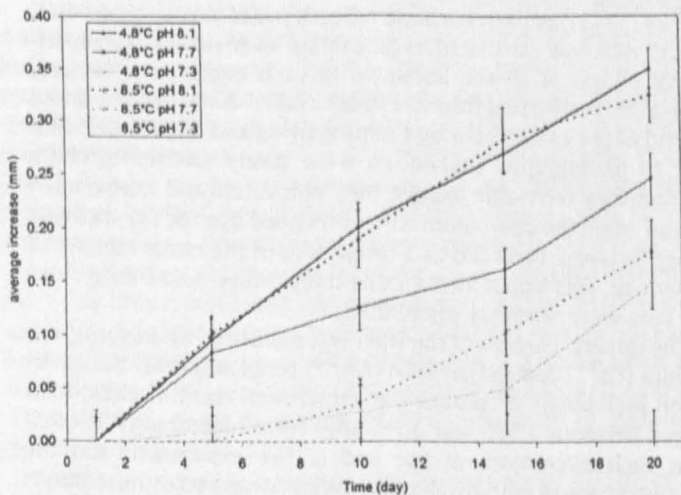


Fig. 1. Mean increase in length (mm) of barnacles over the experimental time period under different conditions of temperature (4.8 °C, lines and 8.5 °C, dashed lines) and pH (pH 8.1, diamonds, pH 7.7, squares, and pH 7.3, triangles). Error bars are 95% confidence intervals.

### 3. Results

The temperatures in the mesocosms were maintained slightly above the nominal temperatures ( $5.2 \pm 0.64$  and  $9.5 \pm 0.35$  °C in the low and high temperatures respectively) because of a small increase in temperature occurring while seawater passed through the tubing. Salinity remained between 33 and 34 in all mesocosms. The carbonate system parameters are provided in Table 1.

The percentage of post-larvae surviving the 20 d exposure period ranged between 85 and 90% and was not affected by either temperature or pH level (Table 2).

All the cyprids had metamorphosed to post-larvae by the start of the experiment. We were able to follow the development of six individual post-larvae in each treatment throughout the experimental period (Fig. 1), as well as measure the overall population growth, which included larger (>1.5 mm operculum length) juveniles, but no adults (>2 mm operculum length) (Fig. 2). The pattern of growth was similar for both the individually monitored post-larvae and the overall average population growth: Post-larvae in the control pH (high and low temperatures) increased in size relatively uniformly over the 20 day exposure period. At pH 7.7 initial growth rate of post-larvae was not significantly different

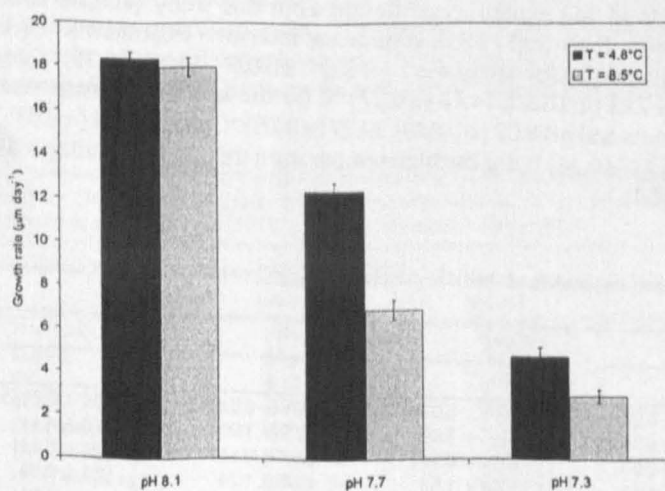


Fig. 2. Mean growth rate ( $\mu\text{m d}^{-1}$ ) over the 20 day experiment period for each pH (pH 8.1, pH 7.7, and pH 7.3) and temperature (4.8 °C, black bars and 8.5 °C, grey bars) treatment. Error bars are 95% confidence intervals.

Table 3

PERMANOVA table of results for average increase in length of post-larvae over the 20 day exposure period testing for differences between fixed factors of pH, temperature (Temp) and over time, and interactions between them. Bold p values indicate significant results ( $p < 0.05$ ).

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
pH	2	2.640	1.320	83.40	<b>0.001</b>	999
Temp	1	0.318	0.318	20.07	<b>0.002</b>	996
Time	4	4.084	1.021	64.51	<b>0.001</b>	999
pHxTemp	2	0.131	6.55E-02	4.141	0.066	998
pHxTime	8	1.183	0.148	9.341	<b>0.002</b>	998
TempxTime	4	0.177	4.43E-02	2.798	0.117	999
Res	8	0.127	1.58E-02			
Total	29	8.659				

from control pH. However after day 10 the growth rate decreased and became significantly slower than the low temperature control after 15 days. At pH 7.30, within the first five days there appeared to be reduced growth and the rate decreased further after 10 days. The individually monitored post-larvae showed significant differences between the average increase in length as a result of pH and temperature effects (Fig. 1, Table 3; PERMANOVA,  $F_{2,29} = 83.404$ ,  $p = 0.001$  and PERMANOVA,  $F_{1,29} = 20.065$ ,  $p = 0.002$  for pH and temperature respectively). The mean growth rate of all the post-larvae in each treatment decreased significantly with decreasing pH (Fig. 2, ANOVA  $F_{2,29} = 4.75$ ,  $p = 0.018$ ) and appeared to decrease with increasing temperature, but this was not significant (Fig. 2, Table 4; ANOVA  $F_{1,29} = 0.82$ ,  $p = 0.375$ ); there was no significant interaction between the temperature and pH.

The calcium and magnesium contents of the shells did not change with either increased temperature or decreased pH (Table 2). Calcium content ranged between 35 and 40% of the shell, while magnesium made up 0.4–0.45% of the shell, irrespective of treatment. There was a relatively low concentration of magnesium in the shells resulting in a relatively high Ca:Mg ratio, between 1:80 and 1:90 (mass: mass).

### 4. Discussion

*Semibalanus balanoides* is a boreal species found on rocky shores across much of northern Europe and North America (Barnes and Barnes, 1976). It is therefore different to true polar species in that it is not restricted (and so presumably is not adapted) specifically to cold waters. Early investigations on *S. balanoides* suggest that a maximum temperature between 10 and 12 °C is required for successful gonad and brood development (Crisp and Clegg, 1960) while embryo and larval development rates are optimal at about 10 °C (Crisp, 1959; Barnes and Barnes, 1976). We therefore did not expect to find a marked effect of increasing temperature from 4 °C to 8 °C on cyprid survival; although we did predict that growth rates would increase with increasing temperature. Surprisingly temperature did not have any significant impacts on the rate of post-larval growth. One hypothesis to explain this lack of growth increase with temperature is that the local population is acclimated (or even adapted) to colder temperatures. Since locally, these populations

Table 4

ANOVA table of results for average growth rate of post-larvae over the experimental period, testing for differences between fixed factors of pH and temperature (Temp), and the interactions between them.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	2	913.740	913.740	456.870	4.750	0.018
Temp	1	78.690	78.690	78.690	0.820	0.375
pHxTemp	2	34.100	34.100	17.050	0.180	0.839
Error	24	2307.250	2307.250	96.140		
Total	29	3333.780				

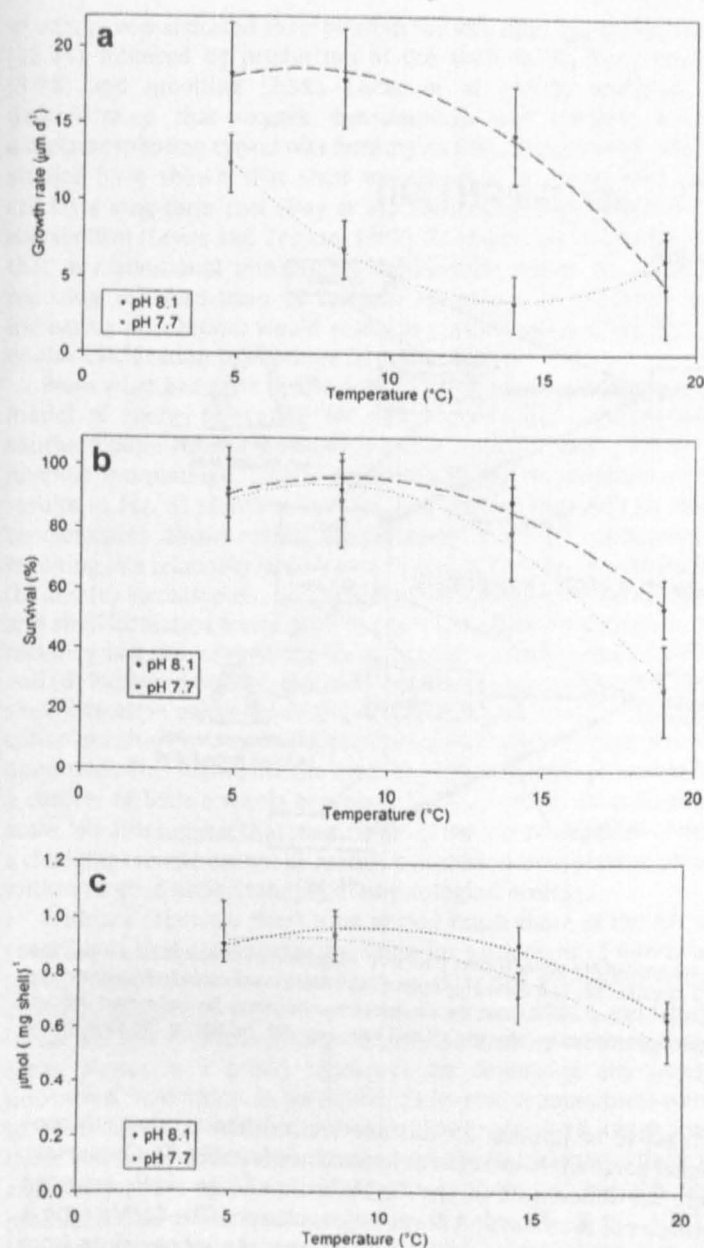


Fig. 3. Impacts of pH (black circles pH 8.1, grey squares pH 7.7) across a range of temperatures on (a) growth rate ( $\mu\text{m d}^{-1}$ ), (b) survival (%) and (c) shell calcium ( $\mu\text{mol (mg of shell)}^{-1}$ ). Error bars are 95% confidence intervals. Dashed lines show a polynomial fit to the data points.

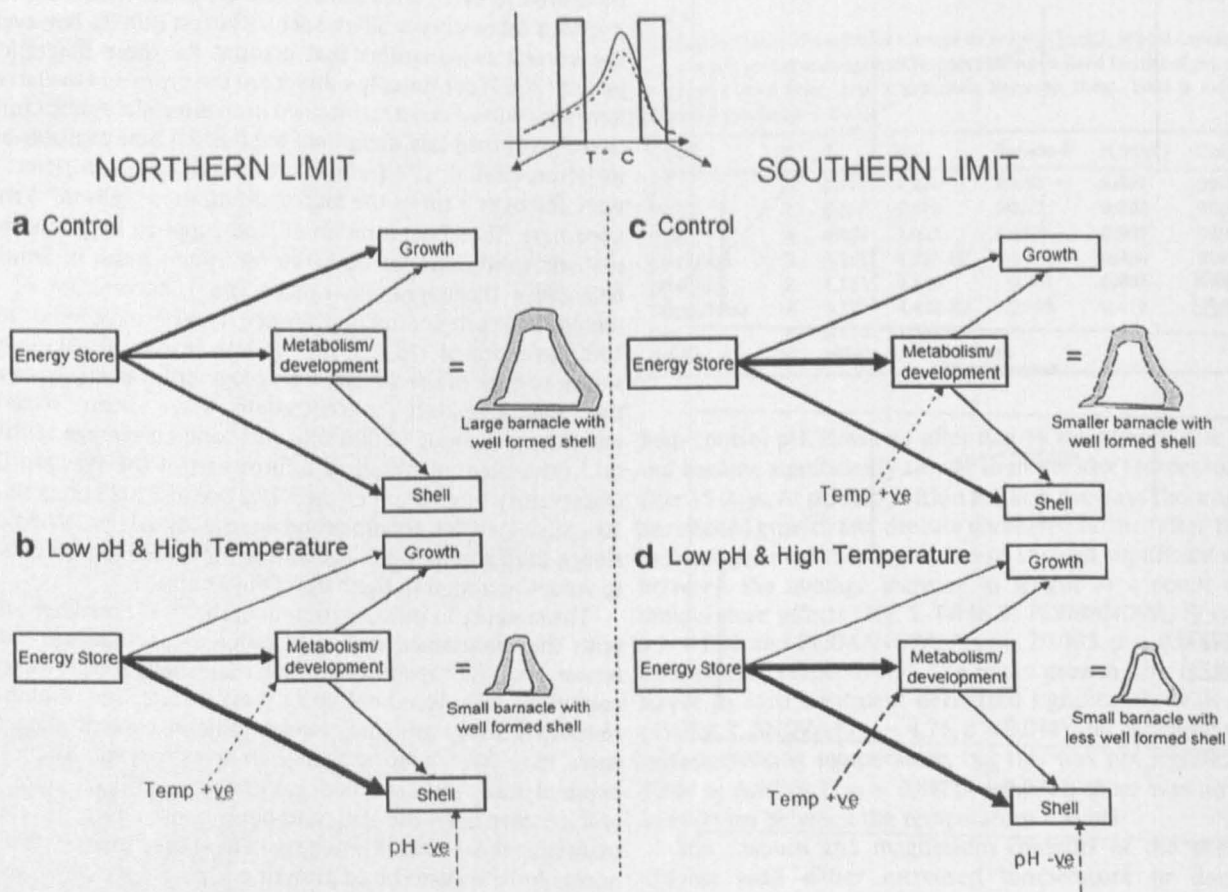
experience a maximum of about 5  $^{\circ}\text{C}$  during the growing season, it would be advantageous for them to have optimal growth rates at the mean temperature that they experience. Hence increases in temperature beyond their normal range may not cause an increase in growth because of the increased cost resulting from elevated metabolism at higher temperature (Levinton, 1983). This 'latitudinal compensation' is well documented among ectotherms (e.g. Dehnel, 1956; Ament, 1979; Levinton, 1983; Lonsdale and Levinton, 1985; Conover and Present, 1990; Arendt, 1997; Blanckenhorn, 2000).

Post-larval growth and development was significantly impacted by lowered pH. All levels of lowered pH caused a significant reduction in growth rate, although neither survival nor shell mineral content was significantly different over the 20 day period. Reduced growth rates as a response to lowered pH have been observed in studies on other marine organisms (e.g. *Halitosis laevigata* and *Halitosis rubra*, Harris et al., 1999; *Mytilus galloprovincialis* Michaelidis et al., 2005; *Mytilus edulis* and *Crassostrea gigas* Gazeau et al., 2007), particularly when shell growth has been

measured. Growth rates shown here are lower than those found in previous laboratory studies, such as Jarrett (2003), however there are several explanations that account for these differences: (1) Jarrett (2003) continually submerged the cyprids in the laboratory; here post-larvae were maintained in an intertidal system and hence these organisms had about half the feeding time available as those in Jarrett (2003); (2) The laboratory individuals in Jarrett (2003) were fed over 3 times the food concentration (cells  $\text{m}^{-1}$ ) than was used here. The concentration of food supplied here was based on realistic conditions for both the north and south of *Semibalanus balanoides* biogeographic range. The concentration of phytoplankton in summer in Kongsfjorden have been recorded (28th July 1989) as a total of 375 cells  $\text{ml}^{-1}$  (Eilertsen et al., 1989) While at the southern edge of the *S. balanoides* geographic range (Plymouth, UK) the phytoplankton concentrations have been recorded at maximum of about 12,000 cells  $\text{ml}^{-1}$  and an average of 3050 cells  $\text{m}^{-1}$  (The phytoplankton data form part of the Western Channel Observatory funded under the NERC Oceans, 2025 program Theme 10 – Sustained Observations and were analysed by C. Widdicombe). Hence food supply was not limiting for these barnacles compared to values recorded in the natural environment.

The stability in mineral content of the shell noted here, together with the maintained net calcification rates, indicates that post-larvae were still capable of forming their shells and growing even in conditions of lowered calcite saturation state. The combination of reduced growth and maintained mineral content suggests that there may have been a change in the energetic balance of the exposed individuals. In undersaturated conditions more mineral will dissolve from the shell and hence more energy is required to maintain the mineral integrity. Therefore, energy that would normally be invested into growth could be reallocated and hence organisms growing in lowered pH grow more slowly and end up smaller than individuals grown in higher pH conditions. If true, it could mean that it is more important that these organisms maintain shell integrity to protect against desiccation, predation and abrasion than that they grow to a larger size. Sessile invertebrates such as intertidal barnacles are unable to avoid mortality from predation or environmental impacts by moving into shelter. The ability to form a hard shell which is thick and strong can deter predators from drilling through the shell and will additionally protect against abrasion by ice or rocks (Barnes, 1999). Although this could be construed as an unnecessary response by *Semibalanus balanoides* which are found in the Arctic and have few predators, it is certainly a critical factor for surviving early life on more temperate rocky shores, where desiccation and predation are two main causes of post-settlement mortality (Connell, 1961; Foster, 1971). Hence it is possible that this may be a feature of *S. balanoides* evolution that has been maintained across their geographical distribution. A recent study by McDonald et al. (2009) on the tropical barnacle *Amphibalanus amphitrite* showed a similar response: barnacles appeared to enhance calcification under elevated  $\text{CO}_2$  conditions although the shells appeared to be weaker as a result of increased dissolution. Adult *S. balanoides* also showed compensatory calcification under elevated  $\text{CO}_2$  conditions over the winter period at the southern range edge (Findlay et al., 2009).

The impact of multiple factors acting on *Semibalanus balanoides* post-larvae concomitantly does appear to increase the result of reduced growth rate. Previous studies investigating both temperature and pH have shown that either an increase in temperature results in an increase in growth rate which is offset by a decrease in growth resulting from lowered pH (Reynaud et al., 2003; Findlay et al., 2009) or both cause an increase in growth rate (Gooding et al., 2009). There are two possible explanations which could account for the further reduction in growth rate seen here. Firstly, the cyprids and post-larvae are likely to be cold-acclimated, and hence



**Fig. 4.** A simple conceptual model of energy allocation in post-larvae from northern and southern populations under control ("normal" conditions of temperature and pH) and under lowered pH and elevated temperature conditions. Arrows indicate possible routes of energy allocation from a finite energy reserve (lipid store in the case of metamorphosing cyprids) to growth, metabolism and development into adult form, and shell formation. The thickness of the arrows denotes the amount of flow of energy. Dashed arrows indicate potential impacts from pH and temperature. Temperature is likely to increase metabolism, requiring more energy, however pH will have negative impacts on the shell (either through direct dissolution) or indirectly through metabolism. See text for further explanation.

experience a short period of heat adjustment and acclimation (Pörtner and Knust, 2007) which impacts on growth in the early period of the experiment (as can be seen in the development data, Fig. 1). Secondly (and possibly related to the previous point), the greater reduction in growth could be the result of a change in energy balance as suggested above. There is a short period during and after metamorphosis when the post-larvae are non-feeding and reliant on energy reserves (Rainbow and Walker, 1977). This period is thought to be particularly energetically costly (Lucas et al., 1979). Elevated metabolism accompanying increased temperature (Newell and Northcroft, 1965) may be detrimental to the post-larvae at this time because it draws essential energy from a limited pool. The larval phase is already a particularly extended period in the Arctic (Petersen, 1966) and hence reserves may not be sufficient to buffer long-term changes in metabolism. Neither of these hypotheses can be advanced by this present study. They require additional studies on cyprid and post-larval biochemistry as well as time of onset of feeding and feeding rates. However, changes to the energy balance may well have long-term effects on the fitness of these barnacles (Thiyagarajan et al., 2005).

Towards the southern edge of its biogeographical range, temperature appears to have a much more significant impact on post-larval *Semibalanus balanoides*, particularly with respect to overall survival (Findlay et al., 2009; Fig. 3). At its southern range edge, *S. balanoides* is already high temperature limited, and hence the effects of increased temperature will be sufficiently abrupt so as to mask the impacts of other stressors. However, at the northern limits, as discussed above, we would not expect a temperature

increase to be limiting for this species and hence other environmental parameters increase in importance (see Fig. 3). Conversely, there are differences in the Ca/Mg ratio of the shells between barnacles of southern and northern populations. The Ca/Mg ratio is  $1:91.4 \pm 8.10$  (mean  $\pm$  s.d.), which is very similar to values from northern Scotland ( $1:92$ , Barnes et al., 1976) but much greater than values from barnacles taken from a southern population (Findlay et al., 2009), which have a ratio  $1:51.5 \pm 8.58$ . The reduction in the ratio from north to south corresponds with an increase in the Mg content. Although barnacles use calcite as the primary form of calcium carbonate in their shells, the decrease in shell Mg indicates a shift from a higher Mg content (making calcite more soluble) in warmer waters to a lower Mg content (less soluble calcite) in colder waters (Andersson et al., 2008). Shell composition is known to vary with environmental conditions, such as temperature (Gussone et al., 2005). There may also be some level of biological control as it is more energetically costly to maintain a more soluble shell in waters where there is a lower level of saturation of carbonate minerals (Freitas et al., 2008) and hence the Mg fraction may be lower under these conditions (Andersson et al., 2008).

An organism is able to allocate energy (resources) to different processes, such as growth, maintenance, reproduction, etc., thus establishing an energy budget which can be altered according to the environmental conditions (Sibly and Calow, 1986). The energy budget of *Semibalanus balanoides* has not been characterised but we can assume it would be similar to energy budgets of other barnacles such as *Balanus glandula* (Wu and Levings, 1978). Wu and Levings (1978) demonstrated that annually, the largest proportion

of energy was allocated to respiration (67.4%), then egg production (12.3%), followed by production of the shell (6.6%), body tissue (3.9%) and moulting (2.3%). Lucas et al. (1979) additionally demonstrated that oxygen consumption was greatest when a metamorphosing cyprid was forming its first calcified shell. Other studies have shown that shell maintenance is continuous and carries a long-term cost (Day et al., 2000) and may be linked to metabolism (Lewis and Cerrato, 1997). Therefore we might expect that any additional impacts on calcification, either by directly reducing the formation of calcium carbonate or indirectly by increasing dissolution, would result in a reallocation of energy to enable calcification to continue (e.g. Bak, 1983).

From what has gone before we can derive a simple conceptual model of energy allocation for post-larvae from northern and southern populations (Fig. 4). This model could be used to predict juvenile morphology. This is presented in Fig. 4 (compare with results in Fig. 3) showing that (a) low energy demands in cold temperatures allows continuous growth and net calcification, resulting in a relatively large barnacle with a well developed shell; (b) and (c) increased energy demand from changes in metabolism and shell formation lower growth rates but allow net calcification, resulting in a slower-growing barnacle with a well developed shell; and (d) increased energy demand from changes in metabolism and shell formation cause lowered growth rates and lower net calcification, resulting in a slower-growing barnacle with a poorly developed shell. This highlights the need to evaluate single processes in a context of both a whole organism but also across an ecological scale. We also suggest that predictions of the fate of a species under a changing climate cannot be readily transferred from place to place without a good understanding of physiological ecology.

Within a relatively short time period much more of the Arctic coastline is likely to become available for settlement of intertidal species. *Semibalanus balanoides* has an extended planktonic larval development period (up to 2 months, Rainbow (1984)) and hence larvae are able to disperse long distance potentially reaching these areas. Hence is a prime candidate for colonising any newly uncovered, 'free' space. In particular, if elevated temperatures were to occur alone, *S. balanoides* would likely expand its range into these new areas. Along with the settlement of *S. balanoides* there is also likely to be settlement of other organisms and possibly the production of a diverse ecosystem (Weslawski et al., 1993). However, with the addition of ocean acidification there may be a much slower extension of this species.

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