PRIFYSGOL
BANGOR
UNIVERSITY

BANGOR UNIVERSITY
School of Ocean Sciences
College of Natural Sciences

Latitudinal and climatic driven changes in local patterns of intertidal macroalgae: implications for biodiversity and ecosystem functioning

by

JOÃO LUÍS GOMES FERREIRA

Internal Supervisors: Dr. Stuart R. Jenkins & Professor Stephen J. Hawkins
External Supervisor: Dr. Michael T. Burrows

Thesis submitted to Bangor University
for the degree of Doctor of Philosophy
February 2012

DECLARATION AND CONSENT

Details of the Work

I hereby agree to deposit the following item in the digital repository maintained by Bangor University and/or in any other repository authorized for use by Bangor University.

Author Name: João Luís Gomes Ferreira

Title: Latitudinal and climatic driven changes in local patterns of intertidal macroalgae: implications for biodiversity and ecosystem functioning

Supervisor/Department: Dr. Stuart Jenkins / School of Ocean Sciences

Funding body (if any): FCT (Fundação para a ciência e tecnologia)

Qualification/Degree obtained: PhD in Marine Ecology

This item is a product of my own research endeavours and is covered by the agreement below in which the item is referred to as "the Work". It is identical in content to that deposited in the Library, subject to point 4 below.

Non-exclusive Rights

Rights granted to the digital repository through this agreement are entirely non-exclusive. I am free to publish the Work in its present version or future versions elsewhere.

I agree that Bangor University may electronically store, copy or translate the Work to any approved medium or format for the purpose of future preservation and accessibility. Bangor University is not under any obligation to reproduce or display the Work in the same formats or resolutions in which it was originally deposited.

Bangor University Digital Repository

I understand that work deposited in the digital repository will be accessible to a wide variety of people and institutions, including automated agents and search engines via the World Wide Web.

I understand that once the Work is deposited, the item and its metadata may be incorporated into public access catalogues or services, national databases of electronic theses and dissertations such as the British Library's EThOS or any service provided by the National Library of Wales.

I understand that the Work may be made available via the National Library of Wales Online Electronic Theses Service under the declared terms and conditions of use (http://www.llgc.org.uk/ index.php?id=4676). I agree that as part of this service the National Library of Wales may electronically store, copy or convert the Work to any approved medium or format for the purpose of future preservation and accessibility. The National Library of Wales is not under any obligation to reproduce or display the Work in the same formats or resolutions in which it was originally deposited.

Statement 1:

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless as agreed by the University for approved dual awards.

Signed ………………………………………………………….… (candidate)

Date 28th of February 2012

Statement 2:

This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s).

All other sources are acknowledged by footnotes and/or a bibliography.

Signed ………………………………………………………….… (candidate)

Date 28th of February 2012

Statement 3:

I hereby give consent for my thesis, if accepted, to be available for photocopying, for inter-library loan and for electronic repositories, and for the title and summary to be made available to outside organisations.

Signed ………………………………………………………….… (candidate)

Date 28th of February 2012

NB: Candidates on whose behalf a bar on access has been approved by the Academic Registry should use the following version of **Statement 3:**

Statement 3 (bar):

I hereby give consent for my thesis, if accepted, to be available for photocopying, for inter-library loans and for electronic repositories after expiry of a bar on access.

Signed ………………………………………………………….… (candidate)

Date ………………………………………………

Statement 4:

Choose **one** of the following options

a)	I agree to deposit an electronic copy of my thesis (the Work) in the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University and where necessary have gained the required permissions for the use of third party material.	X
b)	I agree to deposit an electronic copy of my thesis (the Work) in the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University when the approved **bar on access** has been lifted.	
c)	I agree to submit my thesis (the Work) electronically via Bangor University's e-submission system, however I **opt-out** of the electronic deposit to the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University, due to lack of permissions for use of third party material.	

Options B should only be used if a bar on access has been approved by the University.

In addition to the above I also agree to the following:

1. That I am the author or have the authority of the author(s) to make this agreement and do hereby give Bangor University the right to make available the Work in the way described above.
2. That the electronic copy of the Work deposited in the digital repository and covered by this agreement, is identical in content to the paper copy of the Work deposited in the Bangor University Library, subject to point 4 below.
3. That I have exercised reasonable care to ensure that the Work is original and, to the best of my knowledge, does not breach any laws – including those relating to defamation, libel and copyright.
4. That I have, in instances where the intellectual property of other authors or copyright holders is included in the Work, and where appropriate, gained explicit permission for the inclusion of that material in the Work, and in the electronic form of the Work as accessed through the open access digital repository, *or* that I have identified and removed that material for which adequate and appropriate permission has not been obtained and which will be inaccessible via the digital repository.
5. That Bangor University does not hold any obligation to take legal action on behalf of the Depositor, or other rights holders, in the event of a breach of intellectual property rights, or any other right, in the material deposited.
6. That I will indemnify and keep indemnified Bangor University and the National Library of Wales from and against any loss, liability, claim or damage, including without limitation any related legal fees and court costs (on a full indemnity bases), related to any breach by myself of any term of this agreement.

Signature: ………………………………………………………… Date : 28th of February 2012

This work was supported by “Fundação para a Ciência e a Tecnologia” (FCT) (Foundation for Science and Technology) by a individual PhD scholarship with the reference SFRH / BD / 41541 / 2007, funded by the program POPH - QREN - Typology 4.1 – Advanced formation, co-participated by the European Social Fund and the Portuguese Ministry of Science, Technology and High Education.

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

ABSTRACT

Investigating the relative roles of physical and biological factors in determining community structure across gradients of emersion and wave exposure in the rocky shore intertidal has been one of the principal areas of interest in marine ecology. However, the way in which the role of these factors changes over large latitudinal scales across European shores has not been quantitatively assessed. Using a large scale survey across the European coastline changes in community structure from a local to a European scale were assessed in a quantitative manner, with particular focus on the dominant primary producers, fucoid macroalgae, and key grazers, patellid limpets. Following this quantitative descriptive approach, key controlling processes that ultimately could lead to alterations in the community structure and energy flow between ecosystems were also explored.

The large scale survey gave a broad scale quantitative description of major functional intertidal groups over a latitudinal gradient from northern Scotland to southern Portugal (Chapter 2). This descriptive work explicitly operated at a very coarse scale of resolution to essentially differentiate between algal dominated and sessile invertebrate dominated assemblages. In describing latitudinal patterns, a key confounding variable, wave exposure, was addressed by independently categorising shores into three categories according to the

degree of wave action. This was achieved using a GIS model based on wave fetch. The quantification of patterns of biomass and percentage cover over latitudinal scales resulted in the development of a database for different intertidal functional groups covering a large area of the European rocky intertidal, which can be used by future studies for temporal comparisons and evaluation of the effects of possible climatic alterations. The survey showed a decline in total fucoid biomass and percentage cover in the Portuguese region, but little variation over the extent of the British Isles. The expected corresponding increase in filter feeder cover was not obvious and an increase in *Patella* grazer density and biomass in Portugal was not found.

A number of targeted field experiments and collections were undertaken in Wales and Portugal to ascertain the causal factors leading to the observed gradient in algal-animal balance in the intertidal over a European scale. A factorial experiment (Chapter 3) was undertaken in the field to determine the interactive effect of grazing pressure and physical environment (shade and moisture) on patterns of fucoid recruitment. Amelioration of the physical environment did not improve recruitment at either geographical region, while grazing pressure was found to be an important process regulating fucoid recruitment only on northern European shores.

Adult algal characteristics, specifically stress levels (Chapter 4) and reproductive capacity (Chapter 5), were assessed, through experimental work, to determine their contribution to latitudinal scale patterns. The results showed that stress levels (measured as photosynthetic resilience) increase in fucoid populations when they were subjected to physical conditions characteristic of a summer day in Portugal, with specimens not being able to fully recover even after a 16 hour recovery period. Environmental conditions in southern regions were also found to promote fucoid populations with lower numbers of receptacles and lower reproductive tissue biomass, which indicates reduced reproductive capacity.

Using the data collected and conclusions about patterns and processes from the experiments undertaken during the PhD, changes in algal productivity and filter feeder abundance were discussed, while predicting energy flow from and to the coastal environment at local and large geographical scales. The work demonstrates the importance of undertaking quantitative surveys over large scales whilst controlling for key confounding variables. Whilst many of the patterns expected from previous qualitative descriptions and quantitative work undertaken at local scales were verified, there were important unexpected outcomes. In addition, experimental work undertaken in different geographical regions has provided some insight into key processes determining large scale patterns.

TABLE OF CONTENTS

DECLARATION AND CONSENT .... **I**

ABSTRACT .... **V**

TABLE OF CONTENTS .... **VII**

LIST OF TABLES .... **X**

LIST OF FIGURES .... **XIII**

ACKNOWLEDGMENTS .... **XVI**

CHAPTER 1 GENERAL INTRODUCTION **1**

- 1.1 BRIEF OPENING NOTE 1
- 1.2 SHORE ENVIROMENT 2
  - *1.2.1 Tidal effects* *3*
  - *1.2.2 Effects of exposure to wave action* *4*
  - *1.2.3 Biological interactions* *6*
- 1.3 LARGE SCALE FACTORS AND LATITUDINAL GRADIENTS 12
- 1.4 CLIMATE CHANGE AND ITS IMPACTS 13
- 1.5 GENERAL AIMS 14

CHAPTER 2 BIOGEOGRAPHICAL PATTERNS OF INTERTIDAL ORGANISMS OVER A EUROPEAN LATITUDINAL GRADIENT **16**

- 2.1 INTRODUCTION 16
- 2.2 METHODS 21
  - *2.2.1 Where and when* *21*
  - *2.2.2 Species chosen* *24*
  - *2.2.3 Methodology & design* *25*
- 2.3 RESULTS 26
  - *2.3.1 Fucoid distribution* *26*
  - *2.3.2 Fucoid species results: biomass vs. percentage cover* *35*

2.3.3 Patella spp. distribution *40*

2.3.4 Interaction between grazing pressure and macroalgae biomass *44*

2.3.5 Distribution of other components of the associated community *48*

2.3.6 Within and among shore patterns of variability (patchiness) *54*

2.4 DISCUSSION 58

CHAPTER 3 THE EFFECTS OF GRAZING AND ENVIRONMENTAL CONDITIONS IN FUCOID RECRUITMENT ON NORTHERN AND SOUTHERN EUROPEAN SHORES 63

3.1 INTRODUCTION 63

3.2 METHODS 65

3.2.1 Study areas *65*

3.2.2 Experimental designs *66*

3.2.3 Experimental setup and sampling *66*

3.2.4 Statistical analysis *67*

3.3 RESULTS 68

3.3.1 Experimental artefacts *68*

3.3.2 Densities of fucoids (across the entire period) *68*

3.3.3 Fucoid biomass (after 1 year) *72*

3.4 DISCUSSION 73

CHAPTER 4 PHYSIOLOGICAL RESPONSE OF NORTHERN AND SOUTHERN FUCOID ALGAE TO PHYSICAL STRESS 77

4.1 INTRODUCTION 77

4.2 METHODS 80

4.2.1 Collection *80*

4.2.2 Transport, acclimatization and maintenance *81*

4.2.3 Measurement of response variables *81*

4.2.4 Experimental design and set up *82*

4.2.5 Data analysis *85*

4.3 RESULTS 85

4.3.1 Desiccation resilience *86*

4.3.2 Photosynthetic resilience *93*

4.4 DISCUSSION 104

CHAPTER 5 PATTERNS OF REPRODUCTIVE OUTPUT OF FUCOID SPECIES IN CENTRAL AND RANGE EDGE POPULATIONS 108

5.1 INTRODUCTION 108

5.2 METHODS 112

5.2.1 Collection and preservation of specimens 112

5.2.2 Measurement of response variables 113

5.2.3 Statistical design 113

5.3 RESULTS 114

5.3.1 Fucoid morphology 114

5.3.2 Reproductive indicators and related variables 115

5.4 DISCUSSION 122

CHAPTER 6 CONCLUDING REMARKS 124

6.1 PATTERNS ACROSS LATITUDE (QUANTITATIVE ANALYSIS) 124

6.2 PROCESSES REGULATING FUCOID SURVIVAL AND RECRUITMENT 127

6.3 ENERGETIC CONSEQUENCES AND FUTURE RESEARCH 130

LITERATURE CITED 132

LIST OF TABLES

Table 1: Mix model ANOVA of biomass of total fucoids, *F. serratus* and *F. vesiculosus*. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual.* 31

Table 2: Mix model ANOVA of biomass of *A. nodosum* and *F. spiralis*. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual.* 32

Table 3: Mix model ANOVA of percentage cover of total fucoids, *F. serratus* and *F. vesiculosus*. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual.*... 36

Table 4: Mix model ANOVA of biomass of *A. nodosum* and *F. spiralis*. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual.* 37

Table 5: Mix model ANOVA of density and biomass of *Patella* spp.. Post hoc SNK tests of significant differences are presented. Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual. 41

Table 6: Mix model ANOVA of percentage cover of foliose, turf, crustose and ephemeral algae. Post hoc SNK tests of significant differences are presented. Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual. 49

Table 7: Mix model ANOVA of percentage cover of barnacles, mussels and free space available. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual.*... 52

Table 8: One way ANOVA model testing the effects of geographical region origin on among shore variability of density of *Patella* spp. and biomass of total fucoids and *Patella* spp. (only at mid shore height). *Abbreviations used - Re: Geographical Region; Res: Residual.* 55

Table 9: One way ANOVA model testing the effects of wave exposure on among shore variability of density of *Patella* spp. and biomass of total fucoids and *Patella* spp. (only at mid shore height). Post hoc SNK tests of significant differences are presented. *Abbreviations used - We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Res: Residual*............ 55

Table 10: Two way fix ANOVA model testing the effects of geographical region origin and wave exposure on within shore variability of density of *Patella* spp. and biomass of total fucoids and *Patella* spp. (only at mid shore height). Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Res: Residual.* 56

Table 11: Mix model ANOVA of density and weight of fucoids produced after 1 year on the shore to test the effect of fence. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales); Sh: Shore; Fe: Fence; Res: Residual.*............ 69

Table 12: Mix model ANOVA of density of fucoids produced after 70, 145 and 365 days on the shore. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales); Sh: Shore (Vil: Vila Praia de Âncora; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Li: Light intensity; Gr: Grazing level (-G: Ungrazed; ½G: Half grazing pressure; +G: Grazed at natural densities) Res: Residual.*...... 70

Table 13: Analysis of variance of fucoid biomass after 1 year on the shore. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales); Sh: Shore; Li: Light intensity; Gr: Grazing level (-G: Ungrazed; ½G: Half grazing pressure; +G: Grazed at natural densities) Res: Residual.* 72

Table 14: Mean frond temperatures values (in degree Celsius, ºC) achieved across the experiments during the application of different stress conditions, measured with Easyview 15, Extech Instruments Corp., USA. 83

Table 15: Two way mix model ANOVA of the percentage of H_2O in initial *F. vesiculosus* total weight on experiments 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore; Res: Residual.*............ 87

Table 16: Two way mix model ANOVA of the percentage of H_2O in initial *F. spiralis* total weight on experiments 4.1, 4.2, 5.1 & 5.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region; Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Res: Residual.* 87

Table 17: Mix model ANOVA of the percentage of initial H_2O from *F. vesiculosus* tissues lost due to stress on experiments 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Te: Temperature (War: Warm; Col: Cold); Li: Light intensity (Sha: Shaded; Uns: Unshaded); Res: Residual.* 89

Table 18: Mix model ANOVA of the percentage of initial H_2O lost from *F. spiralis* tissues due to stress on experiments 4.1, 4.2, 5.1 & 5.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Te: Temperature (War: Warm; Col: Cold); Li: Light intensity (Sha: Shaded; Uns: Unshaded); Res: Residual.*............ 90

Table 19: Two way mix model ANOVA of initial Fv/Fm values from *F. vesiculosus* tissues before stress was applied on experiments 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Res: Residual.* 94

Table 20: Two way mix model ANOVA of initial Fv/Fm values of *F. spiralis* tissues before stress was applied on experiments 4.1, 4.2, 5.1 & 5.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Res: Residual.* 94

Table 21: Mix model ANOVA of the percentage of initial Fv/Fm values in *F. vesiculosus* retained after stress on experiments 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Te: Temperature (War: Warm; Col: Cold); Li: Light intensity (Sha: Shaded; Uns: Unshaded); Res: Residual.* 97

Table 22: Mix model ANOVA of the percentage of initial Fv/Fm values in *F. spiralis* retained after stress on experiments 4.1, 4.2, 5.1 & 5.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Te: Temperature (War: Warm; Col: Cold); Li: Light intensity (Sha: Shaded; Uns: Unshaded); Res: Residual.* 98

Table 23: Mix model ANOVA of the percentage of initial Fv/Fm in *F. vesiculosus* working after 16 hours on experiments 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Te: Temperature (War: Warm; Col: Cold); Li: Light intensity (Sha: Shaded; Uns: Unshaded); Res: Residual.* 100

Table 24: Mix model ANOVA of the percentage of initial Fv/Fm in *F. spiralis* working after 16 hours on experiments 4.1, 4.2, 5.1 & 5.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Te: Temperature (War: Warm; Col: Cold); Li: Light intensity (Sha: Shaded; Uns: Unshaded); Res: Residual.* 101

Table 25: Two way mix model ANOVA of *Fucus vesiculosus* length, volume, number of receptacles, mean receptacle dry biomass, dry vegetative biomass, dry reproductive biomass and reproductive effort. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Are: Are; Car: Carreço; Via: Viana do Castelo; Cwy: Porth Cwyfan; Cem: Cemlyn Bay; Tre: Trearddur Bay); Res: Residual.* 118

Table 26: Two way mix model ANOVA of *Fucus spiralis* length, volume, number of receptacles, mean receptacle dry biomass, dry vegetative biomass, dry reproductive biomass and reproductive effort. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Are: Are; Car: Carreço; Via: Viana do Castelo; Cwy: Porth Cwyfan; Cem: Cemlyn Bay; Tre: Trearddur Bay); Res: Residual.* 119

LIST OF FIGURES

Figure 1: Mean sea surface temperature (from February 2000 to February 2008) and mean air temperature at surface (from September 2004 to September 2008) from northern Scotland to southern Portugal. Diagrams extracted and adapted from NASA (http://disc.sci.gsfc.nasa.gov/giovanni/overview/index.html) - TES & MODIS-Aqua missions respectively. 23

Figure 2: Biomass of *F. serratus*, *F. vesiculosus*, *A. nodosum* and *F. spiralis* at Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR). 28

Figure 3: Biomass of total fucoids, *F. serratus* and *F. vesiculosus* at Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR) across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)* and shore height levels *(high, mid and low)*. The top panel shows a histogram, on the right, of total fucoid biomass variation across the four geographical regions where variability within each of the geographical regions is ignored; Error bars = ±1SE. 33

Figure 4: Biomass of *A. nodosum* and *F. spiralis* at Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR) across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)* and shore height levels *(high, mid and low)*; Error bars = ±1SE 34

Figure 5: Percentage cover of total fucoids, *F. serratus* and *F. vesiculosus* at Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR) across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)* and shore height levels *(high, mid and low)*. The top panel shows a histogram, on the right, of total fucoid biomass variation across the four geographical regions where variability within each of the geographical regions is ignored; Error bars = ±1SE. 38

Figure 6: Percentage cover of *A. nodosum* and *F. spiralis* at Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR) across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)* and shore height levels *(high, mid and low)*; Error bars = ±1SE 39

Figure 7: Graphs showing the relationship between *Patella* spp. shell length and dry soft tissue biomass for each wave exposure level at each geographical region; Regression equations and R^2 values are shown. 42

Figure 8: Density and biomass of *Patella* spp. at Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR) across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)* and shore height levels *(high, mid and low)*. On the top right of each panel a histogram of density/biomass variation across the four geographical regions, where variability within each of the geographical regions is ignored, is shown; Error bars = ±1SE 43

Figure 9: Total fucoid biomass, density and biomass of *Patella* spp. at Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR) across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)*; Error bars = ±1SE 45

Figure 10: Graphs showing the relationship between *Patella* spp.density and fucoid biomass levels, when all data is plot together, for each wave exposure level and each geographical region; Regression equations and R^2 values are shown. 46

Figure 11: Graphs showing the relationship between *Patella* spp. dry biomass and fucoid biomass levels, when all data is plot together, for each wave exposure level and each geographical region; Regression equations and R^2 values are shown. 47

Figure 12: Percentage cover of foliose, turf, encrusting and ephemeral algae at Scottish (SCO), Welsh (WAL), English (ENG) and Portuguese (POR) regions across different wave exposures (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered) and shore height levels (high, mid and low). On the top right of each panel a histogram of density/biomass variation across the four geographical regions, where variability within each of the geographical regions is ignored, is shown; Error bars = ±1SE 50

Figure 13: Percentage cover of barnacles, mussels and free rock substrate at Scottish (SCO), Welsh (WAL), English (ENG) and Portuguese (POR) regions across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)* and shore height levels *(high, mid and low)*. On the top right of each panel a histogram of density/biomass variation across the four geographical regions, where variability within each of the geographical regions is ignored, is shown; Error bars = ±1SE 53

Figure 14: a) Among shore variance of total fucoid biomass, total fucoid percentage cover, limpets biomass and limpets density at mid shore height at Scottish (SCO), Welsh (WAL), English (ENG) and Portuguese (POR) regions and across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)*. b) Within shore variance of total fucoid biomass, total fucoid percentage cover, limpets biomass and limpets density at mid shore height at Scottish (SCO), Welsh (WAL), English (ENG) and Portuguese (POR regions across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)*; Error bars = ±1SE 57

Figure 15: Densities of fucoid (ind/m^2) produced after 70 (a), 145 (b) and 365 (c) days on the shore under different light intensities and grazing treatments *(-G: Ungrazed; ½G: Half grazing pressure; +G: Grazed at natural densities)* at two geographical regions each represented by 2 shores *(Sh1: Vila Praia de Âncora (Portugal), Porth Cwyfan (Wales); Sh2: Carreço (Portugal), Cemlyn Bay (Wales)*. Error bars = ±1SE. 71

Figure 16: Total fucoid biomass (g/m^2) produced after 365 days on the shore under different light intensities and grazing treatments *(-G: Ungrazed; ½G: Half grazing pressure; +G: Grazed at natural densities)* at two geographical regions each represented by 2 shores *(Sh1: Vila Praia de Âncora (Portugal), Porth Cwyfan (Wales); Sh2: Carreço (Portugal), Cemlyn Bay (Wales)*. Error bars = ±1SE. 73

Figure 17: Experiment 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2; Error bars = ±1SE. a) Percentage of H_2O in initial *F. vesiculosus* total weight from Portuguese (POR) and Welsh (WAL) shores (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay). b) Percentage of initial H_2O lost from Portuguese (POR) and Welsh (WAL) *F. vesiculosus* tissues due to different light intensity and temperature treatments. 91

Figure 18: Experiment 4.1, 4.2, 5.1 & 5.2; Error bars = ±1SE. a) Percentage of H_2O in initial *F. spiralis* total weight from Portuguese (*POR*) and Welsh (*WAL*) shores (*Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay*). b) Percentage of initial H_2O lost from Portuguese (*POR*) and Welsh (*WAL*) *F. spiralis* tissues due to different light intensity and temperature treatments. 92

Figure 19: Experiment 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2; Error bars = ±1SE. a) *F. vesiculosus* initial Fv/Fm values before stress from Portuguese (*POR*) and Welsh (*WAL*) shores (*Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay*). b) Percentage of initial Fv/Fm, from Portuguese (*POR*) and Welsh (*WAL*) *F. vesiculosus* tissues, retained 30 minutes after the application of light intensity and temperature treatments. c) Percentage of initial Fv/Fm, from Portuguese (POR) and Welsh (WAL) *F. vesiculosus* tissues, working 16 hours after application of light intensity and temperature treatments on experiments. 102

Figure 20: Experiment 4.1, 4.2, 5.1 & 5.2; Error bars = ±1SE. a) *F. spiralis* initial Fv/Fm values before stress from Portuguese (*POR*) and Welsh (*WAL*) shores (*Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay*). b) Percentage of initial Fv/Fm, from Portuguese (*POR*) and Welsh (*WAL*) *F. spiralis* tissues, retained 30 minutes after the application of light intensity and temperature treatments. c) Percentage of initial Fv/Fm, from Portuguese (*POR*) and Welsh (*WAL*) *F. spiralis* tissues, working 16 hours after application of light intensity and temperature treatments. 103

Figure 21: *Fucus vesiculosus* length, volume, number of receptacles, mean receptacle dry biomass, dry vegetative biomass, dry reproductive biomass and reproductive effort at Portugal (POR) and Wales (WAL) across different shores (*Are: Areosa; Car: Carreço; Via: Viana do Castelo; Cwy: Porth Cwyfan; Cem: Cemlyn Bay; Tre: Trearddur Bay*); Error bars = ±1SE............................120

Figure 22: *Fucus spiralis* length, volume, number of receptacles, mean receptacle dry biomass, dry vegetative biomass, dry reproductive biomass and reproductive effort at Portugal (POR) and Wales (WAL) across different shores (*Are: Areosa; Car: Carreço; Via: Viana do Castelo; Cwy: Porth Cwyfan; Cem: Cemlyn Bay; Tre: Trearddur Bay*); Error bars = ±1SE................ 121

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my supervisors, Stuart Jenkins, Steve Hawkins and Mike Burrows for their guidance and support during my studies and for giving me the opportunity to integrate into their research groups, which contributed to a continuous learning process. A special thanks certainly goes to Stuart for his daily support and help throughout my PhD. As a supervisor and friend, he was always willing to answer my doubts and giving me his wise comments for the improvement of my work.

I am very glad and thankful that during this project I had the pleasure to work with so many people, who I am extremely grateful for the time they spend helping me. Without their precious help, the fieldwork would be impossible and definitely not so rewarding and enjoyable. My sincere thank to Ronaldo Christofoletti, Carmen Rodrigues, Anna Grasa, Fábio Matos, Alessandra Fideliss, Patrícia Puig and Raquel Palla for their help during the 120 shores of the survey. This laborious task, by no means small, was also lots of fun. For the completion of the shading and grazing experiments I am very grateful to José Ferreira (Junior and Senior), Sérgio Oliveira, Philip Hollyman, Jorge Godinho and Raquel Palla for their assistance with fieldwork, setting up experiments and removing damaged material not always in the best of weathers but always with a smile, and to Dr. Fernando Lima and Dr. Francisco Arenas for their help in finding suitable areas in Portugal to conduct my experiments. For the input given into the PAM experiments, a special mention has to be made to Dr. Francisco Arenas especially for his guidance and help during the entire period but also for providing all the facilities in the LCB (Laboratory of Coastal Biodiversity) at Ciimar. I would also like to thank Dr. Bruno Jesus and Dr. Martin Skov for their expert PAM advice and to all the members of the LCB, especially to Alba Cisa, for her invaluable help during the frenetic experimental period. During my early days as marine biologist in the University of Azores my first experiences working in marine ecology were done in the group of Ana Neto. The experience was invaluable and made me believe that large surveys are possible to achieve if we have the right team, thanks for all the support and for giving me that opportunity.

My thank also goes to the University of Bangor, School of Ocean Sciences for all technical support given during my studies and to the “Fundação para a Ciência e a Tecnologia” (Foundation for Science and Technology) for the essential financial support. During my time in the Eco Lab, School of Ocean Sciences, I had the joy to contact daily with numerous interesting and friendly people, which make this a pleasurable and interesting place to work. I would like to thank especially to my 201 office mates and closer colleagues, Andrew Johnson, Katrin Bohn, Marija Sciberras, Kate Griffith, Ronaldo Cristofoletti and Raquel Quintã for their continued support and memorable gatherings. A very special mention to Ronaldo is indispensable. Thanks for being such a good friend, always looking on the bright side of things, and for all your guidance and valid comments.

Finally, I would like to express my gratitude to my family who always encouraged me to pursue my goals and provided a refuge to clear my mind. My grandfather was a very precious friendly help after long days working on the experiments, always with some stories to cheer me up. My parents were always the most supportive and were always there for me when I needed. Lastly to Raquel, my girlfriend, I just want to say thanks! for keeping me going and never letting me give up, for the countless hours helping me but most important for sharing with me all the moments.

CHAPTER 1

GENERAL INTRODUCTION

1.1 BRIEF OPENING NOTE

Climate varies with latitude but also fluctuates over time. Climate on Earth varies across temporal scales ranging from seasonal cycles to millennial scales that lead to changes from glacier to inter-glacial transitions (Harley *et al.* 2006). However, over recent years there is evidence of rapid warming. Contemporary climate change has been linked with human activity and data shows that warming patterns observed in past decades clearly exceed the rate observed over the last centuries (Jones *et al.* 2001; Jones & Mann 2004; Hansen *et al.* 2006; IPCC 2007). Climate warming has been observed around the globe with the largest temperature increases occurring at mid to high latitudes in the northern hemisphere (Jones *et al.* 2001; Jones & Mann 2004; Hansen *et al.* 2006). If the rate of climatic warming is to continue, conditions at higher latitude in the future may come to resemble those from lower latitudes today. This potential trend gives great importance to studies made across different latitudes.

As proposed by Parmesan and Yohe (2003), since climatic change is detected through analysis of global patterns of atmospheric gases, its effects should also be observed across large spatial scales and through the analysis of community patterns in order to fully apprehend its global consequences. Therefore, quantitative surveys of European scale patterns spanning several degrees of latitude will be valuable to predict future intertidal community states. The predictive power of the effects of climatic alteration in future will also increase if experiments lead to greater understanding of the processes generating contemporary patterns.

In my thesis I have taken a quantitative approach to measure patterns in rocky shores across Europe, re-examining existing paradigms based on qualitative descriptions at limited sites (Ballantine 1961; Hawkins & Hartnoll 1983; Hawkins *et al.* 1992). I have studied shifts in the balance between filter feeders and large fucoid macroalgae and variation in grazing pressure along horizontal gradients occurring with latitude. The experimental design used has rigorously controlled for wave action and by using multiple shores in a region this study was able to separate local variation from broader scale patterns. Furthermore, in order to obtain a more complete understanding of patterns detected I have undertaken experiments testing hypotheses about the processes that could be leading to pattern formation.

In the rest of this general introductory chapter, I briefly introduce how rocky shore species and assemblages respond to major local environmental gradients and a brief overview of important biological interactions is described in the light of broad changes in balance between fucoid and filter feeder communities. I introduce the more gentle and complex latitudinal gradient, which underlies biogeographical patterns and explores some of the changes caused by climatic differences observed across the European coastline. I conclude by introducing the overall rational of the thesis and the specific objectives of each chapter.

1.2 SHORE ENVIROMENT

The shoreline and its organisms have been the subject of curiosity and study for a long time. Diverse habitats such as sandy beaches, estuarine mudflats and rocky shores, despite presenting a different physical nature, share common environmental and ecological aspects that characterize them as a shore (Raffaelli & Hawkins 1996). The rocky shore community experiences some of the most severe physical conditions in the world. Intertidal organisms are greatly influenced by fluctuation of water level caused by tidal cycles. In addition to the effects of mechanical forces, such as the movement of water, air or sediments, the cyclic emersion periods cause multiple biochemical stresses due to variability in temperature, light, salinity, pressure and moisture levels (Evans 1948; Menge 1976; Underwood & Jernakoff

1984; Carrington 1990). Adding to the physical pressures, intertidal organisms are still exposed to a complex network of biological interactions caused by a biodiverse intertidal community. Specific characteristics and strategies are necessary to overcome abiotic and biotic stresses and the success of organisms will ultimately be reflected in their natural distribution on the shore.

1.2.1 Tidal effects

Environmental conditions on rocky shores are highly influenced by tidal elevation. A sharp unidirectional gradient of physical stress occurs from fully marine conditions up to the highest extent of marine influence, the splash zone. This vertical stress gradient is the main reason for the development of vertical zonation of intertidal algae and animals on rocky shores (Stephenson & Stephenson 1949; Stephenson & Stephenson 1972; Lipkin *et al.* 1993; Raffaelli & Hawkins 1996). The greatest stress occurs in temperate zones in the summer during daylight emersion periods. Extreme levels of stress occur when the low tide happens around mid afternoon on days with clear skies and low influence of waves. This conjunction of factors, promotes an environment with harsh physiological stresses where abnormal high temperatures and solar radiance cause extreme levels of desiccation (Stephenson & Stephenson 1972; Hawkins & Jones 1992; Denny & Wethey 2001). Regarding the vertical position on the shore, upper areas of the shore are classified as more severe mainly due to their greater emersion period and the more variable, unpredictable and extreme physical conditions (Doty 1946; Denny & Wethey 2001).

Several studies exemplify the positive correlation between shore level occupied by intertidal organisms and their tolerance to physiological stresses. The upper and lower vertical distribution limits of species can be dictated by physical factors or biotic interactions (Stephenson & Stephenson 1949; Connell 1972; Wolcott 1973; Menge & Sutherland 1976; Raffaelli & Hawkins 1996).

Various studies observed the impact of physical factors on the vertical distribution of species and some upper shore species have been shown to have improved tolerance to extreme temperatures (Evans 1948; Davies 1970; Somero 2002; Davenport & Davenport 2005), desiccation (Test 1945; Buschmann 1990; Lipkin *et al.* 1993; Gylle *et al.* 2009), osmotic extremes (Smith & Berry 1986; Davison & Pearson 1996) and solar radiance intensity (Lamote *et al.* 2007). The importance of physical factors in regulating upper limits of fucoid species has also been described. Species like *Pelvetia canaliculata*, *Fucus spiralis*, *Ascophyllum nodosum* and *Laminaria digitata* have all shown to be directly affected by physical factors, such as extreme field temperatures and long exposure to sunlight (Schonbeck & Norton 1978; Todd & Lewis 1984; Hawkins & Hartnoll 1985; Norton 1985).

However, physical factors are not the only forces setting vertical limits in the rocky intertidal environment (Wolcott 1973; Lubchenco 1980). The vertical distribution of some intertidal species is not caused by physical limitation but due to the override effect of biological interactions (Connell 1961a; Dayton 1971; Menge 1976; Grosberg 1982). Canopy removal experiments and alga transplant studies made in the United Kingdom demonstrated that the lower limits of fucoid species, like *Pelvetia canaliculata*, *Fucus spiralis*, *Fucus vesiculosus*, *Fucus serratus* and *Himanthalia elongata*, can be established by competition with species that occupy the vertical zone below (Schonbeck & Norton 1980; Hawkins & Hartnoll 1985; Johnson *et al.* 1998b). Similarly to lower levels, upper vertical limits of distribution of some fucoid species, as *Fucus serratus* and *Laminaria digitata*, have also been showed to be set by competition with the species zoned immediately above, *Fucus vesiculosus* and *Fucus serratus* respectively (Hawkins & Hartnoll 1985; Johnson *et al.* 1998b).

From the knowledge acquired so far, the consensus seems to be that both abiotic and biotic processes are possible causes for the vertical distribution limits of species on the shore. A generally accepted consensus is that the upper limits of species are often limited by physical factors, especially high on the shore, whilst the lower limit is generally determined by interactions with other species (Raffaelli & Hawkins 1996).

1.2.2 Effects of exposure to wave action

Wave exposure, defined as the degree of wave action that a shore experiences, has long been described to have a considerable influence on rocky shore communities (Ballantine 1961; Lewis 1964; Jones & Demetropoulos 1968; Stephenson & Stephenson 1972; Denny 1985). Variation in the magnitude, duration and frequency of wave forces create different environmental conditions over a horizontal gradient. Unlike the vertical gradient, the horizontal gradient is not a unidirectional stress gradient (Raffaelli & Hawkins 1996).

The strength and impact of waves is affected by wave height and length, shore topography and coastal bathymetry (Denny 1988, 2006). The swell generated by distant weather systems and the influences of local winds are also very important variables that influence wave height and length (Denny 1988). So, at a large-scale primary wind direction, wind speed and fetch are the factors controlling wave exposure. While at intermediate scales, coastline topography is another factor to consider. On such scales, headlands are considerable areas of wave refraction where the energy converges, whereas embayed areas are regions of divergence of wave energy. At smaller scales, the effects of local bathymetry and shore topography also contribute to the level of wave exposure (Denny 1988). Waves create hydrodynamic forces, such as drag, lift and acceleration that affect the fitness and survival of the littoral community (Carrington 1990). The risk of dislodgment and loss of biomass increases with wave exposure, leading to selection of the intertidal organisms that are able to sustain such forces. Organisms that allocate resources and develop specific characteristics to improve their adhesion to the substratum have better chances of success in wave-exposed intertidal shores.

In the UK, variation in the composition of communities along wave exposure gradients has been well illustrated by Ballantine (1961). The dominant organisms in sheltered bedrock shores are dense canopies of fucoid species, while more exposed areas are represented by a mosaic of mussels, barnacles and limpets. The experiment carried out by Hawkins and Harkin (1985), is a good example of the variation in community structure caused by different levels of wave action. The flourish of *Alaria esculenta* at wave exposed areas at an height of the shore dominated in sheltered conditions by *Laminaria digitata*, illustrates how wave exposure can promote alterations in the community through selection of species better adapted to mechanical disturbance. As with the vertical gradient, not only physical factors are significant, biological processes, like competition and predation explored in the next section, can also determine species horizontal distribution (Jones 1948; Hawkins 1981a; Hawkins *et al.* 1992).

The measurement of wave action levels on intertidal shores has been made through different approaches. Biological indices for the categorization of wave exposure have been used in Europe since the study of Ballantine in 1961. Although practical in the field, biological indices cannot be applied to predict the community composition of shores not yet studied and more crucially have the problem of circularity of reasoning. When the number of sites to study is not large, an alternative method is the deployment of wave force dynamometers on the shore that allow the accurate measurement of the maximum force and maximum water velocity imposed at the place of deployment (Denny 1983; Bell & Denny 1994). Another alternative method is the use of topographical indices (Baardseth 1970; Thomas 1986). This methodology calculates the effective fetch from actual fetch modified by shoreline and offshore extents of shallow water. Crucially, a new approach by Burrows *et al*.

(2008) that uses a combination of geographical information systems, topographical indices and wind energy data has been recently developed. This method allows the rapid calculation of wave exposure indices across large sections of coastline, being the most appropriate to be used in large-scale comparisons studies and as a forecast tool for assessing wave exposure levels of unvisited coastlines.

1.2.3 Biological interactions

Rocky shores are characterized by the formation of species patterns, achieved through a combination of abiotic and biotic processes (Raffaelli & Hawkins 1996; Bertness & Leonard 1997; Harley 2003). The biological intertidal community is affected by multiple physical factors and by a large variety of biological interactions that contribute to the final patterns observed on each shore (Menge *et al.* 1999; Jenkins *et al.* 2008). The strength of different processes is not always similar and varies over different spatial and temporal scales (Thompson *et al.* 1996; Aberg & Pavia 1997; Underwood 1999). Several biological parameters can influence patterns of organisms on a rocky shore, however the most important for macroalgal species are the ones affecting their settlement and recruitment levels. Herbivore pressure (Jenkins *et al.* 2001; Coleman *et al.* 2006) and competition for space (Hawkins & Hartnoll 1985; Johnson *et al.* 1998b) are main factors restricting macroalgae survival. Although biological interactions affect macroalgae abundance, their presence on the shore is also extremely important for other species of the rocky shore community, as they confer vertical structure to a normally two dimensional environment, becoming especially important for facilitation processes (Thompson *et al.* 1996; Bertness & Leonard 1997).

1.2.3.1 Herbivory

The remarkable effect of grazing pressure on macroalgal settlement and recruitment can explain, to some extent, the variation in algal distribution and abundance over vertical, horizontal and latitudinal gradients (Jones 1946; Hawkins 1981a, b; Lubchenco & Gaines 1981; Sousa *et al.* 1981; Hawkins 1983; Johnson *et al.* 1997; Johnson *et al.* 1998a; Jenkins *et al.* 2001; Boaventura *et al.* 2002a; Jenkins *et al.* 2005; Coleman *et al.* 2006).

The impact of grazing pressure can be responsible for the delimitation of algal vertical limits. Experiments excluding patellid limpets performed on moderate wave exposed areas from south and north of Europe help to confirm the concept (Boaventura *et al.* 2002a). Boaventura *et al.* (2002a) showed that after limpet exclusion, turf species could extend their previously set upper limit, proving that herbivory pressure controls algal turf upper vertical limits of distribution on both locations.

Experimental work testing the effect of grazing pressure across shores with different exposures, have too, showed how grazing can influence and control horizontal distributional limits of intertidal algae (Hawkins & Hartnoll 1985; Cervin *et al.* 2005). Patellid limpets are the dominant grazers in north west Europe and have an important role in the control of macroalgal growth on the mid shore at exposed areas over a latitudinal gradient, though their influence decreases on the low shore and at sheltered areas (Jenkins *et al.* 1999a, b; Jenkins *et al.* 2008). These epilithic microphagous grazers can directly control the distribution and abundance of macroalgae by grazing on post-settlement stages (Hawkins 1981a; Lubchenco & Gaines 1981; Jenkins *et al.* 2001; Boaventura *et al.* 2002b) or on mature holdfasts (Hawkins *et al.* 1989; Davies *et al.* 2007). Several experiments, performed in the mid shore at northern moderately exposed areas, areas normally dominated by barnacles and mussels, showed that after removal of patellid limpets those areas become completely covered by macroalgae, which proves the influence of herbivory on the spatial distribution of macroalgae at this latitude (Hawkins 1981a; Jenkins *et al.* 2005; Coleman *et al.* 2006). Coleman *et al.* (2006) also demonstrated that grazing in northern areas could reduce spatial variability of algae. Hence, grazing pressure in northern European rocky shores not only controls macroalgal abundance but also is responsible for its variability, acting as a regulating force for macroalgae diversity. Despite such results, a not so linear outcome occurs in southern European regions. It has been proposed, that with decreasing latitude an increase in grazing pressure could be observed (Ballantine 1961; Hawkins & Jones 1992; Jenkins *et al.* 2001; Jenkins *et al.* 2008). However, in southern locations, probably due to differences in the recruitment of algae and harsher environmental stress, grazing has more unpredictable and unexplained effects (Benedetti-Cecchi *et al.* 2001; Jenkins *et al.* 2005; Coleman *et al.* 2006). Over the European latitudinal gradient the identity of the dominant grazer species also changes (Crisp & Southward 1958; Hawkins & Hartnoll 1983; Boaventura *et al.* 2002b; Jenkins *et al.* 2008). The boreal-cold temperate *Patella vulgata* is the dominant grazer at northern latitudes but its density and importance decreases towards southern latitudes where *Patella depressa* becomes the dominant species (Boaventura *et al.* 2002a; Boaventura *et al.* 2002b).

Herbivore pressure is the result of a combination of intraspecific and interspecific factors. The density of herbivores is significantly correlated with grazing pressure (Jenkins *et al.* 2001; Jenkins & Hartnoll 2001). However, species identity also has a strong impact on algal community composition. The importance of a single species on the ecosystem equilibrium can be enormous. In the Irish intertidal, *Patella ulyssiponensis* has such a significant role upon their ecosystem that its removal leads to a complete alteration of the habitat. In their absence the habitat becomes completely covered by macroalgae, regardless of the presence and abundance of other grazer species (O'Connor & Crowe 2005). In this experiment after a short period, other grazers, *Littorina littorea* and *Gibbula umbilicalis*, seem to gradually compensate and dilute the expected effect created by the *Patella ulyssiponensis* eradication (O'Connor & Crowe 2005), creating a response representative of the redundancy model (Gitay *et al.* 1996) and the biological insurance theory (Yachi & Loreau 1999). However, after approximately one year, compensation disappeared and an increase in macroalgal cover took place. These sequences of events demonstrate how vital is the contribution of a single species on the regulation of patterns in a rocky shore (O'Connor & Crowe 2005). Not only species identity and density are important in determining levels of alga diversity and biomass. The diversity of grazer species can also be correlated with alterations in the efficiency of herbivory and contribute to the control of the algal community. A study developed by Raberg and Kautsky (2007), aimed to clarify the role of nutrient availability and grazer pressure in algal epiphyte diversity, indicated that monocultures of grazers only partially controlled epiphytes growth while multispecies cultures were able to control epiphyte growth. These results show the importance of diversity of grazers for the structure of ecosystems (Raberg & Kautsky 2007).

The strength of herbivory will always be influenced by the probability of an encounter between a plant and a consumer and by the damage caused to the plant, which consequently can modify the plant fitness (Lubchenco & Gaines 1981; Johnson *et al.* 1998a). In addition to the limitations named above, various other factors can cause minimization of grazing damage through avoidance or coexistence mechanisms, such as: occupation of adverse or unavailable areas to grazers; alteration of spatial and temporal distributions; chemical defence; association with unpalatable, territorial or predator animals; rapid grow rates. All these factors plus herbivores densities, diversity and identity are accountable for the maintenance or alteration of patterns in the rocky shore intertidal zone (Hawkins 1981a; Lubchenco & Gaines 1981; Duffy & Hay 1990; Jenkins *et al.* 2001).

1.2.3.2 Settlement & Recruitment

Rocky shores are open systems where many organisms have a planktonic phase within their life cycle. The duration of the larval phase and range of larval dispersal are very variable, extending from hours to months and meters to hundreds of kilometres (Reed & Foster 1984; Santelices 1990). At the conclusion of the larval period, availability of suitable space for settlement in the intertidal becomes critical. The chosen location, with its physical and biological conditions, will regulate survival chances and how successfully organisms can complete their life cycle. This combination of settlement and recruitment success is a fundamental factor in the regulation of littoral communities (Johnson & Brawley 1998; Jenkins *et al.* 2000; Navarrete *et al.* 2008).

The mid-tide region of a moderately exposed British Atlantic shore is a good natural laboratory. The experiments made by Hartnoll and Hawkins (1985) described the importance of biotic factors in the balance of the mosaic composed of fucoids, barnacles, limpets and bare rock. To understand such a mosaic, it is essential to understand the aspects influencing macroalgal settlement and recruitment, as well as the influence of macroalgal canopy on the settlement and recruitment of organisms in the intertidal (Johnson *et al.* 1998a; Johnson & Hawkins 1998).

Several groups of organisms have a clear influence on macroalgal settlement and recruitment success. In areas of high barnacle cover the effectiveness of limpet grazing is reduced. Such a drop in grazing pressure greatly favours recruitment of fucoid plants, as the probability of escaping grazing increases (Hawkins 1981a, b; Hawkins & Hartnoll 1982; Hartnoll & Hawkins 1985; Jenkins *et al.* 1999a). Several studies over the last decades, as previously mentioned, reinforce the link between limpet grazing pressure and the probability of algal settlement and recruitment (Lubchenco & Gaines 1981; Jernakoff 1983; Petraitis 1987; Johnson *et al.* 1997; Cervin *et al.* 2005; Jenkins *et al.* 2005). The settlement and recruitment of algae clearly decreases with increasing grazing activity but can also be dependent on the effects of other algae. For example, the majority of mid shore turfing algae can be dependent on the existence of a fucoid cover (Jenkins *et al.* 1999a). The amelioration of stress provided by the canopy is important for many turfing species, which in turn can cause competition for settlement space with the facilitators (Worm & Chapman 1996). Adding to these biological interactions, several other factors can control or influence algal settlement and recruitment. The levels of propagules arriving on the shore are essential, as shown by Vadas's *et al.* (1992) study, where high levels of propagules of ephemeral algae were reported to swamp herbivores and therefore influence recruitment success. The germination and spore viability are also important factors. Norton's (1983) study confirmed such a notion by showing a decrease in the capacity of *Sargassum* spp. germlings to produce mucilage, that is essential to the attachment after a couple of weeks in the water column. Other characteristics, like the type of substratum and physical factors are also influential for their settlement and recruitment levels (Vadas *et al.* 1990; Vadas *et al.* 1992).

Patterns of algal recruitment are clearly important in determining the dynamics of mid shore intertidal areas. In addition, macroalgae can have important effects on the settlement and recruitment of other organisms. In the north Atlantic, the abundance and recruitment of barnacles changes within the wave-exposure gradient, being higher in exposed and lower in sheltered areas (Jenkins & Hawkins 2003; Burrows *et al.* 2010). This observation prompted some researchers (Bertness *et al.* 1992) to suggest that barnacle larvae supply would follow this pattern of abundance and occur at low concentrations in sheltered shores. However, studies by Jenkins and Hawkins (2003) showed that the concentration of *Semibalanus balanoides* cyprid larvae could be high in canopy free sheltered areas. Numerous others studies illustrate the negative effect that sweeping by the algae fronds has in the settlement of barnacles (Grant 1977; Hawkins 1983; Jenkins *et al.* 1999c). Those studies show that *Fucus spiralis*, *F. vesiculosus*, *Ascophyllum nodosum* and *F. serratus* are species that can negatively influence barnacle recruitment through sweeping, as physical abrasion of the thallus on the surface causes increased mortality of both cyprid and spat. Other factors like predation by the gastropod *Nucella lapillus* should also be taken into consideration, especially in mid-exposed areas (Connell 1961a, b; Menge 1976; Leonard 2000). In exposed conditions, it is predation together with interspecific competition for space and physical factors that regulate settlement and recruitment levels of barnacles (Connell 1961a, b; Burrows & Hughes 1989).

All these biological and physical processes uncovered above, are part of complex interactions between fucoid plants, barnacles, grazers and physical stresses that contribute to the natural spatial variability observed on the rocky intertidal zone.

1.2.3.3 Habitat Facilitation

Facilitative interactions are relationships between organisms where one or more individuals experience a net benefit and none is negatively affected (Bertness & Callaway 1994; Bronstein 1994). This concept can be specified into mutualism, if all the organisms benefit from the interaction, or commensalism, if one organism benefits but the other is not affected (Bruno *et al.* 2003). Many early scientific studies (Dayton 1971) show important demonstrations of facilitation interactions, although these interactions were not described as such until recently. Work of Bertness and co-workers has highlighted the role of facilitation as a structuring process in intertidal communities (Bertness & Callaway 1994; Bruno & Bertness 2001; Bruno *et al.* 2003). The beneficial effects of facilitation are realized in various ways, including aspects of amelioration of environmental stress (Jenkins *et al.* 1999a; Leonard 2000), improved recruitment levels (Eckman *et al.* 1989) and increased growth rates (Irlandi & Peterson 1991).

Facilitation can occur directly or indirectly. Under direct facilitation the simple presence of a species is sufficient to change the local environment and improve neighbouring conditions for other organisms, while in indirect facilitation it is the interaction of two other species that will improve the local environment of a third species (Wootton 1993). Evidence of direct facilitation is easier to recognize and was acknowledged by several studies (Dayton 1971; Dayton 1975; Bertness *et al.* 1999; Jenkins *et al.* 1999a; Moore *et al.* 2007a). A good example of direct facilitation comes from the assemblage of fucoid canopy and limpets in the northwest Atlantic (Jenkins *et al.* 1999a; Moore *et al.* 2007a). Adult fucoid plants have a positive effect on one of the species present, *Patella vulgata*, improving both adult and juvenile survival (Hawkins & Hartnoll 1983). Therefore, this can be seen as direct facilitation as the ecosystem engineers, in this case fucoid plants, are directly ameliorating the physical stress and consequently improving the survival of young and adult *Patella vulgata* (Jones 1948; Hawkins & Hartnoll 1985; Bertness *et al.* 1999; Moore *et al.* 2007a).

The concepts of foundation species (Dayton 1972; Bruno & Bertness 2001) and ecosystem engineers (Jones *et al.* 1997) are closely related with facilitation processes. Such definitions are used to name species that stabilize environmental conditions, increase habitat complexity, and generally create habitat by their mere presence. Foundation species and more generally facilitation processes are directly associated with an increase in habitable space, expanding the amount of areas that meet species niche requirement (Bertness *et al.* 1999). As expected, stress amelioration increases population densities at medium to high levels of stress. The impact that some facilitators (e.g. fucoids, mussels, barnacles or corals) can have in their community makes them extremely important as their removal or exclusion can alter species composition and affect entire community dynamics (Bertness 1989; Bertness *et al.* 1999; Jenkins *et al.* 1999a; Menge 2000a; Bruno & Bertness 2001; Bruno *et al.* 2003). So, the impact of facilitative interactions should not be disregarded and changes in the balance between filter feeder and fucoid community domination must be taken into account as changes in one of this functional groups can lead to change in community structure.

1.3 LARGE SCALE FACTORS AND LATITUDINAL GRADIENTS

On a larger geographical scale, climate variation and the effects of upwelling regimes and ocean currents induce latitudinal dissimilarity on rocky shores (Menge *et al.* 1997a; Menge 2000b; Broitman *et al.* 2001; Connolly *et al.* 2001; Thompson *et al.* 2002). Upwelling areas and ocean currents influence the abundance and spatial distribution of many marine species over a latitudinal gradient. Several examples from North American studies show that bottom-up processes can regulate benthic communities through their influence on filter feeder growth rates and abundance. Food availability for larvae, recruits and adults, produce individuals with better fitness that are more resistant to physical stress, which led to higher levels of space occupation (Menge *et al.* 1997a; Menge *et al.* 1997b; Broitman *et al.* 2001; Connolly *et al.* 2001; Blanchette *et al.* 2006).

Variation in latitude is also normally accompanied by an alteration in the annual mean sea surface temperature. This physical factor can be highly important for determining the abundance of large macroalgae (Peters & Breeman 1993; Broitman *et al.* 2001; Rivadeneira & Fernandez 2005; Hawkins *et al.* 2009). Previous physiological studies confirmed that low temperatures, present at higher latitudes, could positively influence growth, reproduction and algae propagule survival (Lüning & Neushul 1978; Peters & Breeman 1993), creating conditions for the occurrence of areas with high algae abundance (Ballantine 1961; Broitman *et al.* 2001).

In the European northeast Atlantic intertidal coast, annual mean sea surface temperature can vary up to 9 ºC from northern Scottish areas to the southern Portuguese coast. It has been proposed, based on some descriptive and qualitative local studies, that a change in the balance between fucoid algae and sessile invertebrate cover on wave action gradients from northern to southern Europe could match the changes in temperature across latitude (Ballantine 1961; Hawkins *et al.* 1992). Species richness and the extension of fucoid cover are expected to progressively reduce from shores on the north of the United Kingdom (cooler waters) to the Portuguese coast (warmer waters). The effect of wave exposure has also been proposed to vary across latitude. Fucoid cover, which is normally present at UK sheltered and mid-exposed shores, is expected to become restricted to sheltered areas in Portugal where temperature is higher (Ballantine 1961; Hawkins *et al.* 1992). The anticipated change in the balance between filter feeders and fucoid abundance is expect to be promoted by possible increase in grazing pressure and by differences in climatic conditions that can influence early survival, growth and persistence of algae in southern Europe (Ballantine 1961; Hawkins *et al.*

1992; Jenkins *et al.* 2001; Coleman *et al.* 2006). Despite such hypotheses, a clear quantification of patterns along the European coastline is still required in order to clarify the patterns and processes along this latitudinal range.

1.4 CLIMATE CHANGE AND ITS IMPACTS

Intertidal regions are open ecosystems under substantial environmental stress where organisms live under high pressure from abiotic and biotic conditions. So, increased demands derived from climate change or anthropogenic sources can be detected through alteration of the “normal” community pattern. Large scale quantitative surveys and long-term studies that provide a good knowledge of natural community variation are essential to offer a good base for the detection of anthropogenic or climate change alterations in intertidal communities (Thompson *et al.* 2002; Helmuth *et al.* 2006b; Jenkins *et al.* 2008).

Forecasts of climate change follow what was been happening over the last few decades. Warming of the planet has accelerated and changes in the northeast Atlantic community have occurred at a fast pace (Jones *et al.* 2001; Jones & Mann 2004; Hansen *et al.* 2006; IPCC 2007). Changes in the abundance and range limits of some European intertidal species over the last decades have followed the climatic fluctuations in the region (Southward *et al.* 1995; Mieszkowska *et al.* 2005; Mieszkowska *et al.* 2006; Hawkins *et al.* 2008; Hawkins *et al.* 2009). During periods of warming, local abundance and biogeographical range of southern species have been show to increase while northern species have decreased. The opposite was also shown during cooling periods (Southward *et al.* 1995; Hawkins *et al.* 2003; Southward *et al.* 2005). Macroalgal species have been some of the organisms under scrutiny. Studies made on the Portuguese coast showed changes in assemblage composition and distribution of macroalgae. From data comparison between recent and past surveys, a clear northward range expansion of warm-water species was confirmed. Interestingly, the distribution of cold-water macroalgal species did not shrink (Lima *et al.* 2007). These alterations of biogeographical ranges are causing changes in community structure. A long-term survey on the abundance levels of two barnacle species, revealed that over the last years, following the warming of sea surface temperatures, southern species increased their abundance whilst northern species decreased (Southward 1991; Southward *et al.* 1995; Hawkins *et al.* 2003; Poloczanska *et al.* 2008). Curiously, as in the macroalgal study mentioned above, the northern barnacle species did not disappear from the studied area, in this case English southwest coast, being still fairly common despite the detection of some recruitment failure (Hawkins *et al.* 2003; Svensson *et al.* 2005). Changes of such magnitude can clearly alter the strength of trophic interactions and might disturb patterns across large spatial areas with possible repercussions for energy flow between shores and coastal areas.

1.5 GENERAL AIMS

Generally regarded as possessing an oceanic climate, the European rocky coastline spreads over several degrees of latitude and encompasses a great topographic variety, from linear coastlines to enclosed bays. Changeable physical and topographical conditions lead to areas ranging from protected to wave exposed and consequently to an incredible variety of biological communities (Ballantine 1961; Dayton 1971; Jenkins *et al.* 2008). The biological communities that exist nowadays are a consequence of the evolution of physical and biological processes that control the species pool (Jenkins *et al.* 2008), as well as a response to anthropogenic impacts, such as the introduction of non-native species and harvesting/collecting pressures (Carlton & Geller 1993; Hawkins *et al.* 2000; Thompson *et al.* 2002). European rocky shore communities show patterns of distribution at different scales and their quantification and understanding was the central focus of my thesis.

The present study can be divided into two distinct sections. The first large section (Chapter 2), addresses the need to quantitatively describe the intertidal community on vertical and horizontal patterns across a large spatial scale, the European latitudinal gradient. The main goal was to determine changes in the balance between fucoid algae and sessile invertebrate cover on wave action gradients over a European scale (Scotland, northwest Wales, southwest England and north to central Portugal) through targeted field surveys. By accounting for variability across different wave exposure and vertical shore heights, the survey design allowed separation of local scale factors from large scale effects. Distribution patterns for distinct functional groups were assessed giving the first quantitative global picture of community variability across the European coastline.

After determining the patterns, exploration of the processes regulating fucoid recruitment and survival over the European scale of study, and hence the balance between fucoids and sessile invertebrates, was studied by undertaking factorial field and laboratory experiments. In this second section, three experiments were undertaken to ascertain the causal factors leading to the observed gradient in algal-animal balance over the intertidal European scale. Factorial experiments were implemented in mid (Wales) and range edge (Portugal) locations. In the first experiment, manipulations of the physical environment (shade and moisture) and grazing pressure in field conditions were made (Chapter 3). This contributed to clarify the strength and impact of varying physical and biotic conditions upon fucoid recruitment success levels across distinct regions.

In chapter 4, a second factorial experiment was developed in laboratory conditions to test the physiological performance of algae from northern and southern populations. Specimens from both locations where subjected to different levels of physical conditions, variable temperature and shading levels, while macroalgae physiological stress levels were determined.

In order to try to clarify some of the results from these two previous experiments, the final investigation assessed the reproductive capacity of natural populations (Chapter 5). Different morphological and reproductive parameters were assessed to understand the extent to which observed patterns are a consequence of adult fucoid characteristics.

The general discussion (Chapter 6) considers the ability to quantify the patterns and understand some of the processes leading to changes in intertidal community structure across a large latitudinal scale. The topics here presented were addressed with the expectation of providing crucial data for detecting and understanding changes in biomass patterns resulting from future climatic or anthropogenic stresses.

CHAPTER 2

BIOGEOGRAPHICAL PATTERNS OF INTERTIDAL ORGANISMS OVER A EUROPEAN LATITUDINAL GRADIENT

2.1 INTRODUCTION

Physical and biological processes, past and present, set the biogeographic patterns observed nowadays within and across ecosystems (Menge 2000b; Underwood *et al.* 2000; IPCC 2007; Broitman *et al.* 2008; Hawkins *et al.* 2008; Jenkins *et al.* 2008). Patterns of intertidal species distribution, at several scales, have long been the central focus of several ecological studies (Crisp & Southward 1958; Ballantine 1961; Lewis 1964; Southward *et al.* 1995). Characterization of such patterns, in terms of distribution and abundance of species across scales of time and space, and understanding of the processes leading to the development of such patterns are major focal points in ecology, physiology and biogeography research. Early rocky intertidal studies were mainly descriptive and based on qualitative descriptions of whole assemblages (Colman 1933; Stephenson & Stephenson 1949; Southward 1958; Ballantine 1961; Lewis 1964). During this early period, several intertidal studies explored the processes that cause vertical distribution on rocky shores, mainly the role of abiotic factors. Factors like the length of exposure to air during emersion (Doty 1946; Schonbeck & Norton 1978) and its consequences to the levels of desiccation and temperature amplitude felt by organisms in intertidal conditions were explored (Evans 1948; Davies 1970; Sousa 1979). The importance of biological factors, such as predation, competition or facilitation processes, also gained importance after influential studies from Connell (1961b) and Paine (1966; 1974) were published. Over the years the importance of biotic processes, physiological stress and physical disturbance, as independent and interacting factors, have become part of the explanation of pattern formation across the rocky intertidal (Hawkins *et al.* 1992; Chapman 1995).

Rocky shores are normally defined by clear environmental gradients that act at different scales. The change from maritime to terrestrial conditions on the shore occurs across a scale of meters (vertical gradient), while the change in wave exposure levels (horizontal gradient), which is normally observed between headlands and sheltered bays, generally develops at a scale of hundred of meters and above (Jones & Demetropoulos 1968; Vadas *et al.* 1990; Raffaelli & Hawkins 1996). Superimposed on these gradients are numerous microhabitat differences operating at small spatial scales, for example, local variation in wave exposure based on the presence of boulders and reefs and variation in inclination and aspect of the shore, which affects drainage and provides refuges for predators influencing prey patterns (Johnson *et al.* 1998c).

While the consequences of such environmental gradients to the distribution of species and community structure are the focus of innumerable studies, the influence of much larger scales, across latitudes, on community structure is less clear. Over such biogeographic scales the influence of climate on distribution of species and the potential species interactions becomes a major issue. This is, however, a gentle environmental gradient intersecting and interacting with the sharper vertical and horizontal gradients at a particular locality. Over the last decades, several studies have documented latitudinal shifts in species distribution, expansions and contractions, directly linked with climatic driven changes (Southward *et al.* 1995; Hawkins *et al.* 2003; Parmesan & Yohe 2003; Sexton *et al.* 2009). In general, a tendency for a poleward movement in some low latitude species and a retreating and decrease in abundance of higher latitude species due to climate warming has been described. The majority of the studies have been developed in the northern hemisphere (Southward *et al.* 1995; Sagarin *et al.* 1999), but recent work has shown similar shifts in the southern hemisphere (Rivadeneira & Fernandez 2005; Johnson *et al.* 2011). Such movement of species range and abundance variations can lead to shifts in the composition and structure of natural assemblages that could affect ecosystem functioning (Southward *et al.* 1995; Sagarin *et al.* 1999; Harley *et al.* 2006; Helmuth *et al.* 2006b; Lima *et al.* 2007; Hawkins *et al.* 2009).

Intertidal rocky communities are optimal study areas for assessing the effect of climate change due to their extreme physical conditions and accessibility. Many of the organisms inhabiting the intertidal area live close to their thermal tolerance, which make them very sensitive to the impacts of climate change and a good indicator of its effects (Helmuth *et al.* 2006b). So, the development of surveys across large biogeographical regions that examine latitudinal patterns of diversity and abundance of species seems essential to obtain a good understanding of the effects of climate change across this ecosystem. As shown by Helmuth *et al.* (2002; 2006a), thermal stress does not vary consistently with latitude, so surveying at large scale is also essential to get a global picture of the effect of climate change upon populations from the edges and central areas of a species distribution and distinguish local from global effects.

European shores have been well described over space and time and provide an excellent system to test ideas of the influence of latitudinal and temporal shifts in climate on species and assemblages. The balance between fucoid abundance, filter feeder abundance and grazing pressure across vertical and horizontal gradients over the western European coastline has been the focus of several studies (Crisp & Southward 1958; Lewis 1964; Southward *et al.* 1995; Jenkins *et al.* 2005; Coleman *et al.* 2006). Ballantine (1961) described the distribution of the dominant rocky shore organisms and presented some important concepts on community composition modification across the vertical, horizontal and latitudinal gradients.

Across the vertical gradient on the shore, upper levels are generally considered as the most severe mainly because they are exposed to longer emersion periods and more variable, unpredictable and extreme physical conditions (Stephenson & Stephenson 1949; Denny & Wethey 2001). Organisms surviving on these vertical shore levels, like the western European intertidal fucoid species, *Fucus spiralis* and *Pelvetia canaliculata*, normally show higher tolerance to the effects of desiccation (Colman 1933; Stephenson & Stephenson 1949; Schonbeck & Norton 1978; Lubchenco 1980; Hawkins & Hartnoll 1985).

Disturbance in the intertidal zone is not only related to physiological stress due to desiccation. Mechanical forces, caused by waves, can also have negative impacts through dislodgment, biomass reduction or decline of feeding efficiency, leading to the formation of horizontal gradients (Denny 1985; Davenport & Davenport 2005; Jonsson *et al.* 2006; Scrosati & Heaven 2008; Christofoletti *et al.* 2011). The strength and impact of waves is affected by wave height and length, which are a consequence of local and distant weather systems, coastline topography and coastal bathymetry (Denny 1988, 2006). Waves create hydrodynamic forces, such as drag, lift and acceleration that affect the fitness and survival of the littoral community (Carrington 1990; Wolcott 2007). Across the coastline, headlands are areas of considerable wave refraction where the energy converges, whereas embayed areas are regions of divergence of wave energy. The risk of dislodgment and loss of biomass increases with wave exposure, leading to selection of the intertidal organisms that are able to occupy those areas (Hartnoll & Hawkins 1985; Trussell *et al.* 1993; Jenkins & Hawkins 2003; Jonsson *et al.* 2006). Organisms that allocate resources and develop specific characteristics to improve their adhesion to the substratum have better chances of success in wave-exposed intertidal shores. Experimental work developed on north Atlantic shores, demonstrated that fucoid abundance declines with wave exposure while an increase in filter feeders abundance is observed (Lewis 1964; Dayton 1971; Hawkins 1983; Vadas *et al.* 1990; Hawkins & Jones 1992). These works also showed that grazing pressure reduces fucoid abundance on more exposed shores (Jones 1948; Southward & Southward 1978; Hawkins 1981a, 1983; Jenkins *et al.* 2001; Jenkins *et al.* 2005; Coleman *et al.* 2006). Jonsson *et al.* (2006) showed that grazing prevented establishment of fucoids on more exposed shores, but wave action led to lower persistence as plants grew and got dislodged. So, the patterns are reflecting specific characteristics of species and their strategies and capacity to overcome physiological and mechanical stresses correlated with increased wave action on the intertidal area. Although these vertical and horizontal gradients have been described, their strength and consistency of effect across different latitudes is still uncertain.

The clear effect of top-down control by consumers and the effect of bottom-up processes, where increased primary production leads to greater abundance or diversity of grazers, have been illustrated across rocky intertidal communities around the word at large spatial scales (Menge 2000b; Underwood 2000). Parallel to these, the importance of supply of recruits has also been described as the one of the main structuring forces acting across scales from cms to 1000’s of kms (Connolly & Roughgarden 1998). Latitudinal changes in assemblages and species are known worldwide (e.g. Europe: Mieszkowska *et al.* (2005), Lima *et al.* (2007) and Hawkins *et al.* (2008); North America: Connolly & Roughgarden (1998) and Blanchette *et al.* (2008); South America: Broitman *et al.* (2001) and Rivadeneira & Fernandez (2005); New

Zealand: Menge *et al.* (1999; 2003). Superimposed on these latitudinal patterns are recent shifts in distribution prompted by rapid climate change (Hawkins *et al.* 2008). Over such large geographical scales, climate variation and the effects of upwelling regimes and ocean currents can all induce differences in community structure and species abundance levels (Menge *et al.* 1997b; Menge 2000b; Thompson *et al.* 2002).

Based on some descriptive and quantitative local studies, a change in the balance between fucoid algae and sessile invertebrate cover on wave action gradients over a European scale has been proposed (Ballantine 1961; Hawkins *et al.* 1992). For the western European coastline, large scale quantitative surveys from northern to southern European areas, which would allow confirmation of qualitative patterns based on single site surveys at widely separated locations (Ballantine 1961), are absent from the literature. Over this latitudinal gradient a reduction in fucoid levels and a combined increase in sessile invertebrate cover from northern to southern European regions are expected. Confirmation of such a model is important, as quantitative information of biogeographic patterns of intertidal and subtidal species are essential tools in the effort of biodiversity conservation. With growing consensus on the need for protected marine areas for conservation of marine biodiversity, the fundamental knowledge of species distribution and abundance over scales of space and time is indispensable for an informed management of coastal areas. Having a quantitative data set over a large scale is crucial for understanding processes (Underwood *et al.* 2000) and can also be indispensable for long-term studies that provide a good knowledge of natural community variation and offer a good base for the detection of anthropogenic or climate change alterations in these communities (Thompson *et al.* 2002; Helmuth *et al.* 2006b; Hawkins *et al.* 2008).

The present work describes and quantifies the biogeographic patterns of major intertidal functional groups, while accounting for variability across different shore heights (vertical gradient) and levels of wave exposure (horizontal gradient) across the European coast (latitudinal gradient). The main aim was to acquire quantitative data of major intertidal functional groups in order to ultimately be able to determine changes in the balance between fucoid algae and sessile invertebrate cover over wave action gradients at a European scale and relate these with levels of grazing pressure on the shore.

In order to construct this dataset, intertidal transects from Scottish, Welsh, English and Portuguese shores separated by a maximum of 20º of latitude were surveyed to formally test different hypotheses of ecological interest. During the survey, wave exposure was determined for each shore surveyed in order to test the hypothesis that community patterns are maintained over wave exposure and vertical gradients across latitudes. Along the horizontal gradient of exposure it is expected that across latitudes, sheltered areas will always show the highest total biomass of fucoid species, with biomass declining with wave exposure. An inverse gradient of biomass is expected for filter feeder and grazer organisms. In terms of shore height a maximum fucoid biomass is expected to occur at mid shore, declining at high and low shore levels. Based on previous work, it is hypothesised that total fucoid biomass will decline with decreasing latitude but the abundance of filter feeders and the dominant grazers *Patella* spp. will increase.

2.2 METHODS

2.2.1 Where and when

The present large-scale geographic study encompassed four geographical regions, west and northern Scotland, North Wales, southwest England and central and northern Portugal. A total of 120 shores distributed over 19° of latitude were sampled with the aim of quantifying variation in species distribution and abundance along the European rocky intertidal zone from colder to warmer latitudes.

The survey was made between May and October over two consecutive years, 2008 and 2009. Differences encountered among sites were assumed to reflect spatial variation and not temporal variability. This was based on the similar levels of abundance collected over the two years. In addition Burrows *et al.* (2009), resurveyed shores on the west coast of Scotland twice with a three year interval and found high correlation between new and prior abundance scores. Therefore, spatial patterns were considered sufficiently consistent across the two years.

All the chosen shores had fully saline environments with maximal tidal range varying from 6.1 m in North Wales to 3.7m in Portugal (4.8 m and 5.7 m for Scotland and southwest of England, respectively). Within each shore, transects were chosen at random in areas of freely draining rock with a slope of less than 45°. Such criteria allow standardization across locations and guarantee an accurate measurement of all the response variables while microhabitat variation was stratified as much as possible.

Such a large sampling effort implied some logistical constraints and compromise. The focus of the survey was on sampling the maximum number of shores using sufficient sampling effort to characterise the dominant biota. The geographical regions were chosen taking in consideration mean sea surface and air temperature (Figure 1). As a consequence, the survey was spread over the latitudinal scale chosen in an uneven manner but with a more even spread of mean annual sea surface and air temperatures (Mean sea surface temperature: 10° C Scotland, 12° C Wales, 13° C England & 16° C Portugal; Mean air temperature: 9° C Scotland, 10° C Wales, 12° C England & 15° C Portugal). In addition to this data, previous observations over the European latitudinal scale, showing the complex spatial pattern of sea temperature, were also taken into account. Northern areas of Spain and Portugal were not used due to the influence of particular hydrographical features, upwelling and rainfall (Botas *et al.* 1990; Lemos & Pires 2004; Peliz *et al.* 2005; Lima *et al.* 2006). These features were shown to influence sea temperature in these regions (Figure 1) and led to the reappearance of some northern cold water species (Southward *et al.* 1995; Hawkins *et al.* 2009). In contrast, the Bay of Biscay present a high sea surface temperature, similar to the ones observed in the southern Portugal (Crisp & Ficher-Piette 1959; Sanchez & Relvas 2003; Lima *et al.* 2007) and for that reason it was also not included in the survey (Figure 1).

Within each of the geographical regions included in the study, 10 shores were surveyed at each of 3 levels of wave exposure. Thus, 30 shores were surveyed within each geographical region. By formally incorporating in the analyses the response to different shore heights and wave action levels, the survey allowed the detection of alterations in species distribution patterns and community structure caused only by changes in latitude. To achieve this, it was essential to independently and rigorously assess wave exposure levels at each of the 120 shores surveyed.

A wave fetch model, created by Burrows *et al*. (2008), was used to independently determine wave exposure indices for each of the shores. This model determines wave fetch as the distance to the nearest land cell in 16 angular sectors up to a distance of 200 km. Wind energy information, was not used according to the recommendations of Burrows *et al*. (2008). In order to calculate indices of wave exposure across the surveyed area, a high-resolution digital coastline dataset was acquired from freely available sources (Global Self-consistent, Hierarchical, High-resolution Shoreline (GSHHS) dataset available at www.ngdc.noaa.gov/mgg/shorelines/gshhs.html was used) and the wave fetch model downloaded from the Scottish Association for Marine Science website (https://www.sams.ac.uk/Members/MTB/wave-fetch-model).

Using the GIS software package ArcGIS9.3, the vector dataset was converted to a gridded dataset (grain size of 200m) using the British National Grid projection. This gridded dataset was then loaded into the wave fetch model created by Burrows *et al*. (2008). The program identifies sea and land cells, identifying as coastal cells those adjacent to the sea.

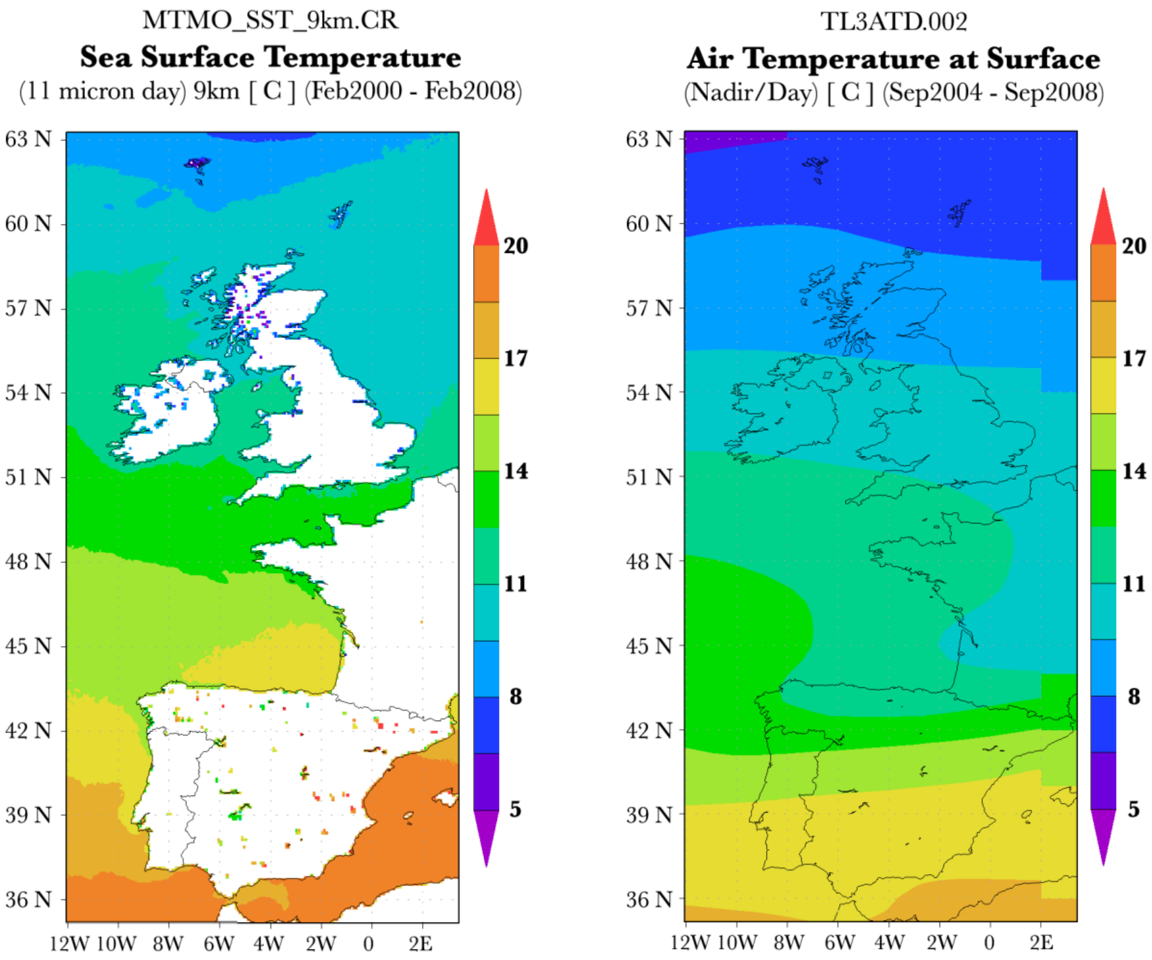


Figure 1: Mean sea surface temperature (from February 2000 to February 2008) and mean air temperature at surface (from September 2004 to September 2008) from northern Scotland to southern Portugal. Diagrams extracted and adapted from NASA (http://disc.sci.gsfc.nasa.gov/giovanni/overview/index.html) - TES & MODIS-Aqua missions respectively.

The next step involved the calculation of fetch for each coastal cell (grain size of 200m). The distance to the closest land cell in each of the 16 equal angular sectors (22.5º) surrounding each coastal cell was calculated. Then the average of wave fetch in km over all 16 sectors was calculated. Once the average wave fetch for each coastal cell was calculated, a file was exported from the wave fetch model, as an ArcGIS ASCII raster file, and further manipulation was carried out in ArcGIS9.3 for better display and manipulation of the exposure categories to be used. With the indices calculated, 3 levels of exposures were created: sheltered shores (wave fetch values between 0 and 1000), intermediate exposed shores (wave fetch values between 1350 and 2350) and exposed shores (wave fetch values between 2700 and 3700). These were created in a non continuous manner to strengthen the difference among shores within each category. In order to ensure that the area surveyed belonged to the expected category of wave exposure, locations were only chosen for survey when they had at least one neighbouring cell belonging to the same wave exposure category.

A greater number of shores within the determined geographical regions and matching the relevant criteria were identified than were required for survey. In order to choose survey sites other practical requirements had also to be taken into account. Factors like accessibility through a nearby road and proximity to other selectable locations were also considered, to allow wherever possible that more than one shore could be surveyed per tidal cycle. When all these requirements were met, the remaining locations were chosen randomly. To guarantee that chosen locations were precisely surveyed, a hand held GPS device (Garmin eTrex H), with a precision of 3m, was used in the field throughout the survey.

Within each of the shores, data were collected at low, mid and high vertical levels on the shore. Biological zones were used to determine vertical levels on the shore. The top, middle and lower area of the barnacle zone were utilized as guides for the vertical position on the shore. After a preliminar study where species area curves were produced and cumulative average densities and percentages of cover where quantified, 8 quadrats (0.5 x 0.5 m) were considered the number that would maximise efficiency in data acquisition while allowing replication to occur and good estimates of average density or percentage cover of common organisms to be obtained. The quadrats were randomly placed on the shore and would only be refused if the criteria previously described were not met. In these cases another random location for the quadrat would be assigned until all the criteria were met.

2.2.2 Species chosen

The focus of the survey was to assess changes in dominant rocky shore biota over latitudinal and local gradients. This large scale approach combined with a requirement to use digital photography as a sampling tool determined that only relatively common, macroscopic fauna and flora were sampled. Cryptic, rare and very small organisms were not sampled and as a consequence the approach does not lend itself to any formal assessment of changes in biodiversity but provided sound data for the abundance and distribution of functional groups. Instead, the main algal species, grazers and space occupying filter feeders were sampled, with the former groups contributing to the general aim of assessing the balance between consumers and their algal resource over latitudinal and wave exposure gradients.

2.2.3 Methodology & design

Digital photographs of 0.5 x 0.5 m quadrats were used for determining animal densities and obtain estimates of algae percentage cover. When multiple layers of cover were present, several photographs were taken before and after removal of the large canopy algae to quantify the canopy and understory communities. Photographic methods, rather than *in situ* identification, were used to allow rapid collection of data across numerous shores at four geographic locations. Photographs were taken using a standard 8 megapixel digital camera (Ricoh Caplio 500G wide) and covered the complete area of the quadrat surveyed. While taking the pictures care was taken to avoid direct sunlight reaching the area being surveyed in order to not over expose the images, which would invalidate or make their use difficult. In addition to photographic sampling, fucoid biomass was determined in the field using 8 haphazardly placed 0.5 x 0.5 m quadrats at each of the shore vertical levels surveyed. All the specimens that had their holdfast inside the quadrats were removed and separated by species to have their biomass weighed with spring balances accurate to the nearest 5 g.

Photographs were analysed in the laboratory. The treatment of all images, involving adjustments of brightness and contrast levels, was undertaken with Adobe Photoshop CS2 software. Before the acquisition of data, a grid of 25 subdivisions was overlaid over the quantification area of each image to help in assessing the total percentage cover of all functional groups. This method was chosen rather than a point intersect method because of its greater adaptability to a photographic survey (Dethier *et al.* 1993). In addition to identification, enumeration and quantification of percentage cover of species, digital photographs were also used to count and measure limpets, using ImageJ 1.38x software. These were later converted to biomass using a non-linear shell length-dry soft tissue mass regression derived from limpets from each location. In order to create such regressions, 50 limpets representing each of the wave exposure groups of shores from each of the areas were collected. The limpets were then blotted dry in the laboratory, their shell lengths were acquired and their hydrated soft tissue weighed before being placed in a drying oven at 60° C. After a 72 hour period a constant weight was obtained, the soft tissue dry weight, and the non linear regression was created.

The biomass, density and percentage of cover data for the functional groups acquired by the survey were analysed using a 4-factor mixed-model ANOVA. Significant results were explored in further detail with SNK (Student Newman Keuls) multiple comparisons. Only

SNK results showing differences are displayed in the results section. Cochran's test was used to test the data for heterogeneity of variance and transformations were done when appropriate. For some analyses homogeneity of variance was not achieved even following transformation. Given no realistic alternative exists for the complex model design, analyses were still undertaken. In such cases caution must be taken when analysing the results owing to increased probability of type I error.

As well as applying ANOVA to means, variability among variances was also examined. Different ANOVA designs were used for testing exclusively shore variability, as only data from the mid shore was used. While testing for the variability among shores, a one-way fixed ANOVA was used for testing the variability across different wave exposures or geographical regions separately. For testing variability within shores, a two way fixed ANOVA model was used for testing the combine effect of geographical region and wave exposure. All these analyses were performed in the statistical package WinGMAV5 (EICC, University of Sydney).

2.3 RESULTS

2.3.1 Fucoid distribution

2.3.1.1 Total Fucoid Biomass

Across the latitudinal range of the survey, four fucoid species were identified. The work was done before the recent separation of *Fucus guiryi* from *Fucus spiralis* (Zardi *et al.* 2011). This only applies at Portuguese shores where *F. spiralis* encompasses both taxa. At a regional scale, the pattern of total fucoid biomass shows, if local variability within each of the geographical regions is ignore, that Portugal has lower fucoid biomass levels than any of the three regions in the United Kingdom. Regions within the United Kingdom (Scotland, Wales and England) showed similar total fucoid biomass levels (Table 1: Total Fucoid - *SNK of Re x We x Vl*, Figure 2 and Figure 3).

Differences in total fucoid biomass between geographical regions varied according to wave action forces and vertical position on the shore (Table 1: Total Fucoid - $F_{(\mathrm{Re\ x\ We\ x\ Vl})\ 12,\ 2520} = 2.6$; $p<0.005$). Nevertheless, a consistent effect of wave exposure intensity on fucoid biomass production was observed across the four geographical regions. The pattern detected shows that sheltered shores always have higher fucoid biomass levels, with increased exposure to wave stress leading to reduce biomass production (Table 1: Total Fucoid - *SNK of*

Re x We x Vl). The effect of vertical position on the shore is less consistent across the four regions. However, some similarities were still captured by the survey. At all geographical regions, wave exposed shores showed similarly low levels of total fucoid biomass across all shore heights. On sheltered and intermediate exposed shores the highest shore level had the lowest fucoid biomass, within each of the exposures across all geographical regions (Table 1: Total Fucoid - *SNK of Re x We x Vl*). The most productive vertical level of the shore varied between the low and mid shore heights depending on the geographical region (Total Fucoid - *SNK of Re x We x Vl*). Considering only sheltered shores, in Scotland and England fucoid biomass was greater at the low shore, while in Portugal and Wales the mid shore level showed the greatest fucoid biomass levels (Figure 3), reflecting the identity and abundance of the species present at each region (Figure 2).

The comparison of fucoid biomass levels, from similar shore heights within similar wave action intensities across the four geographical regions, shows that Portuguese shores consistently had the lowest biomass levels (Table 1: Total Fucoid - *SNK of Re x We x Vl*, Figure 3). Very low levels of fucoid biomass, never exceeding mean values of 65 g/m^2, were also observed on wave exposed sites, independently of shore height and across all the geographical regions (Figure 3). So, differentiation in fucoid biomass across geographical regions was mainly due to differences observed at intermediately exposed and sheltered shores (Table 1: Total Fucoid - *SNK of Re x We x Vl*). At intermediate wave exposed conditions, Scottish shores were the most productive at low and mid shore heights, reaching values of 3119 and 2361 g/m^2 of fucoid biomass, respectively. On the high shore level, Scottish and Welsh shores are the geographical regions with greater fucoid standing stock levels (Table 1: Total Fucoid - *SNK of Re x We x Vl*). In contrast, on sheltered shores the geographical region presenting the highest level of fucoid biomass production varied with shore height (Table 1: Total Fucoid - *SNK of Re x We x Vl*). On the low shore level, the three geographical regions in the UK had similar levels of fucoid biomass. However, at mid shore height, Welsh shores were clearly the most productive, with a mean biomass of 10126 g/m^2, almost twice the mean biomass production of Scottish and English shores, 5237 g/m^2 and 6254 g/m^2, respectively. At the highest level of the shore, where desiccation is most intense, English and Welsh shores were the ones showing highest levels of fucoid biomass, 3192 g/m^2 and 3885 g/m^2, respectively (Figure 3).

The contribution of each species to the total fucoid biomass observed also varied across geographical regions (Figure 2). On Scottish and Portuguese shores *Fucus vesiculosus* dominates, while on Welsh and English shores the highest biomass level is achieved by *Ascophyllum nodosum* and *Fucus serratus*, respectively. A detail description of how the biomass of each of these species varies across latitudinal, horizontal and vertical gradients is given below.

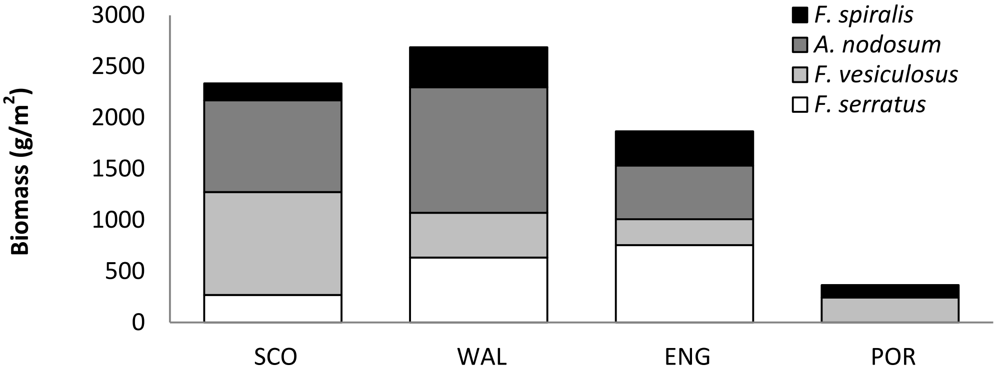


Figure 2: Biomass of *F. serratus*, *F. vesiculosus*, *A. nodosum* and *F. spiralis* in Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR).

2.3.1.2 *Fucus serratus* biomass

The amount of *F. serratus* biomass differed in each geographical region: it was absent from Portuguese shores and within the United Kingdom, Scotland showed the lowest biomass values (Figure 3).

An interaction of geographical regions, wave exposure intensity and vertical position on the shore explained *F. serratus* biomass levels (Table 1*: F. serratus - SNK of Re x We x Vl*). *F. serratus* was absent from Portuguese shores, but also from the highest vertical shore level of all geographical regions, also being rare on exposed and intermediate exposed shores across all geographical regions (Figure 3). The results confirm the patterns proposed for British shores by Ballantine (1961), with *F. serratus* occupying mainly the low shore level on sheltered shores and its abundance declining with an increase of wave exposure across all the geographical regions where it was detected. At the most productive area, the low shore in sheltered conditions, *F. serratus* biomass was highest on English shores, reaching mean values of 6116 g/m^2, significantly greater than 3617 g/m^2 and 1905 g/m^2 of Welsh and Scottish shores, respectively (Figure 3).

2.3.1.3 *Fucus vesiculosus* biomass

Analysis of variance showed four significant interactions that explained *F. vesiculosus* biomass: geographical region x wave exposure; geographical region x vertical level, wave exposure x vertical level and vertical level x shore (Table 1*: F. vesiculosus*).

The wave exposure x vertical level interaction indicates that generally a consistent effect of wave action is present. Exposed shores over these four geographical regions have very low biomass of *F. vesiculosus* across all shore heights with the bladeless form of *F. vesiculosus* var. *evesiculosus* being present. Biomass reached maximal values on sheltered shores, mainly due to values on low and mid shore heights. (Table 1*: F. vesiculosus - SNK of We x Vl*, Figure 3).

Variability in biomass of *F. vesiculosus* across the geographical regions was observed on intermediately exposed and sheltered shores (Table 1: - *SNK of Re x We*) and across low and mid shore heights (Table 1: - *SNK of Re x Vl*). On intermediately exposed and sheltered shores, *F. vesiculosus* biomass was higher on Scottish shores and lower on Portuguese shores v*: F. vesiculosus - SNK of Re x We,* Figure 3). When comparing low or mid shore levels across the four geographical regions, independently of wave exposure levels, ANOVA results indicate that, for both shore levels, Scottish shores were again the most productive, while Portuguese shores had the lowest mean values of *F. vesiculosus* biomass. Other geographical regions, central Welsh and English shores, showed intermediate biomass values (Table 1*: F. vesiculosus - SNK of Re x Li,* Figure 3). Apart from the variability already discussed, interactions between shore and shore vertical levels also occurred showing that other shore characteristics can also be important in controlling the development of *F. vesiculosus* in the intertidal (Table 1*: F. vesiculosus- SNK of Li x Sh (Re x We)*).

2.3.1.4 *Ascophllum nodosum* biomass

The presence of *A. nodusum* was generally restricted to sheltered conditions, with virtual absent from exposed conditions and very low biomass found on intermediately exposed shores (Table 2*: A. nodosum - SNK of Re x We x Vl,* Figure 4). On sheltered shores, however, *A. nodosum* could achieve high biomass levels, with a maximum mean biomass of 7915 g/m^2 occurring at the mid shore height of Welsh sheltered shores (Figure 4). In contrast, *A. nodosum* individuals were not found at any shore height on Portuguese shores. Variability across geographical regions was detected but only between sheltered shores, where *A. nodosum* was absence from Portugal and most abundant on Welsh shores (Figure 4). Post hoc SNK showed that among low shore heights of sheltered shores, Scottish and Welsh shores had a significantly higher standing stock than English and Portuguese shores, while at mid shore height Welsh shores showed the highest values, followed by English, Scottish and finally Portuguese shores (Table 2*: A. nodosum - SNK of Re x We x Vl*).

Although present in the UK, along the three geographical regions surveyed, *A. nodosum* is still absence from the high shore levels. At the most northern area, Scotland, low and mid shore heights were equally productive, while on Welsh and English shores the mid shore level was the most productive (Table 2*: A. nodosum - SNK of Re x We x Vl*).

2.3.1.5 *Fucus spiralis* biomass

The distribution of *F. spiralis* biomass, followed the expected pattern (Ballantine 1961) for the UK populations. At these populations, *F. spiralis* biomass is restricted to high shore levels and their abundance decreases with the increase in wave action (Table 2*: F. spiralis - SNK of Re x We x Vl*, Figure 4). On Portuguese shores the effect of wave action was still observed, so a greater standing stock continued to occur in sheltered shores. However, at this geographical region, the vertical level of the shore with greater biomass was the mid shore height instead of the highest shore level, which shows that *F. spiralis* is replacing *F. vesiculosus* in some coastal areas (Table 2*: F. spiralis - SNK of Re x We x Vl*, Figure 4).

Differences in biomass abundance between geographical regions were detected at the highest vertical level of sheltered shores. On such areas, English and Welsh shores presented significantly higher values than Scottish shores, while Portuguese shores were significantly the less productive (Table 2*: F. spiralis - SNK of Re x We x Vl*).

Table 1: Mix model ANOVA of biomass of total fucoids, *F. serratus* and *F. vesiculosus*. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual.*

Source	**DF**	**Fucoid Total**			***F. serratus***			***F. vesiculosus***			**F ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	3	5563.1	20.5	<0.001	2384.5	19.4	<0.001	1828.4	12.5	<0.001	Sh (Re x We)
We	2	29169.5	107.5	<0.001	7374.1	60.1	<0.001	2395.9	16.4	<0.001	Sh (Re x We)
Sh (Re x We)	108	271.4	40.5	<0.001	122.7	13.2	<0.001	145.9	25.6	<0.001	Res
Vl	2	2084.4	32.4	<0.001	6612.1	106.1	<0.001	1919.4	31.4	<0.001	Vl x Sh (Re x We)
Re x We	6	2223.5	8.2	<0.001	1059.2	8.6	<0.001	330.1	2.3	**0.043**	Sh (Re x We)
Re x Vl	6	487.5	7.6	<0.001	902.3	14.5	<0.001	285.8	4.7	**<0.001**	Vl x Sh (Re x We)
We x Vl	4	439.4	6.8	<0.001	2612.5	41.9	<0.001	358.8	5.9	**<0.001**	Vl x Sh (Re x We)
Vl x Sh (Re x We)	216	64.4	9.6	<0.001	62.3	6.7	<0.001	61.2	10.8	**<0.001**	Res
Re x We x Vl	12	165.7	2.6	**0.003**	371.3	6.0	**<0.001**	85.8	1.4	0.167	Vl x Sh (Re x We)
Res	2520	6.7			9.3			5.7			
Cochran's Test		*C = 0.0268, p > 0.05*			*C = 0.0312, p > 0.05*			*C = 0.0295, p > 0.05*			
SNK		*Re x We x Vl* *IntxLow - Por<Eng=Wal<Sco* *IntxMid - Por=Eng=Wal<Sco* *IntxHigh - Por<Wal, Eng<Wal* *ShexLow - Por<Wal=Sco=Eng* *ShexMid - Por<Sco=Eng<Wal* *ShexHigh - Por<Sco<Eng=Wal* *ScoxLow - Exp<Int<She* *ScoxMid - Exp<Int<She* *ScoxHigh - Exp=Int<She* *WalxLow - Exp<Int<She* *WalxMid - Exp<Int<She* *WalxHigh - Exp=Int<She* *EngxLow - Exp=Int<She* *EngxMid - Exp=Int<She* *EngxHigh - Exp=Int<She* *PorxMid - Exp<Int<She* *ScoxInt - High<Mid=Low* *ScoxShe - High<Mid<Low* *WalxShe - High<Low<Mid* *EngxShe - High<Mid=Low* *PorxShe - Low=High<Mid*			*Re x We x Vl* *IntxLow - Por<Sco=Eng=Wal* *ShexLow - Por<Sco<Wal<Eng* *ShexMid - Por=Sco<Wal=Eng* *ScoxLow - Exp<Int<She* *WalxLow - Exp<Int<She* *WalxMid - Exp=Int<She* *EngxLow - Exp<Int<She* *EngxMid - Exp=Int<She* *ScoxShe - High=Mid<Low* *WalxInt - High=Mid<Low* *WalxShe - High<Mid<Low* *EngxInt - High=Mid<Low* *EngxShe - High<Mid<Low* *Int - High<Low=Mid* *She - High<Low<Mid*			*Re x We* *Int - Por=Eng=Wal<Sco* *She - Por<Sco, Por<Wal, Eng<Sco* *Sco - Exp<Int=She* *Wal - Exp<She* *Re x Vl* *Low - Por<Eng=Wal<Sco* *Mid - Por<Eng=Wal<Sco* *Sco - High<Low=Mid* *Wal - High<Mid* *Eng - High<Low<Mid* *We x Vl* *Low - Exp<Int=She* *Mid - Exp<Int<She* *Int - High<Low=Mid* *She - High<Low<Mid*			

Table 2: Mix model ANOVA of biomass of *A. nodosum* and *F. spiralis*. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual.*

Source	**DF**	***A. nodosum***			***F. spiralis***			**F ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	3	2.0E+08	5.2	0.002	1.2E+07	2.3	0.0786	Sh (Re x We)
We	2	9.7E+08	25.3	<0.001	1.3E+08	24.5	<0.001	Sh (Re x We)
Sh (Re x We)	108	3.8E+07	8.9	<0.001	5.1E+06	19.6	<0.001	Res
Vl	2	3.7E+08	17.8	<0.001	1.3E+08	27.0	<0.001	Vl x Sh (Re x We)
Re x We	6	1.8E+08	4.6	<0.001	8.2E+06	1.6	0.155	Sh (Re x We)
Re x Vl	6	1.1E+08	5.2	<0.001	2.9E+07	6.0	<0.001	Vl x Sh (Re x We)
We x Vl	4	3.3E+08	15.8	<0.001	8.7E+07	18.0	<0.001	Vl x Sh (Re x We)
Vl x Sh (Re x We)	216	2.1E+07	4.8	<0.001	4.8E+06	18.5	<0.001	Res
Re x We x Vl	12	9.0E+07	4.4	**<0.001**	2.0E+07	4.1	**<0.001**	Vl x Sh (Re x We)
Res	2520	4.3E+06			1.2E+07			
Cochran's Test		*C = 0.0820, p < 0.01*			*C = 0.1116, p < 0.01*			
SNK		*Re x We x Vl* *ShexLow - Por=Eng<Wal=Sco* *ShexMid - Por<Sco<Eng<Wal* *ScoxLow - Exp=Int<She* *ScoxMid - Exp=Int<She* *WalxLow - Exp=Int<She* *WalxMid - Exp=Int<She* *EngxMid - Exp=Int<She* *ScoxShe - High<Mid=Low* *WalxShe - High<Low<Mid* *EngxShe - High=Low<Mid*			*Re x We x Vl* *ShexHigh - Por<Sco<Wal=Eng* *ScoxHigh - Exp=Int<She* *WalxHigh - Exp=Int<She* *EngxHigh - Exp=Int<She* *PorxMid - Exp=Int<She* *ScoxShe - Low=Mid<High* *WalxShe - Low=Mid<High* *EngxShe - Low=Mid<High* *PorxShe - Low=High<Mid*			

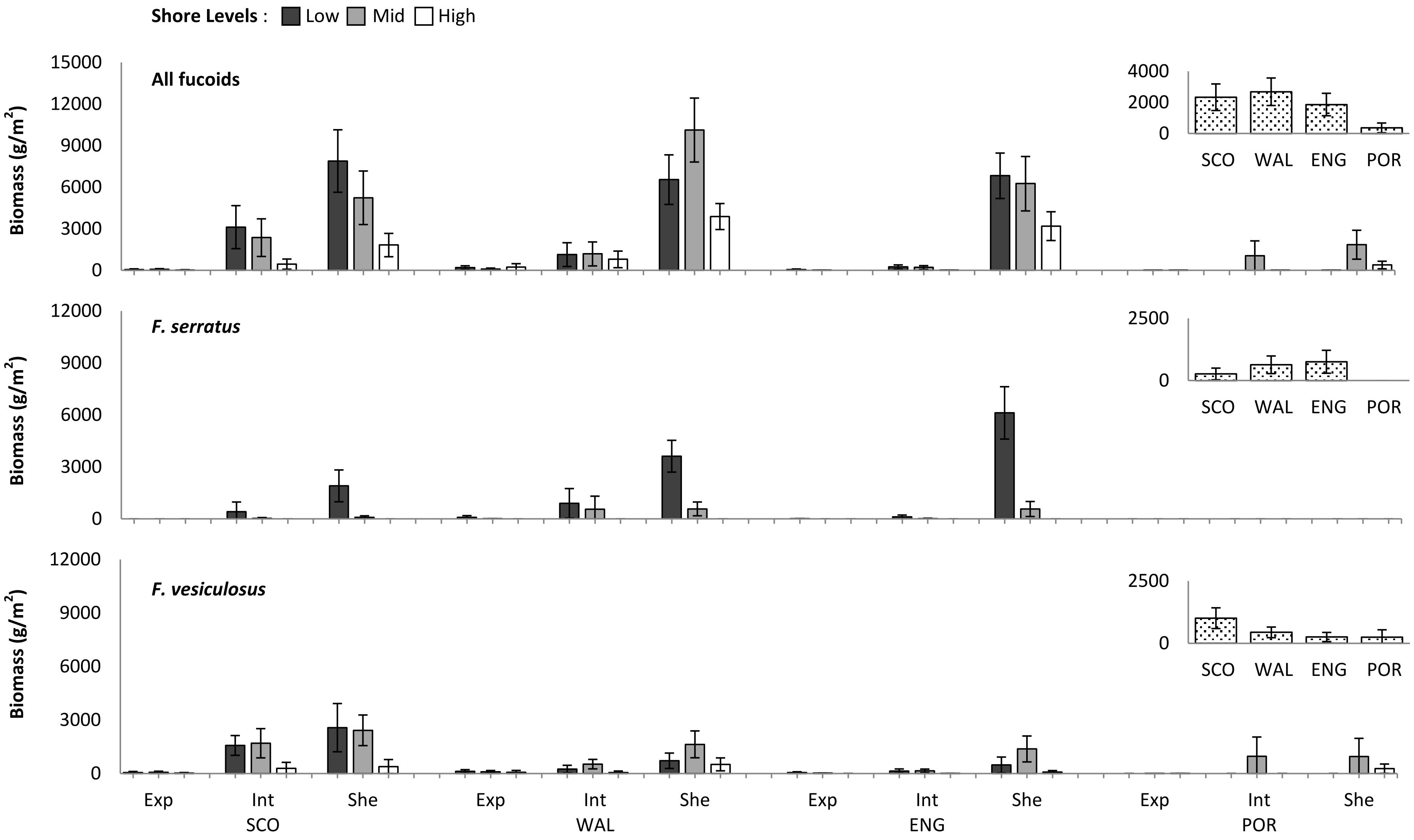


Figure 3: Biomass of total fucoids, *F. serratus* and *F. vesiculosus* in Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR) across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)* and shore height levels *(high, mid and low)*. The top panel shows a histogram, on the right, of total fucoid biomass variation across the four geographical regions where variability within each of the geographical regions is ignored; Error bars = ±1SE.

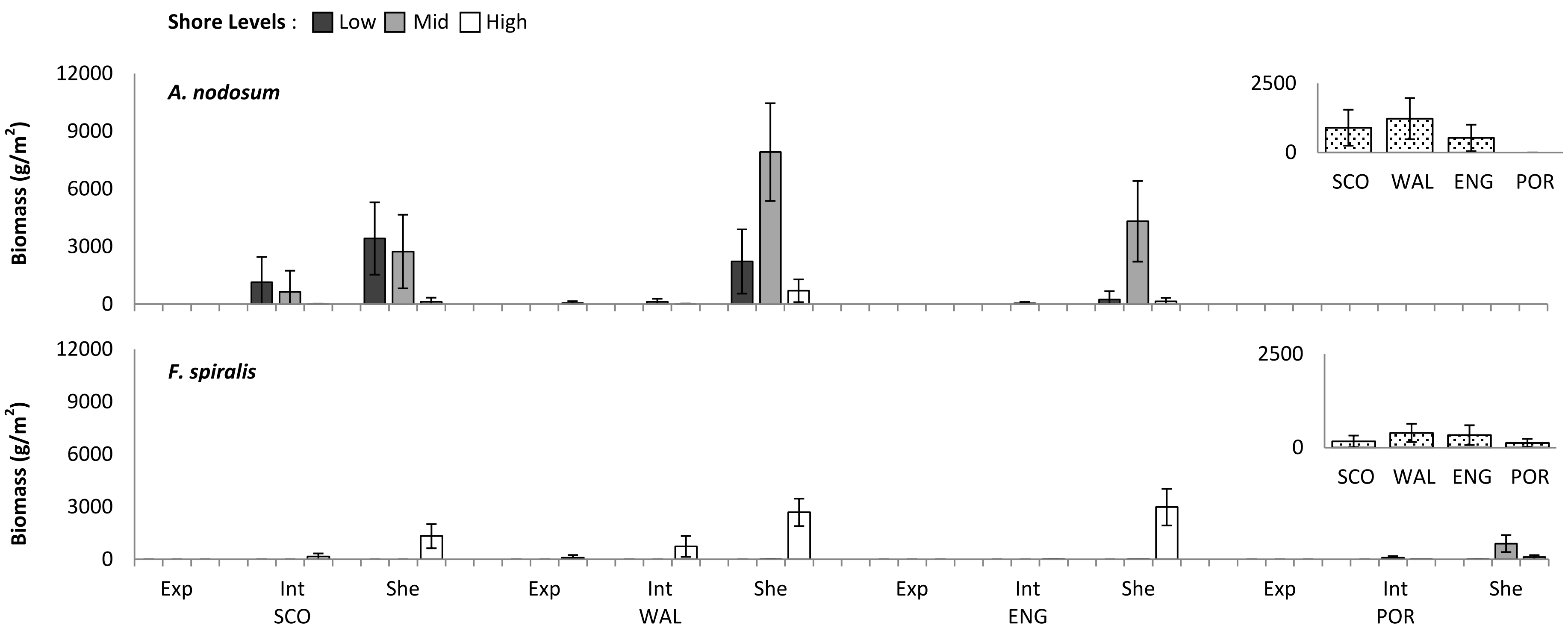


Figure 4: Biomass of *A. nodosum* and *F. spiralis* in Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR) across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)* and shore height levels *(high, mid and low)*; Error bars = ±1SE

2.3.2 Fucoid species results: biomass vs. percentage cover

The methodology used for quantifying percentage cover, allowed different layers of cover, depending on the size of the algae present, to be sampled. This methodology allowed the quantification of not only the biggest algae that normally cover the sampling quadrat but also the smallest individuals that could be living under the larger fucoid species. This contributed to the similarity of ANOVA results conducted on datasets of biomass and percentage cover of fucoid species (see Table 1 to Table 4, differences are highlighted on the Post hoc SNK). So, using percentage cover data it was still possible to observe the decline in total fucoid biomass from the United Kingdom to Portugal and the consistent effect of wave action on total fucoid biomass across all the geographical regions (Figure 5). With smaller dimension algae, such as *F. spiralis*, quantification of percentage cover can be useful to understand that, despite their small size, space occupation by this species can be high (Figure 6). A fact that can be very important when free space is required for colonization by other species.

Despite the similarities encountered, the use of percentage cover is less informative as equal values of percentage cover for different species can be from different biomasses, e.g. a 20 % cover by *F. vesiculosus* at mid shore height of Welsh sheltered shores corresponds to a biomass of approximately 1358 g/m^2 while over the same area a equal 20 % cover of *A. nodosum* corresponds to a biomass of approximately 2595 g/m^2. So, percentage cover is only a very approximate indicator of biomass, since it varies considerably depending on species identity and even on local population characteristics, such as size or bushiness. As a further example, for *F. vesiculosus*, a 20% cover at mid shore height on Welsh sheltered shores corresponded to a mean value of 1358 g/m^2, while at mid shore height on English sheltered shores the same percentage corresponded to a mean value of 1192 g/m^2 (Figure 5).

Table 3: Mix model ANOVA of percentage cover of total fucoids, *F. serratus* and *F. vesiculosus*. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual.*

Source	**DF**	**Fucoid Total**			***F. serratus***			***F. vesiculosus***			**F versus**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	3	183.9	20.3	<0.001	580.0	23.2	<0.001	164.4	14.5	<0.001	Sh (Re x We)
We	2	1116.6	123.3	<0.001	1322.0	52.9	<0.001	214.6	18.9	<0.001	Sh (Re x We)
Sh (Re x We)	108	9.1	52.0	<0.001	25.0	9.9	<0.001	11.4	22.9	<0.001	Res
Vl	2	53.0	23.3	<0.001	1671.7	113.0	<0.001	192.6	40.8	<0.001	Vl x Sh (Re x We)
Re x We	6	82.2	9.1	<0.001	257.7	10.3	<0.001	27.0	2.4	**<0.001**	Sh (Re x We)
Re x Vl	6	18.2	8.0	<0.001	280.2	19.0	<0.001	25.3	5.4	**<0.001**	Vl x Sh (Re x We)
We x Vl	4	10.7	4.7	0.001	687.0	46.5	<0.001	34.6	7.3	**<0.001**	Vl x Sh (Re x We)
Vl x Sh (Re x We)	216	2.3	13.0	<0.001	14.8	5.9	<0.001	4.7	9.5	**<0.001**	Res
Re x We x Vl	12	6.0	2.7	**0.003**	114.6	7.8	**<0.001**	7.1	1.5	0.121	Vl x Sh (Re x We)
Res	2520	0.2			2.5			0.5			
Cochran's Test		*C = 0.0305, p > 0.05*			*C = 0.0297, p > 0.05*			*C = 0.0283, p > 0.05*			
SNK		*Re x We x Vl*			*Re x We x Vl*			*Re x We*			
		IntxLow - Por<Eng=Wal<Sco			*MIDxLow - Por=Sco<Eng=Wal*			*Int - Por=Eng=Wal<Sco*			
		IntxMid - Eng=Por=Wal<Sco			*ShexLow - Por<Sco<Wal<Eng*			*She - Por<Eng=Wal=Sco, Eng<Sco*			
		IntxHigh - Por=Eng<Sco=Wal			*ShexMid - Por=Sco<Wal=Eng*			*Sco - Exp<Int=She*			
		ShexLow - Por<Sco=Eng=Wal			*ScoxLow - Exp=Int<She*			*Wal - Exp=Int<She*			
		ShexMid - Por<Sco=Eng<Wal			*WalxLow - Exp<Int<She*			*Re x Vl*			
		ShexHigh - Por<Sco<Eng=Wal			*WalxMid - Exp<She*			*Low - Por<Eng=Wal<Sco*			
		ScoxLow - Exp<Int<She			*EngxLow - Exp<Int<She*			*Mid - Por<Eng=Wal<Sco*			
		ScoxMid - Exp<Int<She			*EngxMid - Exp=Int<She*			*Sco - High<Low=Mid*			
		ScoxHigh - Exp<Int<She			*ScoxShe - High=Mid<Low*			*Wal - High<Low<Mid*			
		WalxLow - Exp<Int<She			*WalxInt - High<Mid<Low*			*Eng - High<Low<Mid*			
		WalxMid - Exp<Int<She			*WalxShe - High<Mid<Low*			*We x Vl*			
		WalxHigh - Exp=Int<She			*EngxInt - High=Mid<Low*			*Low - Exp<Int=She*			
		EngxLow - Exp=Int<She			*EngxShe - High<Mid<Low*			*Mid - Exp<Int<She*			
		EngxMid - Exp=Int<She						*High - Exp=Int<She*			
		EngxHigh - Exp=Int<She						*Int - High<Low=Mid*			
		PorxMid - Exp<Int<She						*She - High<Low<Mid*			
		ScoxInt - High<Mid<Low									
		ScoxShe - High<Mid<Low									
		WalxShe - High<Mid									
		EngxShe - High<Low									
		PorxShe - Low=High<Mid									

Table 4: Mix model ANOVA of biomass of *A. nodosum* and *F. spiralis*. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual.*

Source	**DF**	***A. nodosum***			***F. spiralis***			**F ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	3	12134.5	6.3	0.001	3034.7	2.6	0.054	Sh (Re x We)
We	2	56663.6	29.3	<0.001	43820.7	37.9	<0.001	Sh (Re x We)
Sh (Re x We)	108	1932.6	28.1	<0.001	1155.5	31.1	<0.001	Res
Vl	2	21028.9	17.4	<0.001	40872.5	40.4	<0.001	Vl x Sh (Re x We)
Re x We	6	10828.2	5.6	<0.001	2278.4	2.0	0.076	Sh (Re x We)
Re x Vl	6	6719.9	5.5	<0.001	10093.4	10.0	<0.001	Vl x Sh (Re x We)
We x Vl	4	18964.1	15.6	<0.001	28275.4	27.9	<0.001	Vl x Sh (Re x We)
Vl x Sh (Re x We)	216	1212.2	17.6	<0.001	1012.6	27.3	<0.001	Res
Re x We x Vl	12	5552.5	4.6	**<0.001**	6909.0	6.8	**<0.001**	Vl x Sh (Re x We)
Res	2520	68.8			37.1			
Cochran's Test		*C = 0.0901, p < 0.01*			*C = 0.0761, p < 0.01*			
SNK		*Re x We x Vl*			*Re x We x Vl*			
		ShexLow - Por=Eng<Wal=Sco			*ShexMid - Sco=Wal=Eng<Por*			
		ShexMid - Por<Sco<Eng<Wal			*ShexHigh - Por<Sco<Eng =Wal*			
		ScoxLow - Exp=Int<She			*ScoxHigh - Exp=Int<She*			
		SCOxMid - Exp=Int<She			*WalxHigh - Exp=Int<She*			
		WALxLow - Exp=Int<She			*EngxHigh - Exp=Int<She*			
		WALxMid - Exp=Int<She			*PorxMid - Exp=Int<She*			
		ENGxMid - Exp=Int<She			*ScoxShe - Low=Mid<High*			
		SCOxShe - High<Mid=Low			*WalxShe - Low=Mid<High*			
		WALxShe - High<Low<Mid			*EngxShe - Low=Mid<High*			
		ENGxShe - Low=High<Mid			*PorxShe - Low=High<Mid*			

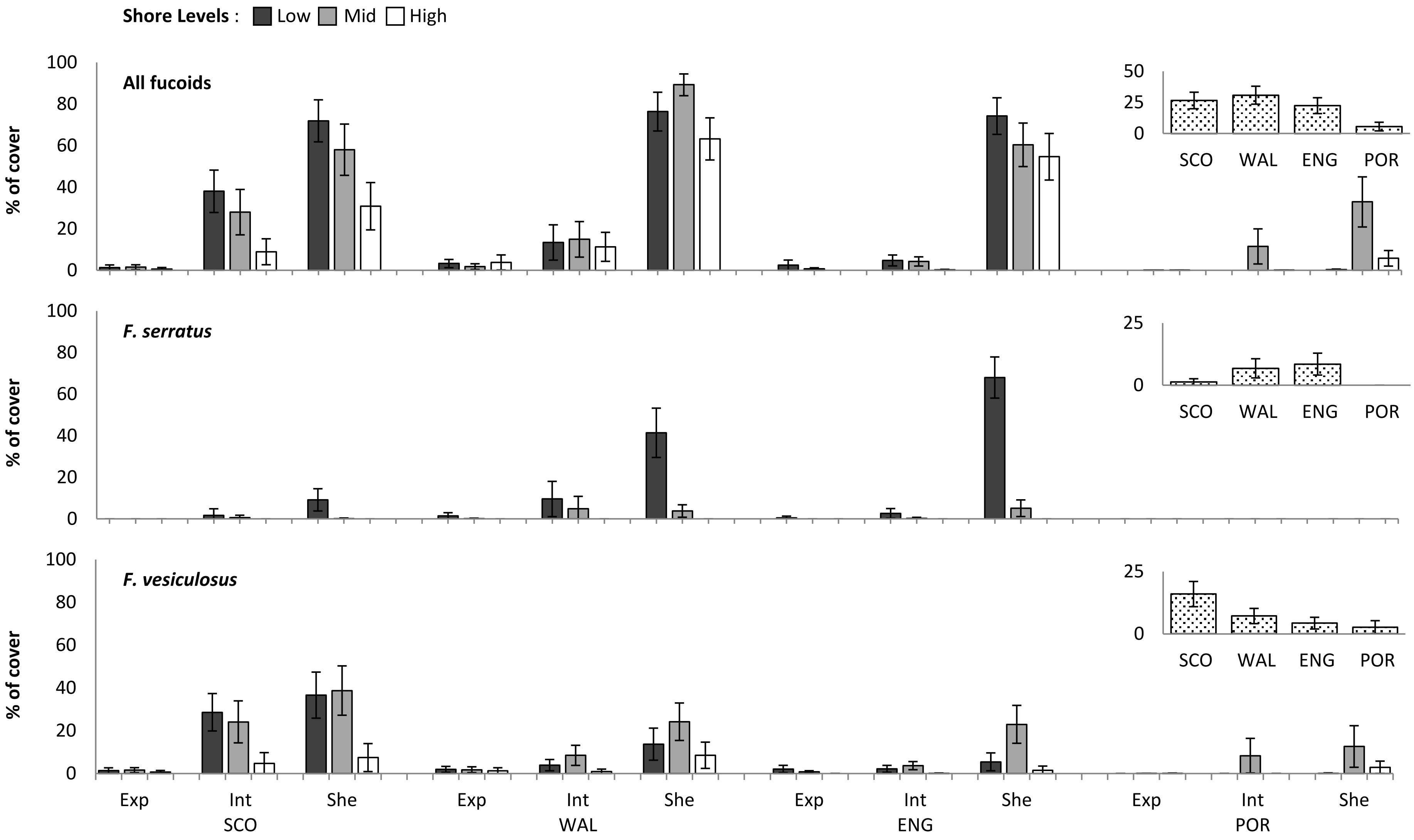


Figure 5: Percentage cover of total fucoids, *F. serratus* and *F. vesiculosus* at Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR) across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)* and shore height levels *(high, mid and low)*. The top panel shows a histogram, on the right, of total fucoid biomass variation across the four geographical regions where variability within each of the geographical regions is ignored; Error bars = ±1SE.

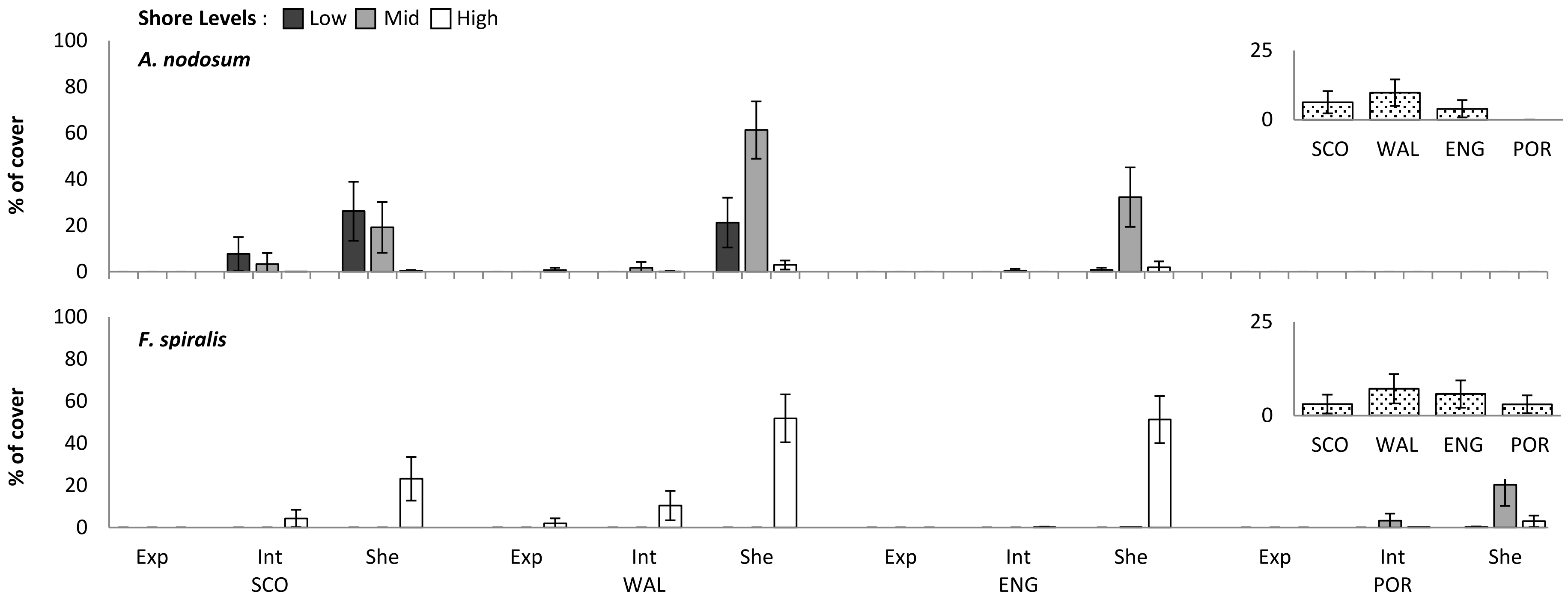


Figure 6: Percentage cover of *A. nodosum* and *F. spiralis* at Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR) across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)* and shore height levels *(high, mid and low)*; Error bars = ±1SE

2.3.3 *Patella* spp. distribution

The collection of 50 limpets, *Patella* species were not distinguished for this exercise, representing each of the wave exposure from each of the geographical areas allowed the development of regression equations that specifically represented the relationship between shell length and dry tissue biomass for each of the conditions described (Figure 7). The equations were combined with limpet length data, collected through digital photographs in the field, to calculate limpet biomass levels in each quadrat sampled. Generally, relationships were best described by power equations. However, for exposed shores from Scotland, Wales and Portugal exponential regressions best fitted the data. The presence of few large limpets in some of the areas, especially at sheltered English shores, heavily influenced the shape of the curves. Despite that, a tight relationship between size and dry soft tissue biomass was obtained across all exposures and geographical regions, with R^2 varying between 0.761 for limpets from wave exposed Portuguese shores to 0.975 for limpets from wave exposed Scottish shores (Figure 7).

The density and biomass of limpets varied according to the interaction between geographical region origin, wave exposure and vertical shore height level (Table 5: Density of limpets - $F_{\text{(Re x We x Vl) 12, 2520}} = 3.2$; $p<0.001$; Biomass of limpets: $F_{\text{(Re x We x Vl) 12, 2520}} = 2.7$; $p<0.005$). Although, if local variability within each of the geographical regions is discarded, the results indicate that the density and biomass of *Patella* spp. in Portugal, as a whole, is lower than at any of the three regions in the United Kingdom, where similar levels of density and biomass of *Patella* spp. are found across the three geographical regions (Figure 8).

Variability in density of limpets within geographical regions followed the expected patterns across wave exposure and vertical shore heights that had been previously proposed (Ballantine 1961). Generally, with exception of the mid shore height in Scotland, mid and low shore heights on sheltered conditions had lower limpet densities than similar shore heights on intermediate or wave exposed shores (Table 5*:* Density of limpets - *SNK of Re x We x Vl*). Density of limpets also varied with vertical shore height, being lowest at high shore levels and generally the highest at mid shore height (Table 5*:* Density of limpets - *SNK of Re x We x Vl*). Maximum densities of *Patella* spp. were observed at mid shore height of wave exposed shores in Welsh and English intertidal (Figure 8).

Although the relationship between shore height and biomass of limpets was similar to the one presented for limpet densities, with lower biomass on higher shore heights, the effect of wave exposure on biomass of limpets within each geographical region was not consistent. For the majority of the geographical regions, where significant differences were detected, sheltered shores presented the lowest limpet biomass levels (Table 5*:* Biomass of limpets - *SNK of Re x We x Vl*). At English shores, however, the reverse relationship was observed, with sheltered areas showing the highest limpet biomass levels (Figure 8). In terms of biomass of limpets, analyses further indicate that higher *Patella* spp. biomass levels were localized at low and mid shore heights from Welsh exposed shores and at low and mid shore heights from English sheltered shores (Table 5*:* Biomass of limpets - *SNK of Re x We x Vl*), (Figure 8)

Table 5: Mix model ANOVA of density and biomass of *Patella* spp.. Post hoc SNK tests of significant differences are presented. Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual.

Source	**DF**	**Density**			**Biomass**			**F ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	3	1336.9	6.4	0.001	98.0	7.5	<0.001	Sh (Re x We)
We	2	8301.9	39.5	<0.001	130.9	10.0	<0.001	Sh (Re x We)
Sh (Re x We)	108	210.3	37.1	<0.001	13.1	20.0	<0.001	Res
Vl	2	6861.1	121.9	<0.001	455.9	105.5	<0.001	Vl x Sh (Re x We)
Re x We	6	685.6	3.3	0.006	72.8	5.6	<0.001	Sh (Re x We)
Re x Vl	6	505.5	9.0	<0.001	19.2	4.4	<0.001	Vl x Sh (Re x We)
We x Vl	4	504.7	9.0	<0.001	12.8	3.0	0.021	Vl x Sh (Re x We)
Vl x Sh (Re x We)	216	56.3	9.9	<0.001	4.3	6.6	<0.001	Res
Re x We x Vl	12	179.4	3.2	**<0.001**	11.5	2.7	**0.002**	Vl x Sh (Re x We)
Res	2520	5.7			0.7			
Cochran's Test		*C = 0.0205, p > 0.05*			*C = 0.0309, p > 0.05*			
SNK		*Re x We x Vl*			*Re x We x Vl*			
		ExpxLow - Por=Sco<Wal=Eng			*ExpxLow - Eng<Wal*			
		ExpxMid - Sco<Por<Wal=Eng			*ExpxMid - Sco=Eng=Por<Wal*			
		IntxLow - Por<Sco=Wal=Eng			*ExpxHigh - Eng<Wal*			
		IntxMid - Sco=Por=Wal<Eng			*IntxLow - Por<Wal=Eng=Sco*			
		ShexLow - Por<Sco, Por<Eng			*IntxHigh - Eng=Por=Sco<Wal*			
		ShexMid - Por=Wal<Eng=Sco			*ShexLow - Por<Wal=Sco<Eng*			
		ShexHigh - Por<Sco			*ShexMid - Por=Wal<Sco=Eng*			
		ScoxLow - She<Exp=Int			*ShexHigh - Por<Wal=Eng=Sco*			
		WalxLow - She<Int<Exp			*ScoxLow - She<Exp=Int*			
		WalxMid - She<Int<Exp			*WalxLow - She<Int<Exp*			
		WalxHigh - She<Exp=Int			*WalxMid - She<Int=Exp*			
		EngxLow - She<Int=Exp			*WalxHigh - She<Int*			
		EngxMid - She<Int=Exp			*PorxLow - She=Int<Exp*			
		PorxLow - She=Int<Exp			*PorxMid - She<Exp=Int*			
		PorxMid - She<Int=Exp			*PorxHigh - She<Int=Exp*			
		PorxHigh - She<Exp=Int			*ScoxExp - High<Mid=Low*			
		ScoxExp - High<Mid=Low			*ScoxInt - High<Mid=Low*			
		ScoxInt - High<Low=Mid			*ScoxShe - High=Low<Mid*			
		ScoxShe - High=Low<Mid			*WalxExp - High<Low=Mid*			
		WalxExp - High<Low<Mid			*EngxExp - High<Low=Mid*			
		WalxInt - High<Low=Mid			*EngxInt - High<Mid=Low*			
		EngxExp - High<Low=Mid			*EngxShe - High<Low=Mid*			
		EngxInt - High<Low=Mid			*PorxExp - High<Low=Mid*			
		EngxShe - High<Low<Mid			*PorxInt - Low=High<Mid*			
		PorxExp - High<Low<Mid			*PorxShe - High<Mid*			
		PorxInt - Low<High<Mid						

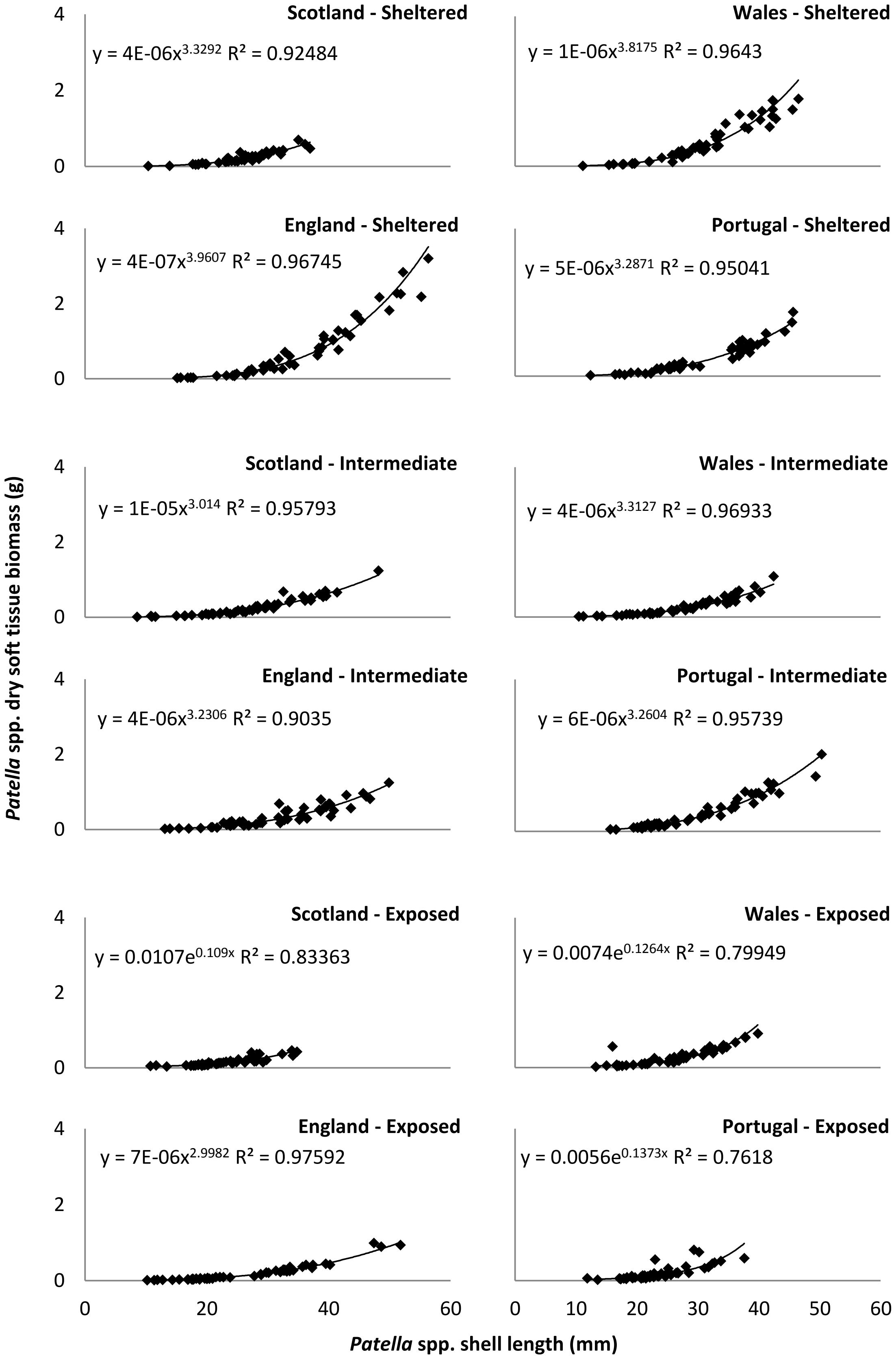


Figure 7: Graphs showing the relationship between *Patella* spp. shell length and dry soft tissue biomass for each wave exposure level at each geographical region; Regression equations and R^2 values are shown.

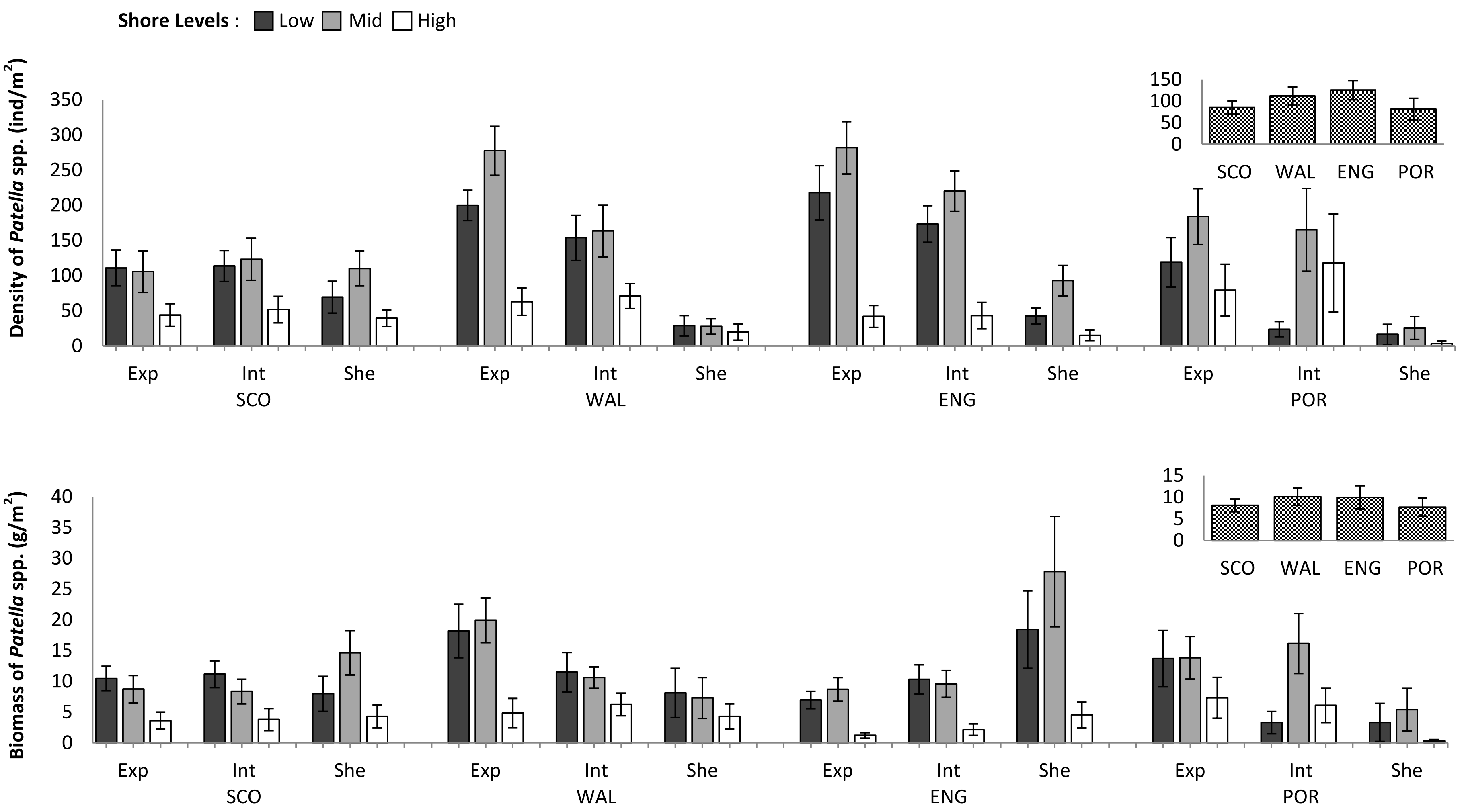


Figure 8: Density and biomass of *Patella* spp. at Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR) across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)* and shore height levels *(high, mid and low)*. On the top right of each panel a histogram of density/biomass variation across the four geographical regions, where variability within each of the geographical regions is ignored, is shown; Error bars = ±1SE

2.3.4 Interaction between grazing pressure and macroalgae biomass

The results obtained for limpet densities and biomass do not seem to support herbivory as the main factor controlling macroalgae abundance on the southern extreme of this latitudinal scale. For the presence of such a scenario, where herbivory would have a major influence on fucoid biomass levels in Portugal, the highest values of limpet biomass or density was expected to be observed on the Portuguese coast, which is not confirmed by the datasets (Figure 9). On the other hand, the importance of grazing pressure cannot be disregarded for Scottish, Welsh and English areas, where the higher abundance of fucoid occurs where limpet density is reduced (Figure 9). However, other factors like the effect of wave exposure also seem to be very important for the presence and abundance levels of fucoid species across different geographical regions. In Scotland for example, where both density and biomass of limpets is similar across several wave exposures, the level of fucoid biomass is still variable, favouring the presence of fucoid species in areas most protected from wave action (Figure 9).

To further explore the possibility of such relationships, graphs displaying the relationship between limpet density and biomass of fucoids were plotted at the scale of the 0.5 x 0.5m quadrat. All quadrat data independent of geographical origin, wave exposure and vertical shore position, were plotted together. This plot shows that as densities of limpets increase across the entire region, fucoid biomass decreases (Figure 10). Although, this tendency is maintained when the data are analysed separately according to geographical region (Scotland, Wales, England, Portugal), when the data are separated by wave exposure level the tendency only appears on intermediately exposed and sheltered shores, (Figure 10). Removing data from the upper and lower areas of the shore does not change the tendencies observed (Figure 10).

A similar tendency is observed in plots displaying a relationship using biomass rather than density of limpets when data are combined across regions and exposure levels (Figure 11). Despite this fact, when data are plotted separately for each geographical region some plots seems to show a variable response to increased grazing. For Scottish, Welsh and Portuguese shores, the increase in limpet biomass seems to lead to a decrease in fucoid biomass, but this tendency is not so clear on English shores (Figure 11). When similar analyses were performed for data from different wave exposure intensities, again for intermediate and exposed shores a decrease in fucoid biomass seems to occur when limpet biomass increases. These tendencies observed are maintained even when only data from the mid shore height is used (Figure 11).

The majority of the graphs presented, generally confirm the expected relationship between limpet density or biomass and levels of fucoid on the shore across the four geographical regions. However, the strength of the relationship seems to be dependent on the geographical region and wave exposure of each shore.

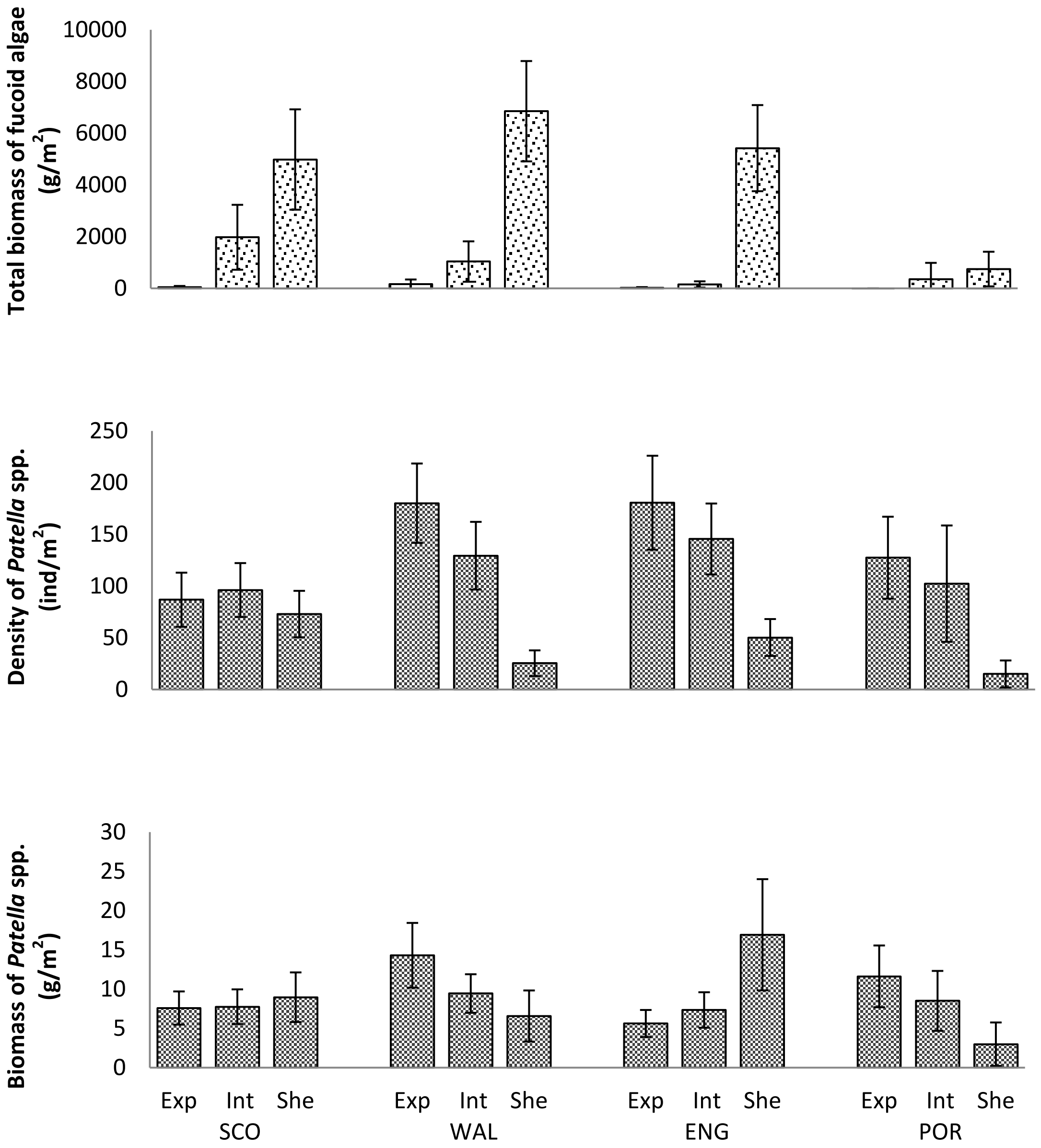


Figure 9: Total fucoid biomass, density and biomass of *Patella* spp. at Scotland (SCO), Wales (WAL), England (ENG) and Portugal (POR) across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)*; Error bars = ±1SE

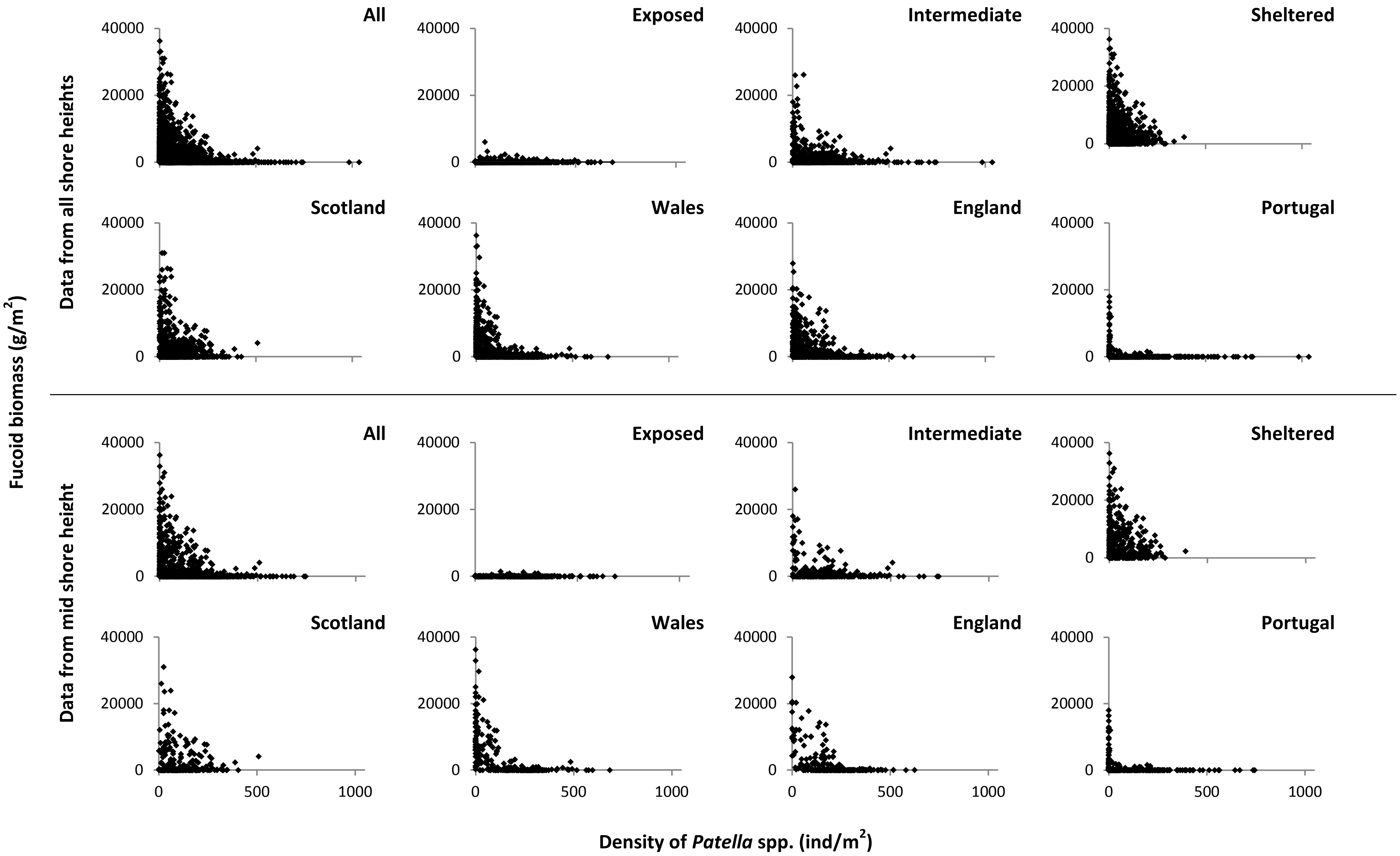


Figure 10: Graphs showing the relationship between *Patella* spp.density and fucoid biomass levels, when all data are plot together, for each wave exposure level and each geographical region; Regression equations and R^2 values are shown.

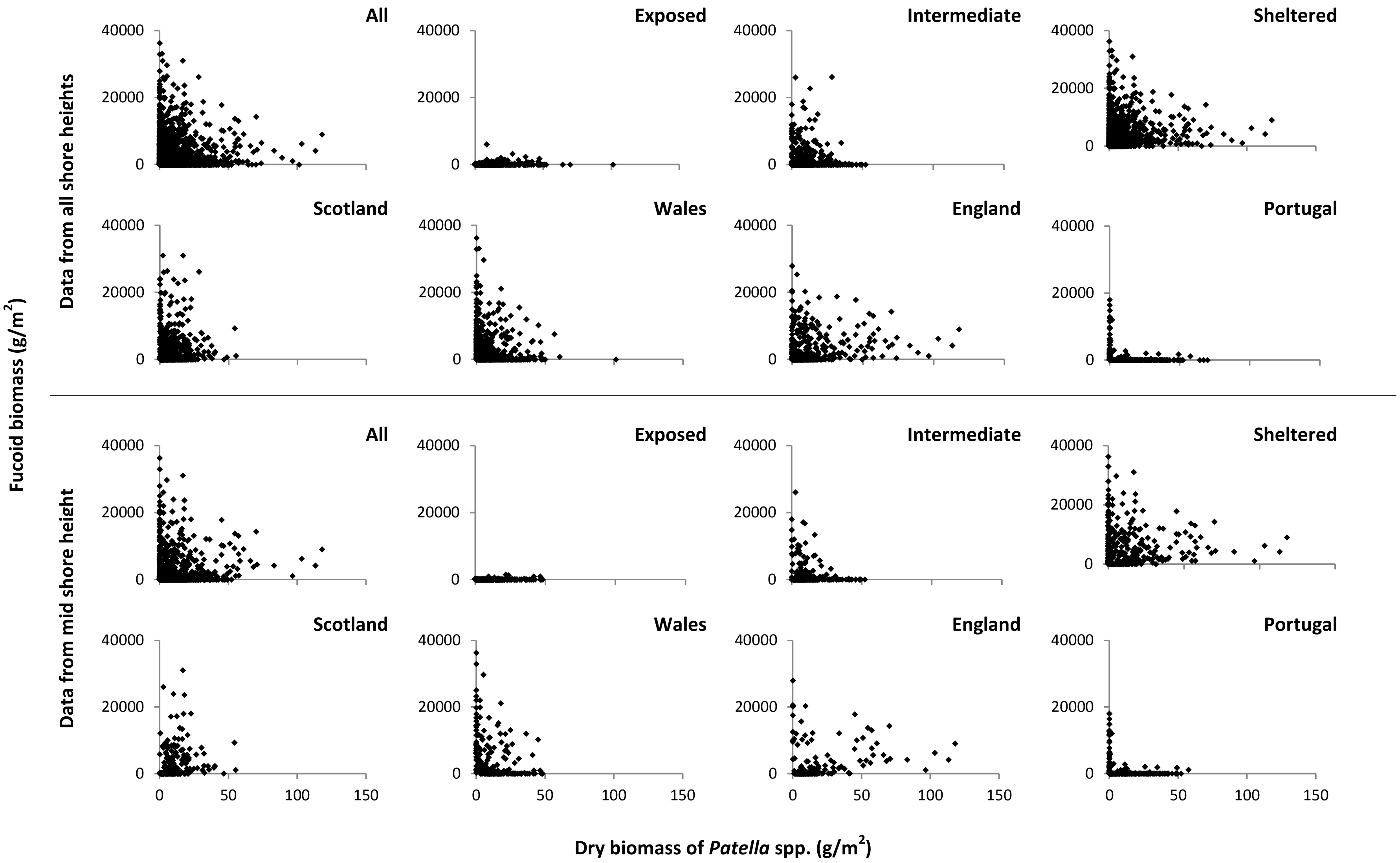


Figure 11: Graphs showing the relationship between *Patella* spp. dry biomass and fucoid biomass levels, when all data are plot together, for each wave exposure level and each geographical region; Regression equations and R^2 values are shown.

2.3.5 Distribution of other components of the associated community

2.3.5.1 Other algal functional groups

Foliose algae community found during the survey was mainly composed of species like *Palmaria palmata*, *Chondrus crispus* or *Mastocarpus stellatus*. In terms of vertical position on the shore, their presence was normally restricted to low shore heights (Table 6: Foliose algae - *SNK of Re x We x VL*). On Portuguese shores, foliose species were present at very low levels but their percentage cover increased across the United Kingdom from northern Scotland to southern England (Figure 12). Across the geographical regions, the influence of wave exposure on levels of foliose algal cover on the shore varied and was not consistent (Figure 12).

Specimens of *Corallina officinalis*, *Cladophora rupestris* and *Osmundea pinnatifida*, among others, comprised the turf community observed across the different geographical regions included in this study. The percentage cover of these specimens progressively increased from northern to southern regions (Figure 12). This community was typically observed on the lower area of the shore across all geographical regions. The largest percentage cover was observed on the Portuguese low shore, especially at intermediate wave exposed conditions where turf algae occupied up to 51 % of the space available (Table 6: Turf algae - *SNK of Re x We x VL*), (Figure 12).

Another functional group also detected at low shore levels along all four geographical regions was the encrusting algal community, represented mainly by specimens belonging to the *Lithophyllum*, *Hildenbrandia* or *Codium* genus. In the United Kingdom, a consistent effect of wave exposure was observed, with encrusting algae coverage reducing as conditions became more exposed to wave action (Table 6: Encrusting algae - *SNK of Re x We x VL*). In contrast, on Portuguese shores, where globally percentage cover of encrusting algae reaches the highest value, the effect of wave exposure was not so obvious (Figure 12).

Ephemeral algae, such as specimens belonging to *Ulva* or *Porphyra* genus, had low percentage cover across the United Kingdom but achieved high percentages on the southern Portuguese shores, particularly on sheltered conditions at low and mid shore heights where algae could avoid the strongest effects of desiccation (Table 6: Ephemeral algae – *SNK of Re x We, Re x Vl and We x Vl*, Figure 12).

Table 6: Mix model ANOVA of percentage cover of foliose, turf, crustose and ephemeral algae. Post hoc SNK tests of significant differences are presented. Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual.

Source	**DF**	**Foliose**			**Turf**			**Crustose**			**Ephemeral**			**F ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	3	74.2	23.8	<0.001	148.9	6.0	<0.001	56.5	1.4	0.253	16.6	40.8	<0.001	Sh (Re x We)
We	2	15.5	5.0	0.009	222.3	9.0	<0.001	439.8	10.7	<0.001	4.9	12.1	<0.001	Sh (Re x We)
Sh (Re x We)	108	3.1	10.7	<0.001	24.7	26.2	<0.001	41.0	22.5	<0.001	0.4	14.4	<0.001	Res
Vl	2	251.3	137.3	<0.001	977.2	78.1	<0.001	153.1	10.2	<0.001	1.5	7.7	0.001	Vl x Sh (Re x We)
Re x We	6	12.2	3.9	0.001	56.6	2.3	0.040	177.7	4.3	<0.001	3.8	9.3	**<0.001**	Sh (Re x We)
Re x Vl	6	35.9	19.6	<0.001	114.8	9.2	<0.001	16.4	1.1	0.372	2.3	11.5	**<0.001**	Vl x Sh (Re x We)
We x Vl	4	6.5	3.5	0.008	49.8	4.0	0.004	10.6	0.7	0.593	0.3	1.4	0.235	Vl x Sh (Re x We)
Vl x Sh (Re x We)	216	1.8	6.3	<0.001	12.5	13.3	<0.001	15.1	8.3	<0.001	0.2	7.2	**<0.001**	Res
Re x We x Vl	12	7.0	3.8	**<0.001**	43.9	3.5	**<0.001**	44.3	2.9	**<0.001**	0.3	1.5	0.123	Vl x Sh (Re x We)
Res	2520	0.3			0.9			1.8			0.0			
Cochran's Test		*C = 0.0299, p > 0.05*			*C = 0.0287, p > 0.05*			*C = 0.0237, p > 0.05*			*C = 0.0311, p > 0.05*			
SNK		*Re x We x Vl* *ExpxLow - Sco=Por<Eng=Wal* *IntxLow - Por=Sco=Eng<Wal, Por<Eng* *ShexLow - Por<Sco<Wal<Eng* *ShexMid - Por<Wal, Por<Eng* *ScoxLow - Exp<Int<She* *EngxLow - Int<Exp<She* *ScoxShe - High=Mid<Low* *WalxExp - High=Mid<Low* *WalxInt - High<Mid<Low* *WalxShe - High<Mid<Low* *EngxExp - High=Mid<Low* *EngxInt - High=Mid<Low* *EngxShe - High<Mid<Low*			*Re x We x Vl* *ExpxLow - Eng=Sco<Por=Wal* *IntxLow - Sco<Wal=Eng<Por* *ShexLow - Wal=Sco=Eng<Por* *ShexMid - Sco<Eng* *ShexHigh - Por=Sco<Eng=Wal* *ScoxLow - Int<She* *WalxLow - She<Exp* *WalxHigh - Exp=Int<She* *EngxLow - Exp<She=Int* *EngxMid - Exp=Int<She* *EngxHigh - Exp=Int<She* *PorxLow - Exp<She<Int* *PorxMid - Exp<She* *ScoxShe - High<Low* *WalxExp - High=Mid<Low* *WalxInt - High<Mid=Low* *EngxInt - High=Mid<Low* *EngxShe - High<Mid* *PorxExp - High=Mid<Low* *PorxInt - High<Mid<Low* *PorxShe - High<Mid<Low*			*Re x We x Vl* *ExpxLow - Sco=Wal=Eng<Por* *ExpxMid - Sco=Wal=Eng<Por* *IntxMid - Eng=Sco=Wal<Por* *IntxHigh - Sco<Por, Eng<Por* *ShexLow - Por<Sco=Eng=Wal* *ShexMid - Por<Wal, Por<Eng, Sco<Wal* *ScoxLow - Exp=Int<She* *ScoxHigh - Int=Exp<She* *WalxLow - Exp=Int<She* *WalxMid - Exp=Int<She* *WalxHigh - Exp<She* *EngxLow - Int=Exp<She* *EngxMid - Int=Exp<She* *PorxLow - She<Int<Exp* *PorxMid - She<Exp=Int* *ScoxShe - Mid<Low* *WalxShe - High<Low* *EngxShe - High=Mid<Low* *PorxExp - High=Mid<Low* *PorxShe - Low=Mid<High*			*Re x We* *Int - Sco=Eng=Wal<Por* *She - Sco=Wal=Eng<Por* *Por - Exp<Int<She* *RexVl* *Low - Sco=Eng=Wal<Por, Sco<Wal* *Mid - Sco=Eng=Wal<Por* *Por - High<Mid<Low* *WexVl* *Low- Exp<Int<She* *mid- Exp=Int<She* *High- Exp=Int<She* *Int- High<Low* *She- High<Low*			

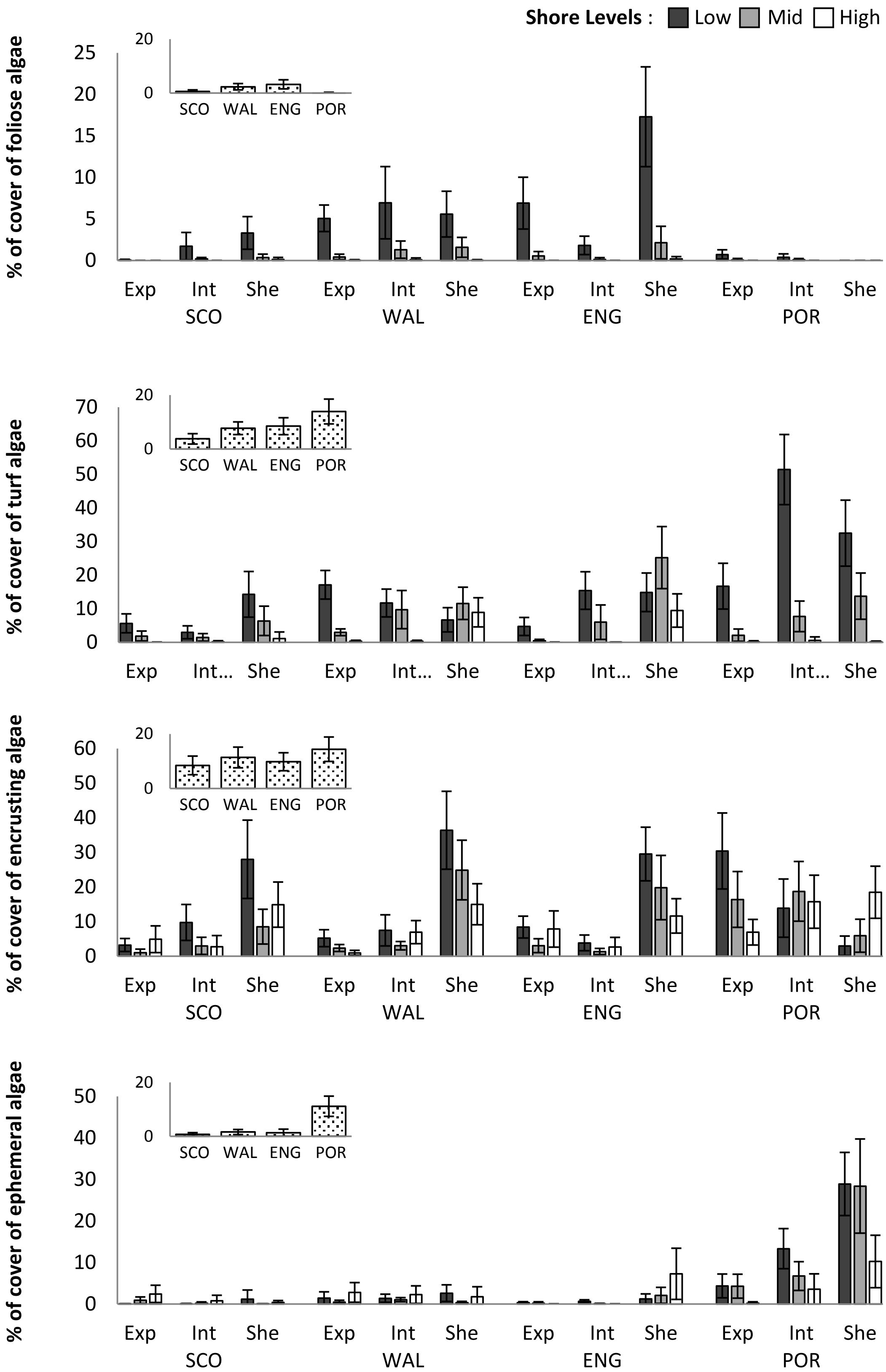


Figure 12: Percentage cover of foliose, turf, encrusting and ephemeral algae at Scottish (SCO), Welsh (WAL), English (ENG) and Portuguese (POR) regions across different wave exposures (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered) and shore height levels (high, mid and low). On the top right of each panel a histogram of density/biomass variation across the four geographical regions, where variability within each of the geographical regions is ignored, is shown; Error bars = ±1SE

2.3.5.2 Filter feeders

Barnacles and mussels were the most abundant filter feeding organisms inhabiting the four geographical regions studied. Barnacle cover was consistently affected by wave exposure intensity across these geographical regions (Table 7: Barnacles - *SNK of Re x We x VL*). Increased percentage of barnacle cover was found on wave exposed shores at all four geographical regions (Figure 13). Regarding the vertical position on the shore, barnacles were present at all shore heights but higher percentage cover normally arose at mid shore height (Table 7: Barnacles - *SNK of Re x We x VL,* Figure 13). Their cover varied across geographical regions and patterns were dependent on wave exposure levels and shore height. Higher space occupancy rates were observed mainly on English and Scottish shores across the different shore heights and wave exposures (Table 7: Barnacles - *SNK of Re x We x VL*). The lowest barnacle percentage cover was detected on the low level of Portuguese shores throughout all wave exposure conditions, where barnacle cover was not greater than 2 % (Figure 13).

For the other abundant filter feeding organism, the mussel, the highest percentage cover was found at regions located on the extremes of the range surveyed, Scotland and Portugal (Table 7: Mussels - *SNK of Re x We x VL*). Although, these organisms were able to occupy shores with different exposures to wave action (Figure 13), no consistent effect of wave action across the four regions was detected. In terms of vertical height, low and mid shore levels usually developed greater mussel percentage cover consistently across all the geographical regions included in this study (Table 7: Mussels - *SNK of Re x We x VL*).

2.3.5.3 Space Available

The upper zone of the shore is the area most subject to large amplitudes of physical conditions, where the physiology of both intertidal animals and algae is most tested. So, as expected, the highest percentage of free rock space occurred here (Table 7: Free space - *SNK of Re x We x VL*). Although, the effect of wave action was not consistent over the four geographical regions, global differences in the total amount of free areas to be colonized across these geographical regions were still detected ((Table 7: Free space - *SNK of Re x We x VL,* Figure 13). Globally, without taking into consideration the variability within geographical region, it is possible to observe that space availability was slightly greater at the two most northern regions, Scotland and Wales, where up to 40% and 50% of the shore, respectively, could be available for colonization (Figure 13).

Table 7: Mix model ANOVA of percentage cover of barnacles, mussels and free space available. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Sh: Shore; Vl: Vertical shore level (high, mid and low); Res: Residual.*

Source	**DF**	**Barnacles**			**Mussels**			**Free Space**			**F ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	3	128728.4	19.7	<0.001	2679.8	12.7	<0.001	46811.1	7.7	<0.001	Sh (Re x We)
We	2	195775.8	29.9	<0.001	1163.7	5.5	0.005	8912.2	1.5	0.234	Sh (Re x We)
Sh (Re x We)	108	6546.2	34.3	<0.001	211.1	46.6	<0.001	6048.7	23.8	<0.001	Res
Vl	2	36141.9	16.5	<0.001	1906.8	31.7	<0.001	162480.9	60.7	<0.001	Vl x Sh (Re x We)
Re x We	6	28551.3	4.4	0.001	274.8	1.3	0.263	29266.7	4.8	<0.001	Sh (Re x We)
Re x Vl	6	13861.1	6.3	<0.001	507.5	8.4	<0.001	6654.3	2.5	0.024	Vl x Sh (Re x We)
We x Vl	4	11303.9	5.2	0.001	179.5	3.0	0.020	6624.0	2.5	0.045	Vl x Sh (Re x We)
Vl x Sh (Re x We)	216	2186.8	11.5	<0.001	60.2	13.3	<0.001	2676.2	10.5	<0.001	Res
Re x We x Vl	12	4254.5	2.0	**0.031**	108.7	1.8	**0.049**	6237.6	2.3	**0.008**	Vl x Sh (Re x We)
Res	2520	190.9			4.5			254.0			
Cochran's Test		*C = 0.0173, p > 0.05*			*C = 0.0306, p > 0.05*			*C = 0.0208, p > 0.05*			
SNK		*Re x We x Vl*			*Re x We x Vl*			*Re x We x Vl*			
		ExpxLow - Por<Wal<Eng=Sco			*ExpxLow - Eng=Wal<Sco=Por*			*ExpxLow - Eng=Sco=Por<Wal*			
		ExpxMid - Wal=Por=Sco<Eng			*ExpxMid - Eng=Wal<Sco=Por*			*ExpxMid - Eng=Por=Sco<Wal*			
		ExpxHigh - Wal<Sco=Por<Eng			*IntxLow - Eng=Wal=Sco<Por*			*ExpxHigh - Eng<Sco=Por<Wal*			
		IntxLow - Por<Wal<Sco=Eng			*IntxHigh - Wal=Eng=Por<Sco*			*IntxLow - Por<Eng<Wal=Sco*			
		IntxMid - Por=Wal=Sco<Eng, Por<Sco			*ShexLow - Eng=Sco=Wal<Por*			*IntxMid - Eng<Wal*			
		IntxHigh - Por=Wal<Sco=Eng			*ScoxLow - She<Int<Exp*			*IntxHigh - Eng=Sco<Por=Wal*			
		ShexLow - Por<Sco			*ScoxMid - She<Int<Exp*			*ShexMid - Wal<Sco, Por<Sco*			
		ShexMid - Por=Eng=Wal<Sco			*PorxMid - She=Int<Exp*			*ScoxLow - Exp=She<Int*			
		ScoxLow - She<Int<Exp			*ScoxExp - High<Low=Mid*			*ScoxMid - Exp<Int=She*			
		ScoxMid - She=Int<Exp			*PorxExp - High<Low=Mid*			*WalxLow - She<Exp=Int*			
		ScoxHigh - She<Exp=Int			*PorxInt - High<Mid=Low*			*WalxMid - She<Int=Exp*			
		WalxMid - She<Exp			*PorxShe - High<Mid<Low*			*WalxHigh - She<Int=Exp*			
		EngxLow - She<Int=Exp						*EngxMid - Exp<She*			
		EngxMid - She<Int=Exp						*EngxHigh - Exp=Int<She*			
		EngxHigh - She<Int=Exp						*ScoxExp - Low=Mid<High*			
		PorxMid - She<Int<Exp						*ScoxShe - Low<Mid=High*			
		ScoxExp - High<Mid=Low						*WalxExp - Low=Mid<High*			
		WalxExp - High<Mid						*WalxInt - Low=Mid<High*			
		EngxExp - High=Low<Mid						*EngxShe - Low=Mid<High*			
		EngxInt - Low<Mid						*PorxExp - Low=Mid<High*			
		PorxExp - Low<High=Mid						*PorxInt - Low<Mid<High*			
		PorxInt - Low<High=Mid						*PorxShe - Low<Mid=High*			
		PorxShe - Low=Mid<High									

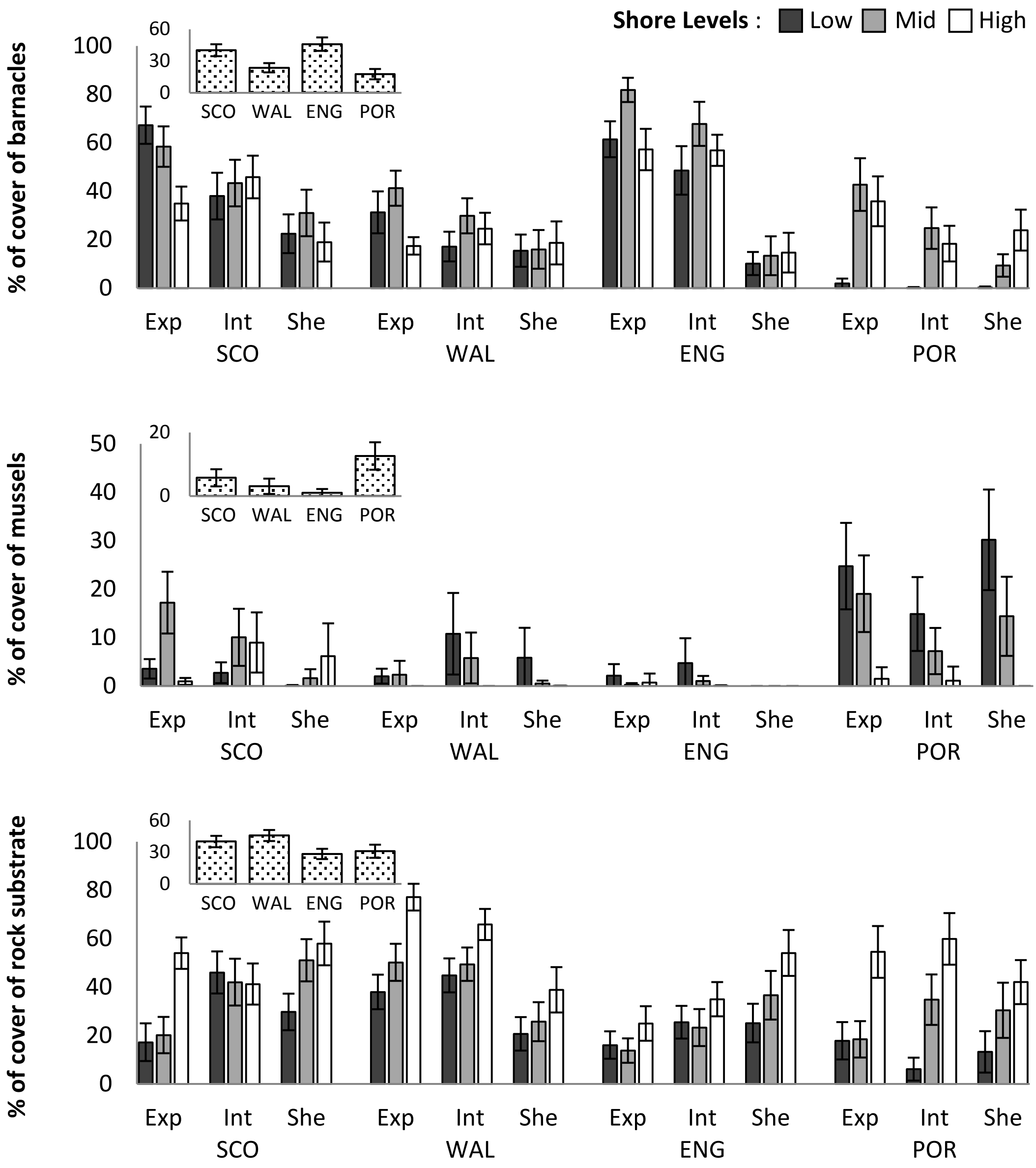


Figure 13: Percentage cover of barnacles, mussels and free rock substrate at Scottish (SCO), Welsh (WAL), English (ENG) and Portuguese (POR) regions across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)* and shore height levels *(high, mid and low)*. On the top right of each panel a histogram of density/biomass variation across the four geographical regions, where variability within each of the geographical regions is ignored, is shown; Error bars = ±1SE

2.3.6 Within and among shore patterns of variability (patchiness)

Total fucoid biomass and percentage cover levels, as well as, *Patella* spp. biomass and density levels were used to quantify variability within shore and variability among shores exposed to similar environmental conditions. To simplify the analysis for this section only the mid shore height data were used, as this shore level presented the greatest abundance of fucoid and limpet species.

The ANOVA's results for among shore variability showed that both fucoid and limpet levels varied similarly across the four geographical regions (Table 8, Figure 14a). However, when analysing amongst shore variability across wave exposure levels, fucoid variability amongst shores was lower on wave exposed conditions than on sheltered shores (Table 9: Fucoid biomass & percentage cover - *SNK of We*). In contrast, the densities of limpets varied less amongst sheltered shores (Table 9: Limpet density - *SNK of We*). This pattern across exposures was not observed for variability of biomass of limpets among shores, which showed similar variability across the three wave exposures (Table 9: Limpet biomass - $F_{(Gr)\ 2,\ 9} = 0.6$, p=0.556, Figure 14a).

Within shore variability of fucoid (biomass and percentage cover) and *Patella* spp. (biomass and density) were tested on shores with equal wave exposed conditions from the same geographical region. For fucoid biomass and percentage cover levels, a pattern across wave exposures is detectable (Figure 14b). So, for these parameters, low wave action promotes greater variability within shore, variability that decreases with the increase of wave action (Table 10: Fucoid biomass - *SNK of Re x We*; Fucoid percentage cover - *SNK of We*). The variability of fucoid biomass and percentage cover within shores, across geographical regions, was also detected (Table 10: Fucoid biomass - *SNK of Re x We*; Fucoid percentage cover - *SNK of Re*). Variability of fucoid percentage cover was generally different between Portuguese and Scottish areas (Table 10: Fucoid percentage cover - *SNK of Re*), while fucoid biomass within shore variability across geographical regions only occurred across sheltered shores (Table 10: FUC bio – SNK of Gr x We). For both parameters, Portuguese shores showed the lowest within shore variability with the highest levels occurring in Wales, for fucoid biomass present in sheltered conditions, and in Scotland for fucoid percentage cover (Figure 14b).

The variability of biomass and density of limpets that occurs within shores, across geographical regions, was only detected on wave protected areas (Table 10: Limpet biomass - *SNK of Re x We*; Limpet density - *SNK of Re x We*). On these sheltered shores, the lowest variability within shore was verified in Portugal, while the highest was detected on English shores for both parameters considered above (Figure 14b). Other factor that influenced within shore variability, on some geographical regions, was wave exposure. Limpet biomass varied similarly across wave exposures on the different geographical regions, apart from English shores, where within shore variability was higher on sheltered conditions (Table 10: Limpet biomass - *SNK of Re x We*, Figure 14b). Limpet density also varied similarly across wave exposures on Scottish and English shores but varied differently across wave exposures on Welsh and Portuguese shores, where sheltered conditions had the lowest variability (Table 10: Limpet density - *SNK of Re x We*, Figure 14b).

Table 8: One way ANOVA model testing the effects of geographical region origin on among shore variability of density of *Patella* spp. and biomass of total fucoids and *Patella* spp. (only at mid shore height). *Abbreviations used - Re: Geographical Region; Res: Residual.*

Source	**DF**	**Fucoid Biomass**			**Fucoid % Cover**			***Patella* spp. Biomass**			***Patella* spp. Density**			**F ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	3	9.0E+12	0.1	0.964	2.0E+05	0.6	0.630	22.4	1.0	0.431	941.5	0.5	0.692	Res
Res	8	1.0E+14			3.4E+05			21.9			1878.5			
Cochran's Test		*C = 0.5963, p > 0.05*			*C = 0.3863, p > 0.05*			*C = 0.7084, p > 0.05*			*C = 0.6320, p > 0.05*			

Table 9: One way ANOVA model testing the effects of wave exposure on among shore variability of density of *Patella* spp. and biomass of total fucoids and *Patella* spp. (only at mid shore height). Post hoc SNK tests of significant differences are presented. *Abbreviations used - We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Res: Residual*

Source	**DF**	**Fucoid Biomass**			**Fucoid % Cover**			***Patella* spp. Biomass**			***Patella* spp. Density**			**F ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
We	2	3.0E+14	11.2	**0.004**	9.1E+05	5.6	**<0.001**	6840.0	0.6	0.556	4.7	8.6	**0.008**	Res
Res	9	2.7E+13			1.6E+05			10898.2			0.5			
Cochran's Test		*C = 0.7026, p > 0.05*			*C = 0.6679, p > 0.05*			*C = 0.6504, p > 0.05*			*C = 0.7849, p > 0.05*			
SNK			*We* *Exp=Int<She*			*We* *Exp <She*						*We* *She<Exp=Int*		

Table 10: Two way fix ANOVA model testing the effects of geographical region origin and wave exposure on within shore variability of density of *Patella* spp. and biomass of total fucoids and *Patella* spp. (only at mid shore height). Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales; Eng: England; Por: Portugal); We: Wave exposure (Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered); Res: Residual.*

Source	**DF**	**Fucoid Biomass**			**Fucoid % Cover**			***Patella* spp. Biomass**			***Patella* spp. Density**			**F ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	3	3330.5	9.4	<0.001	6.9	5.5	**0.002**	0.3	1.6	0.200	2.7	3.6	0.016	Res
We	2	22299.5	19.0	0.003	40.4	31.6	**<0.001**	1.3	1.3	0.352	8.1	3.1	0.118	Re x We
Re x We	6	1174.9	3.3	**0.005**	2.3	1.7	0.109	1.1	6.1	**<0.001**	2.6	3.5	**0.003**	Res
Res	108	355.3			1.3			0.2			0.7			
Cochran's Test		*C = 0.1996, p > 0.05*			*C = 0.1985, p > 0.05*			*C = 0.1867, p > 0.05*			*C = 0.2062, p > 0.05*			
SNK		*Re x We*			*Re*			*Re x We*			*Re x We*			
		She - Por<Eng=Sco<Wal			*Por<Sco*			*She - Por=Wal=Sco<Eng, Por<Sco*			*She - Por=Wal<Sco=Eng*			
		Sco - Exp<Int<She						*Eng - Exp=Int<She*			*Wal - She<Int=Exp*			
		Wal - Exp=Int<She			*We*						*Por - She<Int=Exp*			
		Eng - Exp=Int<She			*Exp<Int<She*									

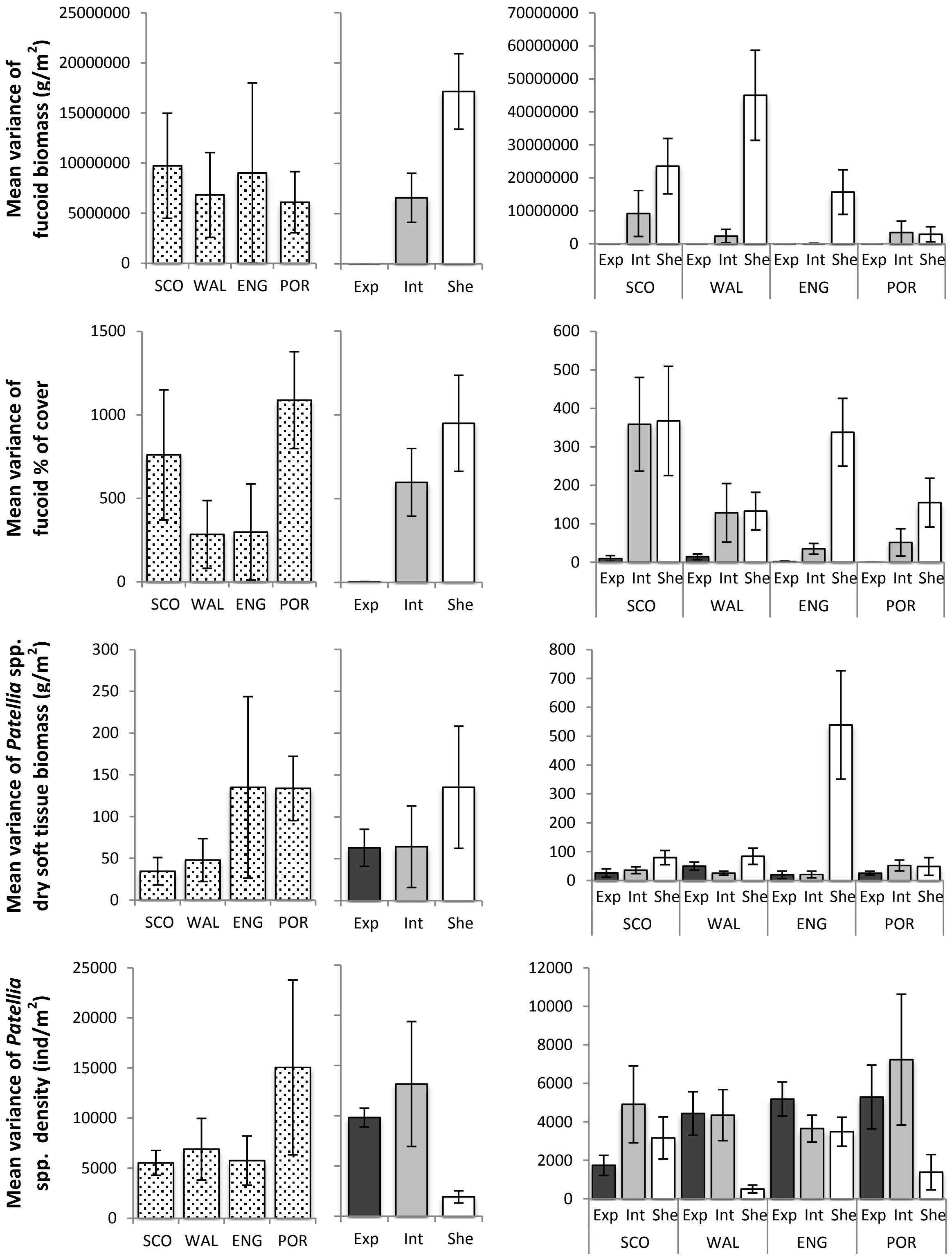


Figure 14: a) Among shore variance of total fucoid biomass, total fucoid percentage cover, limpet biomass and limpet density at mid shore height at Scottish (SCO), Welsh (WAL), English (ENG) and Portuguese (POR) regions and across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)*. b) Within shore variance of total fucoid biomass, total fucoid percentage cover, limpets biomass and limpets density at mid shore height at Scottish (SCO), Welsh (WAL), English (ENG) and Portuguese (POR regions across different wave exposures *(Exp: Wave exposed; Int: Intermediate exposed; She: Sheltered)*; Error bars = ±1SE

2.4 DISCUSSION

The overall results of this large quantitative survey confirmed that fucoid abundance is reduced in southern Europe. However, there was no clear pattern of decline over the length of the British Isles, as expected, and the hypothesised link between grazing pressure and fucoid abundance on southern regions was not apparent. In addition, the results do not confirm the previously described increase in filter feeder abundance at lower latitudes (Ballantine 1961; Hawkins *et al.* 1992; Southward *et al.* 1995). My results show that simple descriptive qualitative studies, although important for getting a general idea of species distribution, are not sufficient to get a complete picture of the complex and variable patterns that occur across large spatial latitudinal scales.

My large scale survey allowed me to disentangle the effects of small, medium and large scale factors that impact community structure, namely vertical height on the shore, the horizontal gradient of wave exposure and latitude. Changes in community composition and structure across large spatial latitudinal scales, as the results show, can be similar to the changes observed at scales of only hundreds of meters, due to changes in wave action levels (e.g. decline of total fucoid biomass from the United Kingdom to Portugal and from sheltered to wave exposed conditions). So, to evaluate the consequences of latitudinal variation it was essential to independently assess wave action on all shores included in the survey and ensure an even distribution of shore selection from exposed to shelter. Biological indices for the categorization of wave exposure have long been used in Europe (Ballantine 1961). Although practical in the field, biological indices were not used in this study for a number of reasons. Firstly, in planning a large scale survey and ensuring coverage across the wave exposure gradient an approach is required which *a priori* defines the level of exposure. Secondly, biological indices have the problem of circularity of reasoning, “a shore is exposed because it has a certain community pattern and it has this pattern because it is exposed” as stated by Ballantine (1961). Another alternative method would be the deployment of accurate wave force dynamometers, simple spring devices that allow the measurement of the maximum force and maximum water velocity imposed at the place of deployment (Denny 1983; Bell & Denny 1994; Denny & Wethey 2001). Although reliable, such an approach proved to be far too expensive and time-consuming to be considered and again suffers from the problem of preventing classification of shores before they are surveyed. In addition, small scale measures of wave force (cms) can be extremely difficult to use in applying classifications of wave exposure over larger scales (100s of meters). Therefore, the use of topographical indices, previously used by Thomas (1986) and recently developed by Burrows *et al.* (2008), proved to be the essential tool to achieve the proposed aim.

Burrows *et al.* (2008) showed that the wave fetch index is very reliable and able to predict and capture the spatial variation in wave exposure, explaining more than 50 % of the variation of species-site abundance matrix over a study encompassing 57 species surveyed at 185 sites throughout west Scotland. Using this index of wave fetch in the survey, allowed the independent quantification of wave exposure across the large geographic area that encompassed the survey, which was an indispensable requirement to be able to independently separate the effect of wave exposure from the effects of large latitudinal variation. During the survey, variability in levels of intertidal organisms along shores of different geographical regions was similar, but differed across wave exposures. This also suggests that the wave exposure index used to independently choose the shores was able to create differences between the classes of exposure, which were maintained across the four geographical regions.

Simplification of data acquisition by using photographic methods also proved to be successful as the survey focused mainly on functional groups. The identification of organisms to species level was only required for large fucoid species that could be identified with the resolution of the images obtained. Due to the short time window for sampling in the intertidal area, the use of photography was essential to extend the number of shores visited, allowing a more comprehensive view of community composition across wave exposures on the four geographical regions included in the survey.

It was hypothesised, based on previous work (Ballantine 1961; Jenkins *et al.* 2001), that one of the key contributory factors in the decline of fucoids at lower latitude is the possible increase of molluscan grazer density, particularly patellid species. However, the expected increase of *Patella* spp. density and biomass in southern regions was not detected in the survey. Grazer exclusion experiments in southern areas present a complex picture with some indicating a limited role for grazing (Benedetti-Cecchi *et al.* 2000; Benedetti-Cecchi *et al.* 2001; Coleman *et al.* 2006) but others indicate that patellid limpets still exert strong top down control (Jenkins *et al.* 2001; Boaventura *et al.* 2002a; Arrontes *et al.* 2004). The absence of a clear response to the removal of grazers in some experiments in southern areas probably reflects the low reproductive output and abundance of fucoid algae in the south (Coleman *et al.* 2006; Viejo *et al.* 2011). One area that requires clarification is the extent to which density or biomass of patellid limpets can be used as a surrogate for grazing pressure. Jenkins *et al.* (2001) demonstrated that grazing pressure detected through the use of wax discs (Thompson *et al.* 1997) could be higher in southern regions. However, although densities of limpets were documented, biomass was not. The present survey showed that density and biomass of limpets is not higher in Portugal than in the United Kingdom. However, the lack of examination of how biomass translates into grazing activity prevents firm conclusions regarding the change in grazing pressure with latitude especially in the light of output from Jenkins et al (2001). Whether the same density/biomass of limpets in Portugal as in Wales, for example, equates to the same level of grazing activity is not clear. Higher temperatures could easily result in a rise in metabolic rates and hence greater grazing pressure to the south. The size distribution of limpets also clouds the issue. Do mature limpets with high biomass exert grazing pressure proportional to their size? Clearly further work is still needed to fully understand how biomass in each of the geographic regions translates into grazing pressure.

While the levels of *Patella* spp. densities and biomass did not seem to be the main reason for the decline of fucoid biomass on southern regions, its effect on northern regions could not be discarded. Their key role in controlling macroalgal development on northern European shores has long been recognised by several studies (Jones 1946; Hawkins 1983; Boaventura *et al.* 2002a; Jenkins *et al.* 2005; Coleman *et al.* 2006; Davies *et al.* 2007). In their absence, huge changes in community structure occur in northern areas with colonization of ephemeral and opportunistic species followed by establishment of fucoid species previously absent from these areas (Jones 1946; Southward & Southward 1978; Hawkins 1981a; Hawkins & Hartnoll 1985; Jenkins *et al.* 2005). The strength of this relationship was also demonstrated by this study when fucoid biomass was compared with limpet density or biomass data from Scottish, Welsh and English shores collected during the survey.

The effects of latitude were detected in the abundance levels of several of the functional groups studied. The decline in fucoid abundance from northern to southern regions and a consequent increase in the dominance of filter feeder community, as proposed (Ballantine 1961; Southward *et al.* 1995; Hawkins *et al.* 2009) were only partly observed. The survey confirmed the expected decline in total fucoid biomass and percentage cover but the expected increase in filter feeder was not verified. The decline in total fucoid biomass was also expected to be progressive from the most northern to the most southern area. However, British regions unexpectedly showed similar levels. There was a steady decline in *F. vesiculosus* biomass from Scotland to England, but when the patterns of biomass of the other fucoid species are combined they counter such a decline, leading to regions within the United Kingdom with similar fucoid abundance. So, total fucoid biomass across the United Kingdom was found to be similar but with different proportions of specific fucoid species.

The analysis of the patterns of individual fucoid species seems to indicate that the decline in total fucoid biomass in the Portuguese shore is mainly due to the absence of *Ascophyllum nodosum* and *Fucus serratus* specimens. The absence of these species, which reach their southern limit of distribution in northern areas of the Portuguese coast (areas outside the surveyed zone) (Lima *et al.* 2007; Araujo *et al.* 2009), could be due to a combination of other abiotic or biotic factors. More demanding weather conditions, with warmer and drier periods that may lead to failure in recruitment (Thompson *et al.* 2004; Viejo *et al.* 2011), or the presence of strong competition for space with other functional groups, like turf species that actually have the greatest percentage cover on Portuguese shores, could lead to the absence of these northern fucoid species, *Ascophyllum nodosum* and *Fucus serratus*.

The absence of a consistent pattern of barnacle abundance along the four geographical regions was also noted, showing that several factors other than changes of latitude are affecting the abundance of these filter feeders. An explanation, could lie in the levels of phytoplankton concentration and larvae supply that are greatly affected by ocean currents and upwelling zones. Burrows *et al.* (2010) recently showed that such factors are especially important for the abundance levels of barnacles across the UK. Despite the inconsistent results across the four regions, the effect of wave exposure within each of the areas was consistent with patterns proposed by Ballantine (1961), showing that barnacle cover consistently increased with rising wave action. The reasons for the decline in barnacle abundance with decreasing wave exposure have been the subject of many studies. Jenkins and Hawkins (2003) show evidence of interference in supply of larvae to the rock surface by dense macroalgal canopies on sheltered shores. Additionally, it is well known that macroalgae have a significant negative impact on barnacle settlement through sweeping effects (Hawkins 1983; Jenkins *et al.* 1999c).

Focusing on the variation across the main gradients being surveyed, it is possible to observe that differences in productivity will be expected as changes in trophic structure are accompanied by changes in energy flow (O'Connor & Crowe 2005). Over larger scales, primary productivity across Europe is also expected to reflect the variability in community structure. The combination of patterns observed across the survey seems to indicate that the most southern intertidal region surveyed, Portugal, displays a lower export of algal based detritus and therefore is expected to act more as a net importer system than the regions from the United Kingdom.

In summary, the survey here presented provides a broad quantitative view of how patterns and community structure varies across European intertidal areas, while taking into consideration variability caused by wave exposure and vertical shore position. The quantification of large scale surveys is time consuming and demands a great input of energy and resources but provides unique opportunities to improve the knowledge of natural patterns of distribution and abundance and are the basic tool for the understanding of more complex ecological processes. The increased amount of work and time spent on the shore are unequivocally important, as acquisition of quantitative data will allow the improvement of comparisons across time and scale in the future. Due to the influence of climate change on the intertidal rocky shores (Harley *et al.* 2006; Helmuth *et al.* 2006b; Lima *et al.* 2007; Hawkins *et al.* 2009) and the increased need for quantitative data to inform conservation projects, the construction of datasets that allow the comparison of data is of the highest importance, as older studies can become a source of historical quantitative datasets for future comparisons. Future experimental work should focus on quantifying the relative importance of processes that act at large, medium and small scales and how these interact with each other to promote and shape the patterns observed.

CHAPTER 3

THE EFFECTS OF GRAZING AND ENVIRONMENTAL CONDITIONS IN FUCOID RECRUITMENT ON NORTHERN AND SOUTHERN EUROPEAN SHORES

3.1 INTRODUCTION

Fucoid species are important intertidal ecosystem engineers and primary producers; hence they are very important for community structure and ecosystem functioning. Due to their size and 3D structure, fucoid canopies are able to influence the physical environment, providing microhabitats that are utilized by other organisms. Fucoid canopy algae have been shown to modify the presence and activity of other organisms in the intertidal through a range of mechanisms including amelioration of temperature and desiccation extremes (Moore *et al.* 2007a), the reduction of wave action (McCook & Chapman 1991) and the reduction of light radiance levels (Jenkins *et al.* 1999a). Sweeping effects of fucoid fronds across the substratum can also have a strong community structuring role (Hawkins 1983; Jenkins *et al.* 1999c).

Fucoid populations occur over much of the European coast, but their abundance declines with declining latitude towards their southern limit of distribution (Chapter 2; Fischer-Piétte 1955, 1957; Ballantine 1961; Ardré 1970; Hawkins & Hartnoll 1983; Hawkins & Jones 1992). These range edge populations live in sub-optimal conditions that normally lead to the development of smaller and more fragmented populations (Guo *et al.* 2005; Bridle & Vines 2007; Araujo *et al.* 2011). The reasons for the decline of fucoid populations and consequent changes in community structure across such latitudinal scales are not clearly understood, but are likely to be the result of a combination of physical and biological processes (Hawkins & Hartnoll 1983; Hawkins & Jones 1992; Menge 2000b; Underwood 2000; Jenkins *et al.* 2008). As sessile organisms living in a tidally dominated environment, fucoid species are adapted to variable physical environmental conditions (Southward 1958; Lubchenco 1980; Norton 1983), although differences in tolerance to stress (Stephenson & Stephenson 1949; Schonbeck & Norton 1978) interacting with competition (Schonbeck & Norton 1980; Hawkins & Hartnoll 1985) and grazing (Hartnoll & Hawkins 1985; Boaventura *et al.* 2002a) set vertical patterns of zonation. The effect of minimum and maximum temperatures combined with extreme climate events, such as unusually cold or warm periods or storms, are important in setting the geographic distribution and range limits of seaweeds (Crisp 1964; Lüning 1984; Underwood 1999; Adey & Steneck 2001; Wethey *et al.* 2011). Other physical factors like solar radiation can also be extremely important. Both low and high solar radiation levels can control macroalgal species distribution by limiting or damaging their photosynthetic system (Powles 1984; Creed *et al.* 1997; Aguilera *et al.* 1999). In addition to physical control of macroalgal distribution and population structure (Arrontes 1993; Altamirano *et al.* 2003), biological interactions, especially top down control by sedentary grazers, can also have a strong effect on the abundance and distribution of algae species. Removal of top down herbivore control can lead to a strong increase in the abundance of prey. Such an increase can lead to further disequilibrium in the ecosystem owing to enhanced primary production (Lubchenco 1978; Dethier & Duggins 1988; Paine 2002). Work developed since the 1940s in Britain (Jones 1946; Hawkins 1981a; Hawkins & Hartnoll 1983; Hartnoll & Hawkins 1985; Jenkins *et al.* 1999b; Boaventura *et al.* 2002a; Thompson *et al.* 2004; Jenkins *et al.* 2005; Jonsson *et al.* 2006) has shown how grazing can be an important factor in controlling fucoid recruitment especially at moderately exposed and exposed shores. In southern Europe however, probably due to differences in the recruitment levels of algae and harsher environmental stress, grazing pressure though influential has more unpredictable effects (Benedetti-Cecchi *et al.* 2001; Jenkins *et al.* 2005; Coleman *et al.* 2006).

Physical and biological processes are especially important in determining survival rates of early fucoid life stages in the rocky intertidal (Thompson *et al.* 2004). These seem to be the most critical stages for the maintenance of viable fucoid populations (Santelices 1990; Vadas *et al.* 1992). Intertidal fucoid recruits are delicate structures that are subjected to severe and variable physical factors and are part of the diet of multiple intertidal grazer species (Lubchenco 1980; Jenkins *et al.* 2001). Their fragile mechanisms of protection and resilience against such environmental stresses, when compared with adult algae, makes them specially susceptible to high mortality leading to a possible bottleneck effect in successful survival of fucoid species (Santelices 1990; Lotze *et al.* 2001; Jenkins *et al.* 2005).

Therefore, it seems the decline of fucoid species abundance from central to range edge populations (Chapter 2) could be due to biological or physical effects, in isolation or combined, and that their impact could be greater on the more vulnerable early recruitment stages. The ability to disentangle the effects of possible controlling mechanisms on early mortality is the basis for the development of this experiment. This study aims to explore the role played by different levels of *Patella* spp. grazing pressure combined with different levels of light radiance in the control of fucoid settlement and recruitment at both northern (central range) and southern (range edge) mid wave-exposed shores of western Europe. I investigated the general hypothesis that removal of limpets at both locations will result in greater macroalgae recruitment success. Many factors can affect the effectiveness of grazers and recruitment of fucoids (Vadas *et al.* 1990; Jenkins & Hartnoll 2001; Arrontes 2002), but taking into consideration information from previous studies (Jenkins *et al.* 2005; Coleman *et al.* 2006) it was predicted that removal of grazers will have a greater impact on fucoid recruitment in northern shores. It was further predicted that the response of fucoid recruitment to reduction of light intensity levels would be higher on the Portuguese shores, where harsher conditions during the summer season are expected to greatly affect fitness of fucoid recruits (Pearson *et al.* 2009).

3.2 METHODS

3.2.1 Study areas

Manipulative experiments were conducted on four rocky shores in two geographical regions separated by 11° of latitude. The geographical regions were northern Portugal and northern Wales, each represented by two shores separated by a minimum of 10 km, Carreço (41.729259°, -8.873483°), Vila Praia de Âncora (41.823255°, -8.875501°), Porth Cwyfan (53.180296°, -4.489920°) and Cemlyn Bay (53.414938°, -4.517396°), respectively. These fully saline rocky shores were selected because of their similar geomorphology, wave exposure and the presence of patches of fucoids and limpets on rocky surfaces with gentle slopes (<45°). Maximal tidal range varied between 3.8 m and 6.3 m on the Portuguese and Welsh shores, respectively. To determine factors controlling fucoid populations under field conditions, plots were spread within the equivalent biological zone in Portugal and Wales (between 2.1 to 4.7 m above Chart Datum in Wales and 2.3 to 3.2 m above Chart Datum in Portugal), so they were emersed for similar periods of time at both locations. *Fucus vesiculosus* and *Fucus spiralis* were the fucoids species present at both locations at these tidal levels.

3.2.2 Experimental designs

To test for the effect of light intensity and grazing level on fucoid recruitment at these two geographical regions an experimental design composed of 9 treatments with 4 replicates each was used. The design allowed testing for the combined effect of 3 different levels of limpet grazing pressure (natural densities of grazers, half densities and no grazers present) across 3 different light intensities (natural radiance levels, shaded plots and a procedural control consisting of a transparent perspex cover). In order to show generality across shores and geographical regions the design was replicated in two shores in Portugal and two shores in Wales.

3.2.3 Experimental setup and sampling

On each shore 36 randomly spread plots were created in areas of rock with no fucoid presence at the specified tidal height. The 9 treatments were randomly allocated to experimental plots. In order to create plots with reduced light intensity, a plastic mesh (model: Figured 45 from Conwed plastics Ltd.) that provided a reduction in photosynthetically active radiation (PAR) of 84 % on cloudy and 80 % on sunny days (Rauh 2006) was used in a third of the plots. To control the effects of this shading apparatus, another third of the plots were covered with a 4 mm perforated clear perspex sheet that acted as a procedural control. This material was used because it allows light to reach the plots, while creating a similar physical barrier to water movement as that created by the plastic mesh. Grazing pressure was manipulated using grazer inclusion fences. After estimating density of *Patella* grazers for each shore, plots with natural, half and no grazing pressure were attained by removal of limpets from the plots. Natural grazing levels varied between shores, with mean values in Portugal reaching 131 limpets/m^2 in Vila Praia de Âncora and 77 limpets/m^2 in Carreço and in Wales reaching 155 limpets/m^2 in Porth Cywfan and 95 limpets/m^2 in Cemlyn Bay. Only limpets larger that 1.3 cm were used in the study. Inclusion fences were created using 3 cm high fences of plastic coated wire mesh (mesh size 13 mm). To determine the effect of limpet inclusion, an additional 4 plots marked only by screws in the corners were created allowing grazing to occur at normal levels. The results from these plots would be compared with results from fenced plots with natural levels of grazing to test for the effect of fencing.

The experiment started at the beginning of May 2010, ahead of the peak in fertility (Knight & Parke 1950; Niemeck & Mathieson 1976; Robertson 1987; Berger *et al.* 2001) and recruitment (Hawkins 1981a; Jenkins *et al.* 2005; Coleman *et al.* 2006) of both *Fucus vesiculosus* and *Fucus spiralis*. From May 2010 to May 2011, macroalgae were allowed to establish and grow on plots under the manipulated grazing and light radiance treatments. Measurements of density of *Fucus* spp. recruits were made at all shores at 3 different dates: 70, 145 and 365 days after deployment. *Fucus* spp. Designation was used due to difficulties in distinguishing juveniles of different fucoid species in the field. After 1 year, all the fucoid individuals were destructively sampled to measure density and dry weight of fucoids from each of the plots. During the entire period of the experiment, plots on every shore were checked, fences and shades repaired if needed and limpet densities assessed and adjusted when required.

3.2.4 Statistical analysis

Differences in total weight after 1 year, plus differences in densities of fucoid species over the three sampling dates were evaluated using a mixed analysis of variance model (ANOVA) with 4 factors. Comparisons were done among geographical regions (fixed, 2 levels), shores (random nested in geographical regions, 2 levels), light intensity (fixed, 3 levels) and grazing pressure (fixed, 3 levels). Results from different sampling dates were not compared due to the non-independence of repeatedly sampled plots. In designing the experiment, procedural controls were also used to determine whether artefacts were introduced by the use of perspex screens and fencing material. These procedural artefacts were tested for with post hoc tests of significant factors. Prior to analysis, heterogeneity of variance of the data was tested with Cochran’s test and when necessary, transformation of heterogeneous data was undertaken. For further analysis of significant factors post hoc Student-Newman-Keuls (SNK) tests were applied. Only SNK results showing differences are displayed in the results section. All the analyses were performed using GMAV5 software (Institute of Marine Biology, University of Sydney, Australia)

3.3 RESULTS

3.3.1 Experimental artefacts

The use of shading apparatus and the control of grazing by use of inclusion cages could introduce experimental artefacts. However, the control areas (natural level of grazing in unfenced plots) did not differ significantly from inclusion plots at natural grazing levels for any of the response variables (fucoid density at 70, 145 and 365 days and fucoid weight at one year), thus indicating that the behaviour of limpets was not modified to a significant extent in inclusion plots (Table 11: Density and Weight of fucoids (1Y) - *non-significant fence treatment*). Similarly, there was no obvious artefact introduced by the use of shading structures; no significant difference was found between unshaded plots and those where the shade procedural control (perspex covers) were used (Table 12 & Table 13: Density of fucoids (70D, 145D and 1Y) and Weight of fucoids (1Y) - *non-significant light intensity treatment*).

3.3.2 Densities of fucoids (across the entire period)

The levels of natural fucoid recruitment varied between geographical regions across all sampling dates (Table 12: Density of fucoids (70D, 145D and 1Y) - *SNK of Re x Gr*, Figure 15). Ungrazed Welsh areas showed significantly greater mean recruitment levels compared to ungrazed Portuguese areas (Table 12: Density of fucoids (70D, 145D and 1Y) - *SNK of Re x Gr*, Figure 15). After 1 year on the shore ungrazed Welsh plots showed mean recruitment levels greater than 1000 /m^2, while for similar areas on Portuguese shores mean recruitment was very low, not exceeding 10 /m^2.

Providing increased shade protection to reduce exposure to light radiance did not promote increased levels of fucoid recruitment at either geographical region (Table 12: Density of fucoids (70D, 145D and 1Y) - *non-significant light intensity treatment*, Figure 15). In Portugal there was also no significant effect of decreased grazing pressure, leading to similar levels of recruitment occurring across all treatments on each of the Portuguese shores (Table 12: Density of fucoids (70D, 145D and 1Y) - *SNK of Re x Gr*, Figure 15). In contrast there was a significant effect of grazing on fucoid densities at all sampling dates on Welsh shores (Table 12: Density of fucoids (70D, 145D and 1Y) - *SNK of Re x Gr,* Figure 15). Absence of grazing pressure at Welsh plots promoted the survival of significantly higher numbers of fucoid recruits (mean densities after 70 days on the shore where > to 700 ind/m^2) compared with grazed plots (mean densities after 70 days on the shore where < to 5 ind/m^2). It is also interesting to point out that the removal of half the limpets from Welsh plots did not significantly increase recruitment levels, when compared with full grazing pressure plots (Table 12: Density of fucoids (70D, 145D and 1Y) - *SNK of Re x Gr*, Figure 15).

Differences in recruitment levels between shores within each geographical region also occurred (Table 12: Density of fucoids (70D, 145D and 1Y) - *SNK of Gr x Sh (Re)*). After one year on the shore in Portugal, higher densities of fucoid recruits occurred at Carreço than at Vila Praia de Âncora across treatments. In Wales, significant differences were only observed between ungrazed plots, showing higher recruitment densities at Cemlyn Bay than at Porth Cwyfan (Table 12: Density of fucoids (1Y) - *Gr x Sh (Re)*, Figure 15).

Table 11**:** Mix model ANOVA of density and weight of fucoids produced after 1 year on the shore to test the effect of fence. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales); Sh: Shore; Fe: Fence; Res: Residual.*

Source	**df**	**Density of fucoids (1Y)**			**Weight of fucoids (1Y)**			***F* ratio vs**
		MS	***F***	**p**	***MS***	***F***	**p**	
Re	1	24.8	0.6	0.535	7.6	0.6	0.518	Sh (Re)
Sh (Re)	2	44.8	5.4	0.012	12.5	4.1	0.031	Res
Fe	1	18.0	1.9	0.306	3.7	1.9	0.305	Fe x Sh (Re)
Re x Fe	1	1.4	0.1	0.743	0.3	0.1	0.752	Fe x Sh (Re)
Fe x Sh (Re)	2	9.7	1.2	0.331	2.0	0.7	0.533	Res
Res	24	8.3			3.1			
Cochran test		*C = 0.4343, p > 0.05*			*C = 0.4300, p > 0.05*			

Table 12: Mix model ANOVA of density of fucoids produced after 70, 145 and 365 days on the shore. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales); Sh: Shore (Vil: Vila Praia de Âncora; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Li: Light intensity; Gr: Grazing level (-G: Ungrazed; ½G: Half grazing pressure; +G: Grazed at natural densities) Res: Residual.*

Source	**df**	**Density of fucoids (70D)**			**Density of fucoids (145D)**			**Density of fucoids (1Y)**			***F* ratio vs**
		MS	***F***	**p**	***MS***	***F***	**p**	***MS***	***F***	**p**	
Re	1	126.9	8.8	0.097	93.7	2.1	0.281	99.1	1.7	0.325	Sh (Re)
Sh (Re)	2	14.5	6.6	0.002	43.7	16.8	<0.001	59.3	15.7	<0.001	Res
Li	2	2.6	3.1	0.149	1.5	2.6	0.191	7.0	2.5	0.199	Li x Sh (Re)
Gr	2	136.1	25.3	0.005	192.4	25.5	0.005	342.5	30.7	0.004	Gr x Sh (Re)
Re x Li	2	0.2	0.2	0.823	0.1	0.2	0.802	6.4	2.3	0.219	Li x Sh (Re)
Re x Gr	2	126.0	23.4	0.006	200.5	26.5	0.005	271.4	24.3	0.006	Gr x Sh (Re)
Li x Sh (Re)	4	0.8	0.4	0.832	0.6	0.2	0.924	2.8	0.8	0.562	Res
Gr x Sh (Re)	4	5.4	2.5	0.050	7.6	2.9	0.025	11.1	2.9	0.024	Res
Li x Gr	4	0.8	0.8	0.573	1.6	1.5	0.298	1.5	0.6	0.650	Gr x Li x Sh (Re)
Re x Li x Gr	4	2.5	2.4	0.136	2.6	2.4	0.140	6.0	2.6	0.120	Gr x Li x Sh (Re)
Gr x Li x Sh (Re)	8	1.0	0.5	0.875	1.1	0.4	0.904	2.4	0.6	0.758	Res
Res	108	2.2			2.6			3.8			
Cochran's test		*C = 0.1414, p > 0.05*			*C = 0.1579, p > 0.05*			*C = 0.157, p > 0.05*			
SNK		*Gr x Sh (Re)*			*Gr x Sh (Re)*			*Gr x Sh (Re)*			
		Cwy / Cem - +G = ½ G < -G			*Cwy / Cem - +G = ½ G < -G*			*Cwy / Cem - +G = ½ G < -G*			
		-G - Vil < Car			*-G - Vil < Car*			*-G - Vil < Car*			
		-G -Cwy < Cem			*½G - Vil < Car*			*½G - Vil < Car*			
		Re x Gr			*+G - Vil < Car*			*+G - Vil < Car*			
		-G - Por < Wal			*-G -Cwy < Cem*			*-G -Cwy < Cem*			
		Wal - +G = ½ G < -G			*Re x Gr*			*Re x Gr*			
					-G - Por < Wal			*-G - Por < Wal*			
					Wal - +G = ½ G < -G			*Wal - +G = ½ G < -G*			

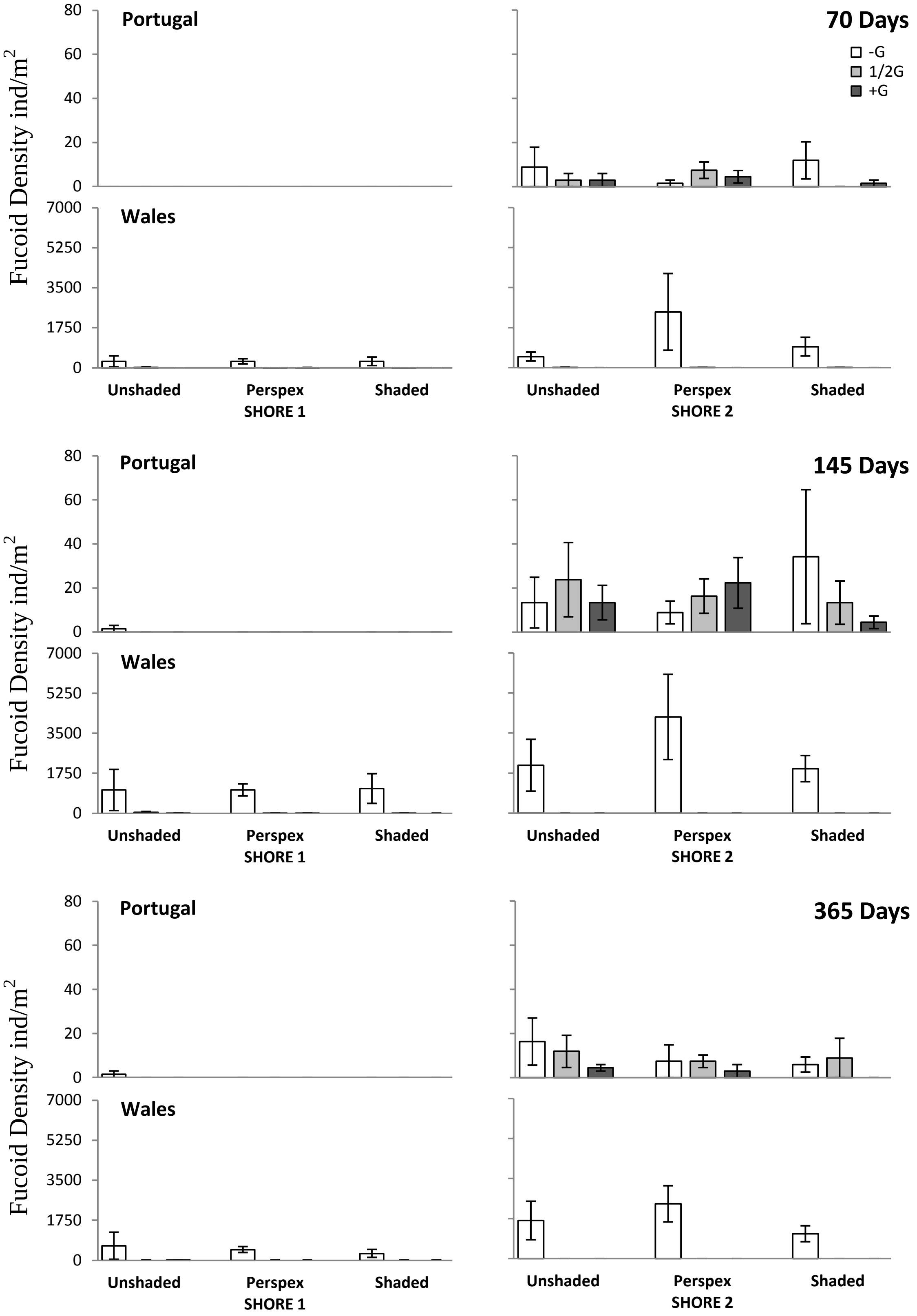


Figure 15: Densities of fucoid (ind/m^2) produced after 70 (a), 145 (b) and 365 (c) days on the shore under different light intensities and grazing treatments *(-G: Ungrazed; ½G: Half grazing pressure; +G: Grazed at natural densities)* at two geographical regions each represented by 2 shores *(Sh1: Vila Praia de Âncora (Portugal), Porth Cwyfan (Wales); Sh2: Carreço (Portugal), Cemlyn Bay (Wales)*. Error bars = ±1SE.

3.3.3 Fucoid biomass (after 1 year)

The data on biomass after one year reflects densities of individual plants: ungrazed plots in Wales had significantly higher levels of fucoid biomass (mean weight 221.27g/m^2) than any other grazing treatment independently of the light intensity applied (Table 13: Weight of fucoids (1Y) - *SNK of Re x Gr*, Figure 16). In Portugal biomass production was low and not significantly different across all the treatments. However in contrast, the complete removal of grazers in Wales greatly enhanced fucoid biomass production; 4.5 times that in ½ grazed or grazed plots (Table 13: Weight of fucoids (1Y) - *SNK of Re x Gr*, Figure 16). Increased shading protection did not, however, promote higher recruitment at either geographical region (Table 13).

Table 13: Analysis of variance of fucoid biomass after 1 year on the shore. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Sco: Scotland; Wal: Wales); Sh: Shore; Li: Light intensity; Gr: Grazing level (-G: Ungrazed; ½G: Half grazing pressure; +G: Grazed at natural densities) Res: Residual.*

Source	**df**	**Weight of fucoids (1Y)**			***F* ratio vs**
		MS	***F***	**p**	
Re	1	98.5	1.5	0.351	Sh (Re)
Sh (Re)	2	67.8	10.4	<0.001	Res
Li	2	4.2	0.6	0.609	Li x Sh (Re)
Gr	2	320.0	61.1	0.001	Gr x Sh (Re)
Re x Li	2	7.1	0.9	0.463	Li x Sh (Re)
Re x Gr	2	237.4	45.3	**0.002**	Gr x Sh (Re)
Li x Sh (Re)	4	7.5	1.2	0.334	Res
Gr x Sh (Re)	4	5.2	0.8	0.525	Res
Li x Gr	4	5.8	0.7	0.616	Gr x Li x Sh (Re)
Re x Li x Gr	4	6.9	0.8	0.545	Gr x Li x Sh (Re)
Gr x Li x Sh (Re)	8	8.4	1.3	0.258	Res
Res	108	6.5			
Cochran's test		*C = 0.43, p > 0.05*			
SNK		*Re x Gr*			
		-G - Por < Wal			
		Wal - +G = ½ G < -G			

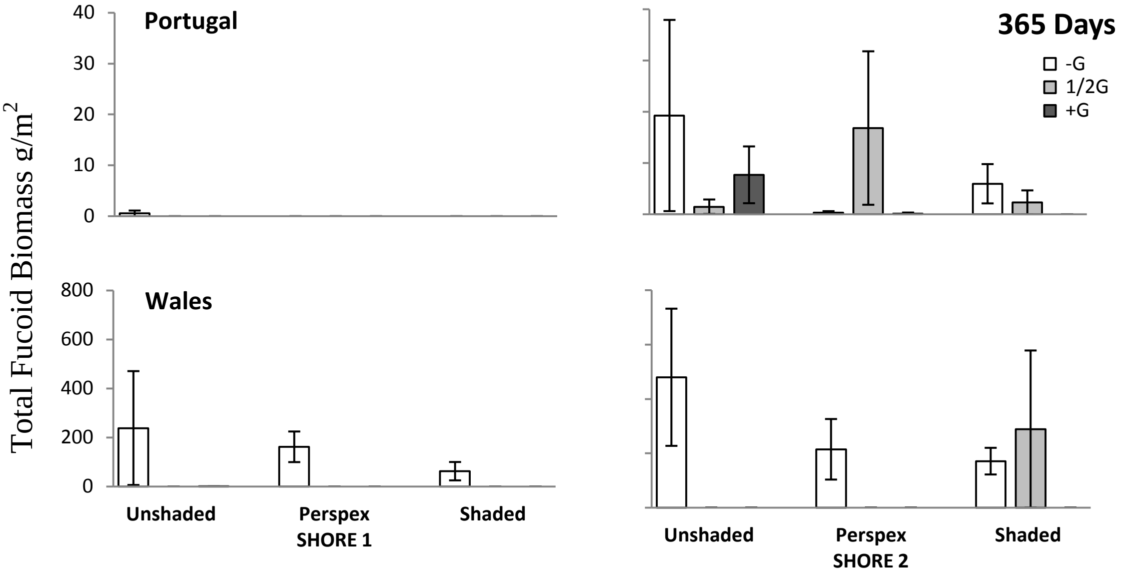


Figure 16: Total fucoid biomass (g/m^2) produced after 365 days on the shore under different light intensities and grazing treatments *(-G: Ungrazed; ½G: Half grazing pressure; +G: Grazed at natural densities)* at two geographical regions each represented by 2 shores *(Sh1: Vila Praia de Âncora (Portugal), Porth Cwyfan (Wales); Sh2: Carreço (Portugal), Cemlyn Bay (Wales)*. Error bars = ±1SE.

3.4 DISCUSSION

The study here presented shows a clear difference in fucoid (*Fucus vesiculosus and Fucus spiralis*) settlement/recruitment levels between Welsh and Portuguese shores. In the absence of grazing pressure by limpets, central populations developed higher numbers of fucoid recruits than range edge populations, independently of the level of light intensity present. These increased levels of recruitment in central populations have also been reported for *F. serratus* growing under natural conditions (Araujo *et al.* 2011), which seems to indicate that these fucoid species have lower recruitment success in range edge populations. Such differences could be promoted by greater abundance or fitness of fucoid adults from central shores, which would result in improved reproductive capacity and higher propagule availability (Mathieson & Guo 1992; Worm *et al.* 2001). They could also be linked with lower survival rate of fucoid recruits in Portugal due to exposure to harsher summer physical environments (Ladah *et al.* 2003; Pearson *et al.* 2009) or even due to the presence of different grazer species affecting recruitment in Portugal (e.g. mobile fish species not controlled by my experimental design, Ruitton *et al.* (2000)). Any of these factors could be causing the differences observed, but could not be resolved with the implemented experimental design. Therefore, further investigation through experimental studies is still needed to understand the cause of such variability.

Assessing the effect of biological and physical processes on fucoid recruitment across Europe proved to be challenging. The ability to reduce solar radiance levels, especially during harsh summer conditions in Portuguese shores, was expected to influence survival of fucoid recruits. Harsher environmental summer conditions have been pointed out as a possible reason for the decline in algae abundance in southern Europe (Jenkins *et al.* 2001; Coleman *et al.* 2006). Controlling solar radiance levels on this experiment was expected to influence fucoid recruitment. Surprisingly, different light intensity levels did not significantly affect fucoid recruitment densities in both geographical regions. In central populations this result was partly expected since fucoid recruits seem well adapted to deal with the levels of summer radiation and temperature characteristics of these intertidal shores, information verified by their dominance at Welsh rocky intertidal sites (Chapter 2; Ballantine 1961; Brazier *et al.* 2007). Still, differences in shading conditions were expected to influence biomass allocation over the different shading treatments, similarly to what was showed for epilithic biofilms by Thompson *et al.* (2004). However that did not occur which indicates that fucoid populations from central regions have the capacity to adjust their photosynthetic apparatus to deal with a range of light intensities and still promote normal levels of growth. In Portugal although the results were not significant, the low levels of recruitment in all the treatments on the two Portuguese shores means that detection of shade effects may not be apparent simply because of a limitation of new individuals. The conclusion that reduced radiance levels were not important for improved fucoid recruitment success in Portuguese shores has to take into account the small number of recruits present during this experimental work. In order to determine whether the physical environment plays a critical role in development of fucoids at the early post settlement period it may be necessary to artificially manipulate the level of early recruits by seeding areas with propagules. By doing this it could be ascertained whether shading of such recruits from harsh physical conditions could lead to enhanced survival and growth of fucoid plants.

The role played by grazers in the control of macroalgae has long been highlighted as a major factor controlling algae abundance and their spatial distribution in different geographical regions (Lubchenco 1978; Southward & Southward 1978; Underwood & Jernakoff 1984; Boaventura *et al.* 2002a). The exclusion of grazers generally promotes an increase of macroalgae recruitment. The presence of grazers can have variable consequences on the spatial organisation of algae depending on the abundance of the macroalgae on the shore (Johnson *et al.* 1997; Coleman *et al.* 2006). The probability of macroalgae escaping grazers, especially during the early post-settlement stages, can determine community composition on the shore (Vadas *et al.* 1992; Burrows & Hawkins 1998; Johnson *et al.* 1998a). The probability of escapes will greatly depend on the identity, abundance and diversity of the grazer community (Jenkins *et al.* 2001; Coleman *et al.* 2006; Moore *et al.* 2007b). Different grazer species can have distinct effects on the establishment of algae (Moore *et al.* 2007b). Some species are not very effective in controlling algae recruitment, as is the case of littorinid grazers on Swedish rocky shores (Lindegarth *et al.* 2001), while other species can be very effective in controlling the spatial distribution of algae (O'Connor & Crowe 2005). Even species that can control algae recruitment in a specific environment can have different impact strengths if the surrounding community or the physical environment changes (Jenkins *et al.* 1999b). In experiment here presented, the effects of *Patella* spp. grazing pressure varied between the central and range edge populations. In Wales, an increase in the recruitment of macroalgae occurred in plots where limpets were absent independently of the levels of radiance arriving at the plots, indicating as previously proposed by several studies (Southward & Southward 1978; Hawkins 1981a; Hawkins & Hartnoll 1983; Jenkins *et al.* 1999b; Boaventura *et al.* 2002a; Jenkins *et al.* 2005) that fucoid recruitment on moderately exposed shores in Britain is greatly affected by the level of limpet grazing pressure on the shore. The results of the experiment seem to indicate that the actual grazing pressure on the Welsh shores is so efficient that even half the natural grazing pressure by limpets is enough to control fucoid recruitment levels. Based on these results, it is possible to conclude that at Welsh mid exposed intertidal rocky areas grazing by *Patella* spp. is one of the most important factors controlling fucoid settlement. In contrast, it has been shown by previous studies (Benedetti-Cecchi *et al.* 2000; Benedetti-Cecchi *et al.* 2001; Coleman *et al.* 2006) that the impact of limpet grazing in southern European algae communities is more variable. It has also been proposed that a possible combination of reduced grazing pressure and amelioration of physical factors may promote fucoid recruitment in these harsher range edge environments (Underwood 1980; Williams 1994; Kaehler & Williams 1998). The experimental design used for the experiment explored both processes simultaneously. However, it is likely that the low levels of supply of new individuals in the form of propagules occurring on the Portuguese shores meant that no response in fucoid recruitment could be detected even in the absence of limpet grazers and with environmental light intensity reduced by 80 %. The fucoid populations in Portugal had lower abundance than in Wales, therefore expected propagule supply probably is also lower (see Chapter 5 for further information on reproductive capacity).

In summary, these series of experiments allowed us to clearly show the strong control that *Patella* spp. grazing pressure can exert on central populations from Welsh shores and how physical factors, in this case radiance levels, are not affecting fucoid recruitment on the Welsh rocky intertidal. Although results for northern areas were clear, on Portuguese shores due to the low natural levels of fucoid recruitment the influence of reduced radiance levels and limpet grazing pressure could not be conclusively determined. Even though the processes controlling the abundance of fucoids are not completely clarified for southern regions, my results still seem to indicate that northern intertidal areas are greater net producers than southern intertidal shores. The results of this research further suggest that propagule supply seems to be crucial and that further experimental work is required on these range edge populations to clarify what processes are limiting settlement and controlling recruitment on these intertidal rocky shores.

CHAPTER 4

PHYSIOLOGICAL RESPONSE OF NORTHERN AND SOUTHERN FUCOID ALGAE TO PHYSICAL STRESS

4.1 INTRODUCTION

Fucoid algae are important primary producers on rocky intertidal shores of the European and American Atlantic coast (Raffaelli & Hawkins 1996). In the intertidal zone of the northeast Atlantic, fucoid cover and biomass decreases with latitude ((Ballantine 1961; Hawkins & Jones 1992). While work presented in this thesis (Chapter 2) shows the pattern of decline southward is less gradual than previously thought, my quantitative assessment of community structure through the British Isles and into Portugal also shows that fucoid taxa decrease significantly in Portugal. Portugal is known as a biogeographical transition zone (Ardré 1970; Lüning 1990; Southward *et al.* 1995; Ladah *et al.* 2003; Lima *et al.* 2007) where some fucoid species reach or approach their southern limit of distribution. Populations occupying such areas, near the limits of the "realized niche" of a species, experience higher levels of abiotic and/or biotic stresses (Guo *et al.* 2005; Bridle & Vines 2007), which eventually are responsible for the exclusion of the species from these areas (Hutchins 1947; Lewis 1986; Somero 2002; Davenport & Davenport 2005).

The existence, distribution and abundance of fucoid algae in rocky intertidal areas are determined by a combination of physical and biotic factors (Schonbeck & Norton 1978; Lubchenco 1980; Hawkins & Hartnoll 1985; Menge 2000b; Jenkins *et al.* 2008). Physical factors, many of which are associated with tidal emersion, have long been known to influence the position and abundance of organisms in the rocky intertidal (Evans 1948; Ballantine 1961). In temperate zones the peak of environmental stress occurs in the summer, when mid afternoon low tide periods occur on clear, calm days (Helmuth *et al.* 2002). These conditions promote an environment with harsh physiological stresses where high temperatures and solar radiance cause high levels of thermal and desiccation stresses, particularly at the upper levels of the shore (Doty 1946; Stephenson & Stephenson 1972; Bell 1993; Davison & Pearson 1996; Denny & Wethey 2001). The pervasive influence of such adverse physical conditions on algal distribution has been demonstrated numerous times; brown algae species like *Pelvetia canaliculata*, *Fucus spiralis*, *Ascophyllum nodosum* and *Laminaria digitata*, have all been shown to have their upper limit on the shore directly set by physical factors, such as extreme temperatures and harsh exposure to solar radiation (Schonbeck & Norton 1978; Dring & Brown 1982; Todd & Lewis 1984; Hawkins & Hartnoll 1985; Skene 2004). As well as operating on a local scale, such effects are likely to operate in influencing the distribution and southern limit of fucoid taxa as one moves south in Europe.

To understand the role of physical factors in determining algal distribution, a simple but effective method may involve the observation of physical damage in algae under extreme conditions, for example at the upper limit of distribution on a local scale on extreme spring tides in the summer (e.g. Hawkins & Hartnoll 1985). However, such observations are likely to be less useful over biogeographic scales where direct physical limitation is less likely to be important than non lethal effects which influence fitness and hence recruitment at range edges. A more subtle means, which enables a greater understanding of non lethal effects, is to measure stress responses. There are several methods to measure stress in algae; one of the most efficient means is the use of the non-intrusive pulse amplitude modulated (PAM) fluorometer. Its use in fucoid experimental procedures is well documented. Using a PAM fluorometer, Malm & Kautsky (2003) were able to show that different fucoid species, *Fucus serratus* and *Fucus vesiculosus*, had different tolerance levels to wave action and freezing temperatures. Coelho *et al.* (2001) showed that elevated light levels and UVB radiation could be an important stress factor for embryos of *F. spiralis*. Studies developed in the Baltic Sea were able to assess the effects of desiccation and depth on *F. vesiculosus* populations also through the use of PAM fluorometry. This technique was essential to determine that the marine ecotype was more resistant to desiccation than the brackish ecotype and that *F. vesiculosus* specimens living at lower depths in the Baltic Sea had higher capacity of photoinhibition, a factor essential to protect their photosystem (Ekelund *et al.* 2008; Gylle *et al.* 2009). Studies similar to these show how widely versatile the fluorometry technique is and that PAM fluorometers can successfully be used to investigate the physiological response of fucoid algae. PAM fluorometers can measure various aspects of the photosynthetic response to light intensity. Through the measurement of the maximum quantum yield of PSII photochemistry (Fv/Fm values), for example in algae under different environmental stresses, PAM fluorometers allow researchers to assess the effect of stresses on the reaction centres of the PSII and its consequences on the photosynthetic performance of the algae (Consalvey *et al.* 2005; Baker 2008). The ability to assess the photosynthetic performance of algae is extremely important due to its direct impacts on growth rates, reproductive output and tissue health (Davison & Pearson 1996; Somero 2002; Dethier *et al.* 2005).

The distribution of species across ecosystems is closely linked with the environment (Clarke 2003; Lima *et al.* 2007; Hawkins *et al.* 2009; Wethey *et al.* 2011). Changes in temperature, salinity and wave exposure can all contribute to the development of morphologically distinct fucoid populations (Back *et al.* 1993; Kalvas & Kautsky 1993, 1998; Russell *et al.* 1998). Factors like intensity of grazing or wave action have also contributed to differences in reproductive effort observed between populations (Russell 1979). More recently, different populations have also showed different responses to copper due to their historic exposure to the element (Nielsen *et al.* 2003). All these studies indicate that distinct populations can respond differently to similar stress levels. Given that fucoid biomass declines from northern to southern European areas (Chapter 2) and that Portugal is near the southern limit of distribution for the majority of the fucoid species (Lima *et al.* 2007), this study aims to use flourometry to compare the response of different fucoid populations, from central and southern peripheral fucoid populations, to naturally high physical stress levels similar to the ones affecting algae during their emersion period in the summer. To achieve this objective *Fucus spiralis* and *Fucus vesiculosus,* species that occupy respectively the high and mid shore levels of the intertidal, from central (Welsh) and range edge (Portuguese) populations were used. These were chosen as model species since they are present in both geographical regions.

The main aim of the present study was to determine if fucoid populations are locally adapted via a physiological response to the levels of physical stress to which they are normally subjected. To achieve these, fucoid populations from the southern extreme and central areas of distribution were exposed to equal levels of physical stress. The levels of physical stress used, can be naturally observed in both regions during the emersion period. However, the level of physical stress applied occurs with regular frequency on southern regions but only occasionally at northern regions. Therefore, if populations are locally adapted it is expect to observe a greater deterioration of the physiological condition of specimens from the northern populations. The use of stress levels usually found at the southern extreme of distribution, also allows evaluation of how southern populations are dealing with the levels of stress they usually have to sustain during the summer emersion periods. Are southern fucoid populations usually living under physiological stress or does their physiology allows them to easily recover after the emersion periods? Finally, by running the experiments separately on different fucoid species, it was assessed if the patterns of stress observed across geographical regions would be similar in species that normally occupy distinct shore heights. Do high shore species show less difference across their geographic range, since they are naturally adapted to high stress, than mid shore species?

4.2 METHODS

4.2.1 Collection

Fucus vesiculosus and *Fucus spiralis* samples were collected from northern and southern European areas. The two northern populations were from shores located in Wales, Porth Cwyfan (53.182821°, -4.489829°) and Cemlyn Bay (53.407460°, -4.533636°); while in the south the Portuguese populations came from Viana do Castelo (41.690403°, -8.849988°) and Carreço (41.716555°, -8.866798°). The collection of specimens was undertaken throughout August 2010 and was undertaken on shores moderately exposed to wave action and easily accessible. Within each geographical region the shores were at least 3 km apart. They all had a typical mosaic patchy community composed of fucoids, barnacles, bare rock and limpets, were gently sloping and exposed to full salinity. In order to maximize the possible effects of the different physical environments, species from the middle and higher levels of the shore were used, *Fucus vesiculosus* and *Fucus spiralis*, respectively. These species were also chosen due to their geographical range; they are present in both areas of the study. At each of the 4 shores, 72 *F. vesiculosus* individuals and 48 *F. spiralis* individuals, with approximately 10 to 15 cm length, were collected to be used in the photosynthetic and desiccation resilience experiments.

4.2.2 Transport, acclimatization and maintenance

After being collected, the algae were transported in dark, cold and humid conditions to the laboratory. The algae from Welsh shores were transported to Portugal by plane in dark, cold and humid cool boxes wrapped in blotted paper to preserve the macroalgae in a hydrated condition. The transport took no more than 1 hour from the shore to the laboratory and transport of Welsh fucoids to Portugal less than 20 hours. After the transport to Portugal, algae had a 15 day period of acclimatization in laboratory conditions. All the individuals were cultured under ambient day length conditions in aerated and circulating seawater at 16 ºC in 300 L tanks. Seawater was enriched twice a week by adding inorganic nitrogen ($NaNO_3$) and phosphorus (NaH_3PO_4) to a final concentration at least of 50 µM and 5 µM, respectively.

4.2.3 Measurement of response variables

The experimental procedure used involves the assessment of initial fucoid tissue water content and percentage of water lost with stress. To assess both parameters, algae were weighed at the different steps of the experiment. Percentage of water in initial fucoid tissue total weight was determined by the formula:

$$\textbf{ITWC}\ (\%) = \frac{\text{Hydrated Weight} - \text{Dry Weight}}{\text{Hydrated Weight}} \times 100$$

By incorporating the weight of the specimens after the stress period had been applied, the percentage of initial water lost due to the stress period could also be estimated by using the following formula:

$$\textbf{\% H}_2\textbf{O lost} = \frac{\text{Hydrated Weight} - \text{After Stress Weight}}{\text{Hydrated Weight} - \text{Dry Weight}} \times 100$$

In addition to the previous parameters, photosynthetic performance of fucoids during the experiment was also evaluated. The assessment of photosynthetic performance of algae was measured as the ratio of variable to maximal chlorophyll fluorescence: F_v/F_m, where $F_v=F_m-F_0$, F_m is the maximal fluorescence and F_0 is the initial fluorescence in dark-adapted algae (Krause & Weis 1991). Measurements were made with a WATER-PAM Chlorophyll Fluorometer (Heinz Walz GmbH, Germany) using saturating pulse intensities (800 ms, 10000 µmol photons m^{-2} s^{-1}) for both fucoid species. The F_v/F_m ratio is an indicator of the maximal quantum yield of photosystem II photochemistry (Dring *et al.* 1996; Baker 2008). The maximum quantum yield of a specimen is achieved in unstressed conditions when all capable reaction centers are in prime conditions, so alteration of the optimum conditions will lead to a decline of the Fv/Fm ratio indicating that the photosynthetic performance of the algae was affected (Butler 1978; Long *et al.* 1994; Baker & Oxborough 2004). Therefore, the use of PAM fluorometry to assess the levels of the F_v/F_m when algae are exposed to different stress levels is an efficient and sensitive methodology for measuring the fucoid photosynthetic response to stress. To evaluate photosynthetic resilience of the fucoid species, F_v/F_m ratio was quantified three times during each experimental procedure. The maximal chlorophyll fluorescence (F_v/F_m ratio) was measured in algae in hydrated conditions before, after application of stress and after a 16 hour recovery period. The initial levels of F_v/F_m ratio were used directly as a response variable to test for the levels of initial stress presented by fucoid algae from different populations, while the percentage loss of F_v/F_m after stress and after a 16 hours recovery was used as an insight into how the samples responded to the different physical stress levels. The percentage of initial Fv/Fm retained after stress and after a 16 hours recovery were calculated using the formula:

$$\textbf{\% of initial } \boldsymbol{F_v/F_m} \textbf{\textit{ retained}} \textbf{ after stress} = \frac{\text{Initial } F_v/F_m - F_v/F_m \text{ after stress}}{\text{Initial } F_v/F_m} \text{ x } 100$$

(To calculate of the percentage of initial F_v/F_m after working after a 16 hours recovery period, the value of F_v/F_m after stress was replaced by the value of F_v/F_m after a 16 hours recovery period)

4.2.4 Experimental design and set up

During September 2010, a total of ten experiments were performed, six experiments only using *F. vesiculosus* and four using exclusively *F. spiralis*. Each experiment incorporated four different populations, two from Welsh shores (Porth Cwyfan and Cemlyn Bay) and two from Portuguese shores (Viana do Castelo and Carreço). Owing to restrictions in space, experiments were performed simultaneously in two separate experimental systems located on the same rooftop, so only three and two days were necessary to perform all the experiments using *F. vesiculosus* and *F. spiralis*, respectively.

The application of the stress period was carried out in Portugal on warm cloudless summer days on a rooftop location to allow the use of natural sunlight and exposure to temperatures that could vary due to natural combination of all the other physical elements. The experimental design used during the stress period allowed simultaneous testing of the combined effect of two light intensities (natural summer radiance levels and an 80% reduction provided by shading material) and two temperature regimes (warm and cold) across four different fucoid populations from two different geographical region, represented in each experiment. So, on each experiment three replicates of each of the four populations were assigned to each of the four treatments allowing formal comparisons among geographical regions (fixed, 2 levels), shores (random nested in geographical regions, 2 levels), light intensities (fixed, 2 levels) and temperatures (fixed, 2 levels). To create such an experimental system, 16 identical plastic containers were used as experimental units to assess stress in *Fucus* plants. All had transparent perspex covers, but half had an additional cover of mesh, which achieved the 80% reduction of the photosynthetically active radiation previously mentioned. Mean light intensities in the full light treatment, measured with a spherical quantum scalar sensor (QSL-2100, Biospherical Instruments Inc., USA), varied between 2000 and 2200 µmol (photons) m^{-2} s^{-1} during the experiments, while reduced light levels were between 400 and 440 µmol (photons) m^{-2} s^{-1}. Half of the sixteen containers in each experimental set up were also exposed to uncontrolled air temperatures, measured with temperature-humidity data loggers (MicroLog EC650, Fourier Systems Ltd., USA), which varied between 20 and 23ºC during the experiments (warmer treatment), while the other half had temperature controlled at 18 ºC through an air-conditioning unit (cold treatment). The conditions of air temperature and light intensity resulted in clear differences in the mean frond temperatures of the two species across treatments (Table 14)

Table 14: Mean frond temperatures values (in degree Celsius, ºC) achieved across the experiments during the application of different stress conditions, measured with Easyview 15, Extech Instruments Corp., USA.

	Cold Unshaded	**Cold Shaded**	**Warm Unshaded**	**Warm Shaded**
F. vesiculosus	28.7	20.4	35.3	27.4
F. spiralis	27.4	18.5	35.3	27.2

The range of physical conditions, air temperatures and light intensities, utilized in the experiments had been previously observed under field conditions both in the Welsh and Portuguese rocky intertidal areas during the summer season. Thus plants from both regions can naturally be affected by these conditions. However, importantly the number of days during the summer that experienced physical conditions similar to the ones observed on the experimental warmer and lighter treatments is greater in southern than in northern regions (data verified through surface skin temperature and photosynthetically available radiation data from NASA Aqua (http://disc.sci.gsfc.nasa.gov/giovanni/overview/index.html) - AIRS standard & MODIS-Aqua missions respectively).

In order to complete these experiments, only the apices of algae weighing between 1.5 and 2 g were used. Therefore, the day before the stress period was being applied a tip of the fronds from each of the specimen being used was blotted dry, cleaned with seawater, cut and weighed. This first measure of the algae weight was used to determine the previously mentioned initial tissue water content of samples. Only apices of the algae were used to restrict the effects of biomass variation and because the tip of the frond possesses tissues where high photosynthetic performances occur. The apices were then left in culture over night (12 hours) in a 300 L tank with aerated and circulating seawater at 16 ºC in dark conditions before the initial F_v/F_m ratio of the specimens was measured. Apices from the algae were left for a minimum of 12h in dark conditions to guarantee an equilibrium state of the photosynthetic electron transport chain before measuring the initial F_v/F_m. The first measurement of F_v/F_m, indicated the maximum quantum yield of the algae in optimum physiological conditions. Samples were then left for an extra 30 min in the tanks exposed to natural light conditions, so the PSII reaction centres could adapt to the natural sunlight level in a hydrated environment. Subsequently, the physiological resilience of the algae in emerged conditions was tested by subjecting the specimens for 75 minutes to the different conditions of temperatures and light intensity provided by the experimental design implemented on the roof. Afterwards, algae were blotted dry and weighed before being reintroduced to the 300 L tank with aerated and circulating seawater at 16 ºC for a rehydration period of 20 minutes. The fucoid weight measure at this point of the experiment was essential to determine the previously mentioned percentage of water lost due to the stress period. During these 20 minutes of the rehydration period, algae were kept in dark conditions to allow the readaptation of the PSII to dark conditions (Gylle *et al.* 2009) before the measurement of F_v/F_m after stress. After this, algae were left in the tank for a 16 hour period of recovery. For the first 4 hours of this recovery period, ambient light conditions were maintained while during the last 12 hours, the tank was covered so dark conditions could be achieved before the last measurement of F_v/F_m could be recorded. Finally, samples were removed from the tank and dried at 60°C over 48 hours in order to determine their dry weight.

4.2.5 Data analysis

In order to analyse the results, each experiment was considered individually and the results across multiple experiments for each of the two species qualitatively compared. Differences across the majority of response variables were analysed using mixed analysis of variance model (ANOVA) with 4 factors, with significant results being explored further with SNK (Student Newman Keuls) multiple comparisons. Only SNK results showing differences are displayed in the results section. For response variables measured before stress was applied, initial Fv/Fm values and percentage of H_2O in initial fucoid tissue total weight, an analysis of variance model (ANOVA) with only 2 factors, geographical region and shore, was used. Cochran's test was used to test the data for heterogeneity of variance and transformations were done where appropriate. All the analyses were performed using the statistical package WinGMAV5 (EICC, University of Sydney).

4.3 RESULTS

During this study, experiments were repeated multiple times, which led to some observed differences in the results but also to the emerging of general patterns across experiments. Although, the focus in the result sections is put on the general patterns observed across the experiments, reference to the specific experiments is always made. The experiments carried out with *F. vesiculosus* are denominated, experiment 1.1, 1.2, 2.1, 2.2, 3.1 and 3.2, while the experiments carried out with *F. spiralis* are identified as experiment 4.1, 4.2, 5.1 and 5.2.

4.3.1 Desiccation resilience

4.3.1.1 Percentage of H_2O in initial fucoid tissue total weight

Fucus vesiculosus

The hydration of algae tissues prior to application of stress could introduce variability in the response to stress. However, the percentage of water present in algae tissues collected from different shores before experimental exposure to stress was similar, with no significant differences detected between regions in 5 out of 6 experiments (Table 15: Exp. 1.1, 1.2, 2.2, 3.1 & 3.2) and no significant differences between shores within each geographical region detected across all experiments (Table 15). These results thus indicate that algae tissues were equally hydrated and the examination of Figure 17a, indeed shows that there was no consistent pattern between the two regions. Significant differences between specimens from opposing geographical regions were detected by the ANOVA analysis only in experiment 2.1 (Table 15: Exp. 2.1 - *SNK of Re*). However, even in this case the magnitude of the difference was not large (*F. vesiculosus* from Portugal had a mean of 71.3% of their weight composed by H_2O which was only slightly greater than the Welsh *F. vesiculosus* mean of 69.5%) and was within the range observed across other experiments; *F. vesiculosus* from Portugal had mean values between 69.6 and 72.4% while mean values of Welsh *F. vesiculosus* presented were between 68.5 and 70.6% across the 6 experiments (Figure 17a).

Fucus spiralis

As in *F. vesiculosus*, *F. spiralis* algae collected from different geographical regions showed similar levels of hydration across all the four experiments before exposure to stress and no significant effect of geographic region was found (Table 16, Figure 18a). The mean percentage of water in *F. spiralis* from both geographical regions varied between 71 and 73% of their total weight. There was, however, significant variation between shores in 2 experiments (Table 16: Exp. 4.1 & 4.2). Post hoc SNK showed this variation was shown in the two experiments at Portuguese shores and at Welsh shores only in experiment 4.2 (Table 16). Again the amplitude of the differences found was small, never being greater than 2.5% (Figure 18a).

Table 15: Two way mix model ANOVA of the percentage of H_2O in initial *F. vesiculosus* total weight on experiments 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore; Res: Residual.*

Source	**df**	**Exp 1.1**			**Exp 1.2**			**Exp 2.1**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	1	8.0	0.7	0.491	26.2	1.7	0.324	38.7	19.1	**0.049**	Sh (Re)
Sh (Re)	2	11.4	3.0	0.060	15.6	2.9	0.066	2.0	0.4	0.671	Res
Res	44	3.8			5.4			5.0			
Cochran's test		*C = 0.3086, p > 0.05*			*C = 0.4431, p > 0.05*			*C = 0.4741, p > 0.05*			
SNK								*Re*			
								Por > Wal			
Source	**df**	**Exp 2.2**			**Exp 3.1**			**Exp 3.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	1	96.2	7.8	0.108	13.0	1.7	0.323	0.1	0.0	0.933	Sh (Re)
Sh (Re)	2	12.4	2.8	0.070	7.7	1.5	0.236	15.7	2.5	0.098	Res
Res	44	4.4			5.2			6.4			
Cochran's test		*C = 0.3851, p > 0.05*			*C = 0.3335, p > 0.05*			*C = 0.3859, p > 0.05*			

Table 16: Two way mix model ANOVA of the percentage of H_2O in initial *F. spiralis* total weight on experiments 4.1, 4.2, 5.1 & 5.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region; Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Res: Residual.*

Source	**df**	**Exp 4.1**			**Exp 4.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	1	2.8	0.2	0.729	8.2	0.3	0.653	Sh (Re)
Sh (Re)	2	17.3	5.7	**0.006**	30.1	7.5	**0.002**	Res
Res	44	3.0			4.0			
Cochran's test		*C = 0.3478, p > 0.05*			*C = 0.3404, p > 0.05*			
SNK		*Sh (Re)*			*Sh (Re)*			
		Cem > Cwy			*Via > Car*			
					Cem > Cwy			
Source	**df**	**Exp 5.1**			**Exp 5.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	1	2.5	0.8	0.460	0.0	0.0	0.972	Sh (Re)
Sh (Re)	2	3.1	0.7	0.490	6.1	1.2	0.317	Res
Res	44	4.2			5.1			
Cochran's test		*C = 0.5093, p < 0.05*			*C = 0.4127, p > 0.05*			

4.3.1.2 Percentage of initial H_2O lost due to the stress period

Fucus vesiculosus

The loss of water was monitored after the period of stress to assess which factors caused greater desiccation and if similar levels of stress had different impacts on algae from different locations. The main factor causing water loss was light intensity. Plants exposed to full light showed consistently higher water loss than shaded plants across all experiments (Figure 17b). Four out of six analyses showed light as a significant factor across all shores (Table 17: Exp. 1.2, 2.1, 2.2 & 3.1 - *SNK of Li and Li x Sh (Re)*). Variability in the response across spatial scales also occurred, with algae from different shores within the same geographical region in 4 out of 6 experiments showing significantly different levels of water loss (Table 17: Exp. 2.1, 2.2, 3.1 & 3.2 - *SNK of Sh (Re) and Li x Sh (Re)*). However, significant differences in the amount of water lost were only detected once between algae from distinct geographical regions. Significantly more water was lost in Portuguese specimens than Welsh, although the magnitude of the differences was small (mean H_2O lost: Wales 68.48%; Portugal 69.95%), (Table 17: Exp. 1.2 - *SNK of Re*). Focusing on the effect of temperature, the results show it did not consistently affect the percentage of water lost through the experiments. However, at least in experiment 1.2 and 3.1 warm conditions promoted greater loss of water on fucoid tissues (Table 17: Exp. 1.2 & 3.1 - *SNK of Te*). These results seem to indicate that temperature levels are important but probably higher temperatures will be needed to establish a clear result.

Fucus spiralis

Light intensity during the stress period was again the most important factor contributing to the final levels of hydration throughout all experiments (Figure 18b). *F. spiralis* individuals exposed to higher light intensity levels retained lower water levels (Table 18: Exp. 4.1, 4.2, 5.1 & 5.2 - *SNK of Re x Te, Li and Te x Li, Li*, Figure 18b). As in the experiments using *F. vesiculosus*, the effect of temperature on the percentage of water loss by *F. spiralis* throughout the experiments was not consistent. However, in half of the experiments it was shown, once again, that specimens that were subjected to warmer temperatures presented lower water content than those exposed to cooler conditions during the stress period (Table 18: Exp. 4.1 & 5.1 - *SNK of Re x Te and Te x Li*). In addition, spatial variability between shores within the same geographical region was also detected in 3 out of the 4 experiments (Table 18: Exp. 4.1, 4.2 & 5.1 - *SNK of Sh (Re) and Li x Sh (Re)*) with geographical differentiation only detected in experiment 4.1 (Table 18 - *SNK of Re x Te*). In this experiment, Post hoc SNK showed that Portuguese *F. spiralis* lost slightly more water content than Welsh specimens during the stress period when subjected to warm conditions (mean H_2O lost: Wales 71.25%; Portugal 71.73%), (Table 18 - *SNK of Re x Te*, Figure 18b).

Table 17: Mix model ANOVA of the percentage of initial H_2O from *F. vesiculosus* tissues lost due to stress on experiments 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Te: Temperature (War: Warm; Col: Cold); Li: Light intensity (Sha: Shaded; Uns: Unshaded); Res: Residual.*

Source	**df**	**Exp 1.1**			**Exp 1.2**			**Exp 2.1**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	1	497.4	9.0	0.095	333.8	30.6	**0.031**	16.1	0.0	0.907	Sh (Re)
Sh (Re)	2	55.2	1.6	0.214	10.9	0.6	0.555	915.1	33.6	<0.001	Res
Te	1	33.2	0.4	0.593	544.7	33.5	**0.029**	1025.7	65.4	0.015	Li x Sh (Re)
Li	1	748.0	11.8	0.075	946.4	994.1	**0.001**	3564.7	36.4	0.026	Gr x Sh (Re)
Re x Te	1	12.4	0.2	0.737	4.5	0.3	0.652	91.4	5.8	0.137	Li x Sh (Re)
Re x Li	1	4.3	0.1	0.819	0.5	0.5	0.562	9.1	0.1	0.790	Gr x Sh (Re)
Te x Sh (Re)	2	83.6	2.5	0.102	16.3	0.9	0.419	15.7	0.6	0.568	Res
Li x Sh (Re)	2	63.3	1.9	0.172	1.0	0.1	0.949	98.1	3.6	**0.039**	Res
Te x Li	1	8.5	0.6	0.519	226.6	6.0	0.135	386.1	9.6	0.090	Gr x Li x Sh (Re)
Re x Te x Li	1	140.1	10.0	0.087	1.9	0.1	0.843	4.2	0.1	0.777	Gr x Li x Sh (Re)
Li x Te x Sh (Re)	2	14.1	0.4	0.666	38.0	2.1	0.141	40.3	1.5	0.244	Res
Res	32	34.1			18.2			27.3			
Cochran's test			*C = 0.3142, p > 0.05*			*C = 0.2912, p > 0.05*			*C = 0.2518, p > 0.05*		
SNK						*Re*			*Li x Sh (Re)*		
						Por > Wal			*All Sh – Uns > Sha*		
						Te			*Por x Sha – Via > Car*		
						War > Col			*Wal x All Li – Cwy > Cem*		
						Li					
						Uns > Sha					

Source	**df**	**Exp 2.2**			**Exp 3.1**			**Exp 3.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	1	272.7	0.3	0.623	450.8	4.7	0.162	391.0	1.0	0.429	Sh (Re)
Sh (Re)	2	1641.9	10.8	**<0.001**	95.4	4.0	**0.028**	403.5	15.9	**<0.001**	Res
Te	1	187.0	4.9	0.157	326.5	21.1	**0.044**	23.3	0.6	0.526	Li x Sh (Re)
Li	1	3182.1	66.6	**0.015**	1115.0	25.7	**0.037**	205.0	5.9	0.136	Gr x Sh (Re)
Re x Te	1	135.7	3.6	0.199	65.1	4.2	0.177	1.5	0.0	0.865	Li x Sh (Re)
Re x Li	1	414.7	8.7	0.099	22.6	0.5	0.546	186.8	5.4	0.146	Gr x Sh (Re)
Te x Sh (Re)	2	75.9	0.5	0.610	15.5	0.7	0.530	40.3	1.6	0.221	Res
Li x Sh (Re)	2	95.5	0.6	0.538	43.5	1.8	0.178	34.8	1.4	0.270	Res
Te x Li	1	0.3	0.0	0.966	194.1	14.7	0.062	357.0	13.6	0.066	Gr x Li x Sh (Re)
Re x Te x Li	1	14.2	0.1	0.780	27.6	2.1	0.286	6.4	0.2	0.670	Gr x Li x Sh (Re)
Li x Te x Sh (Re)	2	277.2	1.8	0.176	13.3	0.6	0.579	26.3	1.0	0.368	Res
Res	32	2415.4			23.9			25.4			
Cochran's test			*C = 0.2296, p > 0.05*			*C = 0.2481, p > 0.05*			*C = 0.2547, p > 0.05*		
SNK			*Sh (Re)*			*Sh (Re)*			*Sh (Re)*		
			Cwy > Cem			*Via > Car*			*Car > Via*		
			Li			*Te*					
			Uns > Sha			*War > Col*					
						Li					
						Uns > Sha					

Table 18: Mix model ANOVA of the percentage of initial H_2O lost from *F. spiralis* tissues due to stress on experiments 4.1, 4.2, 5.1 & 5.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Te: Temperature (War: Warm; Col: Cold); Li: Light intensity (Sha: Shaded; Uns: Unshaded); Res: Residual.*

Source	**df**	**Exp 4.1**			**Exp 4.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	1	5.3	0.1	0.820	3.2E+14	0.0	0.967	Sh (Re)
Sh (Re)	2	79.3	3.7	0.037	1.5E+17	5.3	**0.010**	Res
Te	1	188.2	800.4	0.001	2.3E+17	13.5	0.067	Li x Sh (Re)
Li	1	1196.4	16.1	0.057	2.4E+18	99.0	**0.010**	Gr x Sh (Re)
Re x Te	1	19.5	83.0	**0.012**	2.2E+16	1.3	0.375	Li x Sh (Re)
Re x Li	1	0.6	0.0	0.939	3.4E+16	1.4	0.359	Gr x Sh (Re)
Te x Sh (Re)	2	0.2	0.0	0.989	1.7E+16	0.6	0.540	Res
Li x Sh (Re)	2	74.2	3.4	**0.045**	2.4E+16	0.9	0.424	Res
Te x Li	1	79.8	4.9	0.156	1.3E+17	3.5	0.201	Gr x Li x Sh (Re)
Re x Te x Li	1	0.4	0.0	0.886	6.1E+16	1.6	0.335	Gr x Li x Sh (Re)
Li x Te x Sh (Re)	2	6.2	0.8	0.482	3.8E+16	1.4	0.265	Res
Res	32	21.6			2.8E+16			
Cochran's test		*C = 0.0280, p > 0.05*			*C = 0.0333, p > 0.05*			
SNK		*Re x Te*			*Sh (Re)*			
		All Re – War > Col			*Cwy > Cem*			
		War – Por > Wal			*Li*			
		Li x Sh (Re)			*Uns > Sha*			
		Car/Cwy/Cem – Uns > Sha						
		Por x Sha – Via > Car						

Source	**df**	**Exp 5.1**			**Exp 5.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	1	4.2	0.1	0.809	92.0	4.0	0.185	Sh (Re)
Sh (Re)	2	54.8	5.7	0.008	23.3	2.4	0.112	Res
Te	1	136.5	7.5	0.112	0.5	0.0	0.881	Li x Sh (Re)
Li	1	410.8	6.4	0.128	643.6	140.3	**0.007**	Gr x Sh (Re)
Re x Te	1	28.4	1.6	0.339	117.6	6.9	0.120	Li x Sh (Re)
Re x Li	1	11.6	0.2	0.713	9.6	2.1	0.285	Gr x Sh (Re)
Te x Sh (Re)	2	18.2	1.9	0.169	17.1	1.7	0.193	Res
Li x Sh (Re)	2	64.5	6.7	**0.004**	4.6	0.5	0.632	Res
Te x Li	1	29.2	32.8	**0.029**	16.8	0.7	0.498	Gr x Li x Sh (Re)
Re x Te x Li	1	6.0	6.7	0.122	230.7	9.3	0.093	Gr x Li x Sh (Re)
Li x Te x Sh (Re)	2	0.9	0.1	0.912	24.9	2.5	0.096	Res
Res	32	9.7			9.9			
Cochran's test		*C = 0.0244, p > 0.05*			*C = 0.2988, p > 0.05*			
SNK		*Li x Sh (Re)*			*Li*			
		Car/Cwy/Cem – Uns > Sha			*Uns > Sha*			
		Por x Sha – Via > Car						
		Te x Li						
		All Li – War > Col						
		All Te – Uns > Sha						

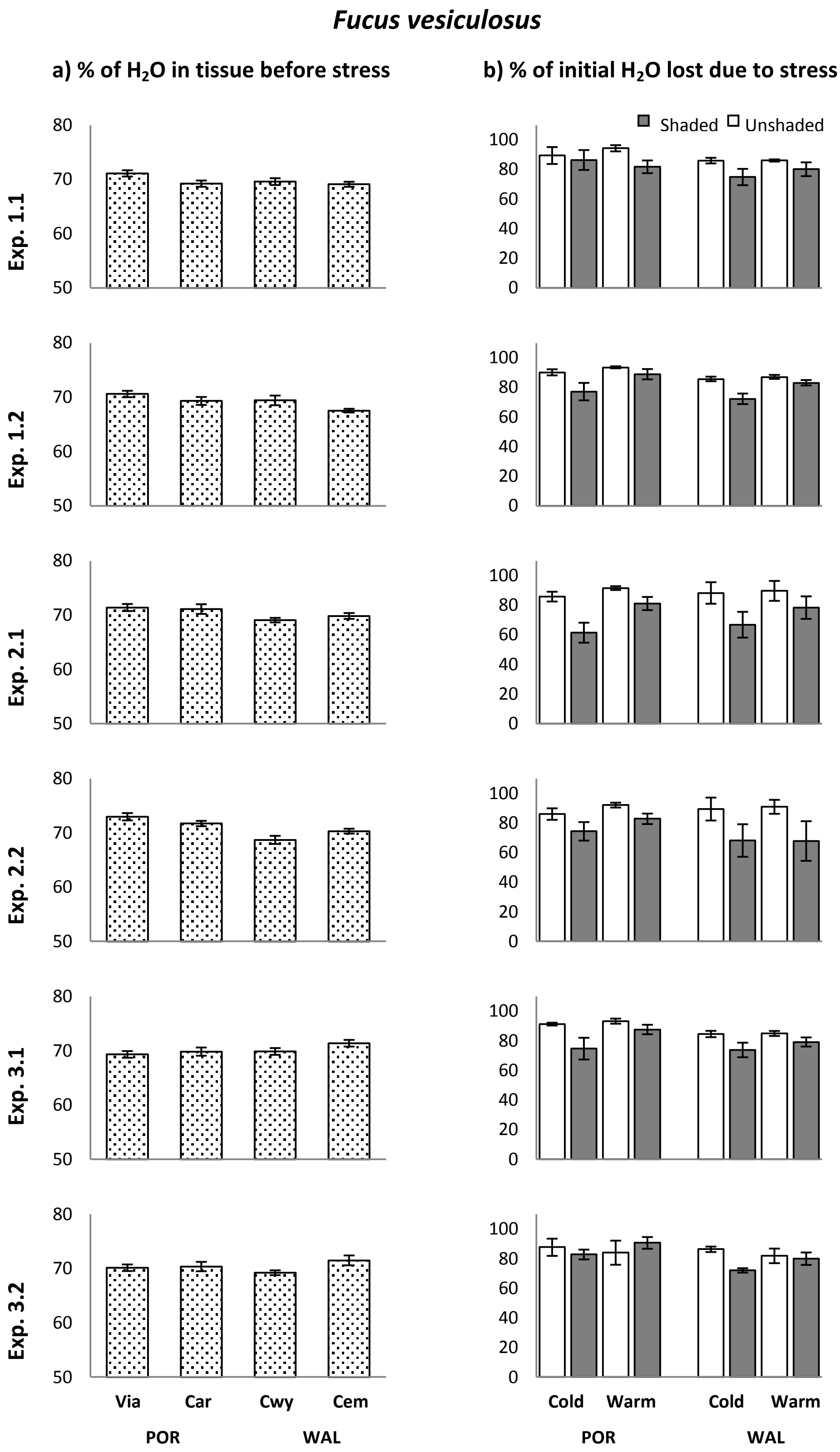
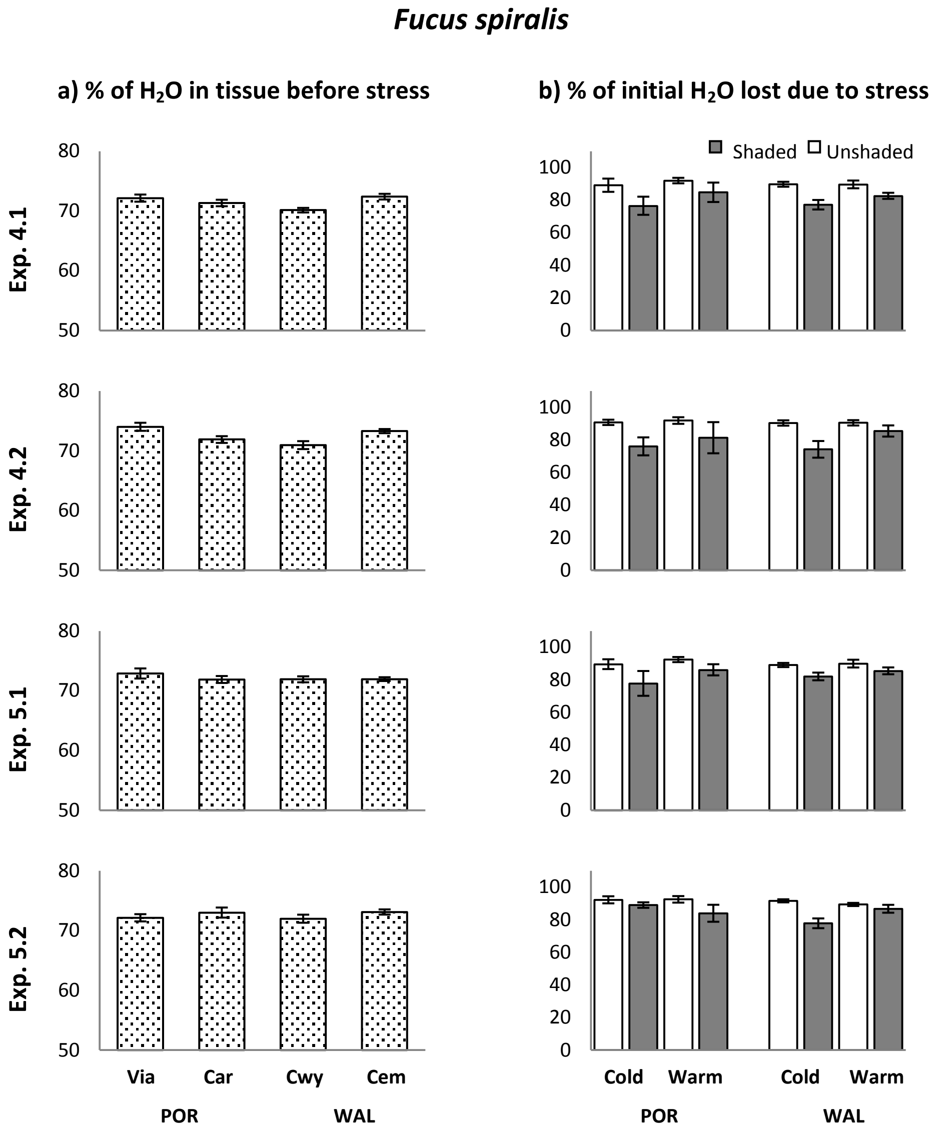


Figure 17: Experiment 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2; Error bars = ±1SE. a) Percentage of H_2O in initial *F. vesiculosus* total weight from Portuguese (POR) and Welsh (WAL) shores (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay). b) Percentage of initial H_2O lost from Portuguese (POR) and Welsh (WAL) *F. vesiculosus* tissues due to different light intensity and temperature treatments.



Fucus spiralis

a) % of H_2O in tissue before stress

b) % of initial H_2O lost due to stress

Figure 18: Experiment 4.1, 4.2, 5.1 & 5.2; Error bars = ±1SE. a) Percentage of H_2O in initial *F. spiralis* total weight from Portuguese (*POR*) and Welsh (*WAL*) shores (*Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay*). b) Percentage of initial H_2O lost from Portuguese (*POR*) and Welsh (*WAL*) *F. spiralis* tissues due to different light intensity and temperature treatments.

4.3.2 Photosynthetic resilience

4.3.2.1 Initial Fv/Fm values before stress

Fucus vesiculosus

The measure of the Fv/Fm parameter is important to assess the health status of algae prior to stress exposure. Mean initial values of Fv/Fm for *F. vesiculosus* from different shores were always between 0.72 and 0.78 (Figure 19a). Such high values, similar to the ones observed in other studies (Magnusson 1997; Pearson *et al.* 2000; Skene 2004; Gylle *et al.* 2009), indicate that the photosystems of algae used were in a good state.

However, Fv/Fm parameters of *F. vesiculosus* algae still varied significantly between different shores and geographical regions. Algae collected in Carreço presented significantly lower Fv/Fm values than algae from Viana do Castelo in 3 experiments (Table 19: Exp. 1.1, 1.2 & 2.1 - SNK of Sh (Re)). Differences between geographical regions in Fv/Fm values were also detected in 3 other experiments (Table 19: Exp. 2.2, 3.1 & 3.2 - SNK of Re), where Portuguese algae showed lower Fv/Fm values when compared with Welsh algae (Mean initial Fv/Fm values: Wales: 0.78, 0.78 & 0.77; Portugal: 0.75, 0.74 & 0.75 for Exp. 2.2, 3.1 & 3.2 respectively). The slightly lower values in Portugal indicate that algae were in a marginally lower status of health. However, as the magnitude of the difference between geographical regions was not large (0.03, 0.04 and 0.02 for exp. 2.2, 3.1 & 3.2, respectively) and Fv/Fm values were high for both geographical regions, algae were all considered to be in good photosynthetic conditions, confirming that the transport of Welsh fucoids to Portugal was achieved without negatively impacting the algae.

Fucus spiralis

Significant differences in initial Fv/Fm values in *F. spiralis* specimens from different geographical regions were only detected in 1 out of 4 experiments (Table 20: Exp. 5.1). In this experiment Welsh *F. spiralis* specimens showed mean Fv/Fm values of 0.75, slightly superior to the 0.74 mean of Portuguese *F. spiralis* (Table 20: Exp. 5.1 - *SNK Re*, Figure 20a). Across all experiments initial mean Fv/Fm value of Welsh *F. spiralis* was 0.75 while Portuguese *F. spiralis* presented mean values varying between 0.73 and 0.74 across the 4 experiments (Figure 20a). The consistency and high Fv/Fm ratios, similar to the ones observed in other studies (Skene 2004; Vinegla *et al.* 2006), indicate that the *F. spiralis* photosystems were in good condition prior to experimental stress and that Welsh *F. spiralis* were not damaged by transportation. Spatial variability in initial Fv/Fm values from *F. spiralis* from Portuguese shores was also noticed, but in only one of the four experiments (Table 20: Exp. 5.2 - *SNK of Sh (Re)*).

Table 19: Two way mix model ANOVA of initial Fv/Fm values from *F. vesiculosus* tissues before stress was applied on experiments 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Res: Residual*.

Source	**df**	**Exp 1.1**			**Exp 1.2**			**Exp 2.1**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**P**	**MS**	***F***	**p**	
Re	1	0.0	0.5	0.557	0.0	0.8	0.458	0.0	1.7	0.319	Sh (Re)
Sh (Re)	2	0.0	10.6	**<0.001**	0.0	6.5	**0.003**	0.0	13.7	**<0.001**	Res
Res	44	0.0			0.0			0.0			
Cochran's test		*C = 0.4793, p > 0.05*			*C = 0.4789, p > 0.05*			*C = 0.3372, p > 0.05*			
SNK		*Sh (Re)* *Via > Car*			*Sh (Re)* *Via > Car*			*Sh (Re)* *Via > Car*			

Source	**df**	**Exp 2.2**			**Exp 3.1**			**Exp 3.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	1	0.0	19.0	**0.049**	0.0	34.9	**0.028**	0.0	200.1	**0.005**	Sh (Re)
Sh (Re)	2	0.0	1.4	0.247	0.0	0.8	0.455	0.0	0.1	0.943	Res
Res	44	0.0			0.0			0.0			
Cochran's test		*C = 0.2965, p > 0.05*			*C = 0.4797, p > 0.05*			*C = 0.3779, p > 0.05*			
SNK		*Re* *Wal > Por*			*Re* *Wal > Por*			*Re* *Wal > Por*			

Table 20: Two way mix model ANOVA of initial Fv/Fm values of *F. spiralis* tissues before stress was applied on experiments 4.1, 4.2, 5.1 & 5.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Res: Residual*.

Source	**df**	**Exp 4.1**			**Exp 4.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**P**	
Re	1	0.0	10.2	0.086	0.0	18.1	0.051	Sh (Re)
Sh (Re)	2	0.0	1.9	0.165	0.0	0.4	0.684	Res
Res	44	0.0			0.0			
Cochran's test		*C = 0.4782, p > 0.05*			*C = 0.3455, p > 0.05*			

Source	**df**	**Exp 5.1**			**Exp 5.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**P**	
Re	1	0.0	46.5	**0.021**	0.0	3.1	0.223	Sh (Re)
Sh (Re)	2	0.0	0.6	0.572	0.0	3.8	**0.030**	Res
Res	44	0.0			0.0			
Cochran's test		*C = 0.3104, p > 0.05*			*C = 0.3501, p > 0.05*			
SNK		*Re* *Wal > Por*			*Sh (Re)* *Car > Via*			

4.3.2.2 Percentage of initial Fv/Fm retained after stress

Fucus vesiculosus

Due to lack of time and resources during one of the days, this parameter was only measured on 4 of the 6 experiments made using *F. vesiculosus*. Across all experiments carried out as expected, thirty minutes after application of stress, Fv/Fm levels in *F. vesiculosus* declined as a response to desiccation across all treatments, but mostly under unshaded conditions (Figure 19b). In all experiments where the measurement was conducted, with the exception of data for the Viana shore in experiment 2.1, specimens subjected to higher light intensities showed significantly lower Fv/Fm values than those confined to shaded compartments (Table 21: Exp. 1.1, 1.2, 2.1 & 2.2 - *SNK of Re x Te x Li, Li, Li x Sh (Re) and Li x Te x Sh (Re)*).

Significant differences in Fv/Fm levels among other factors or interactions of factors also occurred in isolated experiments, but not consistently across the 4 experiments (Table 21). Temperature levels had a partial effect on some experiments when combined with other factors, with warmer conditions in some combination of treatments contributing to a greater reduction of Fv/Fm values than cooler conditions (Table 21: Exp. 1.1 & 2.2 - *SNK of Re x Te x Li and Li x Te x Sh (Re)*, Figure 19b).

At this point of the experiment, differences in the percentage of initial Fv/Fm retained after stress between fucoids from different geographical regions were observed on 2 of the 4 experiments (Table 21: Exp. 1.1 & 1.2 - *SNK of Re x Te x Li and Re*). In these two experiments, Portuguese specimens of *F. vesiculosus* were less affected by the stress being applied, especially when subjected to the full light treatment, than Welsh specimens (Figure 19b).

Fucus spiralis

In a similar manner to the results of *F. vesiculosus*, Fv/Fm levels of *F. spiralis* were also lower after the application of stress. Once again the highest levels of light intensity applied to the algae greatly decreased the levels of Fv/Fm presented immediately after the stress period consistently across the 4 experiments conducted (Table 22: Exp. 4.1, 4.2, 5.1 & 5.2 - *SNK of Li x Sh (Re), Te x Li, Re x Li and Re x Te x Li,* Figure 20b). A partial effect of temperature was also detected across the 4 experiments with Fv/Fm levels generally less reduced in algae subjected to stress periods in cooler conditions (Table 22: Exp. 4.1, 4.2, 5.1 & 5.2 - *SNK of Te x*

Sh (Re), Te x Li, Re x Te & Re and Te x Li). The effect of temperature was not consistent, affecting the Fv/Fm outcome at different spatial scales, within *F. spiralis* from different shores (Table 22: Exp. 4.1) to specimens from distinct geographical regions (Table 22: Exp. 5.1 & 5.2).

Differences in the percentage of initial Fv/Fm retained after stress between *F. spiralis* from different geographical regions were observed on 2 of the 4 experiments (Table 22: Exp. 5.1 & 5.2 - *SNK of Re x Te, Re x Li and Re x Te x Li*, Figure 20b). In these two experiments Portuguese specimens of *F. spiralis* were less affected than Welsh specimens, but results were also dependent of interaction between geographical region origin and other factors controlled during the experimental procedure.

Table 21: Mix model ANOVA of the percentage of initial Fv/Fm values in *F. vesiculosus* retained after stress on experiments 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Te: Temperature (War: Warm; Col: Cold); Li: Light intensity (Sha: Shaded; Uns: Unshaded); Res: Residual.*

Source	**df**	**Exp 1.1**			**Exp 1.2**			**Exp 2.1**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	1	316.5	1.7	0.321	929.5	210.5	**0.005**	912.0	0.6	0.507	Sh (Re)
Sh (Re)	2	184.8	0.7	0.508	4.4	0.0	0.987	1418.3	7.7	0.002	Res
Te	1	336.8	2.0	0.296	3711.3	12.0	0.074	5.7	0.0	0.938	Li x Sh (Re)
Li	1	42579.4	763.3	0.001	23257.1	61.6	**0.016**	16080.2	25.3	0.037	Gr x Sh (Re)
Re x Te	1	402.6	2.4	0.265	0.5	0.0	0.972	222.1	0.3	0.637	Li x Sh (Re)
Re x Li	1	614.5	11.0	0.080	700.0	1.9	0.307	5038.3	7.9	0.107	Gr x Sh (Re)
Te x Sh (Re)	2	171.3	0.6	0.533	310.0	0.9	0.406	729.5	4.0	**0.029**	Res
Li x Sh (Re)	2	55.8	0.2	0.813	377.8	1.1	0.336	636.7	3.5	**0.043**	Res
Te x Li	1	324.2	9.2	0.094	303.3	2.0	0.294	277.8	16.5	0.056	Gr x Li x Sh (Re)
Re x Te x Li	1	998.6	28.3	**0.034**	2.0	0.0	0.919	246.9	16.2	0.057	Gr x Li x Sh (Re)
Li x Te x Sh (Re)	2	35.3	0.1	0.877	152.1	0.5	0.639	126.2	0.7	0.510	Res
Res	32	266.9			334.6			183.7			
Cochran's test		*C = 0.2693, p > 0.05*			*C = 0.2282, p > 0.05*			*C = 0.2500, p > 0.05*			
SNK		*Re x Te x Li*			*Re*			*Te x Sh (Re)*			
		Col x Uns – Por > Wal			*Por > Wal*			*Via – War > Col*			
		Por x Uns – Col > War			*Li*			*Por x Col – Car >Via*			
		All Re at all Te – Sha > Uns			*Sha > Uns*			*Li x Sh (Re)*			
								Car/Cwy/Cem – Sha > Uns			
								Por x Sha – Car > Via			
								Wal x Uns – Cem > Cwy			

Source	**df**	**Exp 2.2**			**Exp 3.1**			**Exp 3.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	1	3654.4	193.6	0.005							Sh (Re)
Sh (Re)	2	18.9	0.1	0.896							Res
Te	1	1375.8	4.8	0.160							Li x Sh (Re)
Li	1	24815.7	2036.3	<0.001							Gr x Sh (Re)
Re x Te	1	1.0	0.0	0.958		No			No		Li x Sh (Re)
Re x Li	1	2093.5	171.8	0.006		Data			Data		Gr x Sh (Re)
Te x Sh (Re)	2	287.7	1.7	0.202		Collected			Collected		Res
Li x Sh (Re)	2	12.2	0.1	0.931							Res
Te x Li	1	319.7	0.4	0.604							Gr x Li x Sh (Re)
Re x Te x Li	1	1062.2	1.2	0.382							Gr x Li x Sh (Re)
Li x Te x Sh (Re)	2	857.3	5.0	**0.013**							Res
Res	32	171.0									
Cochran's test		*C = 0.2821, p > 0.05*									
SNK		*Li x Te x Sh (Re)*									
		All Sh at all Te (except Via x War) – Sha > Uns									
		Car/Cwy x Uns – Col > War									
		Por x War x Uns - Via > Car									

Table 22: Mix model ANOVA of the percentage of initial Fv/Fm values in *F. spiralis* retained after stress on experiments 4.1, 4.2, 5.1 & 5.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Te: Temperature (War: Warm; Col: Cold); Li: Light intensity (Sha: Shaded; Uns: Unshaded); Res: Residual.*

Source	**df**	**Exp 4.1**			**Exp 4.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	1	1949.5	7.3	0.115	349.1	3.3	0.212	Sh (Re)
Sh (Re)	2	248.9	1.6	0.212	106.2	0.5	0.609	Res
Te	1	58.7	0.1	0.801	2753.2	12.0	0.074	Li x Sh (Re)
Li	1	20104.8	34.3	0.028	15590.5	29.2	0.033	Gr x Sh (Re)
Re x Te	1	177.4	0.3	0.667	50.2	0.2	0.686	Li x Sh (Re)
Re x Li	1	127.9	0.2	0.686	208.9	0.4	0.595	Gr x Sh (Re)
Te x Sh (Re)	2	711.3	4.3	**0.022**	229.1	1.2	0.349	Res
Li x Sh (Re)	2	585.6	3.6	**0.041**	533.3	2.5	0.095	Res
Te x Li	1	521.5	1.3	0.369	1126.1	36.0	**0.027**	Gr x Li x Sh (Re)
Re x Te x Li	1	80.8	0.2	0.695	112.0	3.6	0.199	Gr x Li x Sh (Re)
Li x Te x Sh (Re)	2	393.7	2.4	0.108	31.3	0.2	0.862	Res
Res	32	165.0			210.6			
Cochran's test		*C = 0.1839, p > 0.05*			*C = 0.1979, p > 0.05*			
SNK		*Te x Sh (Re)*			*Te x Li*			
		Cem – Col > War			*Uns – Col > War*			
		Wal x Col – Cem >Cwyl			*All Te – Sha > Uns*			
		Li x Sh (Re)						
		All Sh – Sha > Uns						
		Wal x Uns – Cem >Cwyl						

Source	**df**	**Exp 5.1**			**Exp 5.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	1	109.7	0.3	0.663	197.7	0.3	0.620	Sh (Re)
Sh (Re)	2	428.0	4.3	0.022	587.2	4.1	0.025	Res
Te	1	174.7	9.6	0.090	2052.8	3.5	0.203	Li x Sh (Re)
Li	1	29864.7	1436.0	<0.001	25052.7	1149.2	<0.001	Gr x Sh (Re)
Re x Te	1	667.0	36.5	**0.026**	4.5	0.0	0.938	Li x Sh (Re)
Re x Li	1	918.8	44.2	**0.022**	1450.7	66.6	0.015	Gr x Sh (Re)
Te x Sh (Re)	2	18.3	0.2	0.834	587.6	4.2	0.025	Res
Li x Sh (Re)	2	20.8	0.2	0.813	21.8	0.2	0.851	Res
Te x Li	1	104.8	3.0	0.230	318.7	8.0	0.101	Gr x Li x Sh (Re)
Re x Te x Li	1	629.8	17.5	0.053	593.2	20.0	**0.047**	Gr x Li x Sh (Re)
Li x Te x Sh (Re)	2	36.0	0.4	0.700	39.9	0.3	0.751	Res
Res	32	99.9			141.7			
Cochran's test		*C = 0.2099, p > 0.05*			*C = 0.2045, p > 0.05*			
SNK		*Re x Te*			*Re x Te x Li*			
		Col – Por > Wal			*Col x Uns – Por > Wal*			
		Por – Col > War			*Por x Uns – Col > War*			
		Re x Li			*All Re at all Te – Sha > Uns*			
		Sha – Por > Wal						
		All Re – Sha > Uns						

4.3.2.3 Percentage of initial Fv/Fm working 16 hours after stress

Fucus vesiculosus

In order to assess the permanent effect of the different stress levels a measure of Fv/Fm after a 16 hour recovery period was undertaken. Although Fv/Fm values improved from the values observed after stress, only algae exposed to reduced light intensities achieved complete recovery (Figure 19c). The level of previous exposure to light was once more a significant factor affecting the photosynthetic performance/recovery of the algae across the majority of the experiments (5 out of 6) (Table 23: Exp. 1.1, 1.2, 2.1, 3.1 & 3.2 - *SNK of Re x Li, Li and Te x Li*). Variation in the temperatures provided during stress was also influential for the levels of recovery but only in 2 experiments (Table 23: Exp. 2.2 & 3.1). In such experiments, cooler conditions during the stress period promoted greater recovery across all treatments (Table 23: Exp. 3.1 - SNK of Te x Li) or only at some shores (Table 23: Exp. 2.2 - *SNK of Te x Sh (Re)*).

However, the most intriguing results occurred in experiments 1.1, 1.2 and 3.2. In these experiments, the interaction between geographical origin of *F. vesiculosus* individuals and light intensity provided during the stress period influenced the degree of recovery achieved (Figure 19c). In all these 3 experiments *F. vesiculosus* specimens collected in Portugal, which had previously been stressed in unshaded conditions, were able to significantly achieve greater levels of recovery than Welsh *F. vesiculosus* individuals subjected to equal levels of stress (mean Fv/Fm levels of recovery on specimens previously subjected to unshaded stress conditions, independently of the temperature provided: Wales - 59.3, 64.1 & 64.1 %; Portugal - 81.9, 81.9 & 81.8 % for Exp. 1.1, 1.2 & 3.2, respectively (Table 23: Exp. 1.1, 1.2 & 3.2 - *SNK of Re x Li)*).

Fucus spiralis

In contrast to the results from the *F. vesiculosus* experiments, there was no discernible influence of geographical origin of *F. spiralis* individuals on the degree of recovery achieved (Table 24, Figure 20c). The levels of recovery depended mainly on light intensity exposure history (Table 24). In 3 of 4 experiments, specimens that had been exposed to higher light intensities were less able to recover from the stress period (Table 24: Exp. 4.2, 5.1 & 5.2 - *SNK of Te x Li, Li and Li x Te x Sh (Re)*, Figure 20c). Other factors, such as temperature and shore identity, also had an impact on Fv/Fm recovery (Table 24). The effect of shore identity was not consistent across experiments (Table 24: Exp. 4.1 & 5.2 - *SNK of Te x Sh (Re) and Li x Te xSh (Re)*). Cooler conditions during the stress period generally promoted greater Fv/Fm recovery after 16 hours, although its impact was not evident throughout treatments across the 4 experiments (Table 24: Exp. 4.1, 4.2, 5.1 & 5.2 - *SNK of Sh (Re) x Te, Te x Li, Te and Li x Te x Sh (Re)*).

Table 23: Mix model ANOVA of the percentage of initial Fv/Fm in *F. vesiculosus* working after 16 hours on experiments 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Te: Temperature (War: Warm; Col: Cold); Li: Light intensity (Sha: Shaded; Uns: Unshaded); Res: Residual.*

Source	**df**	**Exp 1.1**			**Exp 1.2**			**Exp 2.1**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	1	2.8E+05	152.2	0.007	1126.1	12.2	0.073	648.1	1.7	0.323	Sh (Re)
Sh (Re)	2	1845	0.1	0.919	92.0	1.1	0.338	382.0	3.7	**0.037**	Res
Te	1	3161.9	0.1	0.785	175.2	84.5	0.012	4.1	0.0	0.884	Li x Sh (Re)
Li	1	1.6E+06	509.3	0.002	8156.0	280.7	0.004	8049.7	26.6	**0.036**	Gr x Sh (Re)
Re x Te	1	44041.6	1.2	0.365	45.1	2.2	0.274	0.0	0.0	0.991	Li x Sh (Re)
Re x Li	1	1.9E+05	58.7	**0.017**	790.0	27.2	**0.035**	243.7	0.8	0.464	Gr x Sh (Re)
Te x Sh (Re)	2	32637.9	1.5	0.239	20.2	0.3	0.783	150.9	1.5	0.250	Res
Li x Sh (Re)	2	3236.2	0.2	0.862	29.1	0.4	0.704	302.5	2.9	0.070	Res
Te x Li	1	5988.5	1.0	0.423	392.2	8.2	0.104	34.5	0.2	0.696	Gr x Li x Sh (Re)
Re x Te x Li	1	337.4	0.1	0.835	23.6	0.5	0.556	413.5	2.4	0.259	Gr x Li x Sh (Re)
Li x Te x Sh (Re)	2	5989.8	0.3	0.761	48.1	0.6	0.562	169.6	1.6	0.212	Res
Res	32	21763.5			82.0			104.1			
Cochran's test		*C = 0.3282, p > 0.05*			*C = 0.2267, p > 0.05*			*C = 0.2243, p > 0.05*			
SNK		*Re x Li*			*Re x Li*			*Sh (Re)*			
		Uns – Por > Wal			*Uns – Por > Wal*			*Cem > Cwy*			
		All Re – Sha > Uns			*All Re – Sha > Uns*			*Li*			
								Sha > Uns			

Source	**df**	**Exp 2.2**			**Exp 3.1**			**Exp 3.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	1	3.9E+07	12.8	0.070	2430.5	40.2	0.024	873.8	9.2	0.094	Sh (Re)
Sh (Re)	2	3.1E+06	0.6	0.575	60.4	0.9	0.427	95.4	0.9	0.411	Res
Te	1	5.5E+07	1.2	0.382	2811.5	54.7	0.018	947.3	4.5	0.169	Li x Sh (Re)
Li	1	4.8E+08	30.5	0.031	8885.2	39.5	0.024	6389.5	5409.0	<0.001	Gr x Sh (Re)
Re x Te	1	2.1E+07	0.5	0.567	3.0	0.1	0.833	0.2	0.0	0.978	Li x Sh (Re)
Re x Li	1	1.5E+07	1.0	0.428	1013.5	4.5	0.168	1016.2	860.3	**0.001**	Gr x Sh (Re)
Te x Sh (Re)	2	4.5E+06	8.2	**0.001**	51.4	0.7	0.484	212.0	2.0	0.147	Res
Li x Sh (Re)	2	1.6E+07	2.9	0.072	224.7	3.3	0.052	1.2	0.0	0.989	Res
Te x Li	1	76198.6	0.0	0.952	902.0	129.9	**0.008**	540.7	2.0	0.293	Gr x Li x Sh (Re)
Re x Te x Li	1	4.8E+06	0.3	0.645	45.6	6.6	0.125	404.8	1.5	0.346	Gr x Li x Sh (Re)
Li x Te x Sh (Re)	2	1.7E+07	3.1	0.059	6.9	0.1	0.095	270.7	2.6	0.090	Res
Res	32	5.4E+06			69.1			104.2			
Cochran's test		*C = 0.3262, p > 0.05*			*C = 0.2546, p > 0.05*			*C = 0.2501, p > 0.05*			
SNK		*Te x Sh (Re)*			*Te x Li*			*Re x Li*			
		Car – Col > War			*All Li – Col > War*			*Uns – Por > Wal*			
		Cwy – Col > War			*All Te – Sha > Uns*			*All Re – Sha > Uns*			
		Por x Col – Car > Via									
		Por x War – Via > Car									
		Wal x Col – Cwy > Cem									

Table 24: Mix model ANOVA of the percentage of initial Fv/Fm in *F. spiralis* working after 16 hours on experiments 4.1, 4.2, 5.1 & 5.2. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay); Te: Temperature (War: Warm; Col: Cold); Li: Light intensity (Sha: Shaded; Uns: Unshaded); Res: Residual.*

Source	**df**	**Exp 4.1**			**Exp 4.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	1	45.5	0.4	0.586	2.4E+07	30.1	0.032	Sh (Re)
Sh (Re)	2	110.2	2.2	0.127	8.1E+05	0.1	0.949	Res
Te	1	111.1	0.6	0.535	2.1E+08	14.8	0.061	Li x Sh (Re)
Li	1	5843.9	198.4	0.005	9.0E+08	2712.8	<0.001	Gr x Sh (Re)
Re x Te	1	0.0	0.0	0.997	7.6E+06	0.5	0.541	Li x Sh (Re)
Re x Li	1	3.0	0.1	0.780	4.6E+06	13.7	0.066	Gr x Sh (Re)
Te x Sh (Re)	2	200.9	4.0	**0.028**	1.4E+07	0.9	0.408	Res
Li x Sh (Re)	2	29.5	0.6	0.561	3.3E+05	0.0	0.979	Res
Te x Li	1	173.3	2.7	0.240	2.5E+06	19.7	**0.047**	Gr x Li x Sh (Re)
Re x Te x Li	1	36.9	0.6	0.525	9.3E+06	1.5	0.345	Gr x Li x Sh (Re)
Li x Te x Sh (Re)	2	63.3	1.3	0.296	6.2E+06	0.4	0.671	Res
Res	32	50.1			1.5E+07			
Cochran's test		*C = 0.2046, p > 0.05*			*C = 0.3038, p > 0.05*			
SNK		*Te x Sh (Re)*			*Te x Li*			
		Cem – Col > War			*Uns – Col > War*			
		Por x War – Car > Via			*All Te – Sha > Uns*			
		Wal x War – Cwy > Cem						

Source	**df**	**Exp 5.1**			**Exp 5.2**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	1	295.6	17.3	0.053	2.3E+30	1.5	0.340	Sh (Re)
Sh (Re)	2	17.1	0.2	0.834	1.5E+30	5.4	0.010	Res
Te	1	673.4	23.3	**0.040**	1.1E+30	1.7	0.323	Li x Sh (Re)
Li	1	4967.4	85.3	**0.012**	9.0E+31	558.0	0.002	Gr x Sh (Re)
Re x Te	1	5.8	0.2	0.697	4.4E+29	0.7	0.489	Li x Sh (Re)
Re x Li	1	83.4	1.4	0.354	4.5E+28	0.3	0.651	Gr x Sh (Re)
Te x Sh (Re)	2	29.0	0.3	0.736	6.2E+29	2.3	0.122	Res
Li x Sh (Re)	2	58.2	0.6	0.544	1.6E+29	0.6	0.564	Res
Te x Li	1	424.6	7.2	0.115	3.7E+27	0.0	0.965	Gr x Li x Sh (Re)
Re x Te x Li	1	269.6	4.6	0.166	7.5E+28	0.1	0.846	Gr x Li x Sh (Re)
Li x Te x Sh (Re)	2	58.9	0.6	0.540	1.5E+30	5.6	**0.008**	Res
Res	32	93.7			2.8E+29			
Cochran's test		*C = 0.2603, p > 0.05*			*C = 0.3325, p > 0.05*			
SNK		*Te*			*Li x Te x Sh (Re)*			
		Col > War			*All Sh at all Te – Sha > Uns*			
		Li			*Via x Uns – Col > War*			
		Sha > Uns			*Por x Col x Uns – Via > Car*			
					Por x War x Sha – Via > Car			

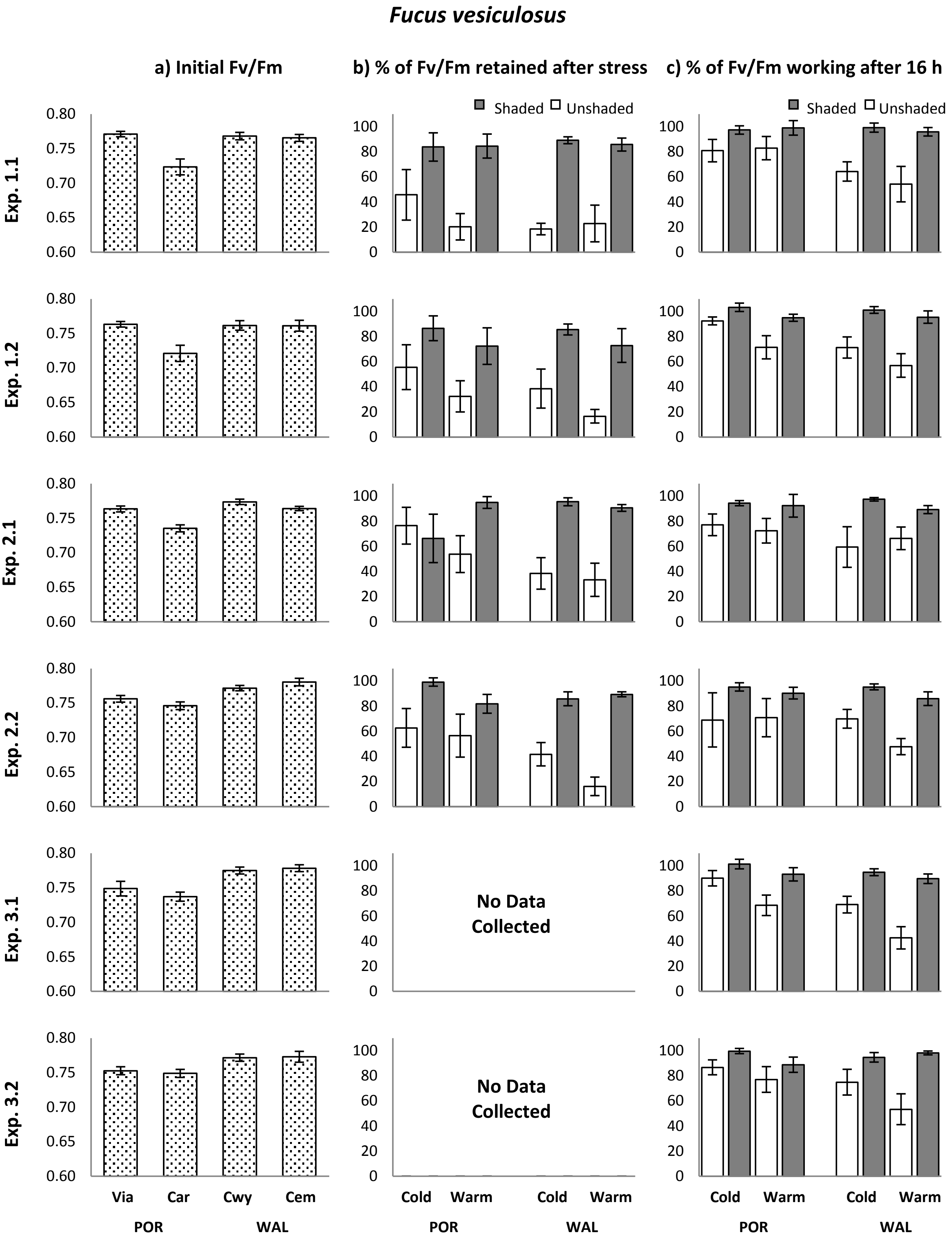


Figure 19: Experiment 1.1, 1.2, 2.1, 2.2, 3.1 & 3.2; Error bars = ±1SE. a) *F. vesiculosus* initial Fv/Fm values before stress from Portuguese (*POR*) and Welsh (*WAL*) shores (*Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay*). b) Percentage of initial Fv/Fm, from Portuguese (*POR*) and Welsh (*WAL*) *F. vesiculosus* tissues, retained 30 minutes after the application of light intensity and temperature treatments. c) Percentage of initial Fv/Fm, from Portuguese (POR) and Welsh (WAL) *F. vesiculosus* tissues, working 16 hours after application of light intensity and temperature treatments on experiments.

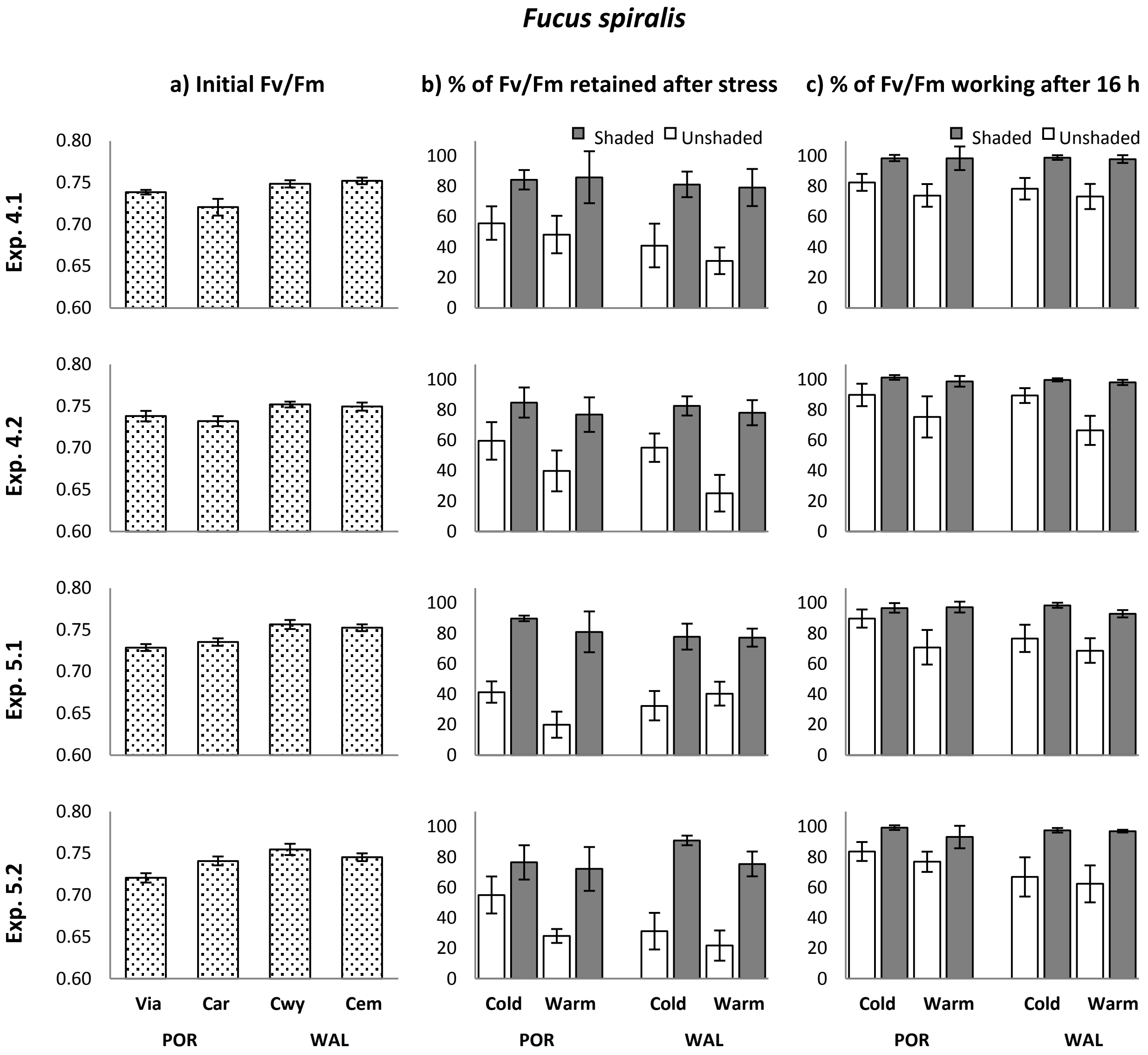


Figure 20: Experiment 4.1, 4.2, 5.1 & 5.2; Error bars = ±1SE. a) *F. spiralis* initial Fv/Fm values before stress from Portuguese (*POR*) and Welsh (*WAL*) shores (*Via: Viana do Castelo; Car: Carreço; Cwy: Porth Cwyfan; Cem: Cemlyn Bay*). b) Percentage of initial Fv/Fm, from Portuguese (*POR*) and Welsh (*WAL*) *F. spiralis* tissues, retained 30 minutes after the application of light intensity and temperature treatments. c) Percentage of initial Fv/Fm, from Portuguese (*POR*) and Welsh (*WAL*) *F. spiralis* tissues, working 16 hours after application of light intensity and temperature treatments.

4.4 DISCUSSION

The warming of the Earth's climate system is unequivocal and reflected in the increases in global average air and ocean temperatures during the last 50 years (IPCC 2007). The response of marine and terrestrial organisms to recent warming has been observed with species from both central and extreme ranges of distribution being affected (Parmesan 1996; Sagarin *et al.* 1999; Helmuth *et al.* 2002; Sagarin & Gaines 2002; Helmuth *et al.* 2006b; Hawkins *et al.* 2008; Hawkins *et al.* 2009). Studies undertaken in the Portuguese intertidal seem to indicate that some species ranges are shifting in this biogeographical transition zone. Lima *et al.* (2007) interestingly reported on distributional shifts of species in Portugal, showing an expansion of the range of warm-water species northwards, while northern species showed no particular shifting trend. The abundance of fucoid algae declines from the northern to southern coasts of the northeast Atlantic and some species reach their southern limit of distribution in Portugal or in nearby coastal areas (Ballantine 1961; Ardré 1970; Hawkins & Jones 1992; Lima *et al.* 2007). In northern Portugal, where this study was undertaken many fucoid species are present but their abundance (Chapter 2) and reproductive output (Chapter 5; Viejo *et al.* 2011) is limited. Can this decline in the abundance of fucoid species be linked with a difficulty in dealing with the physical environment? Are the harsher summer conditions limiting the survival of species like *F. vesiculosus* and *F. spiralis* in Portugal or are the local populations adapted to deal with those levels of stress? In order to test such hypotheses the photophysiological response of algae to stressful conditions based on temperature and light regimes found naturally on Portuguese shores was measured. As a comparison, plants from North Wales were exposed to the same regime to explore if fucoid populations are adapted to local climatic conditions. Although the levels of stress (temperature and light) used on the experiments were at a level experienced by Welsh plants, they would typically not experience them regularly.

Results presented in this study indicate that different populations of *F. vesiculosus* and *F. spiralis* have similar potential to use the available excitation energy for photochemistry in PSII reaction centers and that algae collected from all shores, in central and southern range geographical regions, were in healthy conditions (measurements of Fv/Fm made before application of stress). However, the observations and results of these experiments also show that the ability to tolerate such levels of summer emersion stress varies depending on the intensity of the stress provided, e.g. the levels of light and temperature, and on the geographical origin of the populations.

Differences in the ability to deal with emersion stress between fucoid populations of the same species have previously been observed. Comparisons between marine and brackish populations of *F. vesiculosus,* for example, showed that Baltic brackish algae are less able to recover maximum photochemical yield (Fv/Fm) after a freezing emersion period than North Sea marine algae (Pearson *et al.* 2000). Even when subjected to milder temperatures, between 0ºC and 20ºC during emersion periods, it is only the brackish algae population that shows a decrease in Fv/Fm values during stress, values that are only recovered in treatments where Baltic algae had been subjected to 0ºC (Gylle *et al.* 2009). In contrast, the experiments presented here focus on the southern extreme of distribution, therefore on the effect of high light intensity levels and higher temperatures. The fact that the photosynthetic system of northern populations of *F. vesiculosus* was significantly more affected than the ones from southern populations, when subjected to physical conditions recreating a southern summer period of emersion, shows that populations of *F. vesiculosus* are physiologically adapted to the levels of physical stress to which they are normally subjected. When strong light intensity levels were applied, the effect of these stressful summer emersion conditions in *F. vesiculosus* was more marked in Welsh than in Portuguese populations. These results indicate that populations from the central range are greatly affected by episodic warm cloudless days. Welsh *F. vesiculosus* populations showed lower recovery potential when high light intensities had been applied (the trend was always present across experiments and was significant in 3 out of 6 experiments) and as such demonstrated lower capacity to use the available excitation energy for photochemistry after the recovery period compared to the Portuguese *F. vesiculosus* specimens. The fact that Fv/Fm values did not completely recover, suggests damage to the electron transport chain, probably due to the insufficient capacity of photoprotection mechanisms. Although, similar results have been explained by higher rates of water loss (Dring & Brown 1982; Ji & Tanaka 2002; Gylle *et al.* 2009), in this case the percentage of water loss resulting from stress was not significantly different between algae from distinct geographical regions. So, could this pattern of stress be observed across *F. vesiculosus* populations from distinct geographical regions be similar in species that normally occupy a different shore height?

The analysis of *F. spiralis* results still shows that populations are adapted to different levels of physical conditions that they sustain in their natural environment. However, results for *F. spiralis* are more complex reflecting the broader “functional niche” that this species possesses. A range of previous work has shown that the tolerance to climatic variability and capacity to tolerate desiccation of these sessile species is greater than the ones living on lower areas of the shore

(Schonbeck & Norton 1978; Dring & Brown 1982; Todd & Lewis 1984). Dring & Brown (1982) showed that *F. spiralis* specimens were capable of a complete recovery of photosynthetic levels after 2 hours, even when subjected to tissue water loss of 80 to 90%, a result similar to the ones presented here for specimens subjected to shaded conditions. On the shore, *F. spiralis* sustains longer periods of exposure to greater amplitudes of climatic conditions than *F vesiculosus.* Its fundamental niche is therefore wider than that of *F. vesiculosus* or other intertidal algae species living lower on the shore. The recovery levels of *F. spiralis* presented after 16 hours were similar, independent of their geographical origin across all experiments, indicating that if damage to the electron transport chain occurred it affected similarly fucoid specimens from both geographical regions. However, the results of half of the experiments also showed that geographical region of origin of *F. spiralis* influenced the degree of loss of photosynthetic capacity 20 minutes after the application of the stress period. The fact that results obtained 20 minutes after the application of the stress period, when specimens had not had time to physiologically recover, still show significant differences between populations from distinct geographical regions, indicates that different populations from this species have their photosystem adapted to local climate as well. Overall, the combination of the results from all the experiments indicates that both fucoid species studied have their photosynthetic system adapted to local climatic conditions. However, the levels of physical conditions provided seem to affect less *F. spiralis* than *F. vesiculosus,* a result in accordance with the vertical position of the shore normally occupied by these species, which reflects the differences in their “fundamental niche” sizes.

Northern populations of both fucoid species were the most affected. However, the results also indicate that physical factors in southern regions can be one of the reasons for the decline in fucoid abundance levels observed in chapter 2, as none of the specimens exposed to unshaded conditions was able to fully regain the initial Fv/Fm ratio. As previously shown by other studies on photosynthetic depth limitation (Duarte 1991; Rohde *et al.* 2008), light intensity can be a main factor determining the distribution and abundance of photosynthetic species. The results presented here, confirm that light intensity is a main factor affecting the photosystem of both species. The importance of light intensity was greater than temperature variation across experiments, affecting the loss of tissue water content and the photosynthetic performance of algae while subjected to stress, as well as, limiting the photosynthetic recovery success over time. Specimens of either *F. vesiculosus* or *F. spiralis,* exposed to higher light intensities, during emersion stress, lost a greater amount of tissue water content and photosynthetic capacity than the ones subjected to shade treatments. When subjected to low light intensity levels (80% reduction) both fucoid species were capable of regaining levels of photosynthetic performance equal or very similar to the ones presented before the application of stress. However, specimens of any population from either species subjected to the high light intensity treatment were unable to fully regain the Fv/Fm values experienced before stress, even after the 16 hours recovery period. Temperature effects were not consistent throughout the experiments, but when significant, treatments with warmer conditions generally favoured the decline of Fv/Fm values after stress and reduced the recovery even after the 16 hour period for specimens from both species. This is in accordance with previous results from Pearson *et al.* (2009). Globally, these results show that southern fucoid populations, if subjected consecutively to levels of stress provided during the experiments, can have their photosynthetic performance diminished, leading to algae with reduced fitness that ultimately could affect growth rates, reproductive output and tissue health putting at risk the survival of these populations.

In summary, the results of this study contribute to clarify which ecological processes may be leading to the decline in abundance of some fucoid species near their southern limits of distribution. Biological processes, like grazing pressure and facilitation, are probably important for the decline of fucoid abundance at their southern limits of distribution. However, the experiments performed here clearly demonstrate that physical stress, caused by emersion on warm cloudless summer days, can be an important factor limiting the abundance of some fucoid species by directly affecting their photosynthetic performance. Portuguese specimens exposed to unshaded conditions were not able to fully regain the initial Fv/Fm ratio, even after a 16 hour recovery period, which proves the previous idea. By demonstrating that populations inhabiting different geographical regions can show adaptation of their photosynthetic system to local climatic conditions and be affected differently when exposed to equal levels of physical stress, this work also informs future studies of the importance of local historical climatic background. This often disregarded characteristic can be an important factor to take into consideration, especially when analysing possible effects of climate change or local anthropogenic stresses. As showed by Helmuth *et al.* (2002), the interaction of climate and timing of low tides can seriously influence the thermal environment and promote a complex network of hotspots in which northern sites can be more stressful than southern sites. So, the expected effects of climate change may not lead to a smooth gradient of stress being observed, making the capacity of local populations very important to avoid localized extinctions in the areas most affected by climate change. Therefore, in order to fully understand and predict the effects of change in climatic conditions local capacity of populations should also be included in future models being developed.

CHAPTER 5

PATTERNS OF REPRODUCTIVE OUTPUT OF FUCOID SPECIES IN CENTRAL AND RANGE EDGE POPULATIONS

5.1 INTRODUCTION

Large brown macroalgae, particularly fucoid species, dominate rocky intertidal areas, especially wave protected sites, across the north Atlantic. They play an important community structuring role, providing a three-dimensional habitat and influencing the associated understory community in a number of different ways (Hawkins 1983; Hawkins & Harkin 1985; Chapman & Johnson 1990; McCook & Chapman 1991; Jenkins *et al.* 1999a). Macroalgae are essential for the survival of a large associated community through facilitation, by providing amelioration of extreme environmental factors during emersion (McCook & Chapman 1991; Jenkins *et al.* 1999a). Although present throughout Europe, their biomass levels decline from northern to southern European regions (Chapter 2; Ballantine 1961; Hawkins *et al.* 1992). The processes behind this decline are not fully understood, although factors such as grazing pressure, emersion stress and recruitment failure are probably important (Chapter 4; Jenkins *et al.* 2005; Viejo *et al.* 2011). Towards their range limits in southern Europe, fucoid macroalgae are likely to be greatly impacted by rising seawater temperatures and air temperatures as a consequence of climate warming, although there is a lack of quantitative studies in this area (Lima *et al.* 2007). In general, it is known that these ’northern’ species decrease their abundance in southern Europe (Chapter 2) but the extent to which the environment has a negative effect on their fitness is still largely undetermined (see Chapter 4 for environmental effects on photophysiology).

Maintenance of a viable population is dependent on the density of adults forming that population (Hixon *et al.* 2002). A population is considered viable when it is persistent, defined as able to survive over several generations (Hixon *et al.* 2002). Macroalgal species can show complex density dependent relationships (Schiel & Foster 2006). Density levels can at one end, when very low, lead to extinction, if mortality rates exceed reproductive replacement over a long enough period (Sodhi *et al.* 2009). When not leading to extinction, density levels can promote positive and negative effects (Reed 1990; Creed *et al.* 1998; Scrosati 2005). The effect of density levels of a species can be negative or positive, by promoting survival at one density level or reducing it at another. Reed (1990) showed that low density treatments of the subtidal kelp *Pterygophora californica* had higher growth levels and promoted greater levels of reproductive maturity, while high density treatments prevented recruitment.

Generally, populations with greater density/biomass levels have increased reproductive capacity at least up to the point when resources become scarce. Reproductive effort of algae has been the focus of several studies, although the methods used to measure it have not been consistent. Two of the most widely used measurements are total reproductive effort and annual reproductive effort. Work developed by Aberg (1996), applies annual reproductive effort measurements to define patterns of reproductive effort across two populations of the brown alga, *Ascophyllum nodusum*. This method, requires the identification of current and previous years’ growth in perennial fucoid algae used to calculate the annual reproductive effort, through the following formula:

$$\textbf{Annual reproductive effort \%} = \frac{\text{Receptacular biomass}}{\text{Biomass produced in the last year}} \times 100$$

These annual parameters are difficult to distinguish in some fucoid species. Consequently, due to the morphological characteristics of some species, a broader delineation of reproductive effort, total reproductive effort, has also been adopted. This measurement has been widely used (Back *et al.* 1993; Kalvas & Kautsky 1993; Aberg 1996) and refers to the percentage of receptacular biomass to total biomass, calculated through the following formula:

$$\textbf{Total reproductive Capacity \%} = \frac{\text{Receptacular biomass}}{\text{Total biomass}} \times 100$$

Changes in reproductive effort (measured in various ways) between different fucoid populations have been described in the literature over the last decades (Cousens 1986; Ang 1992; Mathieson & Guo 1992; Back *et al.* 1993; Araujo *et al.* 2011; Viejo *et al.* 2011). Different factors can influence reproductive effort of fucoid species. The influence of algae size on reproductive capacity of fucoid species was described in species like *A. nodosum* (Aberg 1996) and *Sargassum muticum* (Arenas & Fernandez 1998). These studies showed that bigger individuals have greater reproductive effort than smaller specimens. Exposure to different physical environments has also been shown to affect reproductive effort of fucoid species. Comparisons between populations from estuarine and coastal environments have shown that reproductive effort of different species can respond in opposite ways to the decline in salinity (Mathieson & Guo 1992). Mathieson & Guo (1992) showed a decline in the reproductive effort of *Fucus spiralis* when exposed to estuarine waters, whereas populations of *A. nodosum* showed increased reproductive capacity in waters with reduced salinity. Wave action is also greatly important for fucoid species, being described as a major force shaping the horizontal distribution patterns of fucoid species. So, it is no surprise that they too influence levels of reproductive effort. As an example, populations of *F. spiralis* were shown to develop lower reproductive capacity as they become exposed to increasing levels of wave action (Mathieson & Guo 1992).

Variability in abundance of the fucoid community across Europe, more specifically a decline in macroalgae abundance levels from northern to southern Europe, has long been proposed (Ballantine 1961). Through a large quantitative survey (Chapter 2) this proposed decline in fucoid species biomass on the southern European shores was demonstrated, indicating that these populations are under some abiotic or biotic pressure. Although a study from Ladah *et al.* (2003) indicates that fertilization success of *F. spiralis* and *F. vesiculosus* is still high in south Europe, further experimental work also showed that levels of recruitment are low even when *Patella* spp. grazers are excluded (Chapter 3;(Coleman *et al.* 2006). Taking in consideration the lack of recruitment observed on southern populations and that variation in reproductive effort can occur in populations subjected to different environments it is possible that southern fucoid populations may have different reproductive capacity than northern fucoid populations. Therefore, this study aims to evaluate if in addition to the decline in biomass levels, southern fucoid populations also presented diminished reproductive capacity when compared with populations from central areas of their distribution.

For the development of this study, fucoid specimens from North Wales and northern areas of Portugal (above Matosinhos area) were collected. *Ascophyllum nodosum* and *Fucus serratus* species occur in both these geographical regions along with *F. vesiculosus* and *F. spiralis* (Lima *et al.* 2007). However, based on information gathered from the European survey (Chapter 2) specimens of *Ascophyllum nodosum* and *Fucus serratus* were shown to be absent from areas further south in Portugal. These patterns agree with the southern limits defined by Lima *et al.* (2007) for both species and consequently these two species were considered too rare to be used in this experiment. Therefore, the experimental work was developed using only specimens of *F. vesiculosus* and *F. spiralis*, as these were more common and also had previously shown susceptibility to the levels of stress caused by emersion periods during summer along the Portuguese coast (Chapter 4).

To formally test my hypothesis, several variables were quantified for both species. The variables were measured at three populations with high biomass levels from central areas (North Wales) and three populations near the southern extreme of their distribution (Portugal), where a decline in biomass had been observed. Contrary to previous studies, which tested for the effect of size, salinity and wave exposure levels on the reproductive capacity of fucoid species, the approach of this study focused solely on latitudinal effects and minimized variation in local environmental and biotic factors. Thus algae were collected with a fixed and restricted range of frond lengths (20 to 30 cm), exclusively from moderately wave exposed and fully saline shores. This restrictive methodology permitted control of some of the environmental and morphological variability that has been showed to affect reproductive effort (Cousens 1986; Mathieson & Guo 1992; Aberg 1996; Arenas & Fernandez 1998). By controlling parameters that are not dependent of latitudinal variation, it was possible to formally test if specimens of similar length exposed to different geographical environments could develop different morphologies and reproductive capacity levels.

5.2 METHODS

5.2.1 Collection and preservation of specimens

Collection of fertile reproductive *Fucus vesiculosus* and *Fucus spiralis* individuals was made on three shores in north Portugal and North Wales. These geographical regions are separated by 11º of latitude and correspond to areas of high (Wales) and low (Portugal) biomass for these species (Chapter 2). Portuguese populations of both species are near the southern limit of distribution. In North Wales, the collection was made at Porth Cwyfan (53.182821°, -4.489829°), Cemlyn Bay (53.406900°, -4.534340°) and Trearddur Bay (53.271076°, -4.624407°), while in Portugal algae were collected from Viana do Castelo (41.692656°, -8.850554°), Areosa (41.712688°, -8.863659°) and Carreço (41.729574°, -8.873184°) populations. All the locations were moderately exposed to wave action from fully saline environments with maximal tidal range varying from 6.1 m in North Wales to 3.6 m in Portugal.

The reproductive period, more precisely the period of gamete release, takes place for *F. vesiculosus* in the United Kingdom normally between April and July (Knight & Parke 1950), while in Portugal the release of gametes occurs at least as early as April extending until June (Ladah *et al.* 2003; Ladah *et al.* 2008). *Fucus spiralis* show similar but slightly longer periods when reproductive structures are found in algae. In the United Kingdom those periods occur from May to November (Niemeck & Mathieson 1976) and in Portugal it have been shown to happen from at least April to June (Ladah *et al.* 2003; Ladah *et al.* 2008). Due to logistical restrictions, only one sampling period was carried out at each region. To try to maximise possible differences, all the specimens were collected in the last 10 days of May, which is within the peak of the reproductive season of *F. vesiculosus* and within the reproductive season of *Fucus spiralis* (Knight & Parke 1950; Niemeck & Mathieson 1976; Ladah *et al.* 2003; Ladah *et al.* 2008). In order to guarantee that only adult specimens with similar size were collected, all specimens were measured approximately in the field. Quadrats for collection were randomly placed across a stretch of shore, approximately 100 m long, from where only fucoid individuals with length between 20 and 30 cm were collected. In total, forty and thirty specimens of *Fucus vesiculosus* and *Fucus spiralis*, respectively, were collected from each of the shores. After being collected, the algae were transported in cold boxes to the laboratory where they were frozen to avoid tissue deterioration until later assessment.

5.2.2 Measurement of response variables

The length of each specimen was determined in laboratory conditions. In order to understand if populations from different geographical regions have distinct reproductive capacity, numbers of receptacles per individual were determined. This procedure will not only provide information on the reproductive capacity, but also on the strategy adopted through the measurement of mean receptacle biomass. A crude estimate of thallus volume (bushiness) was also achieved by measuring the maximum circumference, as described by Aberg (1990). This method consisted of laying all fronds from one individual in a pile, so that all the stipes were parallel, while measuring the circumference of the bundle of fronds in several places until the maximum value is determined. Using the determined values it is possible to achieve a crude estimate of volume that reflects “bushiness” of each individual using the formula: length x squared circumference. Subsequent to these measurements, the dry biomass of vegetative and reproductive tissues was separately determined after a drying period of 72 hour at 60º C. In order to have an index of reproductive effort (Ang 1992; Back *et al.* 1993) that could be comparable between specimens, a measurement of dry reproductive biomass to total dry biomass was calculated using the following formula:

$$\textbf{Total reproductive effort (\%)} = \frac{\text{Dry reproductive biomass}}{(\text{Dry reproductive biomass} + \text{Dry vegetative biomass})} \times 100$$

Seven different response variables were analyzed for each of the species. One of these, fucoid length, was expected not to vary across geographical regions due to stratification of sampling. To test further morphological variation, volume (bushiness) and vegetative dry tissue biomass data were used, while data on the number of receptacles, mean receptacle dry biomass, reproductive dry tissue biomass and reproductive effort were used to analyze reproductive capacity of populations from both regions.

5.2.3 Statistical design

In order to analyse the effects geographical region and shore identity had on all response variables, a two way nested ANOVA was performed with data from each fucoid species. Geographical origin was treated as a fixed factor, while shore was considered random and nested in geographical origin. Significant results from the two way ANOVA were further investigated with SNK (Student Newman Keuls) multiple comparisons. Only SNK results showing differences are displayed in the results section. The assumption of homogeneity of variances was analysed with Cochran's test and when necessary data transformation was completed. The statistical package WinGMAV5 (EICC, University of Sydney) was chosen to run all the analyses.

5.3 RESULTS

5.3.1 Fucoid morphology

5.3.1.1 Fucoid length

Due to the methodology used in the field, where crude measurements of length were completed before fucoid collection, variation in total length was expected to be limited for both fucoid species (within lengths of 20 to 30 cm). Importantly, no significant differences were detected across distinct geographical regions. However, small amplitude variability across shores was still found for both fucoid species (Table 25 & Table 26: Length - *SNK of Sh (Re)*, Figure 21 & Figure 22). These results probably reflect the frequency of large individuals, with the shores with bigger specimens displaying slightly higher mean values.

5.3.1.2 Volume (Bushiness)

Fucus vesiculosus

The volume of *F. vesiculosus* individuals differed among geographical regions (Table 25: Volume). Individuals that characterized southern populations had a smaller volume than populations from North Wales (Table 25, Figure 21), which indicates that for similar fucoid lengths northern populations have bushier thalli. Variability among shores was also detected ((Table 25: Volume - *Sh (Re)*), but only among Welsh shores, where individuals from Porth Cwyfan showed greater volume (Figure 21).

Fucus spiralis

Similarly to the *F. vesiculosus*, populations of *F. spiralis* from different geographical regions also had significantly different volumes (Table 26), again with Portuguese populations being smaller (Figure 22). Welsh populations showed larger volumes but also volume variability between populations from different shores (Table 26: Volume - *SNK of Sh (Re)*). For this species greater volumes were observed in specimens from Cemlyn Bay (Figure 22).

5.3.1.3 Vegetative dry tissue biomass

Fucus vesiculosus

Although, levels of vegetative dry tissue biomass varied among shores from both geographical regions (Table 25: Dry vegetative biomass - *SNK of Sh (Re),* Figure 21), they were not significantly different between Portuguese and Welsh populations (Table 25). These results, especially the levels obtained by individuals collected from Porth Cwyfan showed that significantly greater vegetative biomass levels occurred on some shores, however the differences occurring at a local scale are not apparent at the larger scales.

Fucus spiralis

As in *F. vesiculosus* populations, levels of vegetative dry tissue biomass in *F. spiralis* populations were not dependent on geographical region (Table 26: Dry vegetative biomass). Variability in this species was only observed across shores within the Welsh region (Table 26: Dry vegetative biomass - *SNK of Sh (Re)*).

5.3.2 Reproductive indicators and related variables

5.3.2.1 Number of receptacles per individual

In an attempt to understand if populations from different geographical regions have distinct reproductive capacity, the number of receptacles per individual was determined. This provides some information on the reproductive capacity but also on the strategy adopted.

Fucus vesiculosus

The number of receptacles present in each individual reached a mean value of 71 and 117 from the Portuguese and Welsh shores, respectively (Figure 21). These values were significantly different (Table 25), which indicates that Portuguese populations are developing fewer receptacles per individual alga. Interestingly, the mean values among shores from each of the geographical regions were similar, which indicates that this might be a consistent pattern for moderately wave exposed rocky shores in both regions (Figure 21).

Fucus spiralis

For this high shore species, the ANOVA result confirms non significant variability between shores and significant differences occurring between geographical regions (Table 26: Number of receptacles). Similar to the results observed for *F. vesiculosus*, Portuguese populations of *F. spiralis* also had significantly lower numbers of reproductive receptacles per individual (Table 26, Figure 22).

5.3.2.2 Mean dry biomass of receptacles

Fucus vesiculosus

The mean dry biomass of receptacles varies with shore but no variation was detected between geographical regions (Table 25). Mean values of 0.05 g were registered for *F. vesiculosus* for Portuguese and Welsh regions. The lowest value was registered at Areosa in Portugal (0.03 g).

Fucus spiralis

Geographic region was not a significant factor but shore identity significantly affected the mean dry biomass of *F. spiralis* receptacles in Portugal (Table 26). All shores apart from Carreço showed mean dry biomass of receptacles between 0.03 and 0.04 g. Whereas receptacles were slightly heavier in Carreço, reaching a mean value of 0.05 g (Table 26: Mean receptacles dry biomass - *SNK of Sh (Re)*, Figure 22).

5.3.2.3 Reproductive dry tissue biomass

Fucus vesiculosus

As shown for the number of receptacles, reproductive dry tissue biomass levels also varied between Welsh and Portuguese shores (Table 25, Figure 21). As a whole, Welsh populations still produced more than twice the amount of reproductive tissue biomass compared with the populations from Portuguese shores (Figure 21). Welsh populations also showed significant variation in levels of reproductive dry tissue biomass among shores (Table 25: Dry reproductive biomass - *SNK of Sh (Re)*).

Fucus spiralis

The variability in levels of *F. spiralis* reproductive dry tissue biomass between Welsh and Portuguese shores was also detected, with Welsh shores once more favouring the development of greater biomass levels (Table 26). For this species, although variability between shores is shown as a significant factor, the post hoc SNK did not identify which shores differed.

5.3.2.4 Reproductive effort

Fucus vesiculosus

The percentage of reproductive tissue per total weight gives an indication of reproductive effort. Differences in reproductive effort of *F. vesiculosus* populations from distinct geographical regions were not detected (Table 25: Reproductive effort), despite some variability observed in populations from different shores within Portuguese and Welsh regions (Table 25: Reproductive effort - *SNK of Sh (Re)*, Figure 21). Overall, the mean reproductive effort ranged between 30 and 39.9% in Portuguese shores and 30 and 51.8% in Welsh shores (Figure 21).

Fucus spiralis

Levels of *F. spiralis* reproductive effort were not significantly different across both geographical regions and within each geographical region (Table 26). Mean reproductive effort was between 39.7 and 46.5% in Portuguese shores and 36.1 and 40.3% in Welsh shores. This indicates that the ratio of reproductive tissue per total weight was similar across populations from different geographical regions even if they had distinct volume, number of receptacles and dry reproductive biomass levels per individual.

Table 25: Two way mix model ANOVA of *Fucus vesiculosus* length, volume, number of receptacles, mean receptacle dry biomass, dry vegetative biomass, dry reproductive biomass and reproductive effort. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Are: Are; Car: Carreço; Via: Viana do Castelo; Cwy: Porth Cwyfan; Cem: Cemlyn Bay; Tre: Trearddur Bay); Res: Residual.*

Source	**df**	**Length**			**Volume (bushiness)**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	1	65.5	0.2	0.665	3890.6	15.9	**0.016**	Sh (Re)
Sh (Re)	4	300.5	19.1	**<0.001**	244.0	2.7	**0.033**	Res
Res	234	15.8			91.3			
Cochran's test		*C = 0.2191, p > 0.05*			*C = 0.2374, p > 0.05*			
SNK		*Sh (Re)*			*Re*			
		Por - Car>Via=Are			*Wal>Por*			
		Wal - Cwy>Tre>Cem			*Sh (Re)*			
					Wal - Cwy>Cem=Tre			

Source	**df**	**Number of receptacles**			**Mean dry biomass of receptacle**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	1	1.3E+05	18.3	**0.013**	0.0044	0.3	0.596	Sh (Re)
Sh (Re)	4	6907.5	1.7	0.161	0.0133	34.7	**<0.001**	Res
Res	234	4166.9			0.0004			
Cochran's test		*C = 0.2393, p > 0.05*			*C = 0.4003, p < 0.01*			
SNK		*Re*			*Sh (Re)*			
		Wal>Por			*Por - Car>Via>Are*			
					Wal - Cem>Cwy>Tre			

Source	**df**	**Dry vegetative biomass**			**Dry reproductive biomass**			**Reproductive Effort**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	1	11.7	5.8	0.074	27.7	13.7	**0.021**	3250.1	0.84	0.412	Sh (Re)
Sh (Re)	4	2.0	9.2	**<0.001**	2.0	6.8	**<0.001**	3886.3	25.9	**<0.001**	Res
Res	234	0.2			0.3			150.1			
Cochran's test		*C = 0.2153, p > 0.05*			*C = 0.2152, p > 0.05*			*C = 0.2850, p < 0.01*			
SNK		*Sh (Re)*			*Re*			*Sh (Re)*			
		Por - Are>Car			*Wal>Por*			*Por - Car>Via>Are*			
		Wal - Cwy> Tre=Cem			*Sh (Re)*			*Wal - Cem>Tre=Cwy*			
					Wal - Cem>Cwy=Tre						

Table 26: Two way mix model ANOVA of *Fucus spiralis* length, volume, number of receptacles, mean receptacle dry biomass, dry vegetative biomass, dry reproductive biomass and reproductive effort. Post hoc SNK tests of significant differences are presented. *Abbreviations used - Re: Geographical Region (Wal: Wales; Por: Portugal); Sh: Shore (Are: Are; Car: Carreço; Via: Viana do Castelo; Cwy: Porth Cwyfan; Cem: Cemlyn Bay; Tre: Trearddur Bay); Res: Residual.*

Source	**df**	**Length**			**Volume (bushiness)**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	1	299.5	4.9	0.092	125.7	10.3	**0.033**	Sh (Re)
Sh (Re)	4	61.6	3.9	**0.005**	12.2	3.7	**0.007**	Res
Res	174	15.9			3.4			
Cochran's test		*C = 0.2706, p > 0.05*			*C = 0.2646, p > 0.05*			
SNK		*Sh (Re)*			*Re*			
		Por - Car=Are>Via			*Wal>Por*			
					Sh (Re)			
					Wal – Cem>Cwy			

Source	**df**	**Number of receptacles**			**Mean dry biomass of receptacle**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	
Re	1	4.2E+04	9.5	**0.037**	<0.0001	0.01	0.939	Sh (Re)
Sh (Re)	4	4455.9	1.4	0.248	0.0010	4.03	**0.004**	Res
Res	174	3265.9			0.0003			
Cochran's test		*Re*			*Sh (Re)*			
SNK		*Wal>Por*			*Por - Car>Via>Are*			
					Wal - Cem>Cwy>Tre			

Source	**df**	**Dry vegetative biomass**			**Dry reproductive biomass**			**Reproductive Effort**			***F* ratio vs**
		MS	***F***	**p**	**MS**	***F***	**p**	**MS**	***F***	**p**	
Re	1	39.0	3.9	0.120	8.3	15.7	**0.017**	720.5	2.6	0.179	Sh (Re)
Sh (Re)	4	10.1	2.6	**0.036**	0.5	2.6	**0.039**	272.7	2.0	0.102	Res
Res	174	3.8			0.2			138.6			
Cochran's test		*C = 0.2524, p > 0.05*			*C = 0.2786, p > 0.05*			*C = 0.2495, p > 0.05*			
SNK		*Sh (Re)*			*Re*						
		Wal - Cem> Tre=Cwy			*Wal>Por*						
					Sh (Re)						
					Por – Are=Car=Via						
					Wal - Cem=Tre=Cwy						

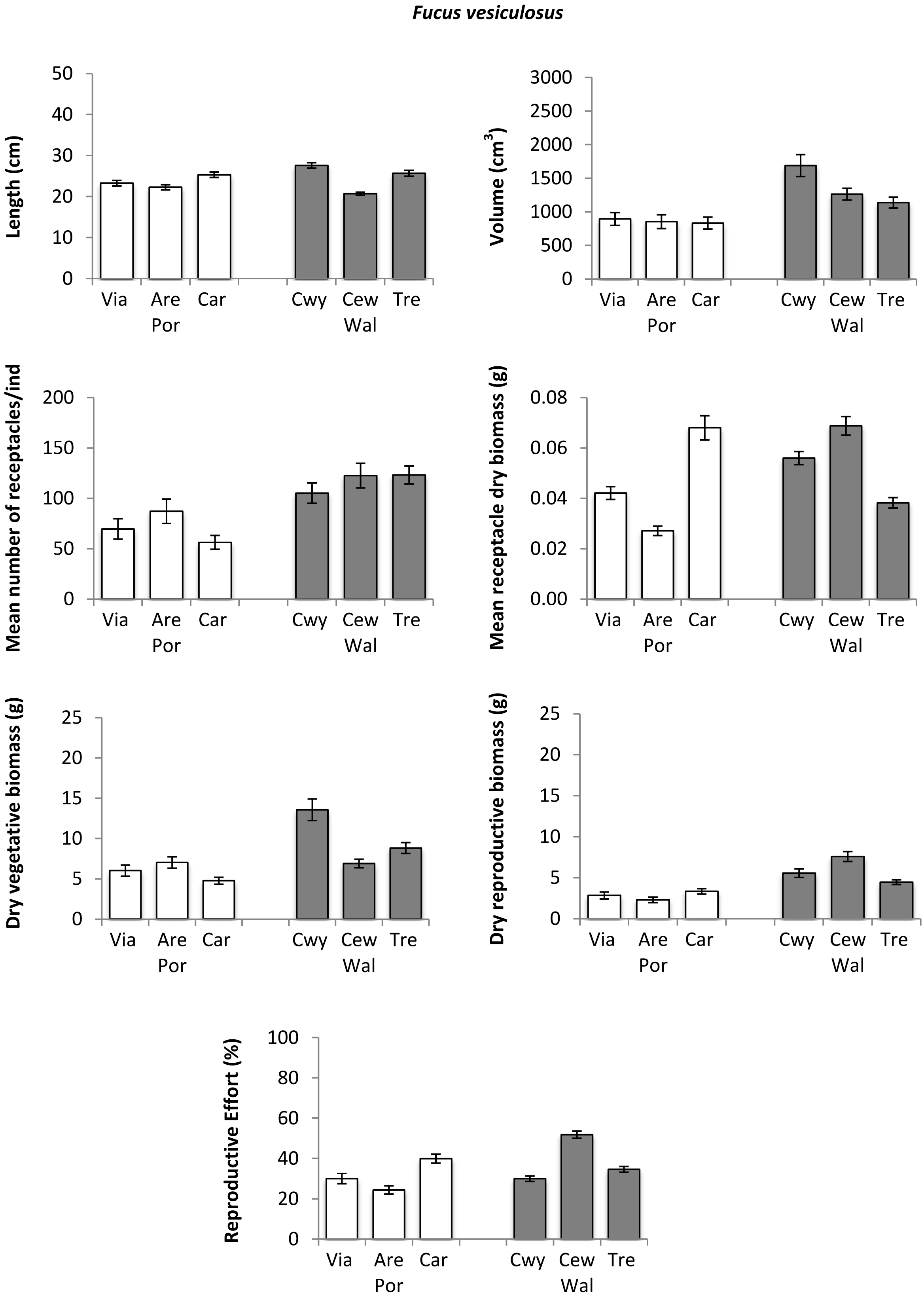


Figure 21: *Fucus vesiculosus* length, volume, number of receptacles, mean receptacle dry biomass, dry vegetative biomass, dry reproductive biomass and reproductive effort at Portugal (POR) and Wales (WAL) across different shores (*Are: Areosa; Car: Carreço; Via: Viana do Castelo; Cwy: Porth Cwyfan; Cem: Cemlyn Bay; Tre: Trearddur Bay*); Error bars = ±1SE.

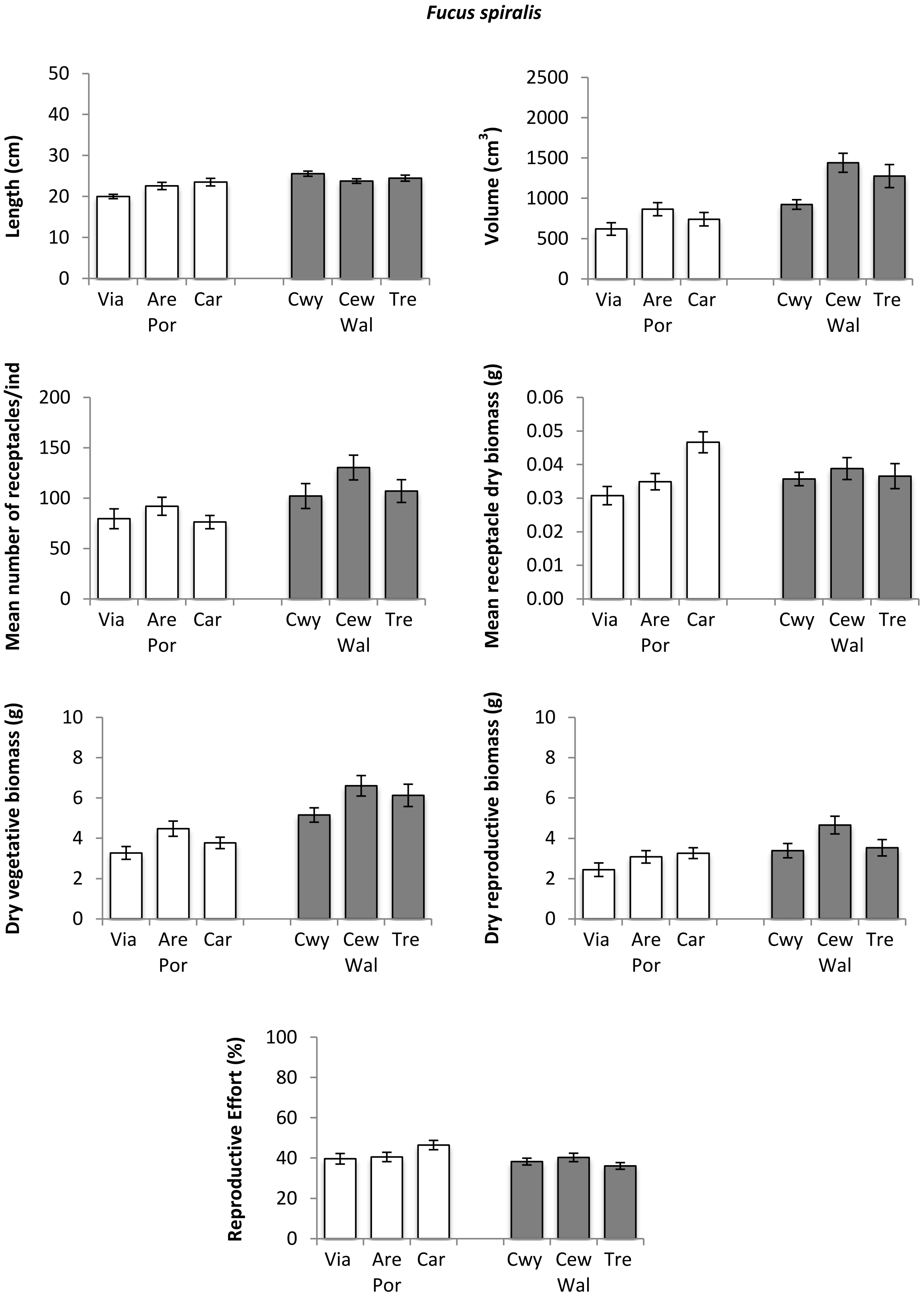


Figure 22: *Fucus spiralis* length, volume, number of receptacles, mean receptacle dry biomass, dry vegetative biomass, dry reproductive biomass and reproductive effort at Portugal (POR) and Wales (WAL) across different shores (*Are: Areosa; Car: Carreço; Via: Viana do Castelo; Cwy: Porth Cwyfan; Cem: Cemlyn Bay; Tre: Trearddur Bay*); Error bars = ±1SE.

5.4 DISCUSSION

I hypothesised that the decline in fucoid biomass levels from northern (Wales) to southern regions (Portugal), described in chapter 2 and the limited recruitment observed in chapter 3 could be dependent on changes in reproductive capacity. Evidence supporting distinct reproductive capacity in populations from the edge of a distribution comes from recent studies developed by Araújo *et al.* (2011) and Viejo *et al.* (2011), in the northern coast of the Iberian Peninsula. Whilst studying reproductive patterns of *F. serratus*, Viejo *et al.* (2011) showed that reproductive capacity of marginal populations in northern Spain was reduced and further suggested that the viability of such populations could be at risk. In contrast, Araújo *et al.* (2011) demonstrated that marginal populations of *A. nodosum* had in fact higher reproductive output than populations from central regions. In order to evaluate if the decline in biomass of *F. vesiculosus* and *F. spiralis* from northern to southern regions could be related with differences in reproductive capacity several variables were tested.

The response of variables in the present study was dependent on spatial scales. Shore variability within each of the geographical regions, measuring variation on the scale of dozens of kilometers, influenced volume (bushiness), dry vegetative tissue biomass and mean receptacle dry biomass of both fucoid species. It also influenced dry reproductive tissue biomass exclusively in *F. spiralis* and total reproductive effort of *F. vesiculosus*. Variability at this spatial scale had also previously been reported at several locations. Some examples include the variability in receptacle dry biomass of *F. vesiculosus* across different shores on Merseyside, UK (Russell 1979) or the variability of *A. nodosum* total reproductive effort observed across shores in New England, USA (Mathieson & Guo 1992). These studies show that variation in reproductive capacity at this spatial scale, due to local changes in environment, is not uncommon.

Despite identifying some variability at smaller scales, the main goal of the study here presented was to identify changes in morphological and reproductive characteristics of *F. spiralis* and *F. vesiculosus* populations across latitudinal scales. Although, the fucoid length values were not significantly different due to the methodology used, other morphological characteristics varied between populations from North Wales and northern Portugal. Vegetative dry tissue biomass levels remained similar across the geographical regions studied. My work, however, on both fucoid species clearly shows that volume (bushiness) is affected by geographical variation with algae of both species showing greater volume in northern areas. This fact could be related to differences observed in levels of reproductive tissue dry biomass, which could influence the measurement of volume.

At this larger spatial scale, reproductive capacity of *F. spiralis* and *F. vesiculosus* populations varied across geographical region. Curiously, the reproductive effort of both species measured, as the percentage of dry reproductive biomass to total dry biomass, did not vary significantly. These results indicate that the reproductive effort of each species per total biomass is equivalent in populations from northern and southern regions. Creed *et al.* (1996) demonstrated a similar effect with reproductive effort of individuals being constant across different densities. Other response variables, such as number of receptacles per individual and dry reproductive tissue biomass, showed differences that crucially can be part of the explanation for the decline in biomass observed near the southern extreme of *F. spiralis* and *F. vesiculosus* distribution (Chapter 2). This study demonstrates for both species that specimens with similar length possessed a higher number of receptacles and crucially greater values of dry reproductive tissue biomass in northern populations. These observations imply that although reproductive effort is maintained in populations from central and southern areas, total reproductive capacity of *F. vesiculosus* and *F. spiralis* is greater in populations from northern areas due to the larger number of receptacles and total reproductive biomass observed per individual (assuming that greater biomass of receptacles is translated into greater levels of propagule output and hence viable recruits). My work on physiological stress in fucoids (Chapter 4), shows that the natural levels of environmental stress occurring during emersion periods in the Portuguese region affect southern populations of *F. vesiculosus* and *F. spiralis*. Combining these results with the data here reported leads me to the conclusion that populations of *F. vesiculosus* and *F. spiralis* could be living, under environmental conditions leading to low reproductive levels in these populations. Similar results were also proposed by Viejo *et al.* (2011) for *F. serratus* populations in northern Spain. Therefore, a continued increase in temperature, similar to the one that has been observed during the last 50 years along the Portuguese coast (Lima *et al.* 2007), would lead me to hypothesize that a shift northwards in the distribution of these species could occur. To assess such a hypothesis a more comprehensive understanding of reproductive capacity in range edge populations combined with recruitment assessment over time and at multiple spatial scales is still necessary, in order to develop a model leading to fuller understanding of the processes leading to the abundance patterns that have been previously detected.

CHAPTER 6

CONCLUDING REMARKS

In this last section, information and findings from the preceding five chapters that constitute the thesis are brought together and interpreted in a holistic manner. Existing knowledge available from the literature is also integrated to further explore the results obtained. The ecological consequences of changes in patterns are explored with special attention to variation in the balance between fucoid and filter feeder across vertical, horizontal and latitudinal gradients.

6.1 PATTERNS ACROSS LATITUDE (QUANTITATIVE ANALYSIS)

In an ecosystem a single species can play several essential functions for the maintenance of the ecosystem in a stable state (Power *et al.* 1996; Bertness *et al.* 1999; Bruno & Bertness 2001). Therefore, in order to comprehend the properties of an ecosystem it is essential to understand the specific role and the interactions between the species that are part of the ecosystem (Hulot *et al.* 2000; Leonard 2000; Loreau *et al.* 2001; Yachi & Loreau 2007).

Changes in the balance between species can lead to alterations in the ecosystem characteristics, in particular when “key” species are affected (Symstad *et al.* 1998; O'Connor & Crowe 2005).

Previous studies have proposed that along the west European rocky intertidal a change in the balance between filter feeder and macroalgae abundance could occur (Ballantine 1961; Hawkins *et al.* 1992). Ballantine (1961) proposed that as climate becomes warmer in southern regions, the dominance of macroalgae will decrease whilst filter feeder abundance will increase. In order to explore variation of the intertidal community caused by latitudinal variation, large areas were quantitatively surveyed from Scotland to Portugal, showing that fucoid biomass declines in southern shores surveyed along the Portuguese coast. As shown in chapter 2, measurements of biomass and percentage cover of the main functional groups were made. The evaluation of percentage cover of functional groups, like the fucoid species, was important to further inform on their structural importance. Studies from Jenkins *et al.* (1999a) and others (Bertness & Callaway 1994; Leonard 2000) have shown the important facilitative role of large fucoid algae. In order to fully assess the impact of the decline of fucoid species, it was also necessary to understand changes in its spatial cover. The decline in biomass of fucoid species from northern to southern regions was combined with a decrease in percentage cover. This drop in percentage cover is probably responsible for the decline of those groups that are dependent on facilitation processes, like the foliose algae. As shown by photosynthetic studies (Bell 1993; Dring *et al.* 2001), these foliose species are greatly affected by temperature and high light intensities. Jenkins *et al.* (1999a) also showed that removal of a fucoid canopy could lead to decreased abundance and survival rates of foliose species due to its dependence on the overlying canopy for amelioration of physical stresses. These combined results justify the use of different measurements that allowed a clear picture of community structure to be obtained.

The ability to differentiate the effects of changes happening at different spatial scales is essential for pattern clarification across latitudinal gradients. This was achieved by using an independent methodology to determine shore exposure to wave action (Burrows *et al.* 2008), allowing the differentiation of local from large scale effects. The methodology developed by Burrows *et al.* (2008), was considered to be the best available for such a large survey. However, improvement in the quantification of wave action probably can be achieved by incorporating bathymetry data to describe length and inclination of offshore zones. Nevertheless, the use of this methodology was indispensable to show that the balance between fucoid abundance and filter feeders along the wave exposure gradient is maintained across different latitudes. These results verify that across the western European coastline, fucoid abundance is higher on sheltered areas and declines at more wave exposed conditions.

The previously proposed decline in fucoid biomass with decreasing latitude was confirmed by the quantitative survey in Chapter 2. However, the expected increase in filter feeder percentage cover was not verified, showing it is essential to quantify patterns at multiple shores to get a clear understanding of changes occurring over such a large spatial scale. The results demonstrated by the survey also showed that the decline in total fucoid biomass was not progressive as expected, since maintenance of fucoid biomass within the shores in the United Kingdom occurred. The insurance hypothesis modelled by Yachi and Loreau (1999), gives a possible explanation for the maintenance of total fucoid biomass levels across the United Kingdom. This intuitive idea declares that the probability of ecosystem functioning to be maintained in the face of environmental fluctuations is higher with increased biodiversity. As species respond differently to environmental pressures, some species may be affected by changes in the environment while others may be able to compensate. This seems to be what occurred with fucoid species within the United Kingdom. Although environmental variation was able to affect species like *Ascohyllum nodosum,* decreasing their abundance in Scotland for example, others like *Fucus vesiculosus* that occupy similar shore heights seem able to compensate such loss by occupying the vacant niche and consequently continuing to provide canopy cover. However, when fucoid diversity is lower, like in the southern Portuguese areas, the loss of a species, for example *F. serratus,* could not be compensated leading to a change in ecosystem functioning. The loss of *F. serratus* from the Portuguese low shore could be one of the reasons for the development of an abundant algal turf community. Such effects of change in species identity and richness are especially important for primary production in benthic marine communities. Studies with macroalgae found that in marine ecosystems species identity has a higher impact on primary production than species richness (Bruno *et al.* 2005; Bruno *et al.* 2006; Stachowicz *et al.* 2007). The level of influence of algal species in the productivity of a system is normally affected by their growth rates (Worm & Chapman 1996; Bruno *et al.* 2005). Therefore, with the loss of *F. serratus* in Portugal and no canopy compensation occurring, changes in ecosystem functioning occurred with the loss of a highly productive canopy environment in the low shore.

The large scale survey was elucidative about how patterns of functional groups varied across European intertidal rocky shores, providing useful data on how the percentage cover of functional groups varied from northern to southern European regions. It showed some interesting patterns that may be related, like the decline of fucoid biomass and the increase of turf community in southern regions. The absence of *F. serratus* from the low shore area in the Portuguese shores surveyed could be caused by competition for space with the increasingly abundant turf community. A similar process, where *Chondrus crispus* prevented the recruitment of *Fucus evanescens*, was described by Worm and Chapman (1996) on wave exposed shores in Nova Scotia, Canada.

The latitudinal survey cannot prove ecological processes but it was indispensable for raising ecological questions to be explored with experimental manipulation. Quantitative large scale studies are particularly important for assessing changes in community over time (Sexton *et al.* 2009). Over the last decades, the importance of large scale studies has been demonstrated. Using such large areas as a natural laboratory allows us to increase knowledge and evaluate some of the possible consequences of global warming as large latitudinal variation may mimic some of the effects of climate warming (Blanchette *et al.* 2008; Jenkins *et al.* 2008; Sexton *et al.* 2009).

6.2 PROCESSES REGULATING FUCOID SURVIVAL AND RECRUITMENT

The use of the large scale survey allowed separation of the effects of latitude from the variability caused by differences in exposure to wave action within each of the geographical regions. To determine which processes are controlling fucoid biomass levels on northern and southern European regions, experimental work in the field and laboratory was developed.

Studies from several authors, completed in the United Kingdom, indicate that grazing pressure can be a very important factor controlling fucoid biomass levels (Hawkins 1981a; Hartnoll & Hawkins 1985; Johnson *et al.* 1997; Jenkins *et al.* 1999a; Thompson *et al.* 2002; Jonsson *et al.* 2006; Moore *et al.* 2007b). Taking this into consideration and the knowledge that physical stresses in summer can also affect algae species, the first experiment focused on the effect of grazing pressure and light intensity on fucoid recruitment at both locations. As expected, in northern regions reduction of light intensity was not influential while grazing pressure effectively controlled fucoid recruitment. By testing the effect of different grazing pressures, it was possible to show that grazers at half the normal densities are still able to control recruitment of fucoids at northern latitudes. The use of reduced light levels and reduced grazing pressure in southern regions was expected to improve fucoid recruitment, but due to low natural settlement levels conclusions about grazing pressure in the south were limited. Nevertheless, grazing could still be an important factor for southern fucoid populations, as proposed by Jenkins *et al.* (2001). This study showed, through the use of wax discs, that grazing activity was higher in southern areas. Differences in mean sea temperature may influence grazers' activity (Jenkins *et al.* 2001) and reported changes in the dominant *Patella* species could also promote different grazing pressures further south (Moore *et al.* 2007b). On top of the grazing effect of *Patella* species other grazers could also be influential. Species like the herbivorous fish *Sarpa salpa* are common in southern regions and become very rare in northern areas. Its effect on seagrass beds has been shown (Havelange *et al.* 1997; Ruitton *et al.* 2000) but its influence on fucoid populations is still not studied, which may prove to be a good research opportunity.

The low recruitment levels, found on southern European regions during my experiment, have previously been reported by Coleman *et al.* (2006) and could be one of the reasons for the decline in fucoid biomass further south. Low recruitment can be caused by low reproductive investment, which in turn can be related with levels of stress an alga has to sustain. It has been shown that algae living under a stressful physiological regime can have reduced survival rates, lower growth rates and lower reproductive capacity due to the use of energetic resources to sustain stressful conditions (Davison & Pearson 1996; Somero 2002; Dethier *et al.* 2005).

To assess stress levels in algae from both locations a widely used technique was employed, the PAM fluoremeter, to measure how the photosystem of *F. vesiculosus* and *F. spiralis* populations from northern and southern regions would react to equal levels of stress. *Fucus spiralis* is regarded as a more resilient species to physical stresses than *F. vesiculosus* due to its vertical position on the shore and its capacity to outcompete *F. vesiculosus* on the highest areas of the shore (Hawkins & Hartnoll 1985). Previous experiments with both fucoid species showed that *F. spiralis* was able to sustain higher desiccation and temperature levels (Dring & Brown 1982; Li & Brawley 2004). Therefore it was expected that *F. spiralis* from both geographical regions would be less affected during the experiment. When both species were eventually subjected to levels of stress occurring during the Portuguese summer, differences between populations from different geographical regions after a recovery period were only observed in *F. vesiculosus*, which is in agreement with their smaller fundamental niche size. This indicates that adaptation of the photosystems to local climatic conditions occurs at least in some fucoid species. However, more importantly these photosynthetic experiments, described in chapter 4, determined that populations from both species also showed difficulty to recover when exposed to levels of stress encountered during the summer on the Portuguese coast. Reduced photosynthetic capacity was observed even after a 16 hour recovery period. This is an important result that could explain the low fucoid biomass levels observed in Portugal, as stressed specimens normally possess reduced survival rates, low growth and decreased reproductive capacity, factors that may lead to decline in biomass levels of a population (Pearson & Brawley 1996; Somero 2002; Dethier *et al.* 2005).

Knowing that fucoid species in Portugal can be subjected to levels of physical environment responsible for a reduction in photosynthetic capacity (Chapter 4), another experiment was developed to understand if the levels of stress observed could be promoting the reduced reproductive capacity in field conditions (Chapter 5). This last experimental procedure showed that reproductive effort measured, as percentage of reproductive biomass in total biomass, and mean biomass per receptacle was similar in populations from northern and southern regions. These results were described for the two fucoid species used, *F. vesiculosus* and *F. spiralis*. Despite the similar reproductive effort, total reproductive capacity was enhanced in populations from the northern regions. These specimens, although of similar length, produced a greater number of receptacles and higher reproductive biomass levels than the ones from southern populations. As proposed by Viejo (2011), such a decline in reproductive capacity in southern regions seems to be a major factor contributing to the decline of fucoid species abundance.

The present work, when the conclusions from the large European survey and results from the experimental work are taken into account, clearly indicate that physical factors, especially during emersion, are important due to their influence in stress levels presented by fucoid species in southern European regions. The levels of stress could also be related with the decline in reproductive capacity observed, although further experimental work is still needed. Nevertheless, higher physical stress levels and lower reproductive capacity are clearly important for populations in southern regions and are most likely among the most important factors contributing to the decline in fucoid abundance in southern regions. In addition to the present knowledge, further studies on *Ascophyllum nodosum* are necessary, due to its role as an extremely important biomass producer in northern rocky intertidal areas. Its abundance is greatly affected in Portugal, where it only exists in smaller populations. Therefore, studies that could provide a better understanding of the processes causing such decline are needed (Araujo *et al.* 2009; Araujo *et al.* 2011). Understanding the processes that lead to the decline of macroalgae abundance is extremely important, especially due to climate warming. Climate warming will probably lead to the expansion of southern communities further north, causing a possible decline in macroalgae abundance, which should be monitored due to their importance as primary producers on the rocky shore.

6.3 ENERGETIC CONSEQUENCES AND FUTURE RESEARCH

Functional diversity of communities has been directly linked with the type of ecological services available in an ecosystem (Loreau *et al.* 2001; Ostfeld & LoGiudice 2003; Arenas *et al.* 2006; Stachowicz *et al.* 2007). Understanding the functions of the different players in an ecosystem may be critical to predicting ecosystem responses in a variety of scenarios, including climate driven global change (Helmuth *et al.* 2006b; Hawkins *et al.* 2008; Jenkins *et al.* 2008). Changes in biota, for example due to inclusion, complete removal or changes in species abundance, like the ones observed by the survey (Chapter 2), can lead to significant alterations in the ecosystem (Jones *et al.* 1997; Bertness *et al.* 1999; Levine *et al.* 2003; Griffin *et al.* 2008). The strength of change will depend on the importance of the ecological functions performed by the affected species and on the existence or absence of other species that can perform similar ecological functions and occupy the vacated niche (Loreau & Hector 2001; Bruno *et al.* 2003; Hooper *et al.* 2005; Raberg & Kautsky 2007; Yachi & Loreau 2007). Across the geographical regions studied, the decline of some fucoid species in Portuguese shores is not compensated, which may lead to changes in community structure and variation in the energetic potential of this intertidal area.

The work here presented, like some others previously proposed (e.g. (Ballantine 1961; Lewis 1964; Raffaelli & Hawkins 1996), shows that the composition of north Atlantic communities changes over a wave exposure gradient, from one dominated by primary producers on sheltered shores to a secondary producer community on exposed sites. As described by the survey, macroalgal primary production dominates sheltered sites while planktonic filter feeders, namely mussels and barnacle, are the principal space occupiers on wave-exposed intertidal areas. The identity of species and complexity of trophic levels can influence the ability of an intertidal system to export energy to coastal areas (O'Connor & Crowe 2005) and therefore this observed change in trophic structure is accompanied by a change in energetic potential of the intertidal (Paine 2002). The production of microalgae at exposed shores on the north Atlantic is much lower than the input of plankton from coastal ecosystems to the dominant filter feeders. Hence, exposed shores function as a net consumer system (Hawkins *et al.* 1992; Raffaelli & Hawkins 1996;

Jenkins *et al.* 2005; Jenkins *et al.* 2008). On the other hand, intertidal sheltered areas in the north Atlantic are areas of high primary production where high levels of macroalgal biomass develop. The uptake of energy from the system by consumers in sheltered intertidal sites is not comparable with the larger amount of algae biomass that is exported to the coastal ecosystem, indicating that canopy dominated areas are a net exporting system (Hawkins *et al.* 1992; Raffaelli & Hawkins 1996; Jenkins *et al.* 2005; Jenkins *et al.* 2008).

On a larger scale, across the European latitudes, variation in community patterns will also be reflected in the energetic production of different latitudinal zones. Such combination of factors indicates that southern Portuguese intertidal shores tend to display an inferior export of fucoid algal based detritus (Chapter 2) and act further as a net importer system than northern intertidal areas. The amplified effect of physical stress on southern Europe leads to decrease in the magnitude of dominance of fucoid species, major primary producers, reducing the ability of this intertidal system, as a whole, to export energy into coastal areas (Jenkins *et al.* 2001; Jenkins *et al.* 2005; Jenkins *et al.* 2008).

The notion that primary production is crucial for all living systems is indispensable for the understanding of intertidal patterns. For such diverse communities to remain energetically viable, links between areas with different productivity are necessary. Rocky shores are the result of this equilibrium and changes in the factors determining patterns need to be evaluated with great care, as they might inform and help to prevent major disequilibrium on the global energetic balance of an entire geographical area.

The work developed through this PhD project allowed exploration of the interaction between physical and biological aspects that together contribute to the development of variability in intertidal community structure across western European rocky shores. The knowledge gained on distribution and abundance levels of major intertidal functional groups across such a large area, combined with the knowledge gained from experimental work on processes causing variation of patterns, is expected to help to construct a base for future comparisons. The need to construct more precise models and improve the understanding of how these culturally and economically important habitats change and respond to natural variability over time and in response to possible global warming pressures, will certainly continue to motivate future research. In my view, future research should focus on clarifying what further processes are operating in edge range areas of the distribution of “key” structural species. Comparisons between these affected populations and others from stable areas are expected to be crucial in revealing which aspects are fundamental for their preservation and maintenance for a long period.

LITERATURE CITED

Aberg P. (1990). Measuring size and choosing category size for a transition matrix study of the seaweed *Ascophyllum nodosum*. *Marine Ecology-Progress Series*, 63, 281-287.

Aberg P. (1996). Patterns of reproductive effort in the brown alga *Ascophyllum nodosum*. *Marine Ecology-Progress Series,* 138, 199-207.

Aberg P. & Pavia H. (1997). Temporal and multiple scale spatial variation in juvenile and adult abundance of the brown alga Ascophyllum nodosum. *Marine Ecology-Progress Series,* 158, 111-119.

Adey W.H. & Steneck R.S. (2001). Thermogeography over time creates biogeographic regions: A temperature/space/time-integrated model and an abundance-weighted test for benthic marine algae. *Journal of Phycology,* 37, 677-698.

Aguilera J., Karsten U., Lippert H., Vogele B., Philipp E., Hanelt D. & Wiencke C. (1999). Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. *Marine Ecology-Progress Series,* 191, 109-119.

Altamirano M., Flores-Moya A., Kuhlenkamp R. & Figueroa F.L. (2003). Stage-dependent sensitivity to ultraviolet radiation in zygotes of the brown alga *Fucus serratus*. *Zygote*, 11, 101-106.

Ang P.O. (1992). Cost of reproduction in *Fucus distichus*. *Marine Ecology-Progress Series*, 89, 25-35.

Araujo R., Serrao E.A., Sousa-Pinto I. & Aberg P. (2011). Phenotypic differentiation at southern limit borders: the case study of two fucoid macroalgal species with different life-history traits. *Journal of Phycology*, 47, 451-462.

Araujo R., Vaselli S., Almeida M., Serrao E. & Sousa-Pinto I. (2009). Effects of disturbance on marginal populations: human trampling on *Ascophyllum nodosum* assemblages at its southern distribution limit. *Marine Ecology-Progress Series*, 378, 81-92.

Ardré F. (1970). Contribution à l'étude des algues marines du Portugal I. La Flore. *Portugaliae Acta Biologica (B)*, 10, 1–423.

Arenas F. & Fernandez C. (1998). Ecology of *Sargassum muticum* (Phaeophyta) on the North coast of Spain - III. Reproductive ecology. *Botanica Marina*, 41, 209-216.

Arenas F., Sanchez I., Hawkins S.J. & Jenkins S.R. (2006). The invasibility of marine algal assemblages: Role of functional diversity and identity. *Ecology*, 87, 2851-2861.

Arrontes J. (1993). Nature of the distributional boundary of *Fucus serratus* on the north shore of Spain. *Marine Ecology-Progress Series*, 93, 183-193.

Arrontes J. (2002). Mechanisms of range expansion in the intertidal brown alga *Fucus serratus* in northern Spain. *Marine Biology*, 141, 1059-1067.

Arrontes J., Arenas F., Fernandez C., Rico J.M., Oliveros J., Martinez B., Viejo R.M. & Alvarez D. (2004). Effect of grazing by limpets on mid-shore species assemblages in northern Spain. *Marine Ecology-Progress Series*, 277, 117-133.

Baardseth E. (1970). A square scanning, two stage sampling method of estimating seaweed quantities. *Norwegian Institute of Seaweed Research*, 33, 1-41.

Back S., Collins J.C. & Russell G. (1993). Comparative reproductive biology of the Gulf of Finland and the Irish Sea *Fucus vesiculosus* L. *Sarsia*, 78, 265-272.

Baker N. (2008). Chlorophyll fluorescence: A probe of photosynthesis in vivo. *Annual Review of Plant Biology*, 89-113.

Baker N.R. & Oxborough K. (2004). Chlorophyll fluorescence as a probe of photosynthetic productivity. In: *Chlorophyll a Fluorescence a Signature of Photosynthesis* (ed. Govindjee GP). Springer Dordrecht, pp. 66-79.

Ballantine W.J. (1961). A biologically defined exposure scale for the comparative description of rocky shores. *Field Studies*, 1, 1-19.

Bell E.C. (1993). Photosynthetic response to temperature and desiccation of the intertidal alga *Mastocarpus papillatus*. *Marine Biology*, 117, 337-346.

Bell E.C. & Denny M.W. (1994). Quantifying wave exposure - a simple device for recording maximum velocity and results of its use at several field sites. *Journal of Experimental Marine Biology and Ecology*, 181, 9-29.

Benedetti-Cecchi L., Bulleri F., Acunto S. & Cinelli F. (2001). Scales of variation in the effects of limpets on rocky shores in the northwest Mediterranean. *Marine Ecology-Progress Series*, 209, 131-141.

Benedetti-Cecchi L., Bulleri F. & Cinelli F. (2000). The interplay of physical and biological factors in maintaining mid-shore and low-shore assemblages on rocky coasts in the north-west Mediterranean. *Oecologia*, 123, 406-417.

Berger R., Malm T. & Kautsky L. (2001). Two reproductive strategies in Baltic *Fucus vesiculosus* (Phaeophyceae). *European Journal of Phycology*, 36, 265-273.

Bertness M.D. (1989). Intraspecific competition and facilitation in a northern acorn barnacle population. *Ecology*, 70, 257-268.

Bertness M.D. & Callaway R. (1994). Positive interactions in communities. *Trends in Ecology & Evolution*, 9, 191-193.

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

Bertness M.D. & Leonard G.H. (1997). The role of positive interactions in communities: Lessons from intertidal habitats. *Ecology*, 78, 1976-1989.

Bertness M.D., Leonard G.H., Levine J.M., Schmidt P.R. & Ingraham A.O. (1999). Testing the relative contribution of positive and negative interactions in rocky intertidal communities. *Ecology*, 80, 2711-2726.

Blanchette C.A., Broitman B.R. & Gaines S.D. (2006). Intertidal community structure and oceanographic patterns around Santa Cruz Island, CA, USA. *Marine Biology*, 149, 689-701.

Blanchette C.A., Miner C.M., Raimondi P.T., Lohse D., Heady K.E.K. & Broitman B.R. (2008). Biogeographical patterns of rocky intertidal communities along the Pacific coast of North America. *Journal of Biogeography*, 35, 1593-1607.

Boaventura D., Alexander M., Della Santina P., Smith N.D., Re P., da Fonseca L.C. & Hawkins S.J. (2002a). The effects of grazing on the distribution and composition of low-shore algal communities on the central coast of Portugal and on the southern coast of Britain. *Journal of Experimental Marine Biology and Ecology*, 267, 185-206.

Boaventura D., da Fonseca L.C. & Hawkins S.J. (2002b). Analysis of competitive interactions between the limpets *Patella depressa* Pennant and *Patella vulgata* L. on the northern coast of Portugal. *Journal of Experimental Marine Biology and Ecology*, 271, 171-188.

Botas J.A., Fernandez E., Bode A. & Anadon R. (1990). A persistent upwelling off the central Cantabrian coast (Bay of Biscay). *Estuarine Coastal and Shelf Science*, 30, 185-199.

Brazier P., Birch K., Brunstrom A., Bunker A., Jones M., Lough N., Salmon L. & Wyn G. (2007). *When the tide goes out. The biodiversity and conservation of the shores of Wales - results from a 10 year intertidal survey of Wales*. The Countryside Council for Wales,, Bangor.

Bridle J.R. & Vines T.H. (2007). Limits to evolution at range margins: when and why does adaptation fail? *Trends in Ecology & Evolution*, 22, 140-147.

Broitman B.R., Mieszkowska N., Helmuth B. & Blanchette C.A. (2008). Climate and recruitment of rocky shore intertidal invertebrates in the Eastern North Atlantic. *Ecology*, 89, S81-S90.

Broitman B.R., Navarrete S.A., Smith F. & Gaines S.D. (2001). Geographic variation of southeastern Pacific intertidal communities. *Marine Ecology-Progress Series*, 224, 21-34.

Bronstein J.L. (1994). Conditional outcomes in mutualistic interactions. *Trends in Ecology & Evolution*, 9, 214-217.

Bruno J.F. & Bertness M.D. (2001). Habitat modification and facilitation in benthic marine communities. In: *Marine community ecology* (eds. Bertness MD, Gaines SD & Hay ME). Sinauer Associates, Inc. Sunderland, pp. 201-218.

Bruno J.F., Boyer K.E., Duffy J.E., Lee S.C. & Kertesz J.S. (2005). Effects of macroalgal species identity and richness on primary production in benthic marine communities. *Ecology Letters*, 8, 1165-1174.

Bruno J.F., Lee S.C., Kertesz J.S., Carpenter R.C., Long Z.T. & Duffy J.E. (2006). Partitioning the effects of algal species identity and richness on benthic marine primary production. *Oikos*, 115, 170-178.

Bruno J.F., Stachowicz J.J. & Bertness M.D. (2003). Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution*, 18, 119-125.

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

Burrows M.T., Harvey R., Robb L., Poloczanska E.S., Mieszkowska N., Moore P., Leaper R., Hawkins S.J. & Benedetti-Cecchi L. (2009). Spatial scales of variance in abundance of intertidal species: effects of region, dispersal mode, and trophic level. *Ecology*, 90, 1242-1254.

Burrows M.T. & Hawkins S.J. (1998). Modelling patch dynamics on rocky shores using deterministic cellular automata. *Marine Ecology-Progress Series*, 167, 1-13.

Burrows M.T. & Hughes R.N. (1989). Natural foraging of the dogwhelk, *Nucella lapillus* (Linnaeus) - the weather and whether to feed. *Journal of Molluscan Studies*, 55, 285-295.

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

Buschmann A.H. (1990). The role of herbivory and desiccation on early successional patterns of intertidal macroalgae in southern Chile. *Journal of Experimental Marine Biology and Ecology*, 139, 221-230.

Butler W. (1978). Energy distribution in photochemical apparatus of photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, 345-378.

Carlton J.T. & Geller J.B. (1993). Ecological roulette: the global transport of nonindigenous marine organisms. *Science*, 261, 78-82.

Carrington E. (1990). Drag and dislodgment of an intertidal macroalga - consequences of morphological variation in *Mastocarpus papillatus* Kutzing. *Journal of Experimental Marine Biology and Ecology*, 139, 185-200.

Cervin G., Aberg P. & Jenkins S.R. (2005). Small-scale disturbance in a stable canopy dominated community: implications for macroalgal recruitment and growth. *Marine Ecology-Progress Series*, 305, 31-40.

Chapman A.R.O. (1995). Functional ecology of fucoid algae - 23 years of progress. *Phycologia*, 34, 1-32.

Chapman A.R.O. & Johnson C.R. (1990). Disturbance and Organization of Macroalgal Assemblages in the Northwest Atlantic. *Hydrobiologia*, 192, 77-121.

Christofoletti R.A., Takahashi C.K., Oliveira D.N. & Flores A.A.V. (2011). Abundance of sedentary consumers and sessile organisms along the wave exposure gradient of subtropical rocky shores of the south-west Atlantic. *Journal of the Marine Biological Association of the United Kingdom*, 91, 961-967.

Clarke A. (2003). Costs and consequences of evolutionary temperature adaptation. *Trends in Ecology & Evolution*, 18, 573-581.

Coelho S., Rijstenbil J., Sousa-Pinto I. & Brown M. (2001). Cellular responses to elevated light levels in *Fucus spiralis* embryos during the first days after fertilization. *Plant Cell and Environment*, 801-810.

Coleman R.A., Underwood A.J., Benedetti-Cecchi L., Aberg P., Arenas F., Arrontes J., Castro J., Hartnoll R.G., Jenkins S.R., Paula J., Della Santina P. & Hawkins S.J. (2006). A continental scale evaluation of the role of limpet grazing on rocky shores. *Oecologia*, 147, 556-564.

Colman J. (1933). The nature of the intertidal zonation of plants and animals. *Journal of the Marine Biological Association of the United Kingdom*, 18, 435-476.

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

Connell J.H. (1961b). The influence of interspecific competition and other factors on the distribution of the barnacle *Chthalamus stellatus*. *Ecology*, 42, 710-723.

Connell J.H. (1972). Community interactions on marine rocky intertidal shores. *Annual Review of Ecology and Systematics*, 3, 169-192.

Connolly S.R., Menge B.A. & Roughgarden J. (2001). A latitudinal gradient in recruitment of intertidal invertebrates in the northeast Pacific Ocean. *Ecology*, 82, 1799-1813.

Connolly S.R. & Roughgarden J. (1998). A latitudinal gradient in northeast Pacific intertidal community structure: Evidence for an oceanographically based synthesis of marine community theory. *American Naturalist*, 151, 311-326.

Consalvey M., Perkins R.G., Paterson D.M. & Underwood G.J.C. (2005). Pam fluorescence: A beginners guide for benthic diatomists. *Diatom Research*, 20, 1-22.

Cousens R. (1986). Quantitative reproduction and reproductive effort by stands of the brown alga *Ascophyllum nodosum* (L) Le Jolis in South-eastern Canada. *Estuarine Coastal and Shelf Science*, 22, 495-507.

Creed J.C., Kain J.M. & Norton T.A. (1998). An experimental evaluation of density and plant size in two large brown seaweeds. *Journal of Phycology*, 34, 39-52.

Creed J.C., Norton T.A. & Kain J.M. (1996). Are neighbours harmful or helpful in *Fucus vesiculosus* populations? *Marine Ecology-Progress Series*, 133, 191-201.

Creed J.C., Norton T.A. & Kain J.M. (1997). Intraspecific competition in *Fucus serratus* germlings: The interaction of light, nutrients and density. *Journal of Experimental Marine Biology and Ecology*, 212, 211-223.

Crisp D.J. (1964). The effects of the severe winter of 1962-63 on marine life in Britain. *Journal of Animal Ecology*, 33, 165-207.

Crisp D.J. & Ficher-Piette E. (1959). Répartition des principales espèces intercotidales de la côte Atlantique Française. 36, 275-388.

Crisp D.J. & Southward A.J. (1958). The distribution of intertidal organisms along the coasts of the English Channel. *Journal of the Marine Biological Association of the United Kingdom*, 37, 157-208.

Davenport J. & Davenport J.L. (2005). Effects of shore height, wave exposure and geographical distance on thermal niche width of intertidal fauna. *Marine Ecology-Progress Series*, 292, 41-50.

Davies A.J., Johnson M.P. & Maggs C.A. (2007). Limpet grazing and loss of Ascophyllum nodosum canopies on decadal time scales. *Marine Ecology-Progress Series*, 339, 131-141.

Davies P.S. (1970). Physiological ecology of *Patella*. IV. Environmental and limpet body temperatures. *Journal of the Marine Biological Association of the United Kingdom*, 50, 1069-1077.

Davison I.R. & Pearson G.A. (1996). Stress tolerance in intertidal seaweeds. *Journal of Phycology*, 32, 197-211.

Dayton P.K. (1971). Competition, disturbance, and community organization: The provision and subsequent utilization of space in a rocky intertidal community. *Ecological Monographs*, 41, 351-389.

Dayton P.K. (1972). Toward an understanding of of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. In: *Proceedings of the Colloquium on Conservation Problems in Antarctica* (ed. Parker BC). Allen Press Kansas, pp. 81-96.

Dayton P.K. (1975). Experimental evaluation of ecological dominance in a rocky intertidal algal community. *Ecological Monographs*, 45, 137-159.

Denny M.W. (1983). A simple device for recording the maximum force exerted on intertidal organisms. *Limnology and Oceanography*, 28, 1269-1274.

Denny M.W. (1985). Wave-forces on intertidal organisms - a case-study. *Limnology and Oceanography*, 30, 1171-1187.

Denny M.W. (1988). *Biology and the Mechanics of the Wave-Swept Environment*. Princeton University Press, Princeton.

Denny M.W. (2006). Ocean waves, nearshore ecology, and natural selection. *Aquatic Ecology*, 40, 439-461.

Denny M.W. & Wethey D.S. (2001). Physical processes that generate patterns in marine communities. In: *Marine community ecology* (eds. Bertness MD, Gaines SD & Hay ME). Sinauer Associates, Inc. Sunderland, pp. 3-37.

Dethier M.N. & Duggins D.O. (1988). Variation in strong-interactions in the intertidal zone along a geographical gradient - A Washington-Alaska comparison. *Marine Ecology-Progress Series*, 97-105.

Dethier M.N., Graham E.S., Cohen S. & Tear L.M. (1993). Visual versus random-point percent cover estimations - objective is not always better. *Marine Ecology-Progress Series*, 96, 93-100.

Dethier M.N., Williams S.L. & Freeman A. (2005). Seaweeds under stress: Manipulated stress and herbivory affect critical life-history functions. *Ecological Monographs*, 75, 403-418.

Doty M.S. (1946). Critical tide factors that are correlated with the vertical distribution of marine algae and other organisms along the Pacific coast. *Ecology*, 27, 315-328.

Dring M., Makarov V., Schoschina E., Lorenz M. & Lüning K. (1996). Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (phaeophyta). *Marine Biology*, 183-191.

Dring M.J. & Brown F.A. (1982). Photosynthesis of intertidal brown algae during and after periods of emersion - a renewed search for physiological causes of zonation. *Marine Ecology-Progress Series*, 8, 301-308.

Dring M.J., Wagner A. & Lüning K. (2001). Contribution of the UV component of natural sunlight to photoinhibition of photosynthesis in six species of subtidal brown and red seaweeds. *Plant Cell and Environment*, 24, 1153-1164.

Duarte C.M. (1991). Seagrass depth limits. *Aquatic Botany*, 40, 363-377.

Duffy J.E. & Hay M.E. (1990). Seaweed adaptations to herbivory. *BioScience*, 40, 368-375.

Eckman J.E., Duggins D.O. & Sewell A.T. (1989). Ecology of understory kelp environments .1. Effects of kelps on flow and particle-transport near the bottom. *Journal of Experimental Marine Biology and Ecology*, 129, 173-187.

Ekelund N., Nygard C., Nordstrom R. & Gylle A. (2008). In situ study of relative electron transport rates in the marine macroalga *Fucus vesiculosus* in the Baltic Sea at different depths and times of year. *Journal of Applied Phycology*, 751-756.

Evans R.G. (1948). The lethal temperatures of some common British littoral Molluscs. *The Journal of Animal Ecology*, 17, 165-173.

Fischer-Piétte E. (1955). Sur le déplacements de frontières biogéographiques intercotidales, observables en Espagne: situation en 1954–1955. *Comptes Rendus de l'Académie des Sciences (Paris)*, 241, 447–449.

Fischer-Piétte E. (1957). Sur les deplacements de frontieres biogeographiques, observes au long des cotes Iberiques dans le domaine intercotidal. *Publicaciones del Instituto de Biologia Aplicada*, 26, 35-40.

Gitay H., Wilson J.B. & Lee W.G. (1996). Species redundancy: A redundant concept? *Journal of Ecology*, 84, 121-124.

Grant W.S. (1977). High intertidal community organization on a rocky headland in Maine, USA. *Marine Biology*, 44, 15-25.

Griffin J.N., De la Haye K.L., Hawkins S.J., Thompson R.C. & Jenkins S.R. (2008). Predator diversity and ecosystem functioning: Density modifies the effect of resource partitioning. *Ecology*, 89, 298-305.

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

Guo Q.F., Taper M., Schoenberger M. & Brandle J. (2005). Spatial-temporal population dynamics across species range: from centre to margin. *Oikos*, 108, 47-57.

Gylle A.M., Nygard C.A. & Ekelund N.G.A. (2009). Desiccation and salinity effects on marine and brackish *Fucus vesiculosus* L. (Phaeophyceae). *Phycologia*, 48, 156-164.

Hansen J., Sato M., Ruedy R., Lo K., Lea D.W. & Medina-Elizade M. (2006). Global temperature change. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 14288-14293.

Harley C.D.G. (2003). Abiotic stress and herbivory interact to set range limits across a two-dimensional stress gradient. *Ecology*, 84, 1477-1488.

Harley C.D.G., Hughes A.R., Hultgren K.M., Miner B.G., Sorte C.J.B., Thornber C.S., Rodriguez L.F., Tomanek L. & Williams S.L. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters*, 9, 228-241.

Hartnoll R.G. & Hawkins S.J. (1985). Patchiness and fluctuations on moderately exposed rocky shores. *Ophelia*, 24, 53-63.

Havelange S., Lepoint G., Dauby P. & Bouquegneau J.M. (1997). Feeding of the sparid fish Sarpa salpa in a seagrass ecosystem: Diet and carbon flux. *Marine Ecology*, 18, 289-297.

Hawkins S.J. (1981a). The influence of *Patella* grazing on the fucoid-barnacle mosaic on moderately exposed rocky shores. *Kieler Meeresforsch., Sonderhelf*, 5, 537-543.

Hawkins S.J. (1981b). The influence of season and barnacles on the algal colonization of *Patella vulgata* exclusion areas. *Journal of the Marine Biological Association of the United Kingdom*, 61, 1-15.

Hawkins S.J. (1983). Interactions of *Patella* and macroalgae with settling *Semibalanus balanoides* (L). *Journal of Experimental Marine Biology and Ecology*, 71, 55-72.

Hawkins S.J., Corte-Real H.B., Pannacciulli F.G., Weber L.C. & Bishop J.D. (2000). Thoughts on the ecology and evolution of the intertidal biota of the Azores and other Atlantic islands. *Hydrobiologia*, 440, 3-17.

Hawkins S.J. & Harkin E. (1985). Preliminary canopy removal experiments in algal dominated communities low on the shore and in the shallow subtidal on the Isle of Man. *Botanica Marina*, 28, 223-230.

Hawkins S.J. & Hartnoll R.G. (1982). The influence of barnacle cover on the numbers, growth and behaviour of *Patella vulgata* on a vertical pier. *Journal of the Marine Biological Association of the United Kingdom*, 62, 855-867.

Hawkins S.J. & Hartnoll R.G. (1983). Changes in a rocky shore community - an evaluation of monitoring. *Marine Environmental Research*, 9, 131-181.

Hawkins S.J. & Hartnoll R.G. (1985). Factors determining the upper limits of intertidal canopy-forming algae. *Marine Ecology-Progress Series*, 20, 265-271.

Hawkins S.J., Hartnoll R.G., Kain J.M. & Norton T.A. (1992). Plant-animal interactions on hard substrata in the north-east Atlantic. In: *Plant-animal interactions in the marine benthos* (eds. John DM, Hawkins SJ & Price JH). Oxford University Press Oxford, pp. 1-32.

Hawkins S.J. & Jones H.D. (1992). *Rocky Shores (Marine Conservation Society, Marine Course Field Guide 1)*. Immel Publishing, London.

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

Hawkins S.J., Southward A.J. & Genner M.J. (2003). Detection of environmental change in a marine ecosystem - evidence from the western English Channel. *Science of the Total Environment*, 310, 245-256.

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

Hawkins S.J., Watson D.C., Hill A.S., Harding S.P., Kyriakides M.A., Hutchinson S. & Norton T.A. (1989). A comparison of feeding mechanisms in microphagous, herbivous, intertidal, prosobranchs in relation to resource partitioning. *J. Mollus. Stud.*, 55, 151-165.

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

Helmuth B., Harley C.D.G., Halpin P.M., O'Donnell M., Hofmann G.E. & Blanchette C.A. (2002). Climate change and latitudinal patterns of intertidal thermal stress. *Science*, 298, 1015-1017.

Helmuth B., Mieszkowska N., Moore P. & Hawkins S.J. (2006b). Living on the edge of two changing worlds: Forecasting the responses of rocky intertidal ecosystems to climate change. *Annual Review of Ecology Evolution and Systematics*, 37, 373-404.

Hixon M.A., Pacala S.W. & Sandin S.A. (2002). Population regulation: Historical context and contemporary challenges of open vs. closed systems. *Ecology*, 83, 1490-1508.

Hooper D.U., Chapin F.S., Ewel J.J., Hector A., Inchausti P., Lavorel S., Lawton J.H., Lodge D.M., Loreau M., Naeem S., Schmid B., Setala H., Symstad A.J., Vandermeer J. & Wardle D.A. (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs*, 75, 3-35.

Hulot F.D., Lacroix G., Lescher-Moutoue F.O. & Loreau M. (2000). Functional diversity governs ecosystem response to nutrient enrichment. *Nature*, 405, 340-344.

Hutchins L. (1947). The bases for temperature zonation in geographical distribution. *Ecological Monographs*, 325-335.

IPCC (2007). *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK.

Irlandi E.A. & Peterson C.H. (1991). Modification of animal habitat by large plants - mechanisms by which seagrasses influence clam growth. *Oecologia*, 87, 307-318.

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

Jenkins S.R., Arenas F., Arrontes J., Bussell J., Castro J., Coleman R.A., Hawkins S.J., Kay S., Martinez B., Oliveros J., Roberts M.F., Sousa S., Thompson R.C. & Hartnoll R.G. (2001). European-scale analysis of seasonal variability in limpet grazing activity and microalgal abundance. *Marine Ecology-Progress Series*, 211, 193-203.

Jenkins S.R., Coleman R.A., Della Santina P., Hawkins S.J., Burrows M.T. & Hartnoll R.G. (2005). Regional scale differences in the determinism of grazing effects in the rocky intertidal. *Marine Ecology-Progress Series*, 287, 77-86.

Jenkins S.R. & Hartnoll R.G. (2001). Food supply, grazing activity and growth rate in the limpet *Patella vulgata* L.: a comparison between exposed and sheltered shores. *Journal of Experimental Marine Biology and Ecology*, 258, 123-139.

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

Jenkins S.R., Hawkins S.J. & Norton T.A. (1999a). Direct and indirect effects of a macroalgal canopy and limpet grazing in structuring a sheltered intertidal community. *Marine Ecology-Progress Series*, 188, 81-92.

Jenkins S.R., Hawkins S.J. & Norton T.A. (1999b). Interaction between a fucoid canopy and limpet grazing in structuring a low shore intertidal community. *Journal of Experimental Marine Biology and Ecology*, 233, 41-63.

Jenkins S.R., Moore P., Burrows M.T., Garbary D.J., Hawkins S.J., Ingolfsson A., Sebens K.P., Snelgrove P.V.R., Wethey D.S. & Woodin S.A. (2008). Comparative ecology of North Atlantic shores: Do differences in players matter for process? *Ecology*, 89, S3-S23.

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

Jernakoff P. (1983). Factors affecting the recruitment of algae in a midshore region dominated by barnacles. *Journal of Experimental Marine Biology and Ecology*, 67, 17-31.

Ji Y. & Tanaka J. (2002). Effect of desiccation on the photosynthesis of seaweeds from the intertidal zone in Honshu, Japan. *Phycological Research*, 50, 145-153.

Johnson C.R., Banks S.C., Barrett N.S., Cazassus F., Dunstan P.K., Edgar G.J., Frusher S.D., Gardner C., Haddon M., Helidoniotis F., Hill K.L., Holbrook N.J., Hosie G.W., Last P.R., Ling S.D., Melbourne-Thomas J., Miller K., Pecl G.T., Richardson A.J., Ridgway K.R., Rintoul S.R., Ritz D.A., Ross D.J., Sanderson J.C., Shepherd S.A., Slotvvinski A., Swadling K.M. & Taw N. (2011). Climate change cascades: Shifts in oceanography, species' ranges and subtidal marine community dynamics in eastern Tasmania. *Journal of Experimental Marine Biology and Ecology*, 400, 17-32.

Johnson L.E. & Brawley S.H. (1998). Dispersal and recruitment of a canopy-forming intertidal alga: the relative roles of propagule availability and post-settlement processes. *Oecologia*, 117, 517-526.

Johnson M.P., Burrows M.T., Hartnoll R.G. & Hawkins S.J. (1997). Spatial structure on moderately exposed rocky shores: patch scales and the interactions between limpets and algae. *Marine Ecology-Progress Series*, 160, 209-215.

Johnson M.P., Burrows M.T. & Hawkins S.J. (1998a). Individual based simulations of the direct and indirect effects of limpets on a rocky shore *Fucus* mosaic. *Marine Ecology-Progress Series*, 169, 179-188.

Johnson M.P. & Hawkins S.J. (1998). Insights from a general model of herbivores and macroalgae on rocky shores. *Oikos*, 82, 603-608.

Johnson M.P., Hawkins S.J., Hartnoll R.G. & Norton T.A. (1998b). The establishment of fucoid zonation on algal-dominated rocky shores: hypotheses derived from a simulation model. *Functional Ecology*, 12, 259-269.

Johnson M.P., Hughes R.N., Burrows M.T. & Hawkins S.J. (1998c). Beyond the predation halo: small scale gradients in barnacle populations affected by the relative refuge value of crevices. *Journal of Experimental Marine Biology and Ecology*, 231, 163-170.

Jones C.G., Lawton J.H. & Shachak M. (1997). Positive and negative effects of organisms as physical ecosystem engineers. *Ecology*, 78, 1946-1957.

Jones N.S. (1946). Browsing on *Patella*. *Nature*, 158, 557.

Jones N.S. (1948). Observations and experiments on the biology of *Patella vulgata* at Port St Mary, Isle of Man. *Proc. Trans. Liverpool Biol. Soc.*, 56, 60-77.

Jones P.D. & Mann M.E. (2004). Climate over past millennia. *Reviews of Geophysics*, 42.

Jones P.D., Osborn T.J. & Briffa K.R. (2001). The evolution of climate over the last millennium. *Science*, 292, 662-667.

Jones W.E. & Demetropoulos A. (1968). Exposure to wave action: measurements of an important ecological parameter on rocky shores in Anglesey. *Journal of Experimental Marine Biology and Ecology*, 2, 46-63.

Jonsson P.R., Granhag L., Moschella P.S., Aberg P., Hawkins S.J. & Thompson R.C. (2006). Interactions between wave action and grazing control the distribution of intertidal macroalgae. *Ecology*, 87, 1169-1178.

Kaehler S. & Williams G.A. (1998). Early development of algal assemblages under different regimes of physical and biotic factors on a seasonal tropical rocky shore. *Marine Ecology-Progress Series*, 172, 61-71.

Kalvas A. & Kautsky L. (1993). Geographical variation in *Fucus vesiculosus* morphology in the Baltic and North Seas. *European Journal of Phycology*, 28, 85-91.

Kalvas A. & Kautsky L. (1998). Morphological variation in *Fucus vesiculosus* populations along temperature and salinity gradients in Iceland. *Journal of the Marine Biological Association of the United Kingdom*, 78, 985-1001.

Knight M. & Parke M. (1950). A biological study of *Fucus vesiculosus* L and *F serratus* L. *Journal of the Marine Biological Association of the United Kingdom*, 29, 439-&.

Krause G. & Weis E. (1991). Chlorophyll fluorescence and photosynthesis - The basics. *Annual Review of Plant Physiology and Plant Molecular Biology*, 313-349.

Ladah L., Bermudez R., Pearson G. & Serrao E. (2003). Fertilization success and recruitment of dioecious and hermaphroditic fucoid seaweeds with contrasting distributions near their southern limit. *Marine Ecology-Progress Series*, 262, 173-183.

Ladah L.B., Feddersen F., Pearson G.A. & Serrao E.A. (2008). Egg release and settlement patterns of dioecious and hermaphroditic fucoid algae during the tidal cycle. *Marine Biology*, 155, 583-591.

Lamote M., Johnson L.E. & Lemoine Y. (2007). Interspecific differences in the response of juvenile stages to physical stress: Fluorometric responses of fucoid embryos to variation in meteorological conditions. *Journal of Phycology*, 43, 1164-1176.

Lemos R.T. & Pires H.O. (2004). The upwelling regime off the west Portuguese coast, 1941-2000. *International Journal of Climatology*, 24, 511-524.

Leonard G.H. (2000). Latitudinal variation in species interactions: A test in the New England rocky intertidal zone. *Ecology*, 81, 1015-1030.

Levine J.M., Vila M., D'Antonio C.M., Dukes J.S., Grigulis K. & Lavorel S. (2003). Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 270, 775-781.

Lewis J.R. (1964). *The Ecology of Rocky Shores*. The English University Press, Ltd., London.

Lewis J.R. (1986). Latitudinal trends in reproduction, recruitment and population characteristics of some rocky littoral Mollusks and Cirripedes. *Hydrobiologia*, 142, 1-13.

Li R. & Brawley S.H. (2004). Improved survival under heat stress in intertidal embryos (*Fucus* spp.) simultaneously exposed to hypersalinity and the effect of parental thermal history. *Marine Biology*, 144, 205-213.

Lima F.P., Queiroz N., Ribeiro P.A., Hawkins S.J. & Santos A.M. (2006). Recent changes in the distribution of a marine gastropod, *Patella rustica* Linnaeus, 1758, and their relationship to unusual climatic events. *Journal of Biogeography*, 33, 812-822.

Lima F.P., Ribeiro P.A., Queiroz N., Hawkins S.J. & Santos A.M. (2007). Do distributional shifts of northern and southern species of algae match the warming pattern? *Global Change Biology*, 13, 2592-2604.

Lindegarth M., Aberg P., Cervin G. & Nilsson P.G. (2001). Effects of grazing on the structure of mid-shore, intertidal assemblages on moderately exposed rocky shores of the Swedish west coast. *Marine Ecology-Progress Series*, 212, 29-38.

Lipkin Y., Beer S. & Eshel A. (1993). The ability of *Porphyra-linearis* (Rhodophyta) to tolerate prolonged periods of desiccation. *Botanica Marina*, 517-523.

Long S., Humphries S. & Falkowski P. (1994). Photoinhibition of photosynthesis in nature. *Annual Review of Plant Physiology and Plant Molecular Biology*, 633-662.

Loreau M. & Hector A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, 412, 72-76.

Loreau M., Naeem S., Inchausti P., Bengtsson J., Grime J.P., Hector A., Hooper D.U., Huston M.A., Raffaelli D., Schmid B., Tilman D. & Wardle D.A. (2001). Ecology - Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science*, 294, 804-808.

Lotze H.K., Worm B. & Sommer U. (2001). Strong bottom-up and top-down control of early life stages of macroalgae. *Limnology and Oceanography*, 46, 749-757.

Lubchenco J. (1978). Plant species diversity in a marine intertidal community: Importance of herbivore food preference and algal competitive abilities. *American Naturalist*, 112, 23-39.

Lubchenco J. (1980). Algal zonation in the New England rocky intertidal community: an experimental-analysis. *Ecology*, 61, 333-344.

Lubchenco J. & Gaines S.D. (1981). A unified approach to marine plant-herbivore interactions .I. Populations and communities. *Annual Review of Ecology and Systematics*, 12, 405-437.

Lüning K. (1984). Temperature tolerance and biogeography of seaweeds - The marine algal flora of Helgoland (North Sea) as an example. *Helgolander Meeresuntersuchungen*, 38, 305-317.

Lüning K. (1990). *Seaweeds: Their Environment, Biogeography, and Ecophysiology*. John Wiley & Sons, Inc, New York.

Lüning K. & Neushul M. (1978). Light and temperature demands for growth and reproduction of Laminarian gametophytes in southern and central California. *Marine Biology*, 45, 297-309.

Magnusson G. (1997). Diurnal measurements of Fv/Fm used to improve productivity estimates in macroalgae. *Marine Biology*, 203-208.

Malm T. & Kautsky L. (2003). Differences in life-history characteristics are consistent with the vertical distribution pattern of *Fucus serratus* and *Fucus vesiculosus* (Fucales, Phaeophyceae) in the central Baltic Sea. *Journal of Phycology*, 39, 880-887.

Mathieson A.C. & Guo Z.Y. (1992). Patterns of fucoid reproductive biomass allocation. *British Phycological Journal*, 27, 271-292.

McCook L.J. & Chapman A.R.O. (1991). Community succession following massive ice-scour on an exposed rocky shore" effects of *Fucus* canopy algae and of mussels during late succession. *Journal of Experimental Marine Biology and Ecology*, 154, 137-169.

Menge B.A. (1976). Organization of the New England rocky intertidal community: role of predation, competition, and environmental heterogeneity. *Ecological Monographs*, 46, 355-393.

Menge B.A. (2000a). Testing the relative importance of positive and negative effects on community structure. *Trends in Ecology & Evolution*, 15, 46-47.

Menge B.A. (2000b). Top-down and bottom-up community regulation in marine rocky intertidal habitats. *Journal of Experimental Marine Biology and Ecology*, 250, 257-289.

Menge B.A., Daley B.A., Lubchenco J., Sanford E., Dahlhoff E., Halpin P.M., Hudson G. & Burnaford J.L. (1999). Top-down and bottom-up regulation of New Zealand rocky intertidal communities. *Ecological Monographs*, 69, 297-330.

Menge B.A., Daley B.A., Wheeler P.A., Dahlhoff E., Sanford E. & Strub P.T. (1997a). Benthic-pelagic links and rocky intertidal communities: Bottom-up effects on top-down control? *Proceedings of the National Academy of Sciences of the United States of America*, 94, 14530-14535.

Menge B.A., Daley B.A., Wheeler P.A. & Strub P.T. (1997b). Rocky intertidal oceanography: An association between community structure and nearshore phytoplankton concentration. *Limnology and Oceanography*, 42, 57-66.

Menge B.A., Lubchenco J., Bracken M.E.S., Chan F., Foley M.M., Freidenburg T.L., Gaines S.D., Hudson G., Krenz C., Leslie H., Menge D.N.L., Russell R. & Webster M.S. (2003). Coastal oceanography sets the pace of rocky intertidal community dynamics. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 12229-12234.

Menge B.A. & Sutherland J.P. (1976). Species-diversity gradients - synthesis of roles of predation, competition, and temporal heterogeneity. *American Naturalist*, 110, 351-369.

Mieszkowska N., Kendall M.A., Hawkins S.J., Leaper R., Williamson P., Hardman-Mountford N.J. & Southward A.J. (2006). Changes in the range of some common rocky shore species in Britain - a response to climate change? *Hydrobiologia*, 555, 241-251.

Mieszkowska N., Leaper R., Moore P., Kendall M.A., Burrows M.T., Lear D., Poloczanska E., Hiscock K., Moschella P.S., Thompson R.C., Herbert R.J., Laffoley D., Baxter J., Southward A.J. & Hawkins S.J. (2005). Marine biodiversity and climate change assessing and predicting the influence of climatic change using intertidal rocky shore biota. Final report for United Kingdom funders. *Marine Biological Association of the United Kingdom Occasional Publication*, 20, 1-53.

Moore P., Hawkins S.J. & Thompson R.C. (2007a). Role of biological habitat amelioration in altering the relative responses of congeneric species to climate change. *Marine Ecology-Progress Series*, 334, 11-19.

Moore P., Thompson R.C. & Hawkins S.J. (2007b). Effects of grazer identity on the probability of escapes by a canopy-forming macroalga. *Journal of Experimental Marine Biology and Ecology*, 344, 170-180.

Navarrete S.A., Broitman B.R. & Menge B.A. (2008). Interhemispheric comparison of recruitment to intertidal communities: Pattern persistence and scales of variation. *Ecology*, 89, 1308-1322.

Nielsen H.D., Brownlee C., Coelho S.M. & Brown M.T. (2003). Interpopulation differences in inherited copper tolerance involve photosynthetic adaptation and exclusion mechanisms in *Fucus serratus*. *New Phytologist*, 160, 157-165.

Niemeck R.A. & Mathieson A.C. (1976). Ecological study of *Fucus spiralis* L. *Journal of Experimental Marine Biology and Ecology*, 33-48.

Norton T.A. (1983). The resistance to dislodgement of *Sargassum muticum* germlings under defined hydrodynamic conditions. *Journal of the Marine Biological Association of the United Kingdom*, 63, 181-193.

Norton T.A. (1985). The zonation of seaweeds on rocky shores. In: *The Ecology of Rocky Coasts* (eds. Moore PG & Seed R). Hodder & Stoughton London, pp. 7-21.

O'Connor N.E. & Crowe T.P. (2005). Biodiversity loss and ecosystem functioning: Distinguishing between number and identity of species. *Ecology*, 86, 1783-1796.

Ostfeld R. & LoGiudice K. (2003). Community disassembly, biodiversity loss, and the erosion of an ecosystem service. *Ecology*, 1421-1427.

Paine R.T. (1966). Food web complexity and species diversity. *The American Naturalist*, 100, 65-75.

Paine R.T. (1974). Intertidal community structure - Experimental studies on relationship between a dominant competitor and its principal predator. *Oecologia*, 15, 93-120.

Paine R.T. (2002). Trophic control of production in a rocky intertidal community. *Science*, 296, 736-739.

Parmesan C. (1996). Climate and species' range. *Nature*, 382, 765-766.

Parmesan C. & Yohe G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421, 37-42.

Pearson G., Kautsky L. & Serrao E. (2000). Recent evolution in Baltic *Fucus vesiculosus*: reduced tolerance to emersion stresses compared to intertidal (North Sea) populations. *Marine Ecology-Progress Series*, 202, 67-79.

Pearson G.A. & Brawley S.H. (1996). Reproductive ecology of *Fucus distichus* (Phaeophyceae): An intertidal alga with successful external fertilization. *Marine Ecology-Progress Series*, 143, 211-223.

Pearson G.A., Lago-Leston A. & Mota C. (2009). Frayed at the edges: selective pressure and adaptive response to abiotic stressors are mismatched in low diversity edge populations. *Journal of Ecology*, 97, 450-462.

Peliz A., Dubert J., Santos A.M.P., Oliveira P.B. & Le Cann B. (2005). Winter upper ocean circulation in the Western Iberian Basin - fronts, eddies and poleward flows: an overview. *Deep-Sea Research Part I-Oceanographic Research Papers*, 52, 621-646.

Peters A.F. & Breeman A.M. (1993). Temperature tolerance and latitudinal range of brown algae from temperate Pacific South-America. *Marine Biology*, 115, 143-150.

Petraitis P.S. (1987). Factors organizing rocky intertidal communities of New England - herbivory and predation in sheltered bays. *Journal of Experimental Marine Biology and Ecology,* 109, 117-136.

Poloczanska E.S., Hawkins S.J., Southward A.J. & Burrows M.T. (2008). Modeling the response of populations of competing species to climate change. *Ecology,* 89, 3138-3149.

Power M.E., Tilman D., Estes J.A., Menge B.A., Bond W.J., Mills L.S., Daily G., Castilla J.C., Lubchenco J. & Paine R.T. (1996). Challenges in the quest for keystones. *Bioscience,* 46, 609-620.

Powles S.B. (1984). Photoinhibition of photosynthesis induced by visible-light. *Annual Review of Plant Physiology and Plant Molecular Biology,* 35, 15-44.

Raberg S. & Kautsky L. (2007). Consumers affect prey biomass and diversity through resource partitioning. *Ecology*, 88, 2468-2473.

Raffaelli D. & Hawkins S.J. (1996). *Intertidal Ecology*. Kluwer Academic Publishers, London.

Rauh N. (2006). *MRes Marine Biology - The interactive effects of grazing and physical stress on epilithic microalgal communities over large spatial spaces,* University of Plymouth.

Reed D.C. (1990). An experimental evaluation of density dependence in a subtidal algal population. *Ecology*, 71, 2286-2296.

Reed D.C. & Foster M.S. (1984). The effects of canopy shading on algal recruitment and growth in a giant-kelp forest. *Ecology*, 65, 937-948.

Rivadeneira M.M. & Fernandez M. (2005). Shifts in southern endpoints of distribution in rocky intertidal species along the south-eastern Pacific coast. *Journal of Biogeography,* 32, 203-209.

Robertson B.L. (1987). Reproductive ecology and canopy structure of *Fucus spiralis* L. *Botanica Marina*, 475-482.

Rohde S., Hiebenthal C., Wahl M., Karez R. & Bischof K. (2008). Decreased depth distribution of *Fucus vesiculosus* (Phaeophyceae) in the Western Baltic: effects of light deficiency and epibionts on growth and photosynthesis. *European Journal of Phycology*, 43, 143 - 150.

Ruitton S., Francour P. & Boudouresque C.F. (2000). Relationships between algae, benthic herbivorous invertebrates and fishes in rocky sublittoral communities of a temperate sea (Mediterranean). *Estuarine Coastal and Shelf Science*, 50, 217-230.

Russell G. (1979). Heavy receptacles in estuarine *Fucus vesiculosus* L. *Estuarine and Coastal Marine Science*, 9, 659-661.

Russell G., Ruuskanen A. & Kiirikki M. (1998). Sunlight, shade and tidal night: Photoadaptation in *Fucus vesiculosus* L. *Sarsia*, 83, 381-386.

Sagarin R.D., Barry J.P., Gilman S.E. & Baxter C.H. (1999). Climate-related change in an intertidal community over short and long time scales. *Ecological Monographs*, 69, 465-490.

Sagarin R.D. & Gaines S.D. (2002). The 'abundant centre' distribution: to what extent is it a biogeographical rule? *Ecology Letters*, 137-147.

Sanchez R.F. & Relvas P. (2003). Spring-summer climatological circulation in the upper layer in the region of Cape St. Vincent, Southwest Portugal. *Ices Journal of Marine Science*, 60, 1232-1250.

Santelices B. (1990). Patterns of Reproduction, Dispersal and Recruitment in Seaweeds. In: *Oceanography and Marine Biology: An Annual Review* (eds. Barnes H & Barnes M). Aberdeen University Press Aberdeen, pp. 177-276.

Schiel D.R. & Foster M.S. (2006). The population biology of large brown seaweeds: ecological consequences of multiphase life histories in dynamic coastal environments. *Annual Review of Ecology, Evolution, and Systematics*, 37, 343-372.

Schonbeck M. & Norton T.A. (1978). Factors controlling upper limits of fucoid algae on shore. *Journal of Experimental Marine Biology and Ecology*, 31, 303-313.

Schonbeck M.W. & Norton T.A. (1980). Factors controlling the lower limits of fucoid algae on the shore. *Journal of Experimental Marine Biology and Ecology*, 43, 131-150.

Scrosati R. (2005). Review of studies on biomass-density relationships (including self-thinning lines) in seaweeds: Main contributions and persisting misconceptions. *Phycological Research*, 53, 224-233.

Scrosati R. & Heaven C. (2008). Trends in abundance of rocky intertidal seaweeds and filter feeders across gradients of elevation, wave exposure, and ice scour in eastern Canada. *Hydrobiologia*, 603, 1-14.

Sexton J., McIntyre P., Angert A. & Rice K. (2009). Evolution and ecology of species range limits. *Annual Review of Ecology Evolution and Systematics*, 415-436.

Skene K.R. (2004). Key differences in photosynthetic characteristics of nine species of intertidal macroalgae are related to their position on the shore. *Canadian Journal of Botany-Revue Canadienne De Botanique*, 82, 177-184.

Smith C.M. & Berry J.A. (1986). Recovery of photosynthesis after exposure of intertidal algae to osmotic and temperature stresses - comparative-studies of species with differing distributional limits. *Oecologia*, 70, 6-12.

Sodhi N.S., Brook B.W. & Bradshaw C.A.J. (2009). Causes and consequences of species extinctions. In: *Princeton Guide to Ecology* (ed. Levin SA). Princeton University Press., pp. 514-520.

Somero G.N. (2002). Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and costs of living. *Integr. Comp. Biol.*, 42, 780-789.

Sousa W.P. (1979). Experimental investigations of disturbance and ecological succession in a rocky intertidal algal community. *Ecological Monographs*, 49, 228-254.

Sousa W.P., Schroeter S.C. & Gaines S.D. (1981). Latitudinal variation in intertidal algal community structure - the influence of grazing and vegetative propagation. *Oecologia*, 48, 297-307.

Southward A.J. (1958). The zonation of plants and animals on rocky sea shores. *Biological Reviews of the Cambridge Philosophical Society*, 33, 137-177.

Southward A.J. (1991). 40 years of changes in species composition and population density of barnacles on a rocky shore near Plymouth. *Journal of the Marine Biological Association of the United Kingdom*, 71, 495-513.

Southward A.J., Hawkins S.J. & Burrows M.T. (1995). 70 years observations of changes in distribution and abundance of zooplankton and intertidal organisms in the Western English-channel in relation to rising sea temperature. *Journal of Thermal Biology*, 20, 127-155.

Southward A.J., Langmead O., Hardman-Mountford N.J., Aiken J., Boalch G.T., Dando P.R., Genner M.J., Joint I., Kendall M.A., Halliday N.C., Harris R.P., Leaper R., Mieszkowska N., Pingree R.D., Richardson A.J., Sims D.W., Smith T., Walne A.W. & Hawkins S.J. (2005). Long-term oceanographic and ecological research in the western English Channel. *Advances in Marine Biology, Vol 47*, 47, 1-105.

Southward A.J. & Southward E.C. (1978). Recolonization of rocky shores in Cornwall after use of toxic dispersants to clean up the Torrey Canyon spill. *Journal of the Fisheries Research Board of Canada*, 35, 682-706.

Stachowicz J.J., Bruno J.F. & Duffy J.E. (2007). Understanding the effects of marine biodiversity on communities and ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, 38, 739-766.

Stephenson T.A. & Stephenson A. (1949). The universal features of zonation between tidemarks on rocky coasts. *Journal of Ecology*, 37, 289-305.

Stephenson T.A. & Stephenson A. (1972). *Life Between Tidemarks on Rocky Shores*. W.H. Freeman and Co., San Francisco.

Svensson C.J., Jenkins S.R., Hawkins S.J. & Aberg P. (2005). Population resistance to climate change: modelling the effects of low recruitment in open populations. *Oecologia*, 142, 117-126.

Symstad A.J., Tilman D., Willson J. & Knops J.M.H. (1998). Species loss and ecosystem functioning: effects of species identity and community composition. *Oikos*, 81, 389-397.

Test A.R.G. (1945). Ecology of California Acmaea. *Ecology*, 26, 395-405.

Thomas M.L.H. (1986). A physically derived exposure index for marine shorelines. *Ophelia*, 25, 1-13.

Thompson R.C., Crowe T.P. & Hawkins S.J. (2002). Rocky intertidal communities: past environmental changes, present status and predictions for the next 25 years. *Environmental Conservation*, 29, 168-191.

Thompson R.C., Johnson L.E. & Hawkins S.J. (1997). A method for spatial and temporal assessment of gastropod grazing intensity in the field: the use of radula scrapes on wax surfaces. *Journal of Experimental Marine Biology and Ecology*, 218, 63-76.

Thompson R.C., Norton T.A. & Hawkins S.J. (2004). Physical stress and biological control regulate the producer-consumer balance in intertidal biofilms. *Ecology*, 85, 1372-1382.

Thompson R.C., Wilson B.J., Tobin M.L., Hill A.S. & Hawkins S.J. (1996). Biologically generated habitat provision and diversity of rocky shore organisms at a hierarchy of spatial scales. *Journal of Experimental Marine Biology and Ecology*, 202, 73-84.

Todd C.D. & Lewis J.R. (1984). Effects of low air temperature on *Laminaria digitata* in southwestern Scotland. *Marine Ecology-Progress Series*, 16, 199-201.

Trussell G.C., Johnson A.S., Rudolph S.G. & Gilfillan E.S. (1993). Resistance to dislodgment - habitat and size specific differences in morphology and tenacity in an intertidal snail. *Marine Ecology-Progress Series*, 100, 135-144.

Underwood A.J. (1980). The effects of grazing by gastropods and physical factors on the upper limits of distribution of intertidal macroalgae. *Oecologia*, 46, 201-213.

Underwood A.J. (1999). Physical disturbances and their direct effect on an indirect effect: responses of an intertidal assemblage to a severe storm. *Journal of Experimental Marine Biology and Ecology*, 232, 125-140.

Underwood A.J. (2000). Experimental ecology of rocky intertidal habitats: what are we learning? *Journal of Experimental Marine Biology and Ecology*, 250, 51-76.

Underwood A.J., Chapman M.G. & Connell S.D. (2000). Observations in ecology: you can't make progress on processes without understanding the patterns. *Journal of Experimental Marine Biology and Ecology*, 250, 97-115.

Underwood A.J. & Jernakoff P. (1984). The effects of tidal height, wave-exposure, seasonality and rock-pools on grazing and the distribution of intertidal macroalgae in New South Wales. *Journal of Experimental Marine Biology and Ecology*, 75, 71-96.

Vadas R.L., Johnson S. & Norton T.A. (1992). Recruitment and mortality of early postsettlement stages of benthic algae. *British Phycological Journal*, 27, 331-351.

Vadas R.L., Wright W.A. & Miller S.L. (1990). Recruitment of *Ascophyllum nodosum* - Wave action as a source of mortality. *Marine Ecology-Progress Series*, 61, 263-272.

Viejo R.M., Martinez B., Arrontes J., Astudillo C. & Hernandez L. (2011). Reproductive patterns in central and marginal populations of a large brown seaweed: drastic changes at the southern range limit. *Ecography*, 34, 75-84.

Vinegla B., Segovia M. & Figueroa F.L. (2006). Effect of artificial UV radiation on carbon and nitrogen metabolism in the macroalgae *Fucus spiralis* L. and *Ulva olivascens* Dangeard. *Hydrobiologia*, 560, 31-42.

Wethey D.S., Woodin S.A., Hilbish T.J., Jones S.J., Lima F.P. & Brannock P.M. (2011). Response of intertidal populations to climate: Effects of extreme events versus long term change. *Journal of Experimental Marine Biology and Ecology*, 400, 132-144.

Williams G.A. (1994). The relationship between shade and molluscan grazing in structuring communities on a moderately-exposed tropical rocky shore. *Journal of Experimental Marine Biology and Ecology*, 178, 79-95.

Wolcott B.D. (2007). Mechanical size limitation and life-history strategy of an intertidal seaweed. *Marine Ecology-Progress Series*, 338, 1-10.

Wolcott T.G. (1973). Physiological ecology and intertidal zonation in limpets (*Acmaea*) - Critical look at limiting factors. *Biological Bulletin*, 145, 389-422.

Wootton J.T. (1993). Indirect effects and habitat use in an intertidal community - interaction chains and interaction modifications. *American Naturalist*, 141, 71-89.

Worm B. & Chapman A.R.O. (1996). Interference competition among two intertidal seaweeds: *Chondrus crispus* strongly affects survival of *Fucus evanescens* recruits. *Marine Ecology-Progress Series*, 145, 297-301.

Worm B., Lotze H.K. & Sommer U. (2001). Algal propagule banks modify competition, consumer and resource control on Baltic rocky shores. *Oecologia*, 128, 281-293.

Yachi S. & Loreau M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 1463-1468.

Yachi S. & Loreau M. (2007). Does complementary resource use enhance ecosystem functioning? A model of light competition in plant communities. *Ecology Letters*, 10, 54-62.

Zardi G.I., Nicastro K.R., Canovas F., Costa J.F., Serrao E.A. & Pearson G.A. (2011). Adaptive traits are maintained on steep selective gradients despite gene flow and hybridization in the intertidal zone. *Plos One*, 6, 13.