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THE IMPLICATION FOR THE MARINE ENVIRONMENT OF CO_2 (IMCO2)

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EXECUTIVE SUMMARY

Growing emissions of carbon dioxide (CO_2) from anthropogenic processes pose a distinct threat to the global environment. However quantifying the consequences of high CO_2 is problematic as many physical and biogeochemical processes combine to create a complex set of interactions. Although many relevant processes are understood, others such as CO_2 induced acidification have only recently emerged as serious issues.

UK policy makers require predictions of the impact of both unmitigated CO_2 emissions and of unplanned CO_2 release from sub-sea geological storage, a prime CO_2 mitigation strategy. Prior to the IMCO2 project, the UK (and international) community did not possess the capacity to answer these questions. This lack of capacity was addressed through:

- Facilitating and promoting the integration of international and national R & D on ocean CO_2 acidification;

- Creating a UK modelling capacity for exploring the effects of high CO_2 on UK shelf waters;

- Developing and applying mesocosm experimental facilities to examining the impacts of CO_2 induced seawater acidification on benthic communities and processes.

Both the modelling and mesocosm work provided initial assessments of high CO_2 impacts whilst the integration program determined and sponsored the continued R & D required to fulfil UK policy maker's requirements.

The IMCO2 project has had made considerable advancements in all three of its core areas.

<u>Integration and Communication</u>
Members of the IMCO2 team have worked effectively at transferring the knowledge gained by the project to users including scientists, policy makers and the general public through a wide range of international and national outreach activities. Their achievements include:

- The topic of ocean acidification has been brought to the climate change, energy and biodiversity scientific and policy communities at national and international levels (e.g. EU, UNFCCC, IPCC 4th Assessment Report, MAPC, IMBER, Stern Review on Economics of Climate Change, UKERC, Avoiding Dangerous Climate Change G8 Presidency initiative, Global Environmental Change Committee, MCCIP, UK Parliamentary and Scientific Committee, UK Ministers and MEP's, Natural England, EA, EEA).

- IMCO2 outreach activities have ensured that ocean acidification is regarded as "the other CO_2 problem" and is as important as climate change in the future health of the planet (e.g. Working Group member of the Royal Society Report on Ocean Acidification, IPCC 4th Assessment Report).

- Ocean acidification is a forceful argument for mitigation of CO_2 emissions and has been used to remove legal obstacles for wide scale geological carbon capture and storage. IMCO2 gave evidence to OSPAR and the London Convention consideration of the legality of CCS and authored the OSPAR intercessional report on ocean acidification and contributed to the UK Energy Research Council, the UK Consortium on Carbon Capture and Storage and the joint Research Council Energy bid for the 2007 Spending Review.

- Media activities (TV programmes, Newspaper articles, BBC1 News and Newsnight features, web articles and a World Ocean Web caste on Ocean acidification, articles in New Scientist, New Yorker) within IMCO2 have promoted the reporting of the science to the general public.

- IMCO2 team have networked and collaborated at the national and international level to help to promote this area of science for future investment (e.g. NERC, DEFRA, BERR, EU, IGBP).

- The Reference User Group of key stakeholders proved to be an effective mechanism of delivery of sound science to the heart of government departments, industry, agencies and NGO's.

<u>Ecosystem Modelling</u>
PML modellers have integrated algorithms that describe CO_2 and pH chemistry into a NW European shelf-wide marine ecosystem simulation model (ERSEM). For the first time this couples environmental CO_2 with the marine ecosystems CO_2 cycle over the continental shelf throughout an annual cycle. The ERSEM model and CO_2 carbonate cycle model have also been successfully coupled in simple 1D applications providing an ideal development and process evaluation tool.

Modelling experiments and observations conducted within IMCO2 suggest that:

- There is spatial heterogeneity in annual pH ranges over the UK shelf and ranges may be as high as 0.3-0.5 pH units in regions of high productivity and seasonal stratification and even higher (>1.0 pH unit) in regions directly influenced by riverine inputs.

- At the current rate of acidification, pH in UK shelf waters will become completely distinct from its historical (20 million years) range should atmospheric concentrations reach 700ppm.

- This concentration is less than that required for disconnection in oceanic waters and may mean that shelf species have more time to adapt to pH change than oceanic ones.

- Changes in the ratio of ammonium to nitrate (key nutrients for phytoplankton) are likely to be provoked by acidification.

- Acidification could change phytoplankton community structure as different phytoplankton species possess different versions of the enzyme concerned with

CO_2 assimilation (Rubisco) and therefore have different uptake:[CO_2] response curves.

- The response of Coccolithophores (a type of phytoplankton) to calcification inhibition is dependant on pH as well as other factors associated with climate change such as temperature and wave mixing.

Laboratory Based Experimentation

PML has built an effective seawater acidification experimental facility. This facility has not only enabled the experiments within IMCO2 to be conducted, it has also stimulated a number of collaborations with other UK and international researchers.

Experiments have shown that:

- The severity of any impact of seawater acidification on benthic organisms will depend on the physiology of the organism concerned, the type of sediment it is associated with and whether it lives above or below the sediment surface.

- Sediment nutrient flux rates would be directly affected by the large changes in seawater acidity likely to occur during leakage from sub-seabed storage but probably not by the small changes predicted as a result of ocean acidification through atmospheric absorption.

- Decreasing seawater pH could lead to a gradual decrease in the diversity of benthic communities. Particularly vulnerable organisms are those that live on the sediment surface and those that depend on calcium carbonate skeletons.

- Macrobenthic communities are likely to be more sensitive to changes in seawater acidity likely to occur during leakage from sub-seabed storage than meiobenthic communities.

- The severity of impacts on both biodiversity and nutrient flux due to seawater acidification is dependant on the nature of the sediment environment concerned.

CONTENTS

1. INTRODUCTION

1.1 THE IMPACT OF RISING ATMOSPHERIC CO_2 CONCENTRATIONS ON EARTH'S OCEANS

Prior to the industrial revolution (circa 1750) the atmospheric concentration of CO_2 was around 280ppm. Since that time, a period of only 250 years, this concentration has increased to more than 380ppm. This increase, caused primarily by the combustion of fossil fuels and the manufacture of cement, is implicated as a primary driver of rising global temperatures and the environmental impacts associated with this "climate change". However, as far as Earth's oceans and the organisms therein are concerned, the biggest threat could come from another impact associated with atmospheric CO_2 increase; ocean acidification and the reduction in dissolved carbonate concentrations (Raven et al. 2005). Surface ocean pH has been maintained between 8.0 and 8.3pH units for the last 25 million years, however, the current rate of increase in CO_2 atmospheric concentration, at approximately 100 times greater than previous naturally induced increases (Blackford & Gilbert in press), is causing seawater pH to decrease (Caldeira & Wickett 2003). Compared to pre-industrial times, seawater pH has fallen by 0.1 pH unit, indicating a 30% increase in the concentration of H^+ ions, whilst the current rate of acidification stands at 0.015 pH units per decade (Haugan & Drange 1996). As further emissions of CO_2 are inevitable it is not unreasonable to assume that concentrations of atmospheric CO_2 will continue to rise. In the long term we face the prospect of atmospheric CO_2 levels exceeding 1500ppm sometime between 2100-2200 (Portner et al. 2004), whilst more immediately, the Intergovernmental Panel on Climate Change (IPCC) has predicted that levels could reach 800ppm by 2100 (Feely et al., 2004). As a result of these increases it is possible that the pH of surface water could fall by up to 0.4 units before 2100 and a reduction of 0.7 units could occur by 2250 (Caldeira & Wickett 2003).

1.2 GEOLOGICAL STORAGE OF CO_2

One potential method suggested for CO_2 abatement is carbon capture and storage (CCS) - the long-term underground containment of CO_2 in suitable geological reservoirs. However, this mitigation strategy will depend on demonstrating acceptable performance, and answering operational, regulatory and public acceptance criteria. Considerable scientific advances have been made in the understanding and implementation of carbon capture, transport and storage using demonstration and pilot studies. However, there is a need for further fundamental generic research concerning environmental issues surrounding geological storage of CO_2. In particular, the effects of long-term leakage, whether slow or through catastrophic release, on marine and subsurface ecosystems have not been explored by the carbon sequestration research community. This project will go some way to rectifying this situation.

1.3 PROJECT AIMS AND OBJECTIVES

This project set out to investigate the potential impact of decreasing seawater pH on the continental shelf marine ecosystem; its processes, biodiversity and health of key organisms. There were 5 main objectives:

Objective 1:
To facilitate and promote integration of international and national R & D on ocean acidification through networking, collaboration and advice.

Objective 2:
To create a UK modelling capacity for exploring the effects of elevated CO_2 (including lowered pH) on the marine ecosystem of UK shelf waters and to make an initial exploration of marine ecosystem response to elevated CO_2.

Objective 3:
To develop a UK facility, within the Plymouth Marine Laboratory (PML), to enable experiments examining the environmental impacts of CO_2 induced seawater acidification.

Objective 4:
To determine the impact of pH change, as a result of CO_2 acidification, on the health and function of key benthic organisms.

Objective 5:
To determine the impact of pH change, as a result of CO_2 acidification, on the biodiversity and community structure of a subtidal soft sediment community.

2. OBJECTIVE 1: NETWORKING, COLLABORATION AND ADVICE

2.1 INTRODUCTION

Limited interaction between research scientists and stakeholders (in particular policy makers, environmental managers, industry, NGOs and the general public) has often limited the relevance and usefulness of findings from research projects. To improve this interaction and therefore maximise the impact of the IMCO2 project, considerable effort was dedicated to communication within and between these groups. Having "networking, collaboration and advice" as the first objective emphasises the importance of this activity to the success of the IMCO2 project.

2.2 INTERNATIONAL NETWORKING AND INTEGRATION

2.2.1 OSPAR and the London Convention

Carol Turley participated in *The Second Trondheim Conference on CO_2 Capture, Transport and Storage*, 24-26 October 2004 and gave a presentation on ocean acidification and acted as panel member at the *OSPAR Workshop on the Environmental Impact of Placement of Carbon Dioxide in Geological Structures in the Maritime Area* on 27 October 2004. A report to OSPAR was an output from the panel members. She also gave a paper at The London Convention (LC) Workshop on Carbon Capture and Sequestration on 20 May 2005, and gave evidence on ocean acidification to the LC Scientific Group the following week at the International Maritime Office. Carol Turley was part of the OSPAR Biodiversity Committee Intercessional corresponding group convened by Norway and the UK to review Effects of Ocean Acidification in OSPAR area. The following "OSPAR report on ocean acidification" was published: Haugan, P.M; Turley, C; Poertner H.O, 2006. Effects on the marine environment of ocean acidification resulting from elevated levels of CO_2 in the atmosphere, DN-utredning 2006-1, 1-36. Steve Widdicombe presented evidence at the "Meeting of the Scientific Group Intercessional Technical Working Group on CO_2 Sequestration within the framework of the Convention on the Prevention of Marine Pollution by Dumping Wastes and Other Matter, 1972" at the International Maritime Organisation, London (3–7 April 2006). This combined evidence over the 3 year period to OSPAR and London Conventions played a substantial role in removal of the legal hurdles for sub seabed CCS. From 10 February 2007 large scale CCS is now legal.

2.2.2 Avoiding Dangerous Climate Change Symposium

Carol Turley and IMCO2 co-authors at PML reviewed the science of ocean acidification at a presentation at the *Avoiding Dangerous Climate Change Symposium* in Exeter 1-3February 2005 (http://www.stabilisation2005.com/). ADDC was part of the government initiative for its presidency of G8 and EU. This was the first time many of the climate change scientists and policy makers became aware of the related issue of ocean acidification. Carol was also a contributory author of the Gibbins et al. presentation on CCS. Both these presentations have been written up and published as Chapters in the Symposium Proceedings, which were published as a book in 2006. The book was launched by DEFRA at The Royal Society on 30 January 2006 and Carol Turley was one of the four contributing authors who gave a presentation at the launch. Significant UK and worldwide media reporting followed this event.

2.2.3 United Nations Framework Convention on Climate Change (UNFCCC)

At the request of DEFRA, Global Change Division a similar paper was presented at the UNFCCC SBSTA Side Event on Outcomes of the International Symposium on Stabilisation of Greenhouse Gases – Latest scientific results on climate change, 19 May 2005, Bonn, Germany.

2.2.4 Intergovernmental Panel on Climate Change (IPCC)

At the UNFCCC meeting in Bonn, discussions were held with Martin Parry, Co-Chair IPCC Working Group II (Impacts, Adaptation and Vulnerability) on including the direct effects of CO_2 on the oceans within the future WG report. Since then PML have supplied further information and understand that this was discussed by WG II. Following this, Carol Turley was invited to become a Lead Author for IPCC WGII Chapter 4 in February 2006 with a specific request to input ocean acidification into the marine section of this chapter, which is on Ecosystems, their Properties, Goods and Services. The WGI report was published in February 2007 and the WGII report is due in April 2007. This process has involved an IPCC meeting in Cape Town and interaction with numerous scientists in the production of the first drafts and several review processes from Experts and Governments.

2.2.5 British Embassies

Carol Turley was invited to a Climate Change Workshop “Tipping Points in the Earth System”, British Embassy, Berlin on 4-6 October 2005. This was part of a UK Government initiative as part of the UK Presidency of the EU. Ocean acidification was considered to be an important tipping point. Mike Kendall gave an invited talk at the “Britain in Norway” symposium organised by the British embassy in Bergen, 11-14 October 2005.

2.2.6 International Global Biosphere Programme (IGBP)

Carol Turley sits on the IGBP sponsored programme Integrated Marine Biogeochemistry and Ecosystem Research (*IMBER*). She has contributed to the writing of the Science and Implementation Plan (http://www.imber.info/), which has now been completed. The SSC

met in Shanghai on 16-22 April 2005 to finalise the implementation of IMBER and since then in Brest, France in 2006, while the 2007 SSG meeting will be in Victoria, Canada. High CO_2 is a priority theme within this programme.

Carol Turley is working with Marie Hood (IGBP) and a group of international scientists on a proposal to the UNGEF/WB on OA.

2.2.7 Advances in Marine Ecosystem Modelling Research (AMEMR)

Jerry Blackford chaired the Scientific Steering Group for the international symposium *Advances in Marine Ecosystem Modelling Research* (http://www.amemr.info/) that was hosted by PML, 27-29 June 2005. One of the symposium themes was the ecosystem effect of high CO_2/low pH, thus bringing the issue to the continued attention of the international science community.

In February 2007, Jerry Blackford followed this success and organised another international workshop on modelling marine response to high CO_2 bringing together experimentalists and modellers.

2.2.8 Europe

Carol Turley was invited to Brussels on 13 December 2004 to present evidence on impacts of ocean acidification and climate change on the marine environment to EU-DG Environment with Director MBA and SAFHOS. PML is one of the partners in the EU-FP6 funded *EUROCEANS* Network of Excellence (NoE) which has a strong interest in ocean CO_2/pH as part of its biogeochemistry programme. Through collaborations with a British Geological Survey (BGS) led project, PML is also involved with another EU NoE *CO_2GeoNet* and submitted an Expression of Interest for a STREP *ECHO* (Environmental and Habitat response to CO_2 leakage) on the sequestration and storage side of CO_2 and its potential impact. Unfortunately, the EU did not select the latter for development into a full programme but PML are still involved with CO2GeoNet contributing a presentation by Steve Widdicombe at the networks workshop on Ecosystem Response to CO_2 Leakage at BGS on 29 June 2005.

Dan Laffoley (NE), Carol Turley and Beth Greenaway (DEFRA) joined forces at EU, Brussels, in an invited lunchtime Seminar on climate change, ocean acidification and policy in the marine environment (October 2006) to heads of DG's and policy makers. During June 2006, Carol Turley acted as a scientific advisor on the EU FP7 science programme and is part of initial planning group for a consortium for an EU FP7 proposal on Ocean Acidification which met in September 2006 and January 2007. Following this a workshop of EU principle scientists met in February 2007 to prepare the consortium proposal for submission in May 2007. There is excellent UK representation in this consortium including the IMCO2 team.

2.2.9 Web activity

We have contributed to the UNESCO website on ocean acidification (http://ioc.unesco.org/ioccp/) which is aimed at being a resource for researchers and to the

climate change debate though Open Democracy (www.opendemocracy.net/home/index). Carol Turley was one of the experts on the live international World Ocean Web Cast on Ocean Acidification, 13 November 2006 (Live presentation, question session and texting session) that involved thousands of 14-18 year olds.

PML's own web site features (http://www.pml.ac.uk/) ocean acidification news and IMCO2 contributes to the UKCCSC web site (www.co2capture.org.uk/). PML's Annual Review frequently gives an update on ocean acidification activities from the IMCO2 team.

2.3 UK NETWORKING AND INTEGRATION

2.3.1 The Royal Society

Carol Turley was one of the working group members of The Royal Society's science policy study on Surface Ocean Acidification. The working group met five times in 12 months and the report from the working group was published on 30 June 2005. This received considerable press attention on the day, including features at the 1pm, 6pm and 10pm BBC1 News, part of which was filmed at PML. Following its release there has been considerable further attention from media, public, government and scientists.

2.3.2 UK Government, DEFRA and Parliament

The Minister for Environment and Climate Change, Elliot Morley and Linda Gilroy (MP for Plymouth Sutton) visited PML on 27 July 2005 to be advised on the issue of ocean acidification, and see the work being carried out by the IMCO2 programme. DEFRA Global Change (David Warrilow and Cathy Johnston) also visited PML to understand better the link between climate change and ocean acidification and the potential synergistic impacts and feedbacks. Discussions are also underway to have closer links between PML and the Hadley Centre, Met Office.

We have supplied information and evidence to DEFRA CSAS on a briefing for the G8 summit and Montreal and also for a response to a Parliamentary Question 0765 05/06 by Norman Baker, Chair of the All-Party Environment Group on the sustainability of current ecosystems at future acidity levels of the world's oceans and current level and direction of research. Giles Chichester (MEP), Linda Gilroy (MP), Alison Seabeck (MP), Barry Gardiner (MP), Emma Beebe (Scottish Parliament) and Jonathon Porritt all received information on ocean acidification or CCS either during visits to PML or via correspondence.

Figure 2.1: Steve Widdicombe explained the problem of Ocean Acidification and the role of Carbon Capture and Storage to Alison Seabeck MP (Labour, Devonport) during her visit to PML on 28 July 2006. He also demonstrated how the work funded through the IMCO2 project was helping to address some of the ecological concerns associated with both OA and CCS.

Plymouth held an Exhibition in the Houses of Commons "Climate Change and the Oceans:

ocean acidification" on 29 January-2 February 2007. Carol Turley gave a short talk to members of the Houses of both Commons and Lords at a breakfast reception during the exhibition and Ben Bradshaw gave a response on the approaches government has to climate change and ocean acidification.

PML also contributed evidence on ocean acidification to the Stern Review on the Economics of Climate Change with the result that the impacts of ocean acidification were included in the report.

Following a presentation by Carol Turley at DEFRA Global Atmosphere, Whitehall, in June 2006 and she was asked to arrange a report on future research needs via co-funding the AMEMR workshop on ocean acidification. Carol Turley led a proposal to Defra on an assessment of pH measurements, modelling vulnerable areas and proposing a monitoring strategy. This was submitted on 2 August 2006 and started in February 2007. PML is the lead organisation collaborating with experts at National Oceanographic Centre (NOC) and University of Easy Anglia (UEA) as sub contractors.

The Parliamentary & Science and Technology Committee invited Carol Turley to give a seminar "Ocean acidification – the other half of the CO_2 problem" and participate in a dinner debate "How can science help to save the marine environment?" at the House of Commons. This is also published in Science in Parliament, 64(1) Spring 2007, 16-17.

DEFRA had a ministerial launch of its new concept of easily accessible and understandable annual report cards on impacts of climate change on marine systems. Carol Turley contributed to the MCCIP ARC section on ocean acidification and attended the Ministerial launch in Whitehall. www.mccip.org.uk.

2.3.3 NERC

NERC UK MARINE SCIENCE AND TECHNOLOGY STRATEGIC RESEARCH PROGRAMME: OCEANS 2025. NERC and the Marine Science Directors have developed one coherent UK strategic programme that looks to the future requirements and issues. Ocean acidification and CCS are both issues that form part of this programme. Oceans 2025 starts in April 2007.
UK-SOLAS: Two expressions of interest were submitted by PML in 2004 but SOLAS decided CO_2 was not its priority.
Environmental Genomics: This programme has been funded by NERC and CO_2 studies using the Bergen seawater mesocosms are part of the programme. PML (Principal Investigator Ian Joint) is leading this UK-wide programme.
QUEST: We have attended a “think tank” workshop in Dartington 14-18 March 2005 to educate the QUEST community on the global significance of ocean acidification including the socio-economical aspects. Our aspiration is that QUEST will consider ocean acidification a priority in its next funding round. Jerry Blackford is a PI on the MARQUEST program, which is focussed on improving Plankton Functional Type models for global simulations. This will underpin our specific remit to model carbonate chemistry and pH.
CASIX: CASIX links NERC Centres, University groups and the Met Office to model ocean circulation and the ocean C-cycle. Jerry Blackford is a CASIX (Centre for observation of Air-Sea Interactions and Fluxes) PI whose focus is to quantify the air-sea flux of CO_2 on global scales.

Responsive mode funding: PML staff submitted 2 major proposals to NERC, one of which was successful. Steve Widdicombe and Dave Lowe, in collaboration with John Spicer (UoP), Dave Billet and Brian Bett (both National Oceanographic Centre Southampton) submitted an application for funding to the NERC standard grant round (unsuccessful). The proposal was entitled 'Faunal response to seawater acidification in shallow and deep seas'. The successful project (by Widdicombe, Lowe and Turley) is entitled "The impact of ocean acidification on the biodiversity and function of coastal marine sediments" and its aims and objectives are complementary to those of IMCO$_2$. This additional funding further enables PML to build its capacity for research in this area.
NERC studentships: over the last 3 years PML has had 4 PhD students working on ocean acidification and 3 MSc/MPhil students.
NERC magazine 'Planet Earth': Steve Widdicombe and Dave Lowe published an article in the summer 2006 edition (pp 14-15) of the NERC magazine 'Planet Earth'. The article was entitled 'Ocean acidification: The other carbon dioxide problem.'

2.3.4 Joint UKRC

TSEC: The *UK Carbon Capture and Storage Consortium* (UKCCSC) is the interface between the environmental impacts aspect of ocean CO_2 and the mitigation method of capture and sub seabed geological sequestration. The consortium, formed in 2004 submitted a £2M proposal to TSEC in October 2004, has 10 partners (*British Geological Survey, Centre for Ecology and Hydrography, Plymouth Marine Laboratory, Imperial College, The Tyndall Centre for Climate Change, Universities of Edinburgh, Leeds, Cambridge, Newcastle and Nottingham*). This consortium, lead by Jon Gibbins (Imperial College London), has been funded and will collaborate with the *UK Energy Research Centre* (UKERC). It started with a kick-off meeting at Imperial College London on 22-23 June 2005. PML leads the environmental impacts part of the consortium and Carol Turley is a member of the consortium steering group. Another UKCCSC workshop was held at UCL on 19-20 September 2006 where it was agreed to use the IMCO2 RUG as the UKCCSC RUG as it was seen to be more effective use of RUG member time. No change in membership will be required. The UKCCSC has 6 mthly workshops with the next planned in April 2007.

2.3.5 Science Advice

In 2006, Carol Turley became a member of the Energy Science Advisory Committee and a Strategic Advisory Team member for EPSRC and a member of the NERC Advisory Committee on 2007SR on Energy the NERC Strategy Panel on Climate Change and an advisor to EU DG Environment on the FP7 Programme. She is also a member of the DEFRA Marine Assessment Policy Committee, the Royal Society Marine Advisory Network and the Royal Society's Climate Change Advisory Network and gave advice to the Arctic Climate Impact Assessment and AMAP on ocean acidification and a presentation and report to the UK Global Environmental Change Committee Biodiversity sub-committee on impacts of ocean acidification on biodiversity. She was also an invited speaker at the UK Energy Research Council meeting on CCS, Edinburgh.

Steve Widdicombe presented evidence at the first meeting of the "Scientific Group Intersessional Technical Working Group on CO_2 Sequestration within the framework of the

Convention on the Prevention of Marine Pollution by Dumping Wastes and Other Matter, 1972" held at the International Maritime Organisation, London (3 – 7 April 2006). He will also participate in the second meeting of this working group to be held in Oslo (Norway) 17–20 April 2007. The purpose of this meeting will be to draft individual sections of the current revised Specific Guidelines for the Assessment of Carbon Dioxide Streams for Disposal into Sub-seabed Geological Formations.

2.3.6 Plymouth Marine Laboratory

PML has built "high CO_2 oceans" in its 10 year science plan "Marine Matters" and plans to expand this area of research through its application to NERC for future funding of its Core Strategic Research Programme within *Oceans 2025* which will start in April 2007.

2.3.7 University of Plymouth

In 2005 we jointly supervised two postdoctoral students, an MRes and MSc, with Dr Jason Hall-Spencer and Dr John Spicer at the University of Plymouth. The masters projects examine the impact of high CO_2 on benthic organisms. The master students have successfully carried out their research experiments using the PML mesocosm samples and one of the studies has now been published in the journal Marine Pollution Bulletin. The other study is preparation for submission. We also supervised 2 undergraduate students in a project that examined the effects of seawater acidification on predator-prey interactions between 2 common intertidal organisms. This work is also being prepared for publication. We have continued to strengthen our collaboration with the UoP through a successful RCUK fellowship proposal on ocean acidification.

2.3.8 Other Universities

We have held discussions with Dr Rod Wilson (University of Exeter) with regard to calcium carbonate production in fish guts, invited him to give a seminar and are collaborators on a NERC proposal (1 December 2006) to develop this area of research. We are also in discussions with Dr Murray Roberts of the Scottish Association of Marine Science concerning a joint proposal on the impact of pH on cold water corals. In collaboration with Toby Tyrell (National Oceanographic Centre Southampton) we have submitted a joint PhD proposal. Carol Turley gave an invited seminar at the Global Environmental Change course at University of East Anglia in February 2006. We also collaborate with a number of other universities within the UK Carbon Capture and Storage Consortium (UKCCSC see above). We collaborate with Dr Daniela Schmidt (University of Bristol) on paleo-indicators of pH in mussel shells, with Profs. Andy Watson (UEA) and Dr David Hydes (NOC) on writing a national monitoring programme for pH.

2.3.9 British Geological Survey

PML has collaborated with BGS within the UKCCSC and the EU NoE *CO_2GeoNet* (see above) and given advice on the BGS position paper on CCS.

2.3.10 UK Agencies, Partnerships and NGOs

A team from PML were invited to present a seminar and lead a workshop on ocean acidification by EN in Peterborough on 28th January 2005. In attendance were members of EN, EA, WWF, RSPB, SNH, CCW, Royal Commission, NHM, Eastern Sea Fisheries Joint Commission, JNCC and DEFRA. It was highly successful and resulted in many follow-up enquiries from agencies for further information and another invitation to present evidence to WWF in summer 2005. The Bellona Foundation and Friends of the Earth have also asked us for advice. Third Generation Environmentalism has been to PML to seek further advice. We gave advice and information used on a poster for the launch of MCCIP in London on 1 March 2005. The EA (Prof. Mike Depledge) requested a short report on CCS and another on ocean acidification this was delivered in February 2006. The Global Environmental Change Committee invited Carol Turley to present evidence on ocean acidification and research needs on 21 February 2006 and has been invited to a Royal Society and UK GECC committee workshop on Biodiversity-Climate Interactions on 12-13 June 2007. Steve Widdicombe gave evidence to English Nature Council on 13-14 March 2006. Through participation in the IMCO2 RUG members receive the latest advances in the field and can impart this to their organisations.

2.4 CONFERENCES AND SYMPOSIA

Carol Turley presented an invited paper at the *Coastal Futures Conference* in London 18-20 January 2005 and presented a paper at the *Avoiding Dangerous Climate Change Symposium* in Exeter 1-3 February 2005 both on impacts of ocean acidification. PML also co-authored another paper at the Exeter conference, on CCS, with other partners from the UKCCSC lead by Jon Gibbins. A further paper was presented at the United Nations Framework Convention on Climate Change, SBSTA Side Event on Outcomes of the International Symposium on Stabilisation of Greenhouse Gases – Latest scientific results on climate change, 19 May, Bonn, Germany and the following day at The London Convention Workshop on CCS at BERR Conference Centre. Carol gave a further presentation at the NERC Regional Conference, 13 June 2005 at Cardiff International Arena. Carol Turley attended the British Embassy Berlin Workshop on Tipping Points in the Earth System on October 5-6 2005. The aim is to assess what are the most important tipping points in the Earth System and under what conditions they will be triggered. She was also invited by the Marine Conservation Society to speak on ocean acidification at Exeter, 5 November 2005. Carol gave an invited presentation on ocean acidification at the intergovernmental (UK & NZ) Climate Change Conference in Wellington, New Zealand in March 2006. She gave a lunchtime Seminar at the EU, Brussels, on Climate Change and Ocean Acidification in the marine environment (Oct 2006) with Dan Lafolley (NE) and Beth Greenaway (DEFRA) and an invited keynote at the UKERC meeting on CCS, Edinburgh (6 July 2006) and at the Climate Change and the Marine Environment Conference, London, (30 November 2006). Interim results from the UK Carbon Capture and Storage Consortium project (Gibbins, J., Hazeldine, S., Holloway, S., Pearse J., Oakey J. Reiner D. and C. Turley 2006) were presented at 8th International Conference on Greenhouse Gases, 19-22 June 2006, Trondheim, Norway. Carol was an invited keynote speaker at the UK Energy Research Council meeting on Carbon Capture and Storage, Edinburgh on Climate Change and Ocean acidification.

Steve Widdicombe was an invited plenary speaker at the Australian Marine Sciences Association meeting on Biodiversity, Biodiscovery and Biosecurity (Darwin, Australia, 10-13 July 2005). During his time in Australia he also gave presentations to the Australian Academy of Science and the Bureau of Rural Sciences, both in Canberra. He also presented a talk introducing the ecological problems associated with ocean acidification to an audience of policy makers and coastal zone managers from Thai government departments and universities in Bangkok, 3 July 2006. Steve presented IMCO2 work to an international audience at the American Society of Limnology and Oceanography (ASLO) 2007 Aquatic Life Sciences meeting held in Santa Fe (USA) 5-9 February. His talk was entitled "Bioturbation in an increasingly acidic ocean: Consequences for sediment nutrient flux. In March 2007, Steve was one of nine invited scientists from the UK and New Zealand who took part in a British Council sponsored event in Wellington (10–16 March) on the theme of "The scientific aspects of anthropogenic climate change and its mitigation to be addressed within the context of climate change in the past, present and the future". During the event he gave two presentations on IMCO$_2$ research to scientists in Wellington and Hamilton. It is hoped the event will lead to a number of joint UK/NZ research projects.

Jerry Blackford presented preliminary modelling results from IMCO2 in a presentation entitled "Marine ecosystem response to pH changes resulting from increasing CO_2 emissions" at the international symposium *Advances in Marine Ecosystem Modelling Research*, Plymouth, 27-29 June 2005. This is published in the symposium proceedings. Jerry, together with Nancy Jones, presented a poster paper at the AGU Symposium, Vienna, April 2006 during a special session on ocean acidification. Jerry gave an oral presentation at the 2006 Challenger conference (Oban), 'CO_2 induced marine acidification and ecological consequences for the North Sea" 12 September 2006. He presented his work at the Earth Systems Science Partnership meeting in Beijing, November 2006. Jerry Blackford also organised an international modelling AMEMR workshop on acidification (12-14 February 2007), with a view to improving model descriptions of a range of pH affects and creating potential research synergies. This benefited from separate NERC and DEFRA GA funding, and has been enabled by the IMCO2 project. Key international researchers attended and presented their research. Four IMCO2 scientists presented their IMCO2 work (Blackford, Jones, Widdicombe and Turley).

Mike Kendall gave an invited talk at the "Britain in Norway" symposium organised by the British embassy in Bergen, 11-14 October 2005. His talk was entitled "The potential environmental impacts of CO_2 sequestration in the North Sea". Mike also represented IMCO2 research at the 2nd East Asian Seas (EAS) Congress in China, where PML was convener and sponsor of a session on Ecosystem Based Management & Forecasting in December 2006.

Hazel Needham presented a poster "The environmental impacts of CO_2 release on marine systems following carbon sequestration" during the "SET for Britain" meeting held at the Houses of Parliament, 13 March 2006.

Dan Laffoley (RUG chairman) will be presenting a paper at Marine Conservation in Europe 2006 entitled "Climate change, Surface Ocean Acidification and their impacts on European seas" by Laffoley, Hawkins and Turley.

Carol Turley, Steve Widdicombe and Jerry Blackford were invited to attend the IGBP-SCOR "Ocean Acidification" workshop in New York (USA) on 28-30 September 2006. All three presented their work on ocean acidification in poster and presentation format.

2.5 REFERENCE USER GROUP (RUG)

The RUG has been formed, with invitations and terms of reference sent out to 14 representatives of UK agencies, industry, programmes, NGOs and the sponsoring government departments, DEFRA and BERR (see Appendices I & II). The RUG is chaired by Dan Laffoley (Natural England) and the first meeting was on the 15 June 2005 at Plymouth Marine Laboratory. The second meeting of the IMCO2 RUG was held on 14 June 2006. This was a one day meeting in Plymouth and the agenda included presentations on the latest BP CCS project by Tony Espie, a presentation on the UKCCSC by Jon Gibbins, an update of the experimental and modelling results from project scientists as well as an update on international science developments and the networking and promotion activities by the project staff. RUG members were asked to give feedback on the projects progress and on their experience of the issues emerging in their own sphere of operation. The PML contribution to the TSEC funded UKCCSC was agreed to be included within the remit of the RUG. The third RUG meeting was held in Plymouth on 28-29 November 2006 at which a stakeholder analysis for CCS was carried out and where the future of IMCO2 was discussed and plans made to implement them. Throughout the project we have maintained communication with the RUG members, keeping them advised of new issues and science developments.

2.6 MEDIA

We have worked with the media to ensure that reporting of this topic is based on sound science. In addition to numerous telephone interviews, we hosted the BBC Newsnight team for a days filming in Plymouth and a World Service reporter working on the Discovery Ocean Blues series. Since the Exeter Conference there has been a substantial media interest in ocean acidification with features by television (eg BBC 6 o'clock News, Newsnight), radio (eg BBC Radio 5 Live, BBC Radio Devon and Scotland, BBC World Service 25 minute programme) at the international, national and local level, broadsheets (eg Guardian, Financial Times, Observer, Telegraph, The Sunday Times, The Independent), scientific magazines (New Scientist, Science and Nature) and on-line news (eg BBC News online, CNN). Since then the "story" has run world-wide with multi-media articles around the world resulting in enquiries from a wide range of interested parties such as the Australian Academy of Science, Japanese Broadcasting Corporation, Embassy of Peru and Lord Norman Blackwell. We have also given information, on enquiry, to the general public and presented the topic as part of the BA Festival of Science on 22 October 2004.

Steve Widdicombe attended a "Communicating Climate Change" workshop at the BBC television centre (26 January 2006). The aim of the workshop was to provide expert opinion to the BBC on subjects relating to climate change and how the BBC could best fulfil its commitment to public communication and education.

On publication of The Royal Society Working Group report on ocean acidification (www.royalsoc.ac.uk/document.asp?id=3249) there was coverage on BBC News (1pm, 6pm and 10pm) as well as articles in broadsheets, scientific magazines and radio. Again this spread worldwide and was based on a days filming in Plymouth. We have aided the accurate reporting of this topic through working with the media.

At the 'Climate Change & Governance Conference' in New Zealand, Carol Turley gave 4 live interviews for radio and newspapers and was involved in filming for a documentary.

IMCO2 science was presented by Steve Widdicombe in the documentary "Can We Save Planet Earth?" presented by David Attenborough. This hour long program was shown on BBC1 at 9pm on 31 May 2006 as part of the BBC Climate Chaos Series.

Carol Turley was interviewed by and supplied images to Olive Heffernan for article in The Marine Scientist (2006), "Souring the Seas", 15, 18—21. She has also been interviewed by:

Jonathan Leake for half page article in Sunday Times 13/3/06.
Martin Bayley from BBC Cornwall
Lynn Dicks from New Scientist leading to an article
BBC Science unit on CCS as mitigation method
Mark Purcell of DNV regarding IEA CCS review of risk
Steven Chipperdale for "Project Ocean" Lister Charitable Trust.

In the last year there have been key general science articles in New Scientist, New Yorker, Marine Scientist, Scientific American. Newspapers, TV and radio have picked up this issue and run different aspects of this on an increasing basis. Scientists at PML have helped increase the profile of this topic through transfer of sound science to authors.

A Google search on "ocean acidification" in 2003 at the start of the project revealed 17 hits, on 28 February 2007 there are 312,000 hits – many of these have been due to outreach activities connected to IMCO2.

2.7 SUMMARY AND CONCLUSIONS

Members of the IMCO2 team have worked effectively at transferring the knowledge gained by the project to users including scientists, policy makers and the general public through a wide range of international and national outreach activities. The topic of ocean acidification has been brought to the climate change, energy and biodiversity scientific and policy communities at national and international levels. IMCO2 outreach activities have ensured that ocean acidification is regarded as "the other CO_2 problem" and is as important as climate change in the future health of the planet, is a forceful argument for mitigation of CO_2 emissions and has been used to remove legal obstacles for wide scale geological carbon capture and storage. PML and the IMCO2 team have networked and collaborated at the national and international level to help to ensure that it is an area of science that will be invested in the future.

3. OBJECTIVE 2: ECOSYSTEM MODELLING

3.1 INTRODUCTION

The ultimate aim of the modelling work is to firstly quantify the ecological impact of a business as usual atmospheric CO_2 build-up scenario and secondly to provide a capability by which we can assess the potential ecological impact of unplanned CO_2 release from geological storage. This requires a significant multi-phase program of model development:

1a Couple existing marine ecosystem, hydrodynamic and CO_2 chemistry models to create a European regional shelf seas coupled 3D modelling capability.

1b Development of geological models that determine likely CO_2 release scenarios including the magnitude, form, location and residence time of release.

2. Develop existing knowledge and ongoing process studies into computationally expressible functional relationships between ecosystem components, CO_2 concentrations and pH. Couple and quantify these process descriptions in the context of European shelf model systems.

3a Assess the influence of the physical impact on the marine system of high CO_2 mediated changes in atmospheric forcing such as temperature, wind speed and storm frequency and test how this interacts with the direct ecosystem effects quantified in phase 2. This would deliver the business as usual quantification.

3b Assess the likely ecological impact of specific CO_2 release scenarios that may result from failure of geological storage. This would quantify the risk from geological storage.

Given the short time frame under which the outputs from such modelling are required in order to inform UK CO_2 abatement policy, it is unrealistic to expect such a substantial financial commitment to come from a single research project. Consequently, the primary aim of the IMCO2 project was to achieve phase 1a and initiate phase 2 described above whilst establishing the capacity and methodology by which to complete phases 2 and 3a. By doing so the work will underpin the developments made by additional projects such as the Joint Research Council funded TSEC project.

3.2 COUPLE EXISTING MARINE ECOSYSTEM, HYDRODYNAMIC AND CO_2 CHEMISTRY MODELS TO CREATE A EUROPEAN REGIONAL SHELF SEAS COUPLED 3D MODELLING CAPABILITY.

Building on existing technologies developed at PML we have integrated a NW European shelf-wide marine ecosystem simulation model with algorithms that describe CO_2 and pH chemistry. For the first time this couples environmental CO_2 with the marine ecosystems CO_2 cycle over the continental shelf throughout an annual cycle. Our current modelling capability is based on the ERSEM ecosystem model which resolves the detailed functional and community structure of the marine system, including benthic chemistry and ecology and the POLCOMS hydrodynamic model which provides a high resolution hydrodynamic model system describing the NW European shelf seas. ERSEM-POLCOMS forms an operationally able model system, which is recognised as state-of-the-art and benefits from substantial development and evaluation funded through other sources. The model requires parallel configured code running on high-performance, multi processor machines. The ERSEM model and CO_2 carbonate cycle model have also been successfully coupled in simpler 1D applications providing an ideal development and process evaluation tool.

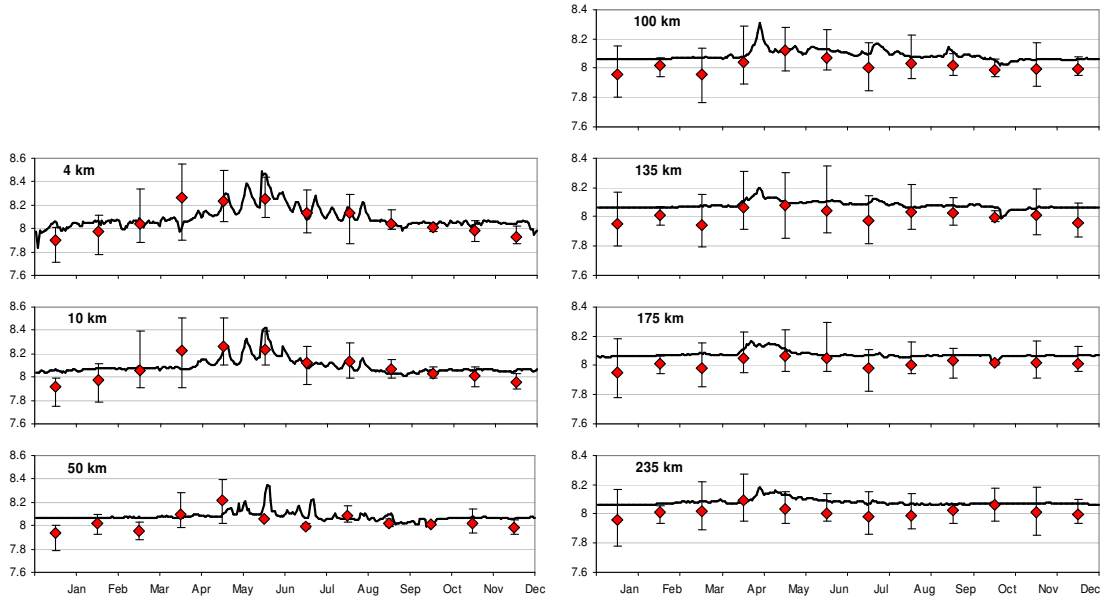

Figure 3.1: Validation of model along a transect from the Dutch coast to the central North Sea (Distances from Dutch coast)

Considerable effort has gone into identifying relevant data sets for validation of the model (eg Ferrybox data, Cavasoo). Because of the sensitivity of the carbonate system to environmental conditions there are significant uncertainties associated with all available data sets. With this proviso the model is shown to capture much of the spatial temporal variability in carbonate parameters (fig 3.1). More international effort in making existing data sets available and creating new observations is urgently required. Our predictions of pH and pCO_2w also agree closely with other model's predictions, giving us some confidence in our models functionality.

Our work suggests that there is much spatial heterogeneity in annual pH ranges over the UK shelf (fig 3.2). Ranges may be as little as 0.2 pH units in areas of low productivity and high mixing, rising to 0.3-0.5 pH units in regions of high productivity and seasonal stratification to ranges >1.0 pH unit in regions directly influenced by riverine inputs. This compares with the predicted maximum pH decrease of 0.7 pH units due to uptake of atmospheric CO_2. In oceanic waters where pH ranges are generally <0.3 units over the annual cycle the current rate of acidification will ensure that pH becomes completely distinct from its historical (20 million years) range by ~2050 or 500ppm atmospheric CO_2. For shelf waters this disconnection, because of the higher range will take longer, until ~700ppm is reached. This might have implications for the ability of shelf dwelling species to adapt.

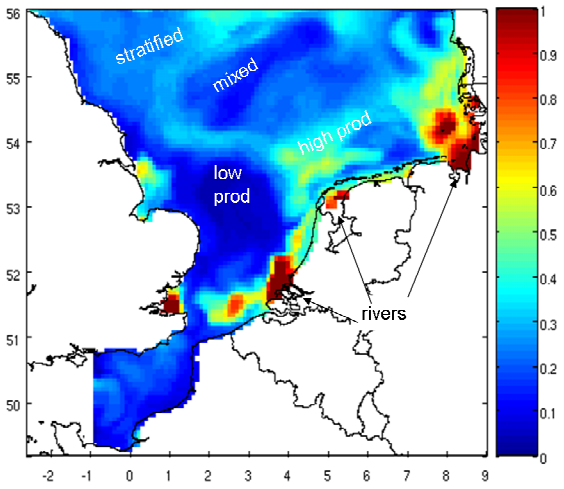

Figure 3.2: Model predicted in-situ pH ranges over the annual cycle in the Southern North Sea.

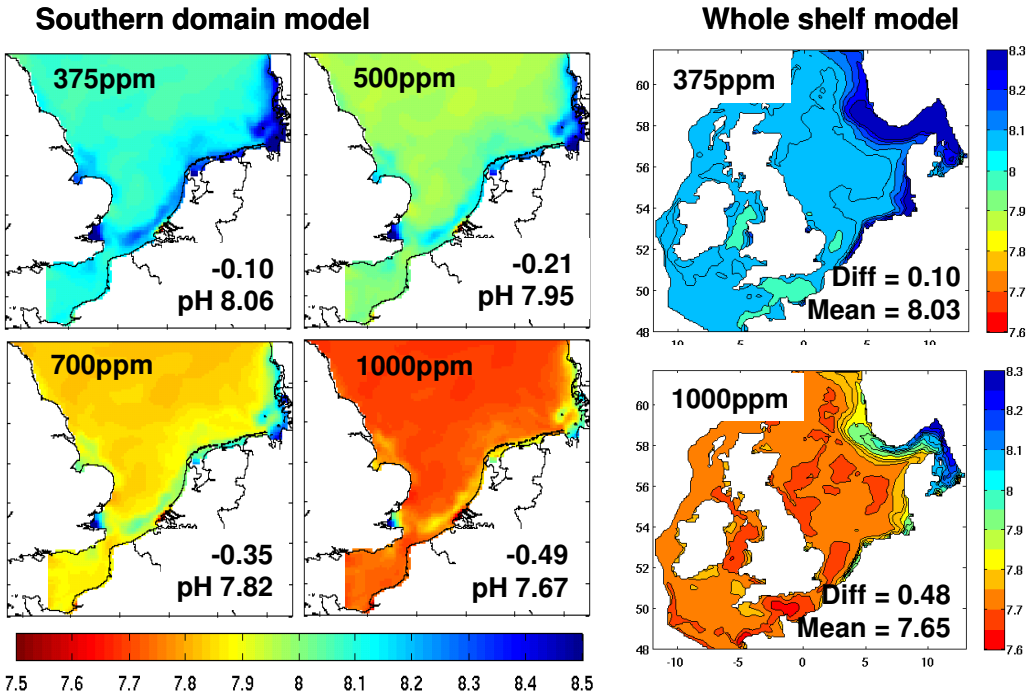

Figure 3.3: pH fields for a range of atmospheric CO_2 concentrations (top left of each panel). The difference from pre-industrial and the mean pH are given to the lower right of each panel.

We have used the model to investigate the rate of acidification across the UK shelf seas (fig 3.3). The model predictions are again consistent with other models (addressing other

domains) and indicate a change of -0.35 pH units from pre-industrial levels associated with atmospheric CO_2 of 700ppm. The rate of acidification is consistent across the domain.

3.3 DEVELOP EXISTING KNOWLEDGE AND ONGOING PROCESS STUDIES INTO COMPUTATIONALLY EXPRESSIBLE FUNCTIONAL RELATIONSHIPS BETWEEN ECOSYSTEM COMPONENTS, CO_2 CONCENTRATIONS AND PH. COUPLE AND QUANTIFY THESE PROCESS DESCRIPTIONS IN THE CONTEXT OF EUROPEAN SHELF MODEL SYSTEMS.

Over the three years of the IMCO2 project the international scientific community has been engaged in a variety of experiments aiming to measure the response of species and ecosystems to increased CO_2. The consensus is that whilst the chemistry of acidification is well determined the ecosystem response is not. Experiments have delivered inconsistent results for some species, and are unable to test the ability of organisms to adapt or evolve. Our approach within this project, given this uncertainty, is to test, singularly some of the observed responses and ascertain if each is able to produce measurable changes in key indicators in the modelled ecosystem. We therefore assess the possibility of response rather than its probability. We have tested three separate processes that are sensitive to the carbonate system.

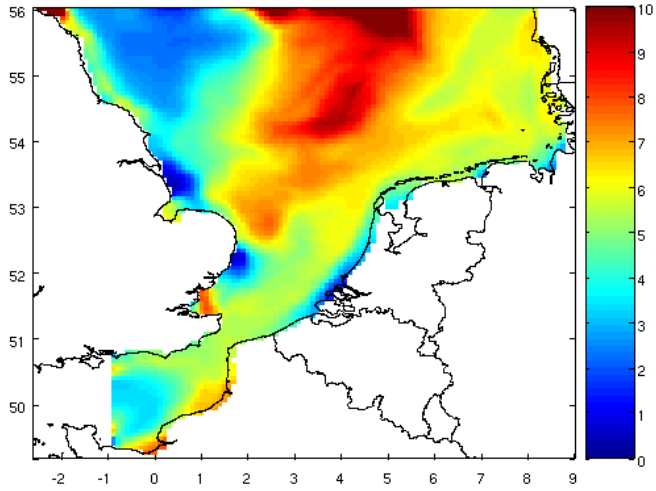

Figure 3.4: Percentage change in the ratio of nitrate to total nitrogen between the 375ppm and 1000ppm simulations.

Nitrification is known to be sensitive to decreasing pH. Using published data we show that measurable and possibly significant changes in the ratio of ammonium to nitrate (key nutrients for phytoplankton) are likely to be provoked by acidification (fig 3.4). The changes to the nitrification rate may lead to a 10% decrease in nitrate and a 20% increase in ammonium in the pelagic. This will influence benthic nutrient cycling but the complexity of the response will require further research; it should be noted that this tests only one process of the many that influence the marine nitrogen cycle.

Phytoplankton use an inefficient enzymatic process to assimilate CO_2 for photosynthesis. As a result the gross rate of primary production is sensitive to the concentration of CO_2 in the water, although CO_2 is never limiting in the sense that nutrients are. Different phytoplankton species process different versions of the enzyme concerned (Rubisco) with have different uptake:[CO_2] response curves. We used the model to asses if changing ambient CO_2 would affect species to a different degree, producing changes in phytoplankton community structure (fig 3.5). The results demonstrate that such changes are possible.

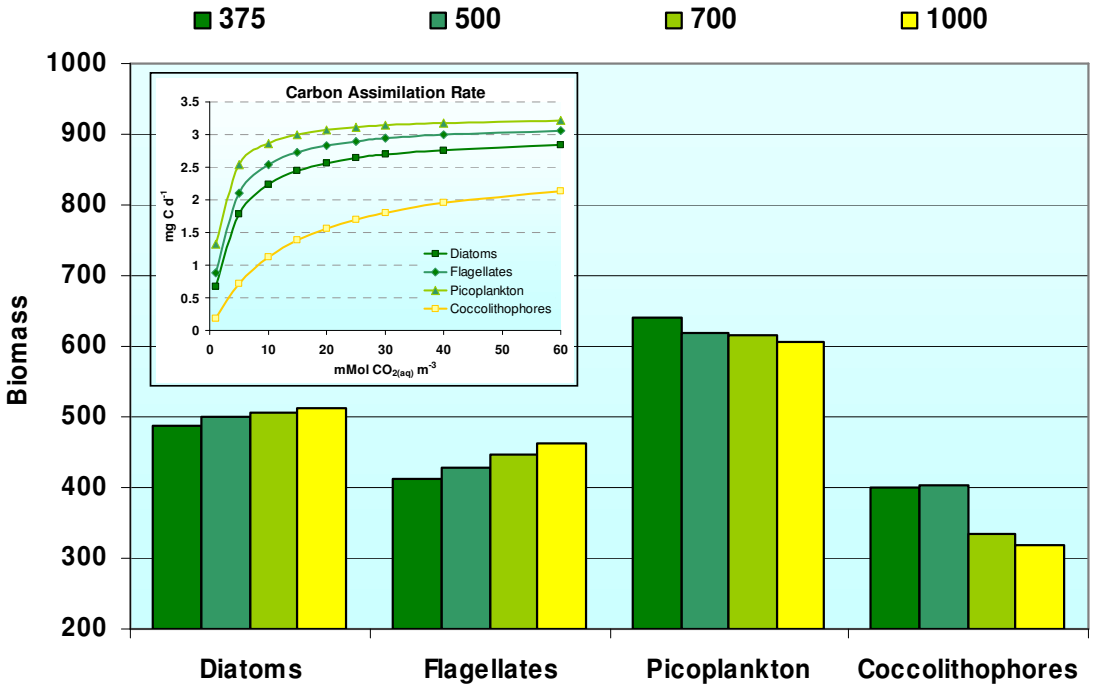

Figure 3.5: Species specific growth response to CO_2 (inset) and resulting modelled community structure suggesting significant changes.

We have simulated coccolithophore (a type of phytoplankton) response to calcification inhibition over the next 100 or so years using two distinct annual weather patterns (fig 3.6). Each year has a different temperature/wind envelope giving different degrees of physical mixing in the water column. Marked inhibition of coccolithophores is seen with decreasing pH. But inhibition strength is very sensitive to the physical conditions prevailing and the resulting dynamics of other species which both alter the calcification window and nutrient availability. We conclude that linking acidification response to climate change response is essential.

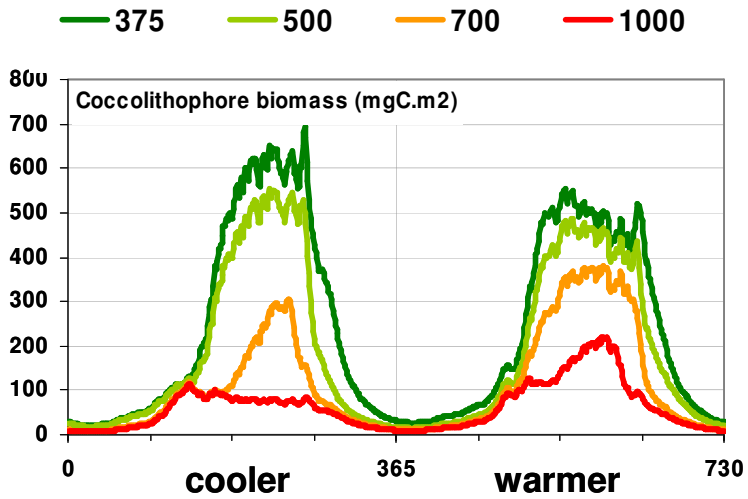

Figure 3.6: Response of coccolithophores in different weather patterns for different atmospheric scenarios of CO_2.

Finally we have begun to examine benthic responses to pH changes building on the mesocosm results described below. As an example figure 3.7 illustrates the significant changes in benthic nutrient content predicted for scenarios up to 1000ppm CO_2 in the atmosphere. These changes are the result of only one process sensitivity (nitrification). A newly structured benthic model will allow us to test the ecosystem sensitivity to loss or inhibition of shallow burrowing calcareous organisms and also allows the model to be more realistically parameterised for different sediment types (mud to sand).

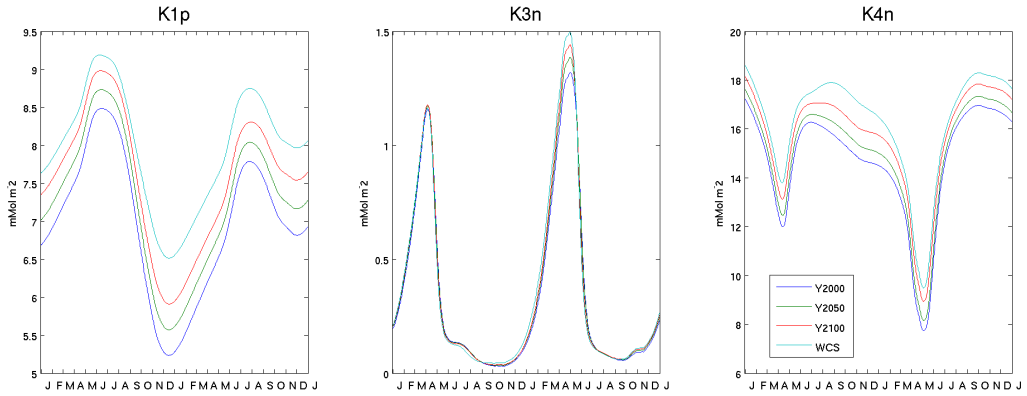

Figure 3.7: Benthic nutrient responses to pH, left to right: Phosphate, Nitrate and Ammonium.

3.4 SUMMARY AND KEY FINDINGS

Algorithms that describe CO_2 and pH chemistry have now been integrated into a NW European shelf-wide marine ecosystem simulation model (ERSEM). For the first time this couples environmental CO_2 with the marine ecosystems CO_2 cycle over the continental shelf throughout an annual cycle. The ERSEM model and CO_2 carbonate cycle model have also been successfully coupled in simple 1D applications providing an ideal development and process evaluation tool.

The model suggests:

- There is spatial heterogeneity in annual pH ranges over the UK shelf and ranges may be as high as 0.3-0.5 pH units in regions of high productivity and seasonal stratification and even higher (>1.0 pH unit) in regions directly influenced by riverine inputs.

- At the current rate of acidification, pH in UK shelf waters will become completely distinct from its historical (20 million years) range should atmospheric concentrations reach 700ppm.

- This concentration is less than that required for disconnection in oceanic waters and may mean that shelf species have more time to adapt to pH change than oceanic ones.

Over the three years of the IMCO2 project the international scientific community has been engaged in a variety of experiments aiming to measure the response of species and ecosystems to increased CO_2. The consensus is that whilst the chemistry of acidification is

well determined the ecosystem response is not. Experiments have delivered inconsistent results for some species, and are unable to test the ability of organisms to adapt or evolve. Our approach within IMCO2, given this uncertainty, was to test, singularly some of the observed responses and ascertain if each is able to produce measurable changes in key indicators in the modelled ecosystem. The modelling experiments suggest:

- Changes in the ratio of ammonium to nitrate (key nutrients for phytoplankton) are likely to be provoked by acidification.

- Acidification could change phytoplankton community structure as different phytoplankton species possess different versions of the enzyme concerned with CO_2 assimilation (Rubisco) and therefore have different uptake:[CO_2] response curves.

- The response of Coccolithophores (a type of phytoplankton) to calcification inhibition is dependant on pH as well as other factors associated with climate change such as temperature and wave mixing.

In order to improve the predictive certainty and policy utility via modelling approaches we recommend:

- Continued investment in national computing infrastructure to enable multiple scenario approaches and experimental coupling of diverse model approaches.

- Funding schemes that couple modelling and observational/experimental research are prioritised. The design of new observational and experimental programmes should be driven by model requirements and statistical rigour.

- Programme structures that couple UK expertise in climate change, acidification, ecosystems and socio-economic modelling are identified.

4. OBJECTIVE 3: SEAWATER ACIDIFICATION FACILITY

4.1 INTRODUCTION

Previous studies have used organic acids to simulate seawater acidification as a consequence of increased levels of CO_2. However, a recent study (Kikkawa et al 2004) has demonstrated that when marine organisms were exposed to seawater acidified to the same pH by the addition of either CO_2 or HCl, CO_2 exposure resulted in significantly higher mortalities than HCl and concluded that the use of acid induced pH change to evaluate CO_2 toxicity was invalid. Consequently, experiments aimed at addressing issues related to ocean acidification and leakage from CO_2 storage should use CO_2 to reduce seawater pH and not acid.

4.2 DEVELOPMENT

In January 2005, four members of the IMCO2 project team visited Oslo (Norway) to observe the temporary seawater acidification system constructed by NIVA staff at the Marine Research Station (MRS) Solbergstrand. The information gathered during this visit helped in the construction of the PML seawater acidification system.

To create acidified seawater of a specific pH, CO_2 is passed through natural seawater contained within a large (450 litres) reservoir tank (fig. 4.1). The CO_2 is passed through the water as very fine bubbles and this allows the CO_2 to pass rapidly into solution. Once the pH has fallen to the required level, the supply of CO_2 is halted. As the acidified water is taken from the reservoir, to supply experimental tanks and aquaria, it is replaced by natural seawater (pH ≈ 8.2) from a separate 16m³ seawater tank causing the pH in the reservoir to increase. This increase triggers the supply of CO_2 to be restarted and CO_2 continues to bubble through the water until the pH has again been reduced to the pre-set level. Using this method it is possible to supply large quantities of CO_2 acidified seawater of a consistent pH.

The PML seawater acidification system consists of 3 such reservoirs which, together with a fourth reservoir containing natural seawater, allow experiments to be conducted using four different pH treatment levels simultaneously (fig. 4.2). The pH in each of the four seawater supply systems will be monitored using a Walchem WebMaster-GI controller, connected to four flat surface combination pH electrodes (S650CD), one in each seawater supply reservoir. The Walchem WebMaster-GI will interface up to four pH sensors, reading temperature-compensated pH to an accuracy of 0.01pH units. This built-in temperature sensing and compensation enables temperature to be logged alongside pH. As is the case in the Norway system, each of the four pH sensor inputs can be assigned to one of four ac relay outputs, allowing control of the CO_2 delivery system to maintain pH within pre-set

bounds. The WebMaster controller is connected directly to the PML LAN. This allows the permanent host computer to be located away from the mesocosm, and also allows access to the system not only from anywhere else in the lab, but also from remote sites.

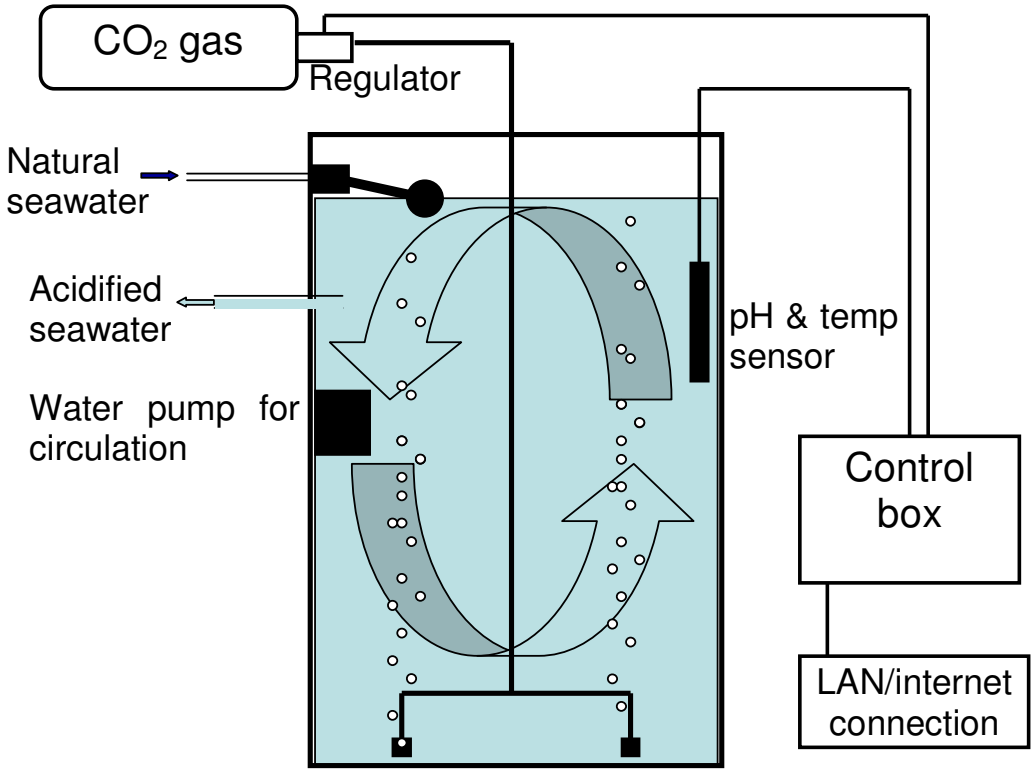

Figure 4.1: Schematic diagram of a seawater acidification tank.

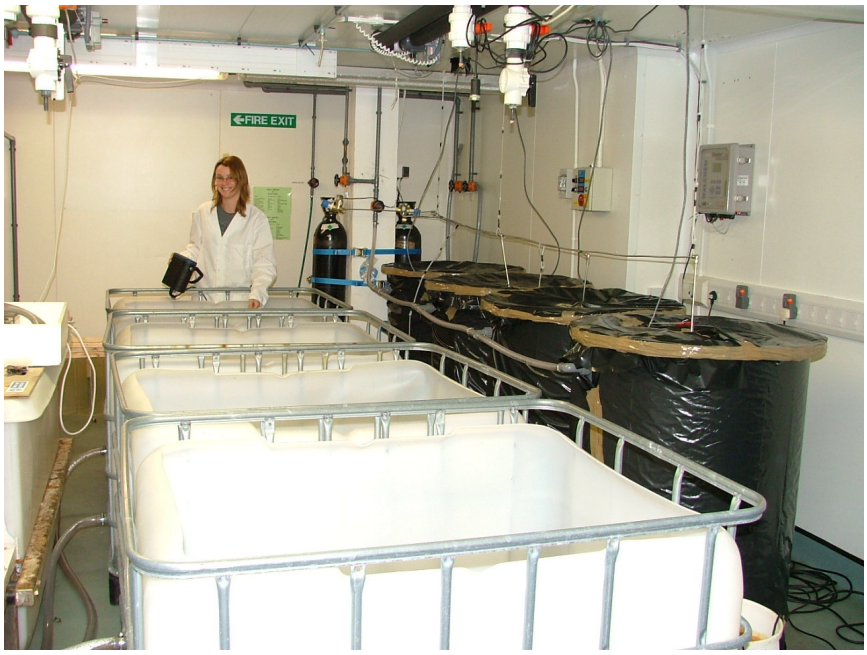

Figure 4.2: The Plymouth Marine Laboratory seawater acidification facility.

4.3 SUMMARY AND CONCLUSIONS

The creation of this facility has not only enabled the experiments within IMCO2 Objective 4 to be conducted, it has also stimulated a number of collaborations with the University of Plymouth. These collaborations have primarily been through jointly supervised PhD and Masters students.

5. OBJECTIVE 4: IMPACT ON KEY BENTHIC ORGANISMS

5.1 BACKGROUND

The sediments that make up the majority of seabed habitats play a crucial role in many important marine processes including the cycling of key nutrients within coastal and shelf sea ecosystems. For example, in shallow (<50m) coastal areas up to 80% of the nitrogen required by phytoplankton may come from the bacterial regeneration of organic matter within the seabed (Dale & Prego 2002). Whilst primarily an activity untaken by microbial organisms, nutrient cycling (nutrient transformations and transport across the sediment water interface) is significantly influenced by the activities of multicellular animals which live and feed within the sediment (e.g. Widdicombe & Austen 1998, Mortimer et al. 1999, Tuominen et al. 1999). In doing so such species may be considered as ecosystem engineers (Lawton, 1994).

5.2 IMPACT ON NEREIS VIRENS

5.2.1 Introduction

With respect to nutrient cycling, organisms which build and irrigate permanent burrows are particularly important (Fenchel 1996 and references therein). These burrows increase both the surface area of sediment across which nutrients can pass as well as the availability of sites for nutrient transformations, such as denitrification (Mayer et al. 1995). In addition, burrow irrigation/ventilation will actively transport oxygen and nutrients between the overlying water and subsurface sediments (Aller 1982, Aller & Yingst 1978). An example of such a species is the polychaete worm *Nereis virens* (Sars 1835) (fig. 5.1). This species is an important burrow building bioturbator in coastal sediments of northern temperate latitudes (Ouellette et al., 2004; and references therein) and creates a semi permanent U-shaped burrow (Bass & Brafield 1972) which it spends 20 – 30% of its time irrigating (Kristensen 1985). In doing so it has been shown to stimulate nutrient transformation processes such as denitrification (Kristensen et al. 1985) and significantly alter the flux of nutrients across the sediment-

Figure 5.1: The polychaete *Nereis virens.*

water interface (Christensen et al. 2000). *Nereis virens* can be locally abundant with densities as high as 2000ind.m^{-2} having been recorded (Hylleberg & Henriksen 1980) although densities between 200-700ind.m^{-2} are more common (Christensen et al. 2000; Kristensen 1984). Where they occur, the burrows of *N. virens* have been shown to increase the total sediment-water interface by up to 150% (Kristensen 1984).

5.2.2 Methodology

Two separate experiments have been conducted. The first examined the impact of seawater acidification on the burrowing activity of *N. virens* and the subsequent implications for nutrient flux. The second experiment determined the impact of acidification on the health of *N. virens.* All worms used in these two experiments were supplied commercially by Seabait Ltd (Northumberland).

5.2.2.1 Sediment collection

Experiment 1: On 20 June 2005, eighty undisturbed cores were collected from an area of moderately sorted sandy mud (median phi = 4.01) 100m north of Plymouth Breakwater (50˚20.090N, 4˚08.520W); water depth was approximately 10m. The cores were collected by sub-sampling from a 0.1m^2 boxcorer. Into each box-core sample, 9 plastic cores (10cm diameter, 20cm long) were pushed into the sediment to a depth of 15cm and capped. Each core was then gently removed from the box-core, sealed on the bottom with a second plastic cap and returned to the Plymouth Marine Laboratory (PML) mesocosm within a few hours of collection. Once in the mesocosm the top caps were removed and the cores were placed randomly in a recirculating seawater system until they were transferred into the experimental set-up.

Experiment 2: On 16 November 2006, 25 undisturbed cores were collected using the same method as described for Experiment 1 (see above) from the same sediment collection site North of Plymouth breakwater. The cores were again held in a recirculating seawater system within the PML mesocosm.

5.2.2.2 Experimental set up

Experiment 1: On 6 July 2005 the sediment cores were placed within the experimental system and each core was individually supplied with seawater at a rate of approximately 5ml.min^{-1} using a peristaltic pump. Cores were randomly assigned to one of four pH treatment levels (7.9 [ambient seawater], 7.3, 6.5, 5.6). The 20 cores within each of the 4 pH treatments were then randomly assigned a *N. virens* addition treatment level (No added worms, small worms [0.5g], medium worms [1.0g], large worms [1.5g], very large worms [2.0g]) to create a 2 factor, multi level, crossed experimental design. Each pH/*N. virens* treatment combination was replicated 4 times. Background faunal biomass at the sediment collection site was 0.22g.core^{-1} (Townsend 2006). This value equated to 15% of the *N. virens* biomass in the 'small worm' treatments (1.5g.core^{-1}) and 4% of the *N. virens* biomass in the 'large worm' treatments (6g.core^{-1}).

After the cores had settled for 24 hours, three *N. virens* of the appropriate size class (g wet weight) were added to each treatment core and allowed to burrow below the sediment surface. This corresponded to a density of 382ind.m^{-2}. To prevent the worms experiencing acidified conditions whilst out of the burrow environment, acidification of the seawater

supply did not begin until 24 hours after the addition of the worms. By this time all worms had burrowed beneath the sediment surface. Miron et al (1991) found that it could take *N. virens* more than 10days to complete a new burrow and that the burrow system may be perpetually reshaped. Consequently, any burrowing in the first 24 hours would have minimal effect on the observations of the final burrow structure. Seawater pH was reduced gradually over a period of 4 days and the experiment started once the final treatment levels had been reached. The experiment ran for 5 weeks during which time the supply water was monitored for pH, temperature (Table 5.1) and water flow. No additional food was added to the cores as this can cause excessive worm activity (Miron et al. 1991).

pH Treatment Level	**7.9**	**7.3**	**6.5**	***5.6***
Average pH	7.89 (±0.02)	7.27 (±0.02)	6.46 (±0.07)	5.58 (±0.01)
pH range (max – min)	7.84 – 7.92	7.21 – 7.30	6.30 – 6.60	5.56 – 5.60
Temperature (ºC)	19.4 (±0.5)	19.5 (±0.5)	19.4 (±0.6)	18.8 (±0.4)

Table 5.1: Measures of pH and temperature (±1 standard deviation) for experiment 1.

Experiment 2: On 22 November 2006, 100 *N. virens* were weighed (blotted wet weight) and added to the 25 sediment cores at a density of 4 individuals per core. The animals were allowed 6 days to construct their burrows before being transferred to the seawater acidification system on 28 November. Each core was individually supplied with seawater at a rate of approximately 5ml.min^{-1} using a peristaltic pump. Cores were randomly assigned to one of five pH treatment levels (7.9 [ambient seawater], 7.7, 7.3, 6.6 and 5.6). Seawater acidification began 30 November and seawater pH levels in the 4 acidified treatments were lowered gradually over a period of 7 days. By 6 December (termed “Day 0”), all pH treatment levels had been reached and the experiment was deemed to have started at this point. The exposure lasted for 40 days and on Monday 15 January 2007 each of the cores was removed from the system and the worms recovered by gently sieving the sediment over a 2mm mesh.

	7.9	**7.7**	**7.3**	**6.6**	***5.6***
pH	7.88 (±0.08)	7.70 (±0.06)	7.36 (±0.13)	6.60 (±0.13)	5.54 (±0.18)
Temp (ºC)	17.0 (±0.5)	16.6 (±0.4)	16.3 (±0.4)	15.9 (±0.5)	15.3 (±0.5)
Salinity	35.9 (±0.5)	36.1 (±0.3)	36.4 (±0.6)	36.2 (±0.7)	36.5 (±0.7)

Table 5.2: Measures of pH, temperature and salinity (± 1 standard deviation) for experiment 2.

5.2.2.3 Measurement of nutrient flux

Experiment 1: The experiment ran for 35 days after which samples of the overlying water were taken from each core and used to determine nutrient flux. Over 3 consecutive days (15-17 August 2005), three 50ml water samples were drawn from each core, filtered through a 47mm ø GF/F filter and stored in an acid washed nalgene bottle. In addition to these ‘core’ samples, 15 ‘inflow’ samples were taken from random selected supply tubes from each of the four header tanks. Each water sample was analysed by a nutrient autoanalyser (Branne & Luebbe Ltd., AAIII) for ammonium, nitrate, nitrite, silicate and phosphate concentrations using standard methods (Brewer & Riley 1965, Grasshoff 1976,

Mantoura & Woodward 1983, Kirkwood 1989, Zhang & Chi 2002). Fluxes were calculated using equation 1 (Austen, 2006).

1) $$F_x = \frac{(C_i - C_o) \times Q}{A}$$

F_x is the flux of nutrient x (μmol.m^{-2}.h^{-1})
C_i is the mean concentration of nutrient x in the inflow water (μM)
C_o is the mean concentration of nutrient x in the core water (μM)
Q is the rate of water flow through the core (l.h^{-1})
A is the area of the core (m²)

A positive flux value indicates a sediment uptake of nutrient whilst a negative value indicates a sediment release.

5.2.2.4 Estimating burrowing activity and morphology

Experiment 1: On 18 August 2005, 24 hours after the completion of the final nutrient sampling, resin casts of all burrows in each core were made by pouring approximately 500ml of catalysed polyester resin onto the sediment surface of each core. The resin used consisted of 84% base resin, 15.5% styrene thinner and 0.5% hardener. Being denser than seawater, the resin flowed down into each of the burrows before setting. The resin at the surface of the core was hard to the touch within a matter of hours. However, the cores were then left for a further 8 days to ensure the resin within the sediment had fully hardened. The resin casts were then removed from the sediment by removing the cap from the base of the core and gently washing away the surrounding sediment. What remained was an intact cast of the burrows attached to a disc of the overlying resin (fig. 5.2).

For each cast, measurements were taken of the maximum depth of burrow penetration and the total length of burrows. The weight of burrow casts was measured after the disc of overlying resin had been removed. From these measurements total burrow volume (equation 2), average burrow diameter (equation 3) and the total burrow surface area (equation 4) were calculated. At the same time as the resin casts were made, random samples of the resin used were poured into 5 separate containers and allowed to set. These 5 resin samples were then cut into blocks of known volume and weighed. From this the resin density was calculated (Resin density = 1.1876g/cm³).

It was not always possible to differentiate between individual worm burrows within each core. Therefore, values for burrow length, volume and surface area were calculated for the total value within each core. This meant the values presented represent the total length, volume and surface area of all burrows within the cast.

2) $$Total\ burrow\ volume = \frac{Total\ weight\ of\ burrow\ cast}{1.1876}$$

3) $$Average\ burrow\ diameter = 2 \times \sqrt{\frac{Total\ burrow\ volume}{Total\ burrow\ length \times \pi}}$$

4) $$Total\ burrow\ surface\ area = Average\ burrow\ diameter \times \pi \times Total\ burrow\ length$$

5.2.2.5 Preparation and analysis of tissue sections

Experiment 2: For the preparation of tissue sections the worms were cut transversely into short lengths so that a complete worm could be accommodated into a single wax block. The cut lengths were immediately placed in Bakers Formal Calcium (10% formalin, 1% calcium chloride and 2.5% sodium chloride) at 4^{O}C and transferred to a refrigerator to complete the fixation process. The preserved specimens were then dehydrated through an ascending alcohol series, cleared in xylene and impregnated in paraffin wax; this procedure was undertaken using an automatic programmable tissue processor. Once wax impregnation was completed the specimens were blocked up in using stainless steel moulds using fresh molten wax and allowed to cool prior to cutting. Sections, 7µm, were cut using disposable steel knives and the sections floated out onto microscope slides coated with ATPS to assist adhesion. Once dry the sections were stained Papanicolaou's and a coverslip placed over the section.

5.2.2.6 Statistical analysis

Experiment 1: Data was manipulated using MS Excel, together with calculations of means, confidence intervals and standard deviations. Statistical analyses were conducted using Minitab 13 for Windows. Two-way crossed analysis of variance (ANOVA) was used to test for significant effects of worm size and seawater acidity on five measures of burrow morphology (maximum depth, total length, average diameter, total volume and total surface area). Where significant effects were observed, mean values were obtained from the ANOVA generated table of means and 95% confidence intervals were calculated using equation 5.

5) $$95\%\ CI = t_{48}(5\%) \times \frac{\sigma}{4} = 2 \times \frac{\sigma}{4}$$

σ is the √ residual Mean Squares from the 2-way ANOVA table

As the flux of nutrients occurs across the sediment/water interface, an analysis of covariance (ANCOVA) was conducted to determine whether a significant relationship existed between burrow surface area and nutrient flux. This analysis was also used to demonstrate whether there was an overall effect of seawater pH on nutrient flux.

Prior to performing the analysis the extent of burrow surface area was calculated as a percentage of the sediment surface area (excluding burrows). This was done so as to allow direct comparisons with other studies which may have used different core sizes or worm densities.

The analyses were conducted within the general linear model function of MINITAB with the nutrient flux as the response variable, pH as the model function and burrow characteristic (e.g. burrow surface area) as the covariate. To further test whether pH had a specific effect on any relationship between nutrient flux and burrow characteristic, the ANCOVA model (H_0) was compared to the regression model (H_1).

H_0: $(y_1 = \alpha_1 + \beta x)$ $(y_2 = \alpha_2 + \beta x)$ $(y_3 = \alpha_3 + \beta x)$ $(y_4 = \alpha_4 + \beta x)$
[Assumes different intercepts but a single slope for each pH treatment]

H_1: $(y_1 = \alpha_1 + \beta_1 x)$ $(y_2 = \alpha_2 + \beta_2 x)$ $(y_3 = \alpha_3 + \beta_3 x)$ $(y_4 = \alpha_4 + \beta_4 x)$
[Assumes different intercepts and different slopes for each pH treatment]

The F ratio was calculated using equation 6 and was referred to the distribution of F and, if F > F (5%), H_0 was rejected. A rejection meant that the slopes were significantly different and therefore pH had a significant effect on the relationship between nutrient flux and burrow characteristic.

6) $$F = \frac{(RSS_{H0} - RSS_{H1})/C}{RSS_{H1}/df}$$

C is the number of constraints imposed by H_0 on H_1
df is the number of degrees of freedom of the residual under H_1

Experiment 2: Paired T tests were conducted in Minitab to identify any changes in worm weight over the experimental period.

5.2.3 Results

5.2.3.1 Mortality

In previous experiments using this organism (Townsend 2006, Batten & Bamber 1996) *N. virens* was seen to come to the sediment surface under hypoxic conditions or before death. Given that no living or dead individuals were observed at the surface during experiment 1 it might be assumed that there was low mortality in response to changes in seawater pH. In addition, during resin casting many worms came to the surface when resin was poured onto the sediment. On recovery of the resin casts the bodies of the worms were observed in the sediment or encapsulated in the resin. Given the relatively good condition of the bodies it would be fair to assume that most worms were alive at the time of resin casting. The results from experiment 2 supported this assumption as in this experiment there were no mortalities in any of the treatments. Evidence from both experiments would therefore suggest that *N. virens* is extremely tolerant to low pH conditions and is able to survive for at least 40 days in extremely acidified seawater (pH 5.6).

5.2.3.2 Weight

In experiment 2 there was no significant difference in the average weight of worms in each treatment at start of experiment (F=1.81, p=0.133). This average starting weight was 1.159g (± 0.429). Worms in pH treatments 6.5 and 5.6 were significantly lighter at the end of the exposure period than at the start (pH 6.5 p-value = 0.028, pH 5.6 p-value = 0.036). Worms in the control and in pH treatments 7.7 and 7.3 showed no weight loss during the experiment (pH 8 p-value = 0.112, pH 7.7 p-value = 0.749, pH 7.3 p-value = 0.433).

5.2.3.3 Reproduction

In experiment 2 there was no evidence of germ cell development in any of the animals sampled and so it is not possible to draw any conclusions as to likely effects.

5.2.3.4 Digestive system

In experiment 2, examination of the stained tissue sections indicated no significant differences in gut structure between the various treatment groups. Furthermore there was no evidence of any differences between treatment groups on the dermal surfaces or its underlying musculature.

5.2.3.5 Burrowing activity

In experiment 1 the resin casts recovered from the sediment (fig. 5.3) demonstrated the significant impact that the burrows of *N. virens* could have on the sediment environment. Analysis of these casts also showed that the size of the worms creating the burrows had a significant effect on all aspects of burrow morphology (table 5.3). However, changes in pH had no significant effect on any of the measures of burrow morphology (table 5.3). In addition, no significant interactions were observed (table 5.3) which confirmed that there were no size dependent effects of pH on *N. virens*.

	Max depth		Length		Diameter		Volume		Surface area	
	F	p	F	p	F	p	F	p	F	p
Worm Size	**9.44**	**0.000**	**4.77**	**0.005**	**44.48**	**0.000**	**33.82**	**0.000**	**22.31**	**0.000**
pH	1.84	0.153	1.70	0.180	2.41	0.078	1.72	0.176	1.62	0.198
Interaction	1.15	0.351	1.27	0.275	1.19	0.326	0.72	0.691	0.63	0.762

Table 5.3: Results from 2-way crossed ANOVA analyses of worm size and pH effects on five measures of burrow morphology. (Significant values highlighted in bold)

As ANOVA demonstrated no significant effects of pH, values for burrow morphology could be pooled across all pH treatments. Figure 5.3 demonstrated that the larger the worm, the longer, wider and deeper are its burrows.

Figure 5.2: Typical resin cast of N. virens burrow

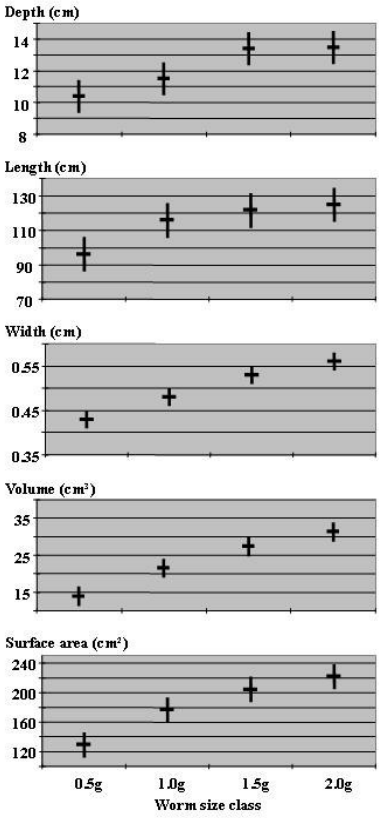

Figure 5.3: Impact of worm size on burrow morphology

5.2.3.6 Nutrient fluxes

In experiment 1 the sediment used in this experiment acted as a sink for nitrate (figure 5.4) and phosphate (figure 5.5) as well as a source of nitrite, ammonia and silicate (figure 5.4). There were significant linear relationships between burrow surface area and the flux of nitrate, nitrite, ammonia and silicate (table 5.4). The numerical properties of these relationships are described in table 5.5. An increase in the burrow surface area caused an increase in the sediment uptake of nitrate and a decrease in the release of nitrite, ammonia and silicate (table 5.4, figure 5.4). There was no significant relationship observed between phosphate flux and burrow surface area.

Whilst a decrease in seawater pH did affect the fluxes of nitrate (increased uptake), nitrite (decreased release) and ammonia (increased release), as demonstrated by the variations from the average intercept shown in table 5.4, pH did not change the relationship (slope) between burrow surface area and the flux of these nutrients (table 5.4). There was no observed effect of seawater pH on silicate flux (table 5.4). Table 5.4 also shows a significant impact of seawater pH on the flux of phosphate.

For nitrite flux, variations from the average intercept (table 5.4) indicate that changes occur when pH falls from 8.0 to 7.3. However, in the case of nitrate and ammonia much greater changes in seawater pH, between 7.3 and 6.5, were needed before a response was observed. This was also true for phosphate where a significant decrease in sediment uptake was only observed when the pH was less than 7.3 (figure 5.5).

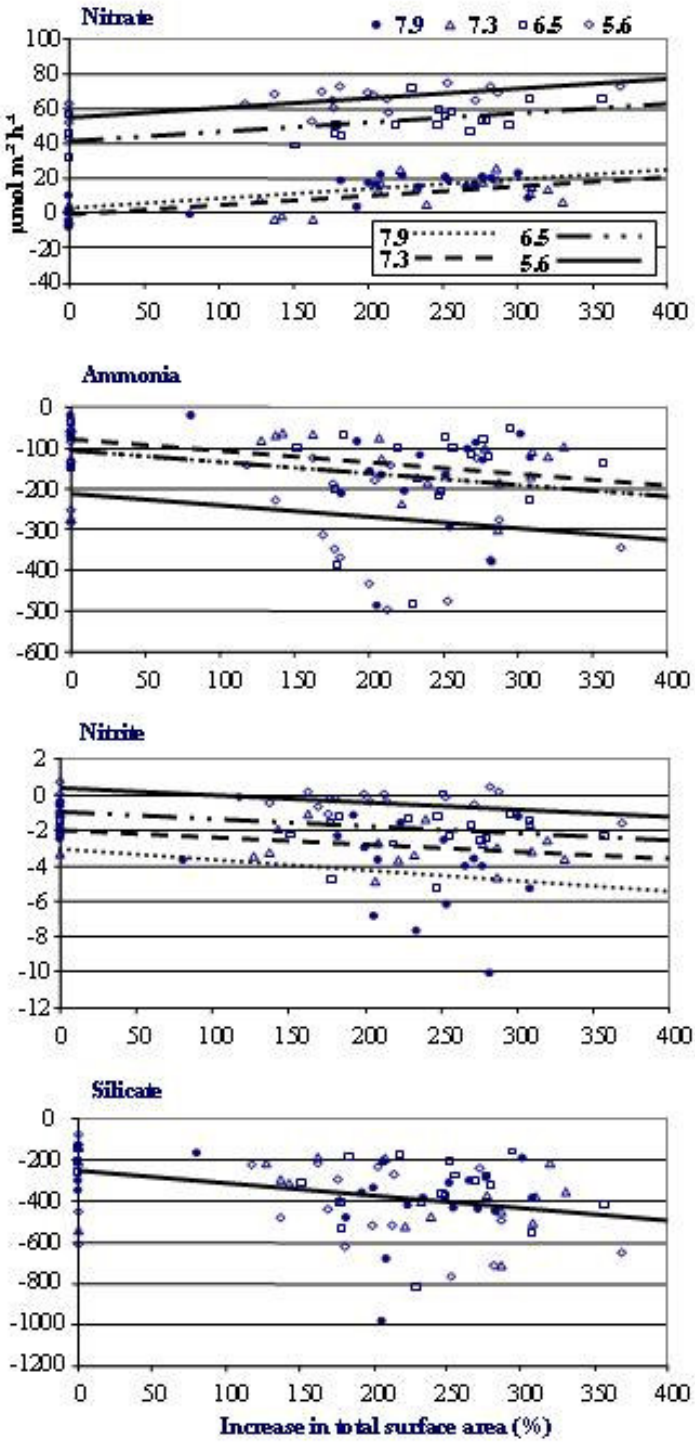

Figure 5.4: Effect of seawater pH and burrow surface area on the flux of nitrate, nitrite, ammonium and silicate

	df	Burrow surface area		pH		Slope
		F	p	F	p	F
Nitrate	3, 71	**31.44**	**0.000**	**320.21**	**0.000**	0.42
Nitrite	3, 69	**10.92**	**0.001**	**21.52**	**0.000**	2.118
Ammonia	3, 71	**4.43**	**0.039**	**6.37**	**0.001**	0.72
Silicate	3, 71	**10.42**	**0.002**	1.62	0.193	0.20
Phosphate	3, 71	2.03	0.159	**14.08**	**0.000**	na

Table 5.4: Impact of burrow surface area and seawater pH on nutrient flux. (Significant values highlighted in bold)

	Intercept	Variation from intercept due to pH				Slope
		pH 8.0	pH 7.3	pH 6.5	pH 5.6	
Nitrate	24.417	-21.76	-25.33	+16.70	+30.39	0.055
Nitrite	-1.3797	-1.6295	-0.5914	+0.4529	+1.7680	-0.004
Ammonia	-124.66	+19.05	+44.73	+25.46	-89.24	-0.283
Silicate	-250.12	ns	ns	ns	ns	-0.605

Table 5.5: Numerical descriptions of burrow surface area - nutrient flux relationships for different seawater pH treatments.

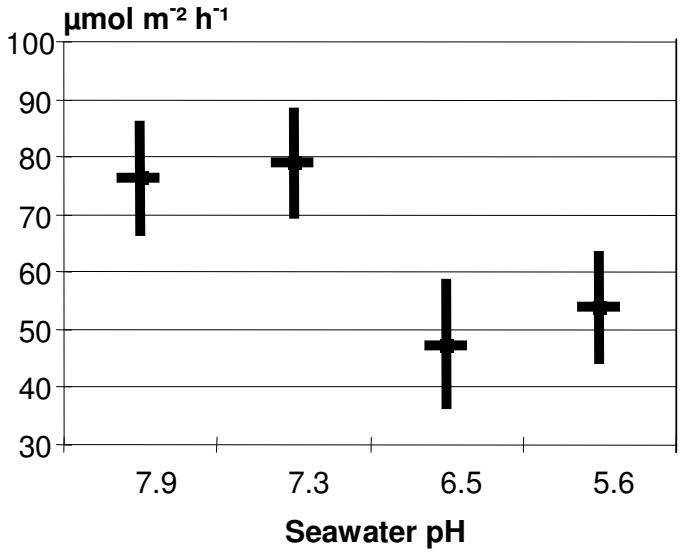

Figure 5.5: Impact of pH on phosphate flux.

5.2.4 Conclusions

No effects of seawater acidification were observed on *N. virens* mortality, reproductive, digestive and dermal tissue morphology or on burrowing activity. Batten & Bamber (1996) however observed significantly reduced burrowing activity (number of burrows created) for N. virens at pH 7.5. These authors also observed mortality in worms exposed to seawater with a pH of 6.5 or less within 10days. The results obtained by Batten & Bamber (1996) are therefore in contrast to those of the current study. The most likely explanation for this discrepancy is due to the medium in which the worms were retained. Batten & Bamber (1996) established *N. virens* in artificial sediment made of 1mm glass beads whilst the

current study used natural sediment. Given the known pH buffering capacity of fine sediment particles (Fenchel & Blackburn 1979) it would seem likely that the natural sediment used in the current study may have reduced the impact of seawater acidification within the burrow environment. It could also reflect a lack of physiological dependency on calcium.

Batten & Bamber (1996) also reported that surviving worms displayed significant repression of growth and metabolism within 30 days at pH levels at or below 6.5. Whilst no growth was observed in the current study, significant declines in weight were observed at pH levels at or below 6.5. In response to hypercapnia, acid-base and ion-equilibria in many marine invertebrates will reach new steady state values. This comes at a metabolic cost and whilst not acutely life threatening is still expected to hamper growth (reduced protein biosynthesis) and reproduction during long-term exposures (Langenbuch & Pörtner 2002). Some marine invertebrates are also known to produce an infusion of the neuromodulator adenosine when exposed to elevated levels of CO_2 (Reipschläger et al. 1997). This adaptive strategy, known as metabolic depression, suppresses aerobic energy turnover rate. Again, this response may be beneficial in the short term but may be detrimental to whole organism functions during long-term exposure. Given that leakage events from sub-seabed storage are likely to last for short periods of time, physiological response in *N. virens*, such as metabolic depression, are likely to protect this species from damage during these short exposures.

Our study demonstrates that the effects of leakage from sub-seabed storage on nutrient cycling could be considerable. Huesemann et al. (2002) demonstrated that rates of ammonium oxidation to nitrite or nitrate (nitrification) were reduced by approximately 50% at pH 7, by more than 90% at pH 6.5 and were completely inhibited at pH 6. As the majority of nitrate used to fuel denitrification comes from nitrification rather than from the overlying water, particularly within the burrow environment, it could be predicted that a reduction in pH would cause a reduction in the supply of nitrate through nitrification and therefore a greater reliance on the uptake of nitrate from the overlying water. The results of the current study would support this hypothesis in that the uptake of nitrate increases significantly as seawater pH decreases. This hypothesis assumes that the process of denitrification is less affected by pH change than nitrification and as yet that assumption remains to be tested.

The release of ammonium from the sediment changes very little between the controls and the treatments with seawater pH of 7.3 or 6.5. If nitrification had been reduced at a pH of around 7, it might be expected that ammonium efflux would increase. However, it is possible that instead of being released, the ammonium is oxidised anaerobically. Anaerobic ammonium oxidation (anammox) is now recognised as a significant process in the conversion of fixed nitrogen into atmospheric nitrogen (N_2) gas (den Camp, 2006). Recent studies have shown that this process could account for nearly 80% of the total N_2 production in some coastal sediment (Engstrom 2005). The anammox process occurs when ammonia is oxidized with nitrite as the primary electron acceptor and is catalysed by a specialised group of planctomycete-like bacteria (den Camp 2006). Consequently, observations of the nitrite data generated from the current study may provide indirect evidence to support the hypothesis that in acidic conditions ammonium oxidation occurs more through anammox than through nitrification. The progressive decrease in nitrite release in response to increasing pH, results in nitrite uptake in non-burrowed sediment

exposed to pH 5.6. This could indicate an increased demand for nitrite to fuel the anammox process. Whilst this hypothesis would seem to explain the changes observed in both ammonium and nitrite flux, the assumption that the anammox process is more tolerant to pH change than nitrification is highly speculative and remains to be tested. The dramatic increase in ammonium release at pH 5.6 was probably caused by a combination of stress induced protein catabolism in the natural fauna within the cores together with fatalities in this natural fauna which may have stimulated bacterial production of ammonia. Cessation of the anammox process may also have contributed.

The uptake of phosphate by surface sediments occurs via assimilation by microphytobenthos (Sundback et al. 1991) and by adsorption of PO_4^{3-} onto hydrated metal oxides under oxic conditions (Hartikainen et al. 1996). As the anions PO_4^{3-} and SiO_4^{3-} adsorb onto the same components in the sediment and have common chemical reactions (Hartikainen et al. 1996), the lack of any pH effects on the flux of silicate would indicate that the impact of pH on phosphate flux was not due to any changes in the oxic condition of surface sediments. It is most likely therefore, that acidification of the seawater to a pH of less than 7.3 had a detrimental effect on surface dwelling microphytobenthos resulting in the observed decrease in sediment uptake of phosphate.

5.2.5 Key Findings

- The presence and structure of *Nereis virens* burrows have a significant influence on the flux rates of a number of key nutrient species.

- *Nereis virens* is likely to be able to survive short term exposure (40 days or less) to extreme changes (up to 2.5 pH units) in seawater acidity with little or no damage to body tissues.

- Nutrient flux rates would be directly affected by the large changes in seawater acidity likely to occur during leakage from sub-seabed storage but not by the small changes predicted as a result of ocean acidification through atmospheric absorption.

5.3 IMPACT ON MYTILUS EDULIS

5.3.1 Introduction

Mussels are a valuable food source globally for both, mussels have also been used widely for many years as an indicator species for studies on anthropogenic impacts and have been shown to be sensitive to petroleum derived hydrocarbons, PCBs and some metals. Mussels beds act as a ‘safe haven’ for a multitude of small benthic invertebrates, so called cryptic fauna, that reside within the byssus threads that hold the mussels onto a diverse array of substrate types; as such mussels represent an important component of the ecosystem.

5.3.2 Methodology

5.3.2.1 Mussel collection and experimental setup

Mytilus edulis, measuring between 45 and 55mm, were collected from Trebarwith Strand, North Cornwall. Animals were cleaned of epibionts and thirty-two were placed into each of eight tanks holding 50 litres of seawater, held within a flow-through system, in a mesocosm environment. Tanks were fed with seawater from one of four header tanks, adjusted to pH 6.5, 7.6, 7.8, or 8.0, through the addition of CO_2. Each header tank supplied two replicate tanks, at a rate of approximately 60ml.min^{-1}. Mussels were fed with *Isochrysis galbana*, or *Pavlova sp.*, to provide 30mg of dry weight algae per mussel per day. The feed was prepared in seawater and pumped into the tanks at a rate of 1ml.min^{-1}.

5.3.2.2 Experimental conditions and monitoring

Daily measurements were recorded, to monitor pH, temperature and salinity in the header tanks, exposure tanks and samples from the water feed tubes. Total CO_2 concentration (TCO_2) in header and exposure tanks was measured 3 times a week by analysing 10µl sub-samples using a CO_2 analyser (Ciba-Corning 965, England). The partial pressure of CO_2 (PCO_2) in these sub-samples was then calculated using values for CCO_2 and pH using a modified form of the Henderson-Hasselbalch equation (Spicer et al. 1988):

Equation 1: $$PCO_2 = CCO_2/\alpha\ (10^{pH-pKi} + 1)$$

α is the carbon dioxide solubility coefficient in sea water at 15°C and 35ppm and has a value of 0.0499mmol^{-1}torr^{-1}.

pKi is the negative log of the dissociation constant of carbonic acid and has a value of 6.04 for sea water at 15°C (Truchot 1976).

Using the value calculated for PCO_2, bicarbonate ion concentration ($[HCO_3^-]$) in sub-samples was calculated using equation 2 (Spicer et al. 1988)

Equation 2: $$[HCO_3^-] = CCO_2 - \alpha\ (PCO_2)$$

5.3.2.3 Sampling regime

On Day 0, sixteen mussels collected at the same time as the animals placed in the acidified seawater exposure system were sampled for determine the health of mussles at the start of the experiment. Blood was taken from these animals in order to carry out the Neutral Red Retention Assay. The same mussels were fixed in Baker's Formol Calcium and processed for wax histopathology. The shells were retained for further analysis. Subsequent sampling from the exposure tanks was carried out on days 6, 13, 31, and 60. Eight mussels were removed from each of the tanks on the four sampling days. These animals were replaced with fresh mussels on days 6, 13, and 31, in order to keep the number of animals constant. The replacement animals were collected from Trebarwith Strand before each sampling day and separated from the exposure mussels by being held in baskets within the tanks.

5.3.2.4 Measuring the health of M. edulis using the Neutral Red Retention Assay

A haemolymph sample was removed from each animal as follows. The valves were carefully prised apart with a broad backed solid scalpel that was held in position whilst 100 microlitres of haemolymph was withdrawn from the anterior adductor muscle into a 1.0ml hypodermic syringe, fitted with a 21 gauge needle, containing 100 microlitres of a mussel physiological saline (20mM HEPES, 436mM NaCl, 53mM $MgSO_4$, 10mM KCl, 10mM $CaCl_2$, gassed for 10min with 95% O_2:5% CO_2 and adjusted to pH7.3 with 1N NaOH, Peek & Gabbot, 1989). In order to reduce shearing forces the needle was then removed and the contents of the syringe ejected into a 2ml siliconised (Sigmacote, Sigma Chemical Co., Poole, UK.) Eppendorf tube which was held in water ice until required. The neutral red dye stock solution was made by dissolving 28.8mg of dye (C.I.50040, Sigma) in 1ml of DMSO (dimethyl sulphoxide) and the working solution prepared by diluting 10µl of the stock solution with 5ml of the mussel physiological saline. A 50µl aliquot of the cell suspension was dispensed onto a 76x26mm microscope slide for 15mins, excess solution was then carefully tipped off, 40µl of the neutral red working solution was added and a 18x18mm coverslip applied. The preparations were then examined under the microscope at 15, 30, 60, 90, 120 and 180 minutes for evidence of damage to the lysosomes. When it was observed that >50% of the blood cells in a sample exhibited damage lysosomes the test was terminated.

5.3.2.5 Preparation and analysis of tissue sections

For the preparation of tissue sections of mussels the soft tissues were removed and a transverse slice removed taking in the mid region of the digestive gland and its associated tissues. The samples were immediately placed in Bakers Formal Calcium (10% formalin, 1% calcium chloride and 2.5% sodium chloride) at $4^{O}C$ and transferred to a refrigerator to complete the fixation process. The preserved specimens were then dehydrated through an ascending alcohol series, cleared in xylene and impregnated in paraffin wax; this procedure was undertaken using an automatic programmable tissue processor. Once wax impregnation was completed the specimens were blocked up in using stainless steel moulds using fresh molten wax and allowed to cool prior to cutting. Sections, 7µm, were cut using disposable steel knives and the sections floated out onto microscope slides coated with ATPS to assist adhesion. Once dry the sections were stained Papanicolaou's and coverslipped.

5.3.3 Results

5.3.3.1 Experimental conditions

	Header Tanks			
	8.0	**7.8**	**7.6**	**6.5**
pH	8.08 (±0.09)	7.72 (±0.12)	7.54 (±0.09)	6.41 (±0.22)
TCO_2 (µmol.l⁻¹)	1.88 (±0.65)	1.95 (±0.62)	1.97 (±0.70)	2.94 (±0.97)
pCO_2 (Torr)	0.04 (±0.02)	0.19 (±0.09)	0.66 (±0.32)	62.82 (±20.51)
$[HCO_3^-]$	1.88 (±0.65)	1.96 (±0.65)	1.96 (±0.70)	2.44 (±0.72)
Temperature (˚C)	17.96 (±0.78)	17.83 (±0.86)	17.76 (±0.88)	17.41 (±0.92)
Salinity	35.13 (±0.47)	35.11 (±0.42)	35.21 (±0.39)	35.05 (±0.52)
	Exposure Tanks			
	8.0	**7.8**	**7.6**	**6.5**
pH	8.00 (±0.09)	7.84 (±0.08)	7.68 (±0.08)	7.38 (±0.24)
TCO_2 (µmol.l⁻¹)	1.84 (±0.72)	1.92 (±0.70)	2.01 (±0.74)	1.96 (±0.70)
pCO_2 (Torr)	0.03 (±0.02)	0.11 (±0.05)	0.17 (±0.08)	5.70 (±7.87)
$[HCO_3^-]$	1.84 (±0.72)	1.92 (±0.70)	2.01 (±0.74)	1.93 (±0.71)
Temperature (˚C)	16.11 (±0.12)	16.05 (±1.07)	16.06 (±0.94)	16.06 (±0.84)
Salinity	35.38 (±0.17)	35.43 (±0.25)	35.27 (±0.39)	35.12 (±0.51)

Table 5.6: Measures of mean pH, TCO_2, pCO_2, $[HCO_3^-]$, temperature and salinity (±1 standard deviation) for experimental period.

The environmental conditions within the header and exposure tanks for each treatment level are summarised in table 5.6. The most notable observation is that the values for pH and pCO_2 in the 6.5 treatment exposure tank are considerably different to those in the 6.5 treatment header tank. Although smaller, similar increases in pH and reductions in pCO_2 in the exposure tanks when compared to the corresponding header tanks were also observed for treatments 7.8 and 7.6. These differences indicate a loss of CO_2 from the water in the exposure tanks. However, it is unlikely this is due to loss of CO_2 into the atmosphere via exchange across the water/air boundary in the exposure tanks. This is primarily because pH data collected during the setting-up period, prior to the addition of the mussels, (table 5.7) demonstrates little change in pH between header and exposure tanks. In addition, this phenomenon of CO_2 loss has not been observed in any of the previous exposure experiments in this system despite using similar, open exposure tanks. It seems more likely that the presence of the mussels in some way buffers the pH changes; probably through the dissolution of their carbonate shells.

	8.0	**7.8**	**7.6**	**6.5**
Header tank	7.94 (±0.13)	7.72 (±0.11)	7.55 (±0.15)	6.45 (±0.60)
Exposure tank	7.84 (±0.15)	7.70 (±0.12)	7.56 (±0.14)	6.82 (±0.41)

Table 5.7: Measures of mean pH (±1 standard deviation) during setting-up period prior to the start of the experiment.

5.3.3.2 Mortality

There were only two mortalities during the course of the exposure, one from the pH 7.8 treatment group on day 6 and one from the Control group on day 13.

5.3.3.3 General health status

Lysosomes are central to many key biological processes including reproduction, digestion and the immune response thus any deleterious changes to their function can have serious consequences for those processes and in the worst case death. The general health status of the mussels was therefore considered in terms of changes to the lysosomal system of the blood cells.

The results of the lysosomal damage assay indicated that there was no significant difference between treatment groups. However, there was a significant difference ($p<0.05$) between all treatment groups and results of the analysis for the day 0 sample. The histological sections indicated that the mussels from all treatment groups were in the latter stages of their reproductive cycle having recently spawned, which is a physiologically stressful event, and this may have contributed to the generally low values observed in the assay for the reference control group. However if reduced pH was a problem to the mussels then the values recorded for the low pH treatment groups should have reflected this fact regardless of the fact that the animals had recently spawned.

The stained sections were analysed for evidence of structural changes to the digestive system, using 5 indicators, and the reproductive system, using 4 indicators. In addition the sections were examined for changes in the gills and for evidence of inflammation to the gut interstitial tissues and evidence of pre-neoplastic lesions. There was no evidence of inflammation of the intestinal tissues, changes to gill structure indication of irritation resulting from exposure to reduced pH or pre neoplastic lesion.

5.3.3.4 Reproduction

There was no evidence to indicate any impact on reproductive processes with all animals exhibiting the same reproductive state and similar levels of reserve tissues to fuel gametogenesis.

5.3.3.5 Digestive system

There was no evidence of impact on digestive processes. The samples scored highly for thinning of the digestive epithelium, however, they were originally collected form a population were this is the norm.

5.3.4 Conclusions

The histological analysis of stained tissue section did not indicate any significant impacts on exposure to reduced pH in the soft tissues of mussels.

5.3.5 Key Findings

- Adult *Mytilus edulis* are likely to be able to survive short term exposure (60 days or less) to extreme changes (up to 1.5 pH units) in seawater acidity with little or no impact on general health or damage to body tissues.

- It is possible the presence of *M. edulis* may act to buffer the effects of seawater acidification due to the partial dissolution of their shells over short exposure periods (less than 60 days). This may act to ameliorate the impacts of a leakage event for any fauna associated with *M. edulis*.

5.4 IMPACT ON OPHIURA OPHIURA

5.4.1 Introduction

The body structure of echinoderms would seem to make them particularly susceptible to changes in pH as they rely on a calcareous skeleton and have no impermeable barrier between ambient seawater and the internal body cavity. Any impact on echinoderms could have important implications for the whole ecosystem. For example, brittle stars, such as Ophiura ophiura and Amphiura filiformis, living on and in the sediment are a major source of food for commercially important species such as cod (Kanapathippillai et al., 1994). Brittlestars are abundant in most coastal sediments around the UK.

5.4.2 Methodology

5.4.2.1 Sediment collection and experimental setup

This experiment was conducted at the Norwegian Institute for Water Research (NIVA) mesocosm facility. On 12 September 2005, four 1.2m x 0.8m plastic trays were filled to a depth of 20cm with sandy mud collected from Bjornhodebukta, a sheltered bay in the inner part of Oslofjord (Norway). The sediment was collected from 60m depth using a $0.1m^2$ vanVeen grab. The trays were returned to the mesocosm and placed in a flow-through holding basin filled with seawater to a depth of 1m. A pipeline situated at 60m in the adjacent fjord continuously supplied the holding basin with natural seawater. On 7 October 2005, 48 specimens of *Ophiura ophiura* (disc diameter 1 – 1.5cm) were collected from an area of sandy mud (59° 41 344N, 10° 32 482E) in 70m of water using a naturalist dredge. These were returned to the mesocosm and 12 individuals were added to each of the 4 trays in the holding basin.

On 14 October 2005 the trays were transferred to the seawater acidification system and each tray was supplied with a constant flow of seawater via a header tank detailed in figure 5.6. On 17 October each of the trays was randomly assigned to one of four pH treatment levels (8, 7.6, 7.2 and 6.8) and seawater acidification began. Acidification was done gradually and target pH levels were reached 7 days later on 24 October

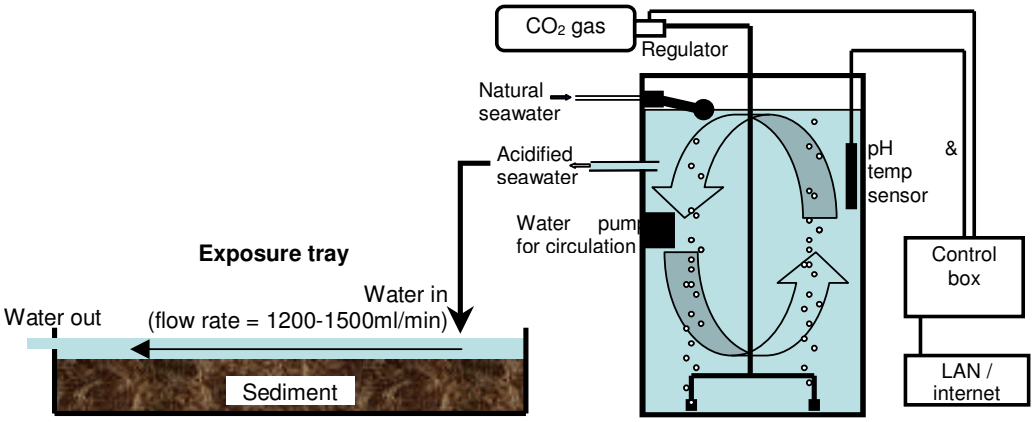

Figure 5.6: Experimental set up consisting of an exposure tray and a header tank for each of the 4 pH treatments.

5.4.2.2 Experimental monitoring and sampling

The experiment then ran for 40 days during which time consistent pH and temperature were maintained in each of the basins (table 5.8).

		Treatment			
		Control	7.6	7.2	6.8
Tray	*pH*	8.00 (±0.03)	7.51 (±0.02)	7.24 (±0.05)	6.84 (±0.04)
	Alkalinity	2.43 (±0.05)	2.43 (±0.04)	2.45 (±0.06)	2.44 (±0.06)
	Temp (°C)	9.35 (±1.09)	9.48 (±0.97)	9.53 (±0.99)	9.60 (±0.96)
Header	*pH*	8.00 (±0.06)	7.52 (±0.02)	7.22 (±0.07)	6.86 (±0.21)
	Alkalinity	2.46 (±0.06)	2.47 (±0.05)	2.45 (±0.05)	2.44 (±0.05)
	Temp (°C)	9.56 (±0.87)	9.26 (±0.90)	9.63 (±0.87)	9.68 (±0.91)
	Oxygen (%)	72.1 (±1.6)	71.7 (±3.2)	70.0 (±2.8)	71.1 (±1.4)

Table 5.8: Experimental conditions within each tray and header tank over the course of the exposure period.

5.4.2.3 Preparation and analysis of tissue sections

On 3 December, the *Ophiura ophi*ura were removed from the trays, placed in 1 litre pots and preserved whole in Bakers formal calcium (10% formalin, 1% calcium chloride). The preserved organisms were then returned to PML for histological analysis. On arrival at PML, the samples were dissected to remove the whole soft tissue mass, dehydrated through an ascending alcohol series, cleared in xylene and embedded in paraffin wax. Seven micron (7µm) sections were cut, mounted on APTS coated microscope slides and stained in Papanicolaou (EA50 and OG6) and Harris's haematoxylin. The stained sections were then examined under the microscope for evidence of changes in the incidence of mucous secretory cells in gut and integumentary tissues and abnormalities in germinal tissues.

5.4.3 Results

5.4.3.1 Mortality

There were no mortalities in any of the treatments during the 40-day exposure period.

5.4.3.2 Reproduction

There was clear evidence in the stained tissue sections that reduced pH had a serious impact on the reproductive process whereby the transfer of nutrients to fuel egg (vitellogenesis) was disrupted, resulting in egg degeneration. It was also apparent from the sections that at pH 7.6 the rate of development was accelerated as compared to the control.

5.4.3.3 Digestive system

As with the reproductive tissues there was clear evidence of a pH effect on digestive processes resulting in a breakdown of the digestive epithelium. The degree of effect increased with decreasing pH with severe disruption exhibited at pH 6.8.

5.4.4 Conclusions

In sharp contrast to both *M. edulis* and *N. virens*, there is clear evidence in the histology to indicate that reduced pH has a significant impact on reproductive and digestive processes in *O. ophiura*.

5.4.5 Key Findings

- The health and function of the brittlestar *Ophiura ophiura* is reduced significantly when exposed to seawater with a pH equal to or less than 7.6.
- Relatively small changes in seawater acidity (≥0.4 pH units) could severely affect the ability of *O. ophiura* to reproduce and consequently this species may be susceptible to the changes in seawater acidity likely to occur from predicted levels of Ocean Acidification.
- Large changes in pH due to leakage from sub-seabed CO_2 storage would cause high levels of mortality to local *O. ophiura* populations.

5.5 IMPACT ON AMPHIURA FILIFORMIS

5.5.1 Introduction

Like *Ophiura ophiura, Amphiura filiformis* is a commonly found brittlestar around the UK coasts. However, unlike the surface dwelling *Ophiura ophiura*, *A. filiformis* lives below the sediment surface. Evidence from the *Nereis virens* studies would indicate that the sediment environment may be able to ameliorate the harmful effects of seawater

acidification and therefore it may be expected that the impacts of acidification on *A. filiformis* may be less pronounced than for *O. ophiura*.

5.5.2 Methodology

5.5.2.1 Sediment collection and experimental setup

On 16 November 2006, sixteen undisturbed cores were collected from an area of moderately sorted sandy mud (median phi = 4.01) 100m north of Plymouth Breakwater (50˚20.090N, 4˚08.520W); water depth was approximately 10m. The cores were collected by sub-sampling from a 0.1m^2 boxcorer. Into each box-core sample, nine plastic cores (10cm diameter, 20cm long) were pushed into the sediment to a depth of 15cm and capped. Each core was then gently removed from the box-core, sealed on the bottom with a second plastic cap and returned to the PML mesocosm within a few hours of collection. Once in the mesocosm the top caps were removed and the cores were placed randomly in a recirculating seawater system. On 28 November 2006, specimens of *Amphiura filiformis* were collected from an area of muddy sand inside Plymouth Sound using a 0.1m^2 vanVeen grab. The individuals were gently hand sorted from the sediment to prevent damage to the brittlestars' delicate arms and were held in large buckets of seawater whilst being transferred to the PML mesocosm. On arrival at the mesocosm each individual was weighed (blotted wet weight) and randomly added to one of the 16 sediment cores being held in the recirculating seawater system. Once five *A. filiformis* had been added to each, the cores were transferred to the seawater acidification system. Each core was individually supplied with natural seawater (pH 8.0) at a rate of approximately 5ml.min^{-1} using a peristaltic pump. After 24 hours all but three *A. filiformis* individuals had burrowed into the sediment. These individuals were replaced by spare animals that had been held in the recirculating seawater system and 24 hours later all individuals in the treatment cores had burrowed into the sediment. Cores were then randomly assigned to one of four pH treatment levels (7.9 [ambient seawater], 7.7, 7.3 and 6.6) and seawater acidification began on 30 November. The pH levels in the 3 acidified treatments were lowered gradually over a period of 7 days. By 6 December (termed "Day 0"), all pH treatment levels had been reached and the experiment was deemed to have started at this point. The experiment lasted for 40 days and on 15 January 2007 each of the cores was removed from the system and the *A. filiformis* were recovered by gently sieving the sediment over a 2mm mesh.

5.5.2.2 Preparation and analysis of tissue sections

On receipt the samples were plunged into cold (4^{0}C) Bakers formal calcium for overnight fixation. The arms were removed and the whole body mass dehydrated in an ascending series of 50, 70 and 95% ethyl alcohol, each for 30 minutes followed by a final change in 95%. The dehydrated samples were then impregnated with 2, hydroxyethyl methacrylate monomer and 95% ethyl alcohol (1:1) and then transferred to pure monomer overnight. Finally the samples were embedded in a monomer plus activator solution. The embedded tissues were allowed to polymerise for 3 hours, removed from the moulds and air dried on the bench prior to cutting. Sections, 3 microns, were cut using glass Ralph knives and the section stained in Lees Methylene Blue/Basic Fuschin.

5.5.3 Results

5.5.3.1 Experimental conditions

The experiment ran for 40 days during which time consistent pH, temperature and salinity were maintained in each of the treatments (table 5.9).

	7.9	**7.7**	**7.3**	**6.6**
pH	7.88 (±0.08)	7.70 (±0.06)	7.36 (±0.13)	6.60 (±0.13)
Temp (ºC)	17.0 (±0.5)	16.6 (±0.4)	16.3 (±0.4)	15.9 (±0.5)
Salinity	35.9 (±0.5)	36.1 (±0.3)	36.4 (±0.6)	36.2 (±0.7)

Table 5.9: Measures of pH, temperature and salinity (±1 standard deviation) for experimental period.

5.5.3.2 Mortality

There were no mortalities in any of the pH treatments indicating that *A. filiformis* can survive exposure to seawater with a pH down to 6.6 for at least 40 days.

5.5.3.3 Reproduction

There was no evidence of damage to the reproductive system, eggs and sperms appear to be developing normally, and there was no indication of follicle asynchrony or acceleration in gamete development.

5.5.3.4 Digestive system

There was no evidence of damage to the digestive epithelium in samples from any of the treatment groups.

5.5.4 Conclusions

In stark contrast to the changes in cellular structure observed in both digestive and germinal tissues in *O. ophiura*, reduced pH (down to pH 6.6) does not appear to impact on the health of *A. filiformis*. This is most probably due to the burrowing behaviour of *A. filiformis* in contrast to the surface dwelling habit of *O. ophiura*.

5.5.5 Key Findings

- *Amphiura filiformis* can survive unharmed in seawater with a pH of 6.6 for at least 40 days.
- Infaunal species could be more tolerant to seawater acidification than surface dwelling species.

6. OBJECTIVE 5: IMPACT ON BIODIVERSITY

The scale of this work was increased significantly thanks to the NERC standard grant (NE/C510016/1) "The impact of ocean acidification on the biodiversity and function of coastal marine sediments." The originally proposed IMCO2 experiment involved the study of only a single sediment type. However, the additional funding allowed the experiment to expand to include a second contrasting sediment type.

6.1 BACKGROUND

The oceans harbour tremendous biological diversity. Of the 29 non-symbiont animal phyla that have been identified so far, all but one has living representatives in the ocean and 13 are represented only in the oceans. With all of these phyla having representatives in the benthos and most having representatives in marine sediments, it is considered that the majority of the species diversity in marine ecosystems consists of invertebrates either residing in (infauna) or on (epifauna) sediments. Recent work has already identified significant variability in pH sensitivity for a number of different benthic taxa (Shirayama et al., 2004; Miles et al., 2007; Spicer et al., 2006). Even amongst organisms that depend on calcium carbonate structures, variability in tolerance has been observed with echinoderms showing less tolerance to pH change than molluscs (Shirayama et al., 2004) or crustaceans (Spicer et al., 2006). Such variability in sensitivity to declining pH could have considerable implications for the structure and diversity of sediment communities. At the 1992 Earth Summit in Rio de Janeiro, world leaders agreed on a comprehensive strategy for sustainable development including the Convention on Biological Diversity (http://www.biodiv.org/convention/articles.asp). The Convention recognised that the loss of biodiversity may change the function of ecosystems and reduce the range of goods and services, from which mankind constantly draws. The convention acknowledged that current levels of biodiversity loss are unsustainable and committed signatory countries to its conservation and the sustainable use of its components.

The sediment environment is characterised by strong geochemical gradients and the pH of pore waters at depths of 30cm may be as much as 1 unit lower than the pH of the overlying water. In the face of such geochemical variability it is difficult to imagine how benthic sediment systems could be affected by the relatively small pH changes resulting from ocean acidification. However, by considering the distribution and activities of sediment dwelling organisms, it is possible to identify areas where these small changes could have potentially large consequences for sediment communities and biogeochemical processes. The communities of benthic sediments are strongly stratified with different species characteristically occupying different depths. The surface layer is the most densely inhabited and is home to the majority of multi-cellular, infaunal organisms, whilst only those species capable of oxygenating their immediate environment are able to dwell below the redox discontinuity depth. The presence of these depth-constrained niches means that

although benthic systems as a whole are already subject to a relatively large range in pH, many of the organisms and processes that exist within them are not. Whilst the majority of macrofaunal species are restricted to the upper oxic layers, others are able to inhabit the deeper sediment layers. They do so through the creation of burrows through which they are able to draw down oxygenated water from above. The burrows created by these macrofauna experience significant oscillation in pH (as much as two pH units) and dissolved oxygen concentration (between saturation and near anoxia) generated by the periodic ventilation of burrows (Furukawa, 2001). Such oscillation is absent at the water/sediment interface. Consequently, animals which use burrows may have a greater tolerance to changes in pH than non-burrow builders. In addition, recent work has already identified significant variability in pH sensitivity for a number of different benthic taxa (Shirayama et al., 2004; Miles et al., 2007; Spicer et al., 2006). Even amongst organisms that depend on calcium carbonate structures, variability in tolerance has been observed with echinoderms showing less tolerance to pH change than molluscs (Shirayama et al., 2004) or crustaceans (Spicer et al., 2006). This potential difference in pH tolerance between different benthic species could lead to the selection of more tolerant species and thereby substantial changes in the structure and function of sediment communities in the face of changing levels of pH.

6.2 METHODOLOGY

6.2.1 Sediment collection and experimental setup

On 9–12 May 2005 sediment was collected from 2 sites situated in the middle Oslofjord (table 6.1). Samples were collected at each site for sediment granulometry and carbon/nitrogen content.

	Site	
	1) Gray Island Sound	2) Solbergstrand
Geographical position	59˚ 41.988N 10˚ 31.506E	59˚ 36.768N 10˚ 38.709E
Sediment type	Mud/Silt	Very fine sand
Median grain size (µm)	14.60 (±0.75)	80.75 (±3.73)
% silt/clay	85.5 (±1.5)	36.0 (±3.3)
% total carbon	2.86 (±0.08)	0.684 (±0.10)
% organic carbon	2.76 (±0.11)	0.511 (±0.04)
% total nitrogen	0.231 (±0.01)	0.045 (±0.01)
% organic nitrogen	0.225 (±0.01)	0.031 (±0.01)
Water depth (m)	36	25

Table 6.1: Sediment collection site description.

At site 1, forty five undisturbed cores were collected by sub-sampling from a 0.1m^2 boxcorer. Into each box-core sample, a stainless steel circular core (26cm diameter) was pushed into the sediment to a depth of 40cm. Each core was then removed from the box-core and transferred to a food grade plastic bucket (30cm diameter, 40cm deep). Whilst being transferred to the mesocosm the cores were covered by seawater to prevent desiccation or temperature change. Once in the mesocosm the buckets were placed in a recirculating seawater system until transferred into the experimental setup.

At site 2, forty five buckets (30cm diameter, 40cm deep) were filled to a depth of 30cm with sediment collected using a 0.1m^2 vanVeen grab. It was not possible to collect undisturbed cores at this site because the box corer could only penetrate the sandier sediment to a depth of 5-10cm. Although the collection of undisturbed cores is the ideal, the use of grab-collected sediment in experiments on soft sediment communities has been shown to produce valid results (Widdicombe & Austen, 1998, 1999 & 2001; Widdicombe et al., 2004). As for the cores collected at site 1, the buckets of sediment collected at site 2 were covered by seawater to prevent desiccation or temperature change and transferred to a flow-through holding basin filled with seawater to a depth of 1m. A pipeline situated at 60m in the adjacent fjord continuously supplied the holding basin with natural seawater. After a few days in the holding basin, 40 buckets from each of the 2 sites were transferred to the experimental system (fig. 6.1) and each bucket was supplied with natural seawater at a rate of approximately 70ml.min^{-1}.

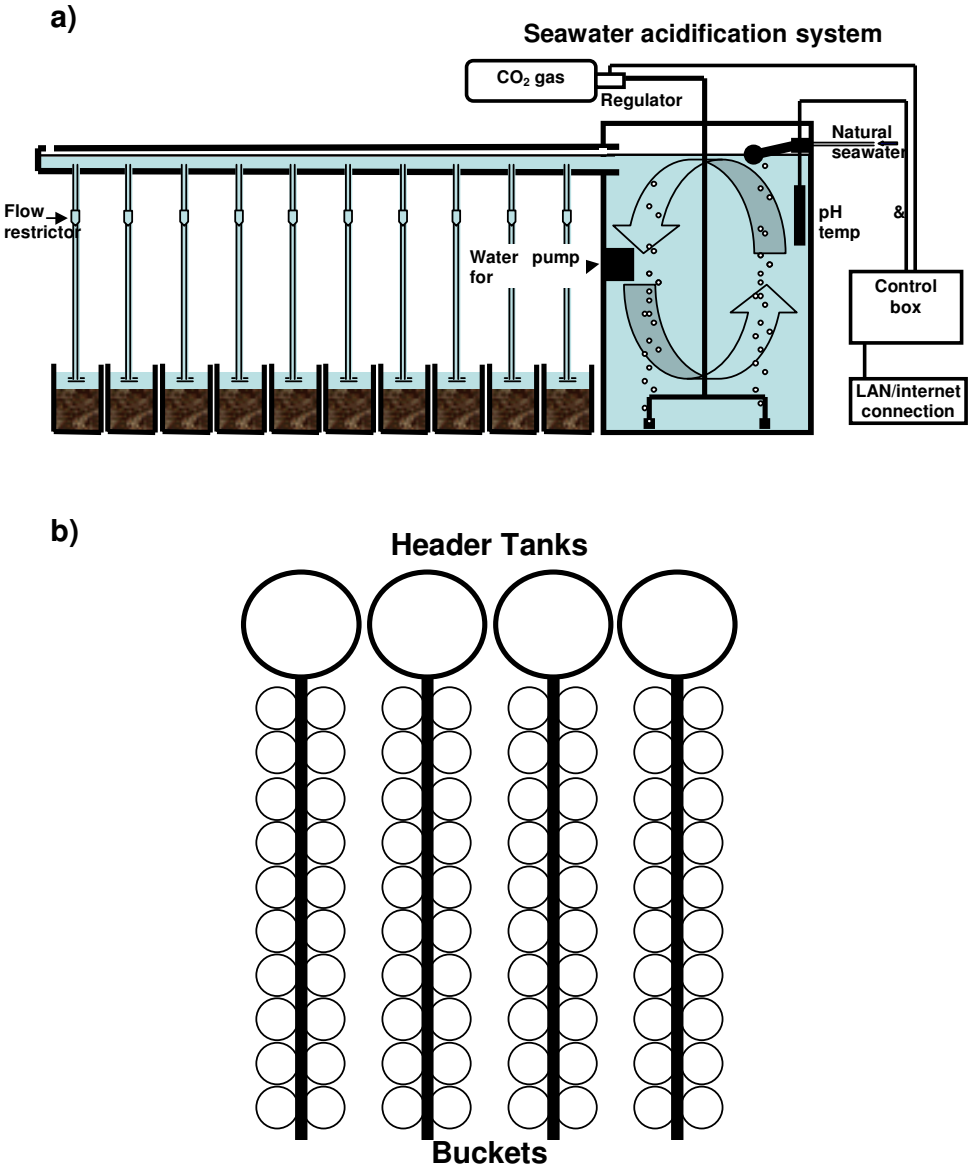

Figure 6.1: Schematic diagram of experimental setup; a) viewed from side, b) viewed from above.

Each of the four pH treatments contained 10 buckets from each of the two sites. Allocation of buckets to a particular pH treatment and the positioning of each bucket within the pH treatment systems were random. The ten unallocated buckets, five from each site, remained in the holding basin were they were sampled to determine the rate of sediment nutrient flux, the carbon and nitrogen content and the infaunal community structure present at the start of the experiment. Data related to these buckets are deemed to describe a start condition and are identified as “Initial” samples.

On the 15 May the water level in the holding basin was lowered until it reached a point approximately 10cm below the rim of the buckets. The time at which the water within the buckets became isolated from the surrounding water was noted and this was deemed to be the start of the incubation period (T_0). At this time a 50ml water samples was drawn from each core, filtered through a 47mm ø GF/F filter and stored in an acid washed nalgene bottle. Samples were stored frozen until analysis. Further samples were taken from each bucket after 6, 12, 18 and 24 hours. Stirring of the overlying water was provided with an air-lift system which maintained an appropriate circulation throughout the incubation periods. After the final water sample was taken a small core of sediment (1.6cm diameter, 6cm deep) was then removed from each bucket and dried at 80˚C for 12 hours. The dried sediment was ground into a powder and stored in moisture proof plastic vials before being analysed for carbon and nitrogen content. Aliquots of the powdered sediment were weighed into aluminium capsules for total carbon and silver capsules for organic carbon. Inorganic carbonates were removed from the samples in silver capsules by addition of two drops of sulphurous acid (Verardo et al., 1990). The capsules were dried at 60℃ for 48h, crimped and analysed on a Thermo Finnegan flash EA1112 elemental analyser using Acetanilide as calibration standard. At the same time as the sample for carbon and nitrogen analysis was being removed, four small (1.6cm diameter, 6cm deep) randomly placed cores were then taken from each bucket and pooled in a 250ml container. These samples were preserved by the addition of 10% formalin and returned to PML where the meiofauna were extracted via flotation using a suspension of colloidal silica (Ludox). In the laboratory nematodes were extracted from the sediment by flotation in Ludox TM with a specific gravity of 1.15, repeated five times and using a 63µm sieve (Somerfield et al. 2005). Extracted nematodes were then suspended in a mixture of 5% glycerol, 20% ethanol and water, which slowly evaporated overnight on a warm heater, to pure glycerol, and then mounted on slides. Nematodes were identified and enumerated, with a compound microscope, to the lowest possible taxonomic level using pictorial keys (Warwick et al. 1998). The remaining sediment in each bucket was sieved over a 0.5mm

Figure 6.2: Sampling the biodiversity experiment.

mesh and the residue was transferred to a 500ml container and preserved by the addition of 10% formalin. On returning to the laboratory in Plymouth, the macrofauna were extracted from this residue under a binocular microscope and identified to species or the lowest taxonomic level possible.

Seawater acidification in the experimental system began on 16 May 2005. To reduce the impact of a sudden acidity shock, the pH was gradually lowered to the required treatment values over the period of one week. The designated pH treatment levels were reached on 23 May and this date was considered to be the start of the experimental exposure period. Seawater acidification followed the methods described in Miles et al (2007) and Widdicombe & Needham (in press) and is summarised here. CO_2 gas was passed through natural seawater contained within large (450 litres) reservoir tanks. The CO_2 was passed through the water as very fine bubbles, which enabled the CO_2 gas to pass rapidly into solution. Once the pH had fallen to the required level, the supply of CO_2 was halted via an automated feedback relay system (Walchem Webmaster-GI controller). As the acidified water was taken from the reservoir, to supply the experimental tanks, it was replaced by natural seawater (pH ≈ 8.0) causing the pH in the reservoir tank to increase. This seawater was collected via a pipe situated at 60m in the fjord adjacent to the marine station and passed over a course filter (grade) before being supplied to the acidification reservoirs. The increase in pH triggered the supply of CO_2 to be restarted and CO_2 continued to bubble through the water until the pH had again been reduced to the pre-set level. Using this method it was possible to supply large quantities of CO_2 acidified seawater of a consistent pH.

Two weeks after the completion of seawater acidification (6 June 2005) 10 buckets (five from each site) from each pH treatment, 40 buckets in total, were randomly selected. These buckets were then sampled, using the methods described above, for nutrient flux, carbon and nitrogen content plus faunal community structure and diversity. The remaining buckets were left undisturbed to run for a further 18 weeks and were sampled for carbon and nitrogen content plus faunal community structure and diversity during 12–14 October 2005, a total exposure of 20 weeks.

6.2.2 Experiment monitoring and sampling

During the entire experimental period the temperature and pH in each reservoir tank was constantly monitored by the Walchem Webmaster-GI controller in connection with four pH electrodes (S650CD), one in each reservoir. In addition point measurements of temperature, pH, alkalinity and oxygen saturation levels in each of the four reservoir tanks were taken every 2-3 days. At these times, point measurements for temperature and pH were also taken from 20 randomly selected buckets, five from each of the four pH treatment levels.

6.2.3 Statistical analysis

The statistical package PRIMERe was used to test for differences in faunal community structure and to generate measures of sample diversity. Potential differences in univariate indices were tested using ANOVA within the Minitab statistical package.

6.3 RESULTS

6.3.1 Experimental conditions

Table 6.2 demonstrates that environmental conditions were well controlled during the experimental exposure period.

	Treatments			
	Control	7.2	6.6	5.7
pH (R)	8.01 (±0.07)	7.23 (±0.11)	6.56 (±0.11)	5.70 (±0.26)
pH (B)	7.99 (±0.04)	7.22 (±0.10)	6.58 (±0.10)	5.64 (±0.05)
Temperature (˚C)(R)	7.8 (±0.7)	8.4 (±0.6)	8.4 (±0.6)	8.4 (±0.6)
Temperature (˚C)(B)	8.5 (±0.6)	9.3 (±0.7)	9.0 (±0.6)	9.0 (±0.6)
O_2 (% saturation)(R)	81.65 (±3.32)	82.75 (±3.48)	80.36 (±3.57)	78.00 (±3.73)
Alkalinity(R)	2.48 (±0.11)	2.52 (±0.05)	2.49 (±0.10)	2.51 (±0.06)

Table 6.2: Chemical and physical properties of reservoir (R) and bucket (B) water (±1 standard deviation) during the experimental exposure period.

6.3.2 Macrofauna

6.3.2.1 Community Structure

Two-way crossed ANOSIM results (table 6.3) show that changes in seawater pH had significant effects on both the relative abundance of numerically dominant species (untransformed data) and the presence or absence of all species. It is also evident that the effects observed after 2 weeks were significantly different from the effects observed after 20 weeks. These significant effects were evident in both muddy and sandy sediments.

	pH (across all Time groups)		Time (across all pH groups)	
	Mud	Sand	Mud	Sand
Untransformed	**0.677**	**0.485**	**0.347**	**0.774**
Presence / Absence	**0.506**	**0.359**	**0.548**	**0.492**

Table 6.3: Global R-values from 2-way ANOSIM indicating changes in macrofaunal community structure in response to pH (significant differences indicated in bold).

One-way ANOSIM results (Table 6.4) show that there were more significant differences after 20 weeks than were evident after 2 weeks. In addition, the equivalent global R values are all higher after 20 weeks than 2 weeks indicating that differences in community structure are getting larger with time. This would indicate that macrofaunal communities contain species with contrasting tolerances to changes in seawater pH. As significant differences were seen when using both untransformed and presence/absence data, the acidification impacts on both the abundance of numerically dominant species and on the loss of rare species and therefore diversity.

Mud	Untransformed		Presence/Absence	
	2 weeks	20 weeks	2 weeks	20 weeks
C v I	-0.176	**0.334**	0.142	0.252
C v 7.3	0.076	-0.024	0.006	-0.162
C v 6.5	0.14	0.148	**0.338**	**0.504**
C v 5.6	0.196	**1**	**0.552**	**1**
7.3 v 6.5	0.216	0.06	0.186	**0.234**
7.3 v 5.6	**0.196**	**0.992**	**0.402**	**1**
6.5 v 5.6	0.092	**0.84**	**0.402**	**1**
Sand	Untransformed		Presence/Absence	
	2 weeks	20 weeks	2 weeks	20 weeks
C v I	**0.996**	**1**	**0.414**	**0.772**
C v 7.3	0.032	-0.008	-0.074	-0.144
C v 6.5	0.22	**0.3**	**0.21**	**0.226**
C v 5.6	**0.924**	**1**	**0.624**	**1**
7.3 v 6.5	0.144	0.084	0.028	**0.282**
7.3 v 5.6	**0.752**	**0.969**	**0.422**	**1**
6.5 v 5.6	**0.888**	**1**	**0.398**	**1**

Table 6.4: Global R-values from 1-way ANOSIM indicating changes in macrofaunal community structure in response to pH (significant differences indicated in bold).

6.3.2.2 Diversity

Visual inspection of the buckets indicated that seawater acidification can have a significant impact on benthic communities. This was most obvious in the buckets containing sandy sediment (fig. 6.3). In both sediment types, diversity was reduced by increasing seawater acidification (fig. 6.4) with the greatest impact being seen at pH treatment level 5.6. In the mud sediment there is little difference between the two week and 20 week samples (apart from the pH 5.6 treatment) indicating that changes in diversity occur quickly in this sediment type. In the sandy sediment, differences can be seen between the two sampling times indicating that in there are both short term and long term impacts of pH change on diversity in this sediment. In addition the overall impacts on diversity appear to be greater in the sandy sediment than in the muddier sediment. It was evident that not all species died at pH 5.6; several species of capatellid polychaetes survived in both the muddy and sandy sediments.

Figure 6.3: Impact of a) pH 6.5 and b) pH 5.6 acidified seawater on a sandy sediment fauna after a 2 week exposure.)

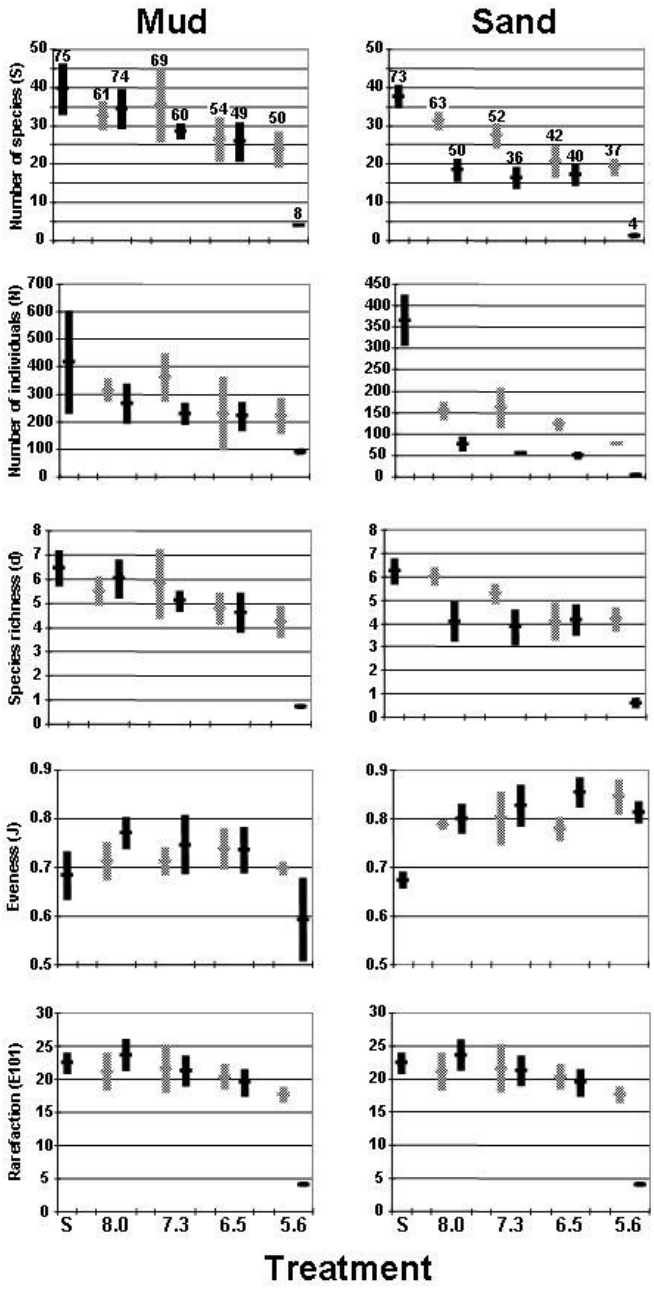

Figure 6.4: Impact of pH on measures of mean macrofaunal diversity (±95% confidence intervals). Grey bars indicate samples taken after 2 weeks exposure, black bars after 20 weeks exposure.

6.3.3 Meiofauna

6.3.3.1 Community structure

Two-way crossed ANOSIM results (table 6.5) show that changes in seawater pH had significant effects on both the relative abundance of numerically dominant species (untransformed data) and the presence or absence of all species. It is also evident that the effects observed after 2 weeks were significantly different from the effects observed after 20 weeks. These significant effects were evident in both muddy and sandy sediments.

	pH (across all Time groups)		Time (across all pH groups)	
	Mud	Sand	Mud	Sand
Untransformed	**0.378**	**0.244**	**0.433**	**0.528**
Presence/Absence	**0.255**	**0.126**	**0.299**	**0.299**

Table 6.5: Global R-values from 2-way ANOSIM indicating changes in meiofaunal community structure in response to pH (significant differences indicated in bold).

One-way ANOSIM results (Table 6.6) show that there were more significant differences after 20 weeks than were evident after 2 weeks. In addition, the equivalent global R values are mostly higher after 20 weeks than 2 weeks indicating that differences in community structure are getting larger with time. This would indicate that meiofaunal communities contain species with contrasting tolerances to changes in seawater pH. In agreement with the macrofauna results, meiofaunal results suggest that both the abundance of numerical dominant species as well as the survival of rare species is significantly impacted by seawater acidification. Comparisons between macrofaunal and meiofaunal results indicates that changes in community structure are larger for macrofaunal communities than for meiofaunal communities.

Mud	Untransformed		Presence/Absence	
	2 weeks	20 weeks	2 weeks	20 weeks
C v I	0.26	**0.32**	0.128	0.07
C v 7.3	**0.312**	0.032	0.152	-0.222
C v 6.5	0.252	**0.396**	**0.374**	0.016
C v 5.6	-0.144	**0.968**	-0.048	**0.704**
7.3 v 6.5	0.188	0.04	**0.258**	**0.38**
7.3 v 5.6	0.22	**0.964**	0.042	**0.78**
6.5 v 5.6	**0.412**	**0.976**	0.024	**0.768**
Sand	Untransformed		Presence/Absence	
	2 weeks	20 weeks	2 weeks	20 weeks
C v I	**0.86**	**0.792**	0.188	**0.43**
C v 7.3	0.048	**0.268**	0.036	0.124
C v 6.5	0.292	-0.176	-0.096	-0.022
C v 5.6	0.096	**0.42**	-0.16	0.298
7.3 v 6.5	0.156	0.172	-0.11	0.198
7.3 v 5.6	0.228	**0.78**	0.118	**0.868**
6.5 v 5.6	0.064	**0.472**	-0.2	**0.724**

Table 6.6: Global R-values from 1-way ANOSIM indicating changes in meiofaunal community structure in response to pH (significant differences indicated in bold).

6.3.3.2 Diversity

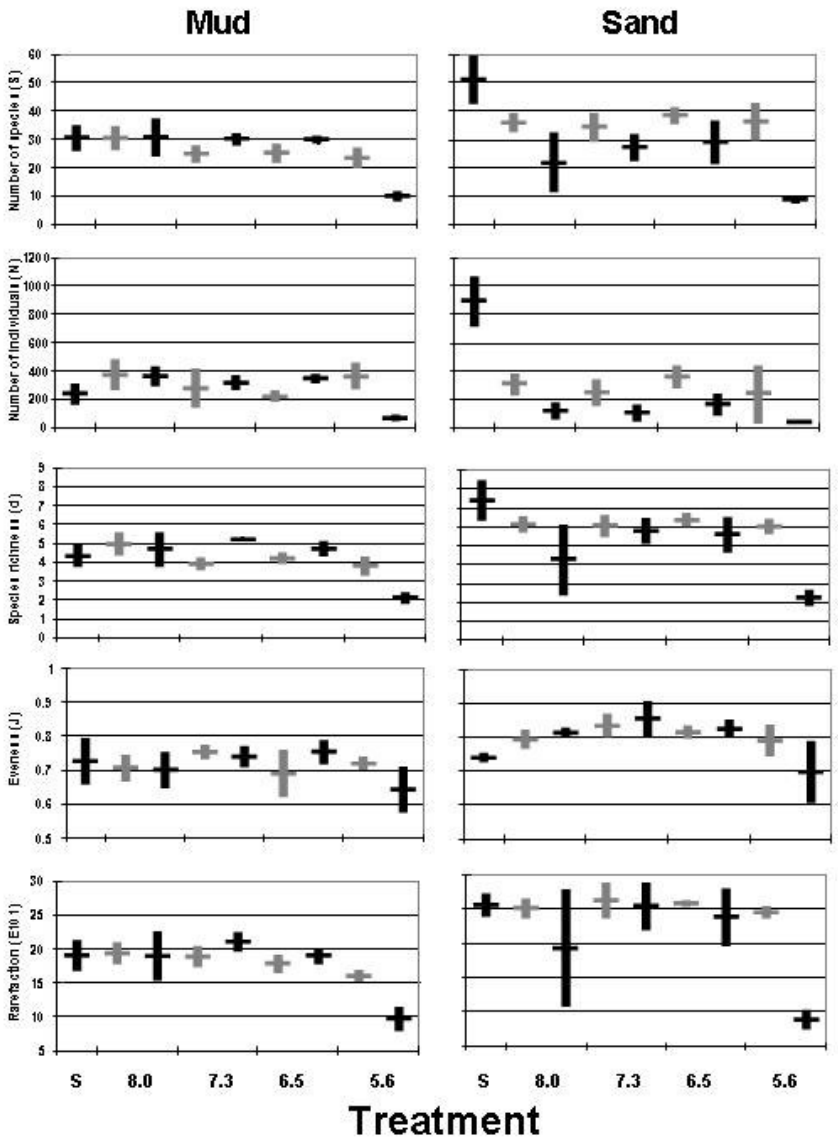

Figure 6.5: Impact of pH on measures of mean meiofaunal diversity (±95% confidence intervals). Grey bars indicate samples taken after 2 weeks exposure, black bars after 20 weeks exposure.

Reductions in meiofaunal diversity in response to seawater acidification were primarily limited to the pH 5.6 treatments after 20 weeks exposure. This supports the conclusions from the community structure analyses that macrofaunal communities are likely to be more impacted by seawater acidification than meiofaunal communities.

6.3.4 Nutrient fluxes

a) 20 weeks **b) 2 weeks**

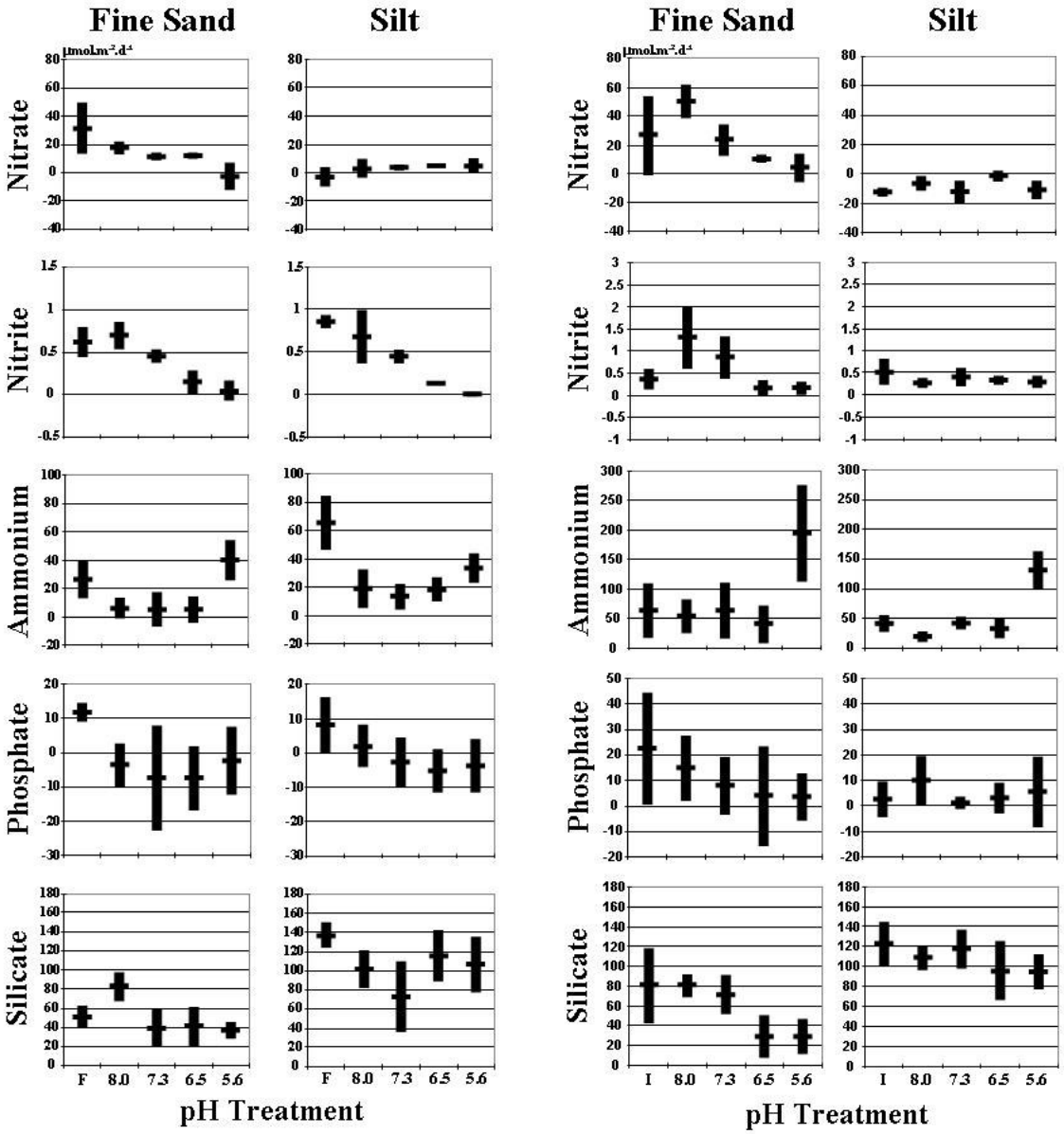

Figure 6.6: Impact of pH on mean sediment nutrient fluxes (±95% confidence intervals) after a) 20 weeks and b) 2 weeks exposure.

After two weeks exposure the mud/silt sediments were a small sink of nitrate and a small source of nitrite. There was no impact of acidification on nitrate or nitrite fluxes. After 20 weeks exposure there is still no impact of pH on nitrate flux although the sediment has become a very slight source of nitrate. However, after 20 weeks exposure an impact of pH on nitrite flux can now be seen with nitrite release becoming less in response to increasing seawater acidity. In contrast the to muddy sediment, the sandy sediment used in this study acted as a source of both nitrate and nitrite, probably as a result of greater nitrification in sandy sediments compared with mud/silt. After 20 weeks exposure total fluxes are less

than after two weeks but a reduction in nitrate and nitrite release due to increasing acidity can still be seen.

In general, both sediment types acted as a source of ammonium throughout the experiment. However, the strength of this source varied with both time and sediment type. In both sediments elevated ammonium release was observed at very low pH (5.6).

There were no significant impacts of pH on phosphate flux in either of the two sediment types.

6.3.5 Carbon and nitrogen sediment concentrations

Changes in seawater pH had no impact on the carbon or nitrogen content of both sediment types after either two or 20 weeks exposure.

6.4 SUMMARY AND CONCLUSIONS

6.4.1 Impact of acidification on biodiversity

It is evident that seawater acidification can have a significant impact on the community structure and biodiversity of sediment communities. This impact comes about primarily through the differential tolerances to changes in seawater pH exhibited by different benthic organisms. However, the largest impacts were observed at very low pH levels. Consequently, it seems unlikely that Ocean Acidification via atmospheric absorption will have a direct effect on many adult benthic organisms. It is far more likely that the effects of Ocean Acidification will be felt by species through impacts on their pelagic larvae and in particular those larvae dependant on calcified structures.

It is likely that leakage from a sub-seabed CO_2 storage site would have a considerable localised effect. However, the presence of capitellid worms in the pH 5.6 treatments after 20 weeks exposure suggests that some organisms would survive in even those areas adjacent to a leak. The presence of these organisms has been known to facilitate the recovery of the sediment community after a disturbance.

6.4.2 Impact of acidification on nutrient flux

The results from this study strongly support the conclusion presented in section 5.2.4, which detailed the impact of acidification and *Nereis virens* on sediment nutrient fluxes.

Both studies demonstrated an impact of acidification nitrogen cycling primarily through effects on nitrification. The “biodiversity” experiment however provided additional insight by demonstrating differences between the responses of nitrate and nitrate fluxes to acidification. These differences may indicate that the bacterial group responsible for the transformation of ammonium to nitrite may be more sensitive to pH change than bacteria transforming nitrite to nitrate. In general, both the muddy and sandy sediments acted as a source of ammonium throughout the experiment. However, the strength of this source varied with both time and sediment type. In both sediments elevated ammonium release

was observed at very low pH (5.6) and as previously discussed (5.2.4) this could be due to a combination of a shutdown in nitrification and a the decay of metazoan animals killed by the initial pH shock. Despite ammonium fluxes being lower after 20 weeks than after two weeks, the elevated fluxes in pH 5.6 treatments were still evident. The large increase in ammonium flux in response to extremely acidified seawater was also observed during the N. virens experiment indicating that this is a consistent response across sediment types. It also indicates that a leakage from sub-seabed storage of CO_2 could result in a large, localised input of ammonium into the pelagic ecosystem. Changes in silicate flux in response to acidification were only observed in the sandy sediment. No impacts were observed in either the muddy sediments used in the "biodiversity" experiments or the sediment used in the "*Nereis*" experiment. This highlights the importance of sediment type when predicting the likely impacts of CO_2 leakage on sediment nutrient fluxes. In the sandy sediment an impact of pH on phosphate release was observed after 2 weeks of exposure but was not event 18 weeks later. In the mud sediment impacts were seen much later with no initial impact (two weeks) evident but instead a marked decline in phosphate release after 20 week exposure. It is likely that the response of sediment phosphate flux to changes in pH is dependant on sediment type with sandy sediments being affected more quickly than muddy ones. In the "*Nereis*" experiment the exposure period was only six weeks and it is possible that the lack of observed response in this muddy sediment could be due too short an exposure period.

6.5 KEY FINDINGS

- The severity of impacts on both biodiversity and nutrient flux due to seawater acidification is dependant on the nature of the sediment environment concerned.

- Decreasing seawater pH could lead to a gradual decrease in the diversity of macrobenthic communities. Particularly vulnerable organisms are those that live on the sediment surface and those that depend on calcium carbonate skeletons.

- Macrobenthic communities are likely to be more sensitive to changes in seawater acidity likely to occur during leakage from sub-seabed storage than meiobenthic communities.

- This experiment supported the conclusions of previous experiments in that nutrient flux rates would be directly affected by the large changes in seawater acidity likely to occur during leakage from sub-seabed storage but not by the small changes predicted as a result of ocean acidification through atmospheric absorption.

- Leakage from a sub-seabed co_2 storage site could have a pronounced but localised effect on benthic biodiversity.

- The survival of some species of polychaete worms even after 20 weeks exposure to ph 5.6 seawater would accelerate the biological recovery of sediments after a leak.

7. CONCLUSIONS

7.1 INTEGRATION AND COMMUNICATION

Previous research projects have often considered the communication of project findings to interested stake holders and end users as an activity to be undertaken in the final few months of the project. IMCO2 was radically different in this respect in that not only did it continously communicate project objectives and findings to various communities (scientific, political, industrial etc) it also established a mechanism by which these communities could communicate their interests, views and concerns back to the project scientists. This was primarily achieved through the Reference User Group (RUG). We recommend that the RUG concept is used more widely within future science projects to ensure policy and science can evolve together with each able to instruct and guide the development of the other. IMCO2 has also demonstrated the power of international and national outreach activities by ensuring that ocean acidification is now accepted as "the other CO_2 problem" and is as important as climate change in the future health of the planet. It is often easy for funding agencies to consider such activities as easy targets when budget cuts need to be made. We would argue however that knowledge transfer and outreach are extremely valuable elements of any research project.

7.2 ECOSYSTEM MODELLING

It is clear from the progress made within the IMCO2 project that a combination of observations, experiments and modelling has the potential to provide a holistic approach to address the potential effects of ocean acidification on marine ecosystems. However, the IMCO2 project should be considered as the first step on a continuing program of model development and refinement. In order to improve the predictive certainty and policy utility via modelling approaches we recommend:

- Continued investment in national computing infrastructure to enable multiple scenario approaches and experimental coupling of diverse model approaches.

- Funding schemes that couple modelling and observational / experimental research are prioritised.

- The design of new observational and experimental programmes should be driven by model requirements and statistical rigour.

- Programme structures that couple UK expertise in climate change, acidification, ecosystems and socio-economic modelling are identified.

7.3 LABORATORY BASED EXPERIMENTATION

Prior to the start of the IMCO2 project very little research had been conducted examining the impact of high CO_2 on marine organisms and processes. What limited knowledge there was had been gained from short term experiments primarily concerned with the impact of emersion on inter-tidal or rockpool species. During the past three years there has been a rapid increase in the number of high CO_2 studies being conducted and the scientific community is now far better placed to make predictions concerning the response of a few species and processes to seawater acidification. However, we are far from possessing the detailed knowledge that would allow accurate predictions for whole ecosystem response. It is essential that future laboratory exposure experiments are encouraged and financially support. It is the opinion of the IMCO2 team that prioritisation of future research topics is not desirable as this invokes a-priori an assessment of relative vulnerabilities, which are as yet unknown. It is however possible to identify some of the key knowledge gaps, the filling of which would substantially advance our understanding of ecosystem functioning in a high CO_2 future. These are:

- The ability of organisms to acclimate or adapt to high CO_2.
- The potential of sediments to buffer shallow continental shelf systems and address benthic – pelagic feedbacks.
- Identify the vulnerable life stages of key species e.g. larvae.
- Dose-response investigations for a wide range of species at realistic CO_2 perturbations identifying the sub-lethal effects and also the mechanism(s) through which the CO_2 response is mediated.
- Multivariate 'matrix' experiments, with numerous variables (e.g. temperature, nutrients, CO_2) would help to identify interactions between key "climate change" effects

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10. APPENDICES

Appendix I: The IMCO2 Reference User Group

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Appendix II: The IMCO2 Reference User Group Terms of Reference

1. Background
   1.1. The Reference User Group (RUG) concept was first developed for the COST-IMPACT programme (Costing the impact of demersal Fisheries on the marine environment), funded by DG FISH, which ran between 2001 and 2004. It was proved to be a highly successful mechanism for ensuring the relevance and user-friendliness of research. The European Commission consider the RUG approach to be best practice and are applying it to future research programmes.

2. Composition of Reference User Group
   2.1. A Reference User Group comprises a number of potential end users of the results of the research project. For the IMCO2 project members will be invited to participate from both commerce and government, with interests spread across relevant environmental, industry and conservation sectors.

3. Terms of Reference

3.1. The RUG will work with the project to examine in detail the user related issues. The specific Terms of Reference for the RUG are therefore:

- To advise on the types of data and analyses and products that will be most useful to managers, policy advisors, decision makers and politicians

- To advise on the format and nature of key messages arising from the research project

- To advise on the dissemination procedures for the project to ensure that the results from the project are disseminated to all potential end users of the information

- To feedback key science developments into their own sector /parent organisation during the life time of the project

4. Operating principles
   4.1. Principal scientists from the project will regularly correspond with the RUG and disseminate information to them concerning progress of the project, co-ordinating their responses and facilitating feedback to other project participants.

5. Frequency and timing of RUG meetings
   5.1. There will be a start up workshop in summer 2005 followed by two annual meeting for the RUG with IMCO2 during the 3-year timescale for the project.

Appendix III: IMCO$_2$ Publications.

Published (Peer-reviewed)

Blackford, J. & Gilbert, F. (In press). Marine ecosystem response to pH changes resulting from increased CO_2 emissions. *Journal of Marine Systems*.

Caldeira, K., Archer, D., Barry, J.P., Bellerby, R.G.J., Brewer, P., Cao, L., Dickson, A.G., Doney, S.C., Elderfield, H., Fabry, V., Feely, R.A., Gattuso, J-P., Hoegh-Guldberg, O., Haugan, P.M., Kleypas, J.A., Langdon, C., Orr, J.C., Ridgwell, A., Sabine, C.L., Seibel, B., **Turley, C**., Watson, A. & Zeebe, R. (in press) Comment on "Modern-age buildup of CO_2 and its effects on seawater acidity and salinity" GRL.

Gibbins, J., Haszeldine, S., Holloway, S., Pearse, J. Oakey, J. Shackley, S. & **Turley, C**. (2006). Scope for Future CO_2 Emission Reductions from Electricity Generation through the Deployment of Carbon Capture and Storage Technologies. In: Avoiding Dangerous Climate Change, Schellnhuber, H J., Cramer,W., Nakicenovic, N., Wigley, T. and Yohe, G (Eds). Cambridge University Press, 40, 379-383.

Miles, H., **Widdicombe, S**., Spicer, J.I. & Hall-Spencer, J. (In press). Acid-base regulation in the sea urchin *Psammechinus miliaris* during chronic exposure to elevated environmental CO_2. *Marine Pollution Bulletin.*

Royal Society Report (2005). Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U., Shepard, J., **Turley, C**. & Watson, A. Ocean acidification due to

increasing atmospheric carbon dioxide. The Clyvedon Press Ltd, Cardiff. (ISBN 0 85403 617 2). The Royal Society Policy document 12/05, pp. 68.

Spicer, J.I., Raffo, A. & **Widdicombe, S**. (in press) Acid-base regulation in the velvet swimming crab *Necora puber* during chronic exposure to elevated environmental CO_2. *Marine Biology*.

Turley, C., Blackford, J., Widdicombe, S., Lowe, D., Nightingale, P.D. & Rees, A.P. (2006) Reviewing the impact of increased atmospheric CO_2 on oceanic pH and the marine ecosystem. In: Avoiding Dangerous Climate Change, Schellnhuber, H J., Cramer,W., Nakicenovic, N., Wigley, T. and Yohe, G (Eds), Cambridge University Press, 8, 65-70.

Widdicombe, S. & **Needham, H.R**. (in press) Impact of CO_2 induced seawater acidification on the burrow morphology of the polychaete *Nereis virens* and the subsequent implications for sediment nutrient flux. *Marine Ecology Progress Series.*

Published (Non peer-reviewed)
Haugan, P.M., **Turley, C**. & Poertner, H.O. (2006). Effects on the marine environment of ocean acidification resulting from elevated levels of CO_2 in the atmosphere, DN-utredning 2006-1, 1-36.

IPCC Forth Assessment Report, Working Group II. Impacts, Adaptation and Vulnerability. Ch 4 Ecosystems. C.M.Turley is one of the Lead Authors (2007).

Laffoley, D., Hawkins, S. & **Turley, C**. Climate change, Surface Ocean Acidification and their impacts on European seas. Proc. of Marine Conservation in Europe 2006.

OSPAR Commission for the Protection of the Marine Environment of the North-East Atlantic Effects on the marine environment of ocean acidification resulting from elevated levels of CO_2 in the atmosphere (2006)Document prepared by an intersessional working group convened by Norway and the United Kingdom (Peter M. Haugan, **Carol Turley** and Hans-Otto Poertner).

Turley, C. (2006). Impacts of Climate Change on Ocean Acidification in Marine Climate Change Impacts Annual Report Card 2006 (Eds. Buckley, P.J, Dye, S.R. and Baxter, J.M), Online Summary Reports, MCCIP, Lowestoft, www.mccip.org.uk

Turley, C. Impacts of a high CO_2 world on marine ecosystems. In: Climate Change and Policy, Standford University Press (in press)

Turley, C.M (2007) How can science help to save the marine environment? Ocean acidification – the other half of the CO_2 problem. Science in Parliament, 64(1) Spring 2007, 16-17.

Turley, C.M. (2005) Case Study 3: Ocean acidification .In: Science into Policy: Taking Part in the Process. NERC Publication, 29-30.

Turley, C.M. (2006) Ocean acidification and its impacts. In: Confronting Climate Change: Critical Issues for New Zealand. Edited by Ralph Chapman, Jonathan Boston and Margot Schwass, Published by Victoria University Press, 125-133.

Turley, C. M. & **Blackford, J.** (2005) Summarising ocean acidification. OpenDemocracy debate on climate change, web article.

Turley, C. M. & **Blackford, J**. (2005) The other CO_2 problem. Marine Conservation, 6 (10), 11.

Turley C and J Henderiks (in press) Learning from the Distant Past: An IGBP/SCOR Workshop on "Ocean Acidification – Modern Observations and Past Experiences" IMBER Newsletter

Turley, C., Nightingale, P., Riley, N., **Widdicombe, S**., Joint, I., Gallienne, C**., Lowe, D**., Goldson, L., Beaumont, N., Mariotte, P., Groom, S., Smerdon, G., Rees, A., **Blackford, J**., Owens, N., West, J., Land, P. & Woodason, E. (2004) Environmental impacts of a gradual or catastrophic release of CO_2 into the marine environment following carbon dioxide capture and storage. DEFRA:MARP 30 (ME2104, 31 March 2004), pp.57.

Widdicombe S. & **Lowe D**. (2006). Ocean acidification The other carbon dioxide problem. Planet Earth magazine (NERC), Summer 2006, pp 14-15.

Submitted

Gibbins, J., Hazeldine, S., Holloway, S., Pearse, J., Oakey, J., Reiner, D. & **Turley, C**. Interim results from the UK Carbon Capture and Storage Consortium project. 8th International Conference on Greenhouse Gases, 19-22 June 2006, Trondheim, Norway (submitted May 2006).

In preparation

Turley, C., Poertner, H.O. & Haugan, P.M. Effects on the marine environment of ocean acidification resulting from elevated levels of CO_2 in the atmosphere. Global Biogeochemical Cycles

Turley, C. M. Summarising ocean acidification. Climate Change and Governance Conference, 28-29 March 2006, Wellington, New Zealand.

Turley C, Roberts M and J Guinotte. Cold water corals – invited perspective paper for Coral Reef's (in prep)

($IMCO_2$ project staff in bold)