

# **Calcifying organisms in changing shallow and deep marine environments**

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Dissertation submitted in partial fulfillment of a  
*Philosophiae Doctor* degree in Earth Science

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Reykjavík, February 2017

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# Abstract

Anthropogenic CO<sub>2</sub> emissions, which are resulting in ocean acidification and a decrease in the saturation state for calcium carbonate ( $\Omega$ ), are a particular threat to calcifying marine biota. The aim of this thesis is to fill important knowledge gaps that limit our understanding of the implication of these anthropogenically driven changes for calcifying organism in intertidal, coastal and deep-sea environments.

*Papers I and II* investigate the intertidal environment where the range and rate of environmental changes are often extreme. *Paper I* describes seasonal and daily fluxes in the inorganic carbon system in tidal pool environments in relation to biological processes in a red calcifying coralline algal species (*Ellisolandia elongata*) through irradiance response curves. *Paper II* describes an experimental study where alga from the same population as studied in *paper I* were grown for 3 weeks at varying  $p\text{CO}_2$  concentrations: 380  $\mu\text{atm}$  (representing modern day atmospheric CO<sub>2</sub> concentration) and 550, 750 and 1000  $\mu\text{atm}$  (representing future atmospheric CO<sub>2</sub> concentrations). Results suggest intertidal algae are less susceptible to future atmospheric CO<sub>2</sub> concentrations compared to coralline alga from the subtidal marine environments.

Data on spatial and temporal variability in the inorganic carbon system in coastal regions is limited, which is an issue for understanding biological responses of coastal species to ocean acidification and for the construction of numerical biogeochemical models. *Paper III* investigates the seasonal variability in the inorganic carbon system in a pristine coastal region of Iceland, Breiðafjörður Bay. The study region was identified as a net sink of atmospheric CO<sub>2</sub> at a rate of 1.8 mol C m<sup>-2</sup> y<sup>-1</sup> with surface  $p\text{CO}_2$  ranging from 212 to 417  $\mu\text{atm}$  from summer to winter.

The deep-sea is a relatively stable environment. Regardless, numerical model predictions suggest the Nordic Seas will be largely undersaturated with respect to aragonite by the year 2100, posing a severe threat to calcifying mollusc in the region. *Paper IV* provides previously lacking information on bivalve and gastropod occurrences and biodiversity in the high latitude North Atlantic, north and south of the Greenland-Iceland-Faroe (GIF) ridge. This information is fundamental for evaluating the implication of environmental changes in these regions for calcifying benthic molluscs.

Altogether, the research presented in this thesis contributes information needed to understand the implications of environmental changes, in particular ocean acidification, for calcifying biota in intertidal, coastal and deep-sea environments.



# Útdráttur

Kalkmyndandi lífverum stafar ógn af þeim umhverfisbreytingum sem eru að verða í hafinu vegna stórtækrar losunar mannkyns á koldíoxíði ( $\text{CO}_2$ ), sem leiðir síðan til súrnunar sjávarins og lækkunar á kalkmettun ( $\Omega$ ) í sjó. Þessari ritgerð er ætlað að fylla upp í mikilvæg göt í þekkingu okkar og efla þannig skilning okkar á afleiðingum súrnunar sjávar fyrir kalkmyndandi lífríki innan þriggja ólíkra búsvæða í og við sjó, þ.e. fjöru, grunnsævi og djúpsjávar.

*Greinar I og II* fjalla um fjöruna þar sem umhverfisbreytingar geta verið bæði hraðar og viðamiklar. *Grein I* lýsir árstíðabundnum og daglegum breytileika í efnajafnvægi ólífræns kolefnis í fjörupollum í tengslum við líffræðilegra ferla hjá kalkmyndandi rauðþörung (*Ellisolandia elongata*). Í *grein II* er lýst tilraun þar sem þörungar úr sama stofni og voru aldri í 3 vikur við mismunandi hlutþrýsting  $\text{CO}_2$  ( $p\text{CO}_2$ ), 380  $\mu\text{atm}$  (núverandi styrk  $\text{CO}_2$  í andrúmslofti), og 550, 750 og 1000  $\mu\text{atm}$ , eða styrk  $\text{CO}_2$  andrúmslofti síðar á 21. öldinni. Niðurstöður tilraunarinnar benda til þess að kalkmyndandi þörungar í fjörum séu minna viðkvæmir fyrir  $\text{CO}_2$  styrk í andrúmslofti framtíðar heldur en tegundir sem finnast helst neðan sjávarborðs.

Það vantar upplýsingar um náttúrulegan breytileika á grunnsævi í tíma og rúmi, sérstaklega hvað varðar ólífræn kolefni. Slíkar upplýsingar eru mikilvægar svo auka megi á skilning á áhrifum súrnunar sjávar á lífríki á grunnsævi og fyrir framleiðslu á reiknilífkönnum um flæði  $\text{CO}_2$  á milli efnageyma. *Grein III* fjallar um árstíðabundinn umhverfisbreytileika á grunnsævi við Ísland, Breiðafirði. Sýnt er fram á upptöku sjávar upp á 1.8 mol C  $\text{m}^{-2}$   $\text{ár}^{-1}$  á rannsóknarsvæðinu en  $p\text{CO}_2$  við yfirborð mældist frá 212  $\mu\text{atm}$  um sumar til 417  $\mu\text{atm}$  um vetur.

Djúpsjórinn er tiltölulega stöðugt búsvæði. Þrátt fyrir það benda reiknilífkön til þess að Norðurhöf verði að mestu undirmettuð með tilliti til kalkgerðarinnar aragónít fyrir árið 2100, sem telja má að ógni kalkmyndandi lindýrum á þessu svæði. Í grein VI er lýst tegundasamsetningu, dreifingu og fjölbreytileika samloka og snigla á háum breiddargráðum í Norður Atlantshafinu, þ.e. norður og suður af Grænlands-Íslands-Færeyja (GIF) hryggnum. Þessar upplýsingar skapa grunn fyrir áframhaldandi rannsóknir á áhrifum umhverfisbreytinga á kalkmyndandi lindýr þeim svæðum sem rannsökuð voru.

Á heildina litið, veita þær rannsóknir sem kynntar eru í þessari ritgerð upplýsingar sem nýta má til þess að öðlast betri skilning á mögulegum áhrifum umhverfisbreytinga, og sér í lagi súrnunar sjávar, á kalkmyndandi lífríki innan þriggja búsvæða í og við sjó, þ.e. fjöru, grunnsævi og djúpsjávar.



*Dedication*

*This thesis is dedicated to Róbert Cabrera for his love and dynamic support!*





# List of Original Papers

This thesis is based on the following papers which will be referred to by their Roman numerals:

- *Paper I: Hronn Egilsdottir*, Jon Olafsson and Sophie Martin. 2016. Photosynthesis and calcification in the articulated coralline alga *Ellisolandia elongata* (Corallinales, Rhodophyta) from intertidal rock pools. *European Journal of Phycology* 51:59-70
- *Paper II: Hronn Egilsdottir*, Fanny Noisette, Laure M-LJ Noël, Jon Olafsson and Sophie Martin. 2013. Effects of  $p\text{CO}_2$  on physiology and skeletal mineralogy in a tidal pool coralline alga *Corallina elongata*. *Marine biology* 160: 2103-2112
- *Paper III: Hrönn Egilsdóttir*, Sólveig Rósa Ólafsdóttir, Jón Ólafsson and Erla B. Örnólfsdóttir. Seasonal variability in inorganic carbon chemistry in a pristine shallow sub-Arctic fjord in Iceland. Manuscript to be submitted to *Ocean Science*.
- *Paper IV: Hrönn Egilsdóttir*, Niall McGinty and Guðmundur Guðmundsson. Diversity of bivalves and gastropods (Mollusca) in the sub-Arctic North Atlantic: Comparing patterns of alpha- and beta-diversity to the north and south of the Greenland-Iceland-Faroe ridge. Manuscript submitted to *Marine Biodiversity*.



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# 1 Introduction

The research presented in this thesis was initiated on account of some of the specific changes humans are inadvertently making to the global oceans and the scarce information available on the implication of these changes for the ocean's inhabitants. Together, the four papers presented in this thesis share a common goal: to fill important knowledge gaps when it comes to understanding the implications of anthropogenically driven changes in the sea, in particular ocean acidification and its effect on calcifying organisms.

## 1.1 Ocean acidification

Although ocean acidification was not realized to be a serious threat to life in the ocean until the end of the 20<sup>th</sup> century (Gattuso & Hansson 2011), the marine biologist Rachel Carson (1951) insightfully wrote in the book 'The Sea Around Us':

*“It is a curious situation that the sea, from which life first arose, should now be threatened by the activities of one form of that life. But the sea, though changed in a sinister way, will continue to exist; the threat is rather to life itself.”*

For centuries, anthropogenic activities have influenced marine life locally or regionally in various ways, e.g. through exploitation, pollution and eutrophication (Roberts 2009). Anthropogenic carbon dioxide (CO<sub>2</sub>) emissions are, however, presently considered a threat to marine life on a global scale (Gattuso et al. 2015). At the beginning of the industrial revolution the concentration of carbon dioxide (CO<sub>2</sub>) in the atmosphere was 280 ppm (parts per million) but in 2014 it rose above 400 ppm. This equals a 40% increase in atmospheric CO<sub>2</sub> and is the direct result of burning of fossil fuels, cement production, deforestation and other land use changes (Le Quere et al. 2009). These are rapid changes when viewed in relation to variability over the last 800 thousand years, during which CO<sub>2</sub> content of the atmosphere varied cyclically between 180 and 280 ppm (EPICA Community Members 2004). In the atmosphere, CO<sub>2</sub> acts as a greenhouse gas by limiting the transparency of the atmosphere to infrared radiation, effectively trapping heat and resulting in global warming and subsequent changes to the Earth's climate systems (Rodhe 1990). The oceans have absorbed roughly 93% of the global temperature increase due to the anthropogenic rise in CO<sub>2</sub> (Roemmich et al. 2015). The world's oceans have also effectively curbed global warming in the last 200 years through the absorption of roughly 30% of anthropogenic CO<sub>2</sub> emissions (Sabine et al. 2004). This is not without cost as the increased concentration of CO<sub>2</sub> affects the inorganic carbon system in seawater causing, for example, an increase in the hydrogen ion concentration ([H<sup>+</sup>]), which translates to a

decrease in seawater pH (Zeebe & Wolf-Gladrow 2001). For this reason, the change in seawater chemistry caused by increasing CO<sub>2</sub> concentration in the atmosphere, and subsequently in the ocean, is generally referred to as ‘ocean acidification’ (Gattuso & Hansson 2011).

The continuing rise in atmospheric CO<sub>2</sub> levels is a threat to marine biodiversity and ecosystems through both ocean warming and ocean acidification (IPCC 2014, Gattuso et al. 2015). These anthropogenic changes are especially adverse in light of the relatively stable ocean pH over the last 20 million years (Pearson & Palmer 2000) and because the global marine environment is now changing at a rate that leaves marine biota with limited time to adapt (Sunday et al. 2011). Together, the results from modern day experimental studies (Kroeker et al. 2013, Wittmann & Portner 2013) along with findings from the paleo record (Kump et al. 2009, Alroy 2010, Hönisch et al. 2012) suggest that a continuing rise in ocean temperatures, ocean acidification or a synergistic effects there between are likely to negatively affect marine biodiversity and result in a profound modification of marine ecosystems, in the next decades and centuries (Cheung et al. 2009, Gattuso et al. 2015).

A change in the concentration of CO<sub>2</sub>, influences the relative concentration of inorganic carbon system components in seawater (see appendix: *Overview of the inorganic carbon system in seawater*). Notable are two parameters, which are especially relevant to biological processes, and influenced by changes in the inorganic carbon system: pH and the concentration of carbonate (CO<sub>2</sub><sup>3-</sup>), but the latter parameter relates to the calcium carbonate saturation states ( $\Omega$ ) (see appendix). The work presented in this thesis mainly considers the effects of the  $\Omega$  on calcifying organisms but a short description of the biological implication of pH is appropriate as the effects of these parameters (and other parameters of the inorganic carbon system) are often difficult to decouple, as discussed in *papers I and II*.

### **1.1.1 Biological implication of seawater pH**

The pH scale describes the concentration of hydrogen ions ([H<sup>+</sup>]) in a solution, i.e. acidity (see appendix). Like temperature, pH is an important regulator of organism biochemistry as it influences the function of many proteins, including enzymes (Pörtner et al. 2004).

Marine species exhibit varying degree of susceptibility and responses to changes in the surrounding seawater pH (Melzner et al. 2009). In general, species with a low capacity to compensate for disturbances in extracellular ion and acid–base status and those with metabolism sensitive to such disturbances are considered vulnerable to pH changes in the surrounding seawater (Widdicombe & Spicer 2008). This includes many marine invertebrates (Pörtner 2008). Some invertebrates are able to successfully compensate for the effects of hypercapnia (i.e. abnormally elevated CO<sub>2</sub> level in blood), generally through actively increasing bicarbonate in their extracellular environment (Pörtner 2008).

However, such compensations may require increased energy allocation and accordingly, can result in reduced overall fitness (Spicer et al. 2007, Wood et al. 2008).

Species that are more mobile and ‘developed’, including vertebrates and cephalopods (molluscs) are generally considered to be less susceptible to changes in seawater pH as a result of being excellent acid-base regulators (Heisler 1984, Gutowska et al. 2010). For example, many fish species have exhibited remarkable tolerance to extremely high CO<sub>2</sub> levels with little or no observed effects on their function (Ishimatsu et al. 2008). However, studies have recorded sub-lethal and lethal effects of more moderately high CO<sub>2</sub> levels on early life forms in fish, including yellowfin tuna (*Thunnus albacares*) (Frommel et al. 2016) and Atlantic cod (*Gadus morhua*) (Frommel et al. 2012, Stiasny et al. 2016).

Indeed, it is recognized that early life stages of organisms are typically more sensitive to environmental conditions than adult life forms, including teleost fish (Sogard 1997) and invertebrates (Pechenik 1987, Gosselin & Qian 1997). Correspondingly, studies on the effects of elevated CO<sub>2</sub> in seawater largely indicate a greater sensitivity in early life stages of various marine organisms (Kurihara 2008, Dupont et al. 2010a, Talmage & Gobler 2010, Hettinger et al. 2012), including in the susceptibility of calcifying organisms to low  $\Omega$  (e.g. Waldbusser et al. 2015).

### **1.1.2 Calcifiers at a disadvantage**

Although pH could potentially affect all marine organisms, the implications of changes in  $\Omega$  relate to calcifying organisms. The continuing absorption of CO<sub>2</sub> by the oceans is leading to a decrease in  $\Omega$  which puts calcifiers at a disadvantage as they compete with non-calcifying organisms for resources, such as food and space (Fabry et al. 2008). Calcification is a trait that has evolved independently within multiple taxa in the marine environment and has undoubtedly contributed the evolutionary success in many of these taxa (Marin et al. 2012). The calcium carbonate shell or skeleton may serve to protect against predators and/or serve a purpose for the anatomy of the organism. An example of the latter is the shell of a bivalve which is a crucial part of the bivalve anatomy, in addition to protecting the animal from predators (Vermeij 1993). The susceptibility of a calcifying organism to  $\Omega$  may depend on various factors, including the type of calcium carbonate they precipitate (Kroeker et al. 2013; see appendix).



## 2 Calcifying biota in contrasting environments

The various functions performed by organisms to survive (growth, behaviour, reproduction, production of secondary metabolites, etc.) come at a prize in the form of energy. This also applies to calcification.

The evolutionary biologist Dr. Geraat J. Vermeij (1993) reflects on the cost of shell production in terms of economics in his book ‘A Natural History of Shells’:

*“Just as it is a major task for economists and entrepreneurs to identify the cost of doing business, it is important for evolutionary biologists to examine the cost in material, energy, and time required to build and maintain biological structures. We must uncover the ways by which these costs have been reduced and ask to what extent and in which circumstances these cost-cutting measures have been implemented by organisms. How are such costs to be measured? In an absolute sense, the currency of the economy of life is a combination of energy and time, but just as the value of money depends on what can be bought with it, so time and energy become important only in the context of benefits that investment brings.”*

Arguably, it may be assumed that the calcium carbonate structures are created by calcifiers because they are beneficial to them in some way or another (e.g. Monteiro et al. 2016). But the importance of these structures may vary between taxa and also depend on ecological factors, such as species interactions in ecosystems. An example of the latter is when a gastropod mollusc shell becomes weaker under acidified conditions, but so does also the claw-strength of a co-existing predator crab, resulting in a no net effects on this predator-prey interaction (e.g. Landes & Zimmer 2012). Thus, although not within the scope of this thesis, it is important to recognize the need to understand ecosystems and ecological interactions, before it is possible to understand the full implications of environmental changes. It is challenging to reliably asses a cost-benefit aspect of calcification in organisms, due to the relatively complex underlying

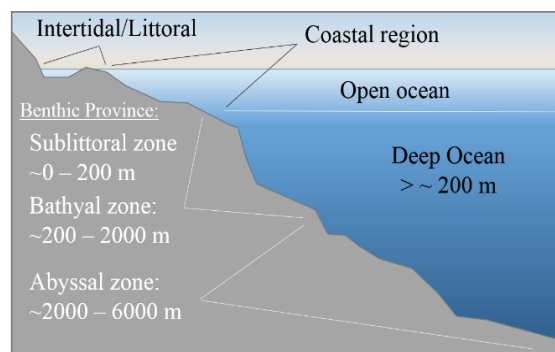


Figure 1. Benthic zones and regions based on Kennish (2000).

physiological and ecological processes, but important for understanding the implication of future ocean changes (Monteiro et al. 2016).

Continuing with Vermeij's analogy, the decrease in  $\Omega$  could be compared to an economic inflation in the price of one product, required by a specific group of consumers: calcifiers. Just like the supply of cement may influence the cost of building a concrete house, the supply of the materials needed to build a skeleton or shell (e.g. calcium ( $\text{Ca}^{2+}$ ) and carbonate ( $\text{CO}_3^{2-}$ )) can influence the energetic cost of calcification. Calcium is abundant in the seawater but this is not the case for carbonate ( $\text{CO}_3^{2-}$ ). The shift in the inorganic carbon system in seawater, caused by increased concentration of  $\text{CO}_2$ , effectively lowers the supply of  $\text{CO}_3^{2-}$  which can make the construction of calcium carbonate increasingly expensive in terms of energy. For example, the negative effects of elevated  $\text{CO}_2$  on shell calcification in the bivalve *Mytilus edulis* were mitigated when food availability was high (Melzner et al. 2011). In other words, the mussels could afford to sustain shell growth when food was abundant but shell growth was slower under food limited conditions (Melzner et al. 2011).

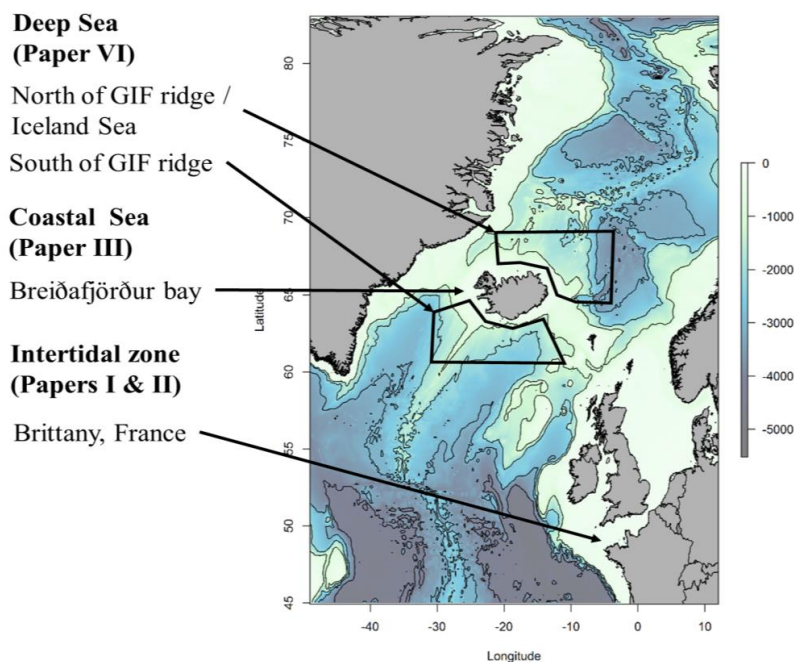


Figure 2. Temperate- and high latitude North Atlantic Ocean bathymetry and the research localities and regions investigated in this thesis.

Molluscs inhabiting energy limited environments such as the deep-sea are likely to be at particular risk under decreasing  $\Omega$  due to their limited capacity for increasing food harvesting efforts to acquire more energy, if shell construction becomes more energy demanding. On top of that, the low temperatures at high latitudes and seasonal food availability make the deep sea at high latitudes a particularly challenging environment to inhabit in general (Bodil et al. 2011). Accordingly, *paper IV* investigates the species assemblages and biodiversity and of bivalve and gastropod molluscs on the Iceland shelf and bathyal zone (Fig. 1) north of the Greenland-Iceland-Faroe (GIF) ridge where  $\Omega$  is decreasing throughout the water column, and compares findings to the region south of the GIF ridge (Fig. 2).

Mussels have been shown to exert biological control of their shell building but some calcifiers, such as calcifying algae, do not actively control calcification rates in the same manner (McCoy & Kamenos 2014). The calcification process in calcifying algae differs from invertebrates as it can be significantly influenced by photosynthesis, with the rates of calcification and photosynthesis generally highly related (e.g. Chisholm 2000). *Papers I* and *II* explore the calcification rates in a calcifying coralline alga together with rates of photosynthesis and respiration under differing environmental conditions, including various irradiance levels (*paper I*) and pH/ $\Omega$  levels (*paper II*). This study was conducted on the coast of Brittany, France (Fig. 2).

An important goal for research on ocean acidification is to understand in which environment the cost of shell building becomes too high so that shell construction is unattainable. In an ecological context the question may be presented as: Does the increased cost of calcification affect an organism overall fitness, including its reproduction capacity and ability to compete for resources against other organisms over space and time?

To answer this question, it is not only important to understand the responses of organisms to differing environmental conditions but also to understand the environmental variability they experience over space and time (Kelly & Hofmann 2013, Waldbusser & Salisbury 2014). The goal of *paper III* is to fill a knowledge gap on the seasonal coastal variability and amplitude of  $\Omega$ , pH and other environmental parameters at high latitudes (Fig. 2).

## **2.1 Ocean acidification at high latitudes**

Surface seawater  $\Omega$  is high in the tropics, which partly explains the existence of massive calcified coral reef structures that are major biodiversity hotspots (Hoegh-Guldberg et al. 2007). Temperatures decrease towards the higher latitudes and so does  $\Omega$  due to the positive relationship between temperature and  $\Omega$  (Orr et al. 2005). In some polar ocean regions the surface seawater is already periodically, or largely, undersaturated with respect to aragonite or high-Mg calcite (Jiang et al. 2015).

High latitude regions are not only characterized by naturally low  $\Omega$  but the inorganic carbon chemistry of surface seawater at high latitudes is also changing at a relatively rapid rate as a result of more rapid absorption of  $\text{CO}_2$  (Takahashi et al. 2009). Ocean warming is also occurring at a relatively rapid rate at high latitudes where organisms do not have the capacity to significantly alter their distribution range, a response to warming that has been observed and predicted in lower latitude species (Mieszkowska & Sugden 2016). Although elevated temperatures partly offset the effect of elevated  $\text{CO}_2$  on the inorganic carbon system, the effect of temperature is minor (e.g. Gattuso et al. 2016). To give an example, the resulting changes in pH and  $\Omega_{\text{aragonite}}$  have been calculated for seawater with a temperature of  $0^\circ\text{C}$  and  $5^\circ\text{C}$ , and based on present atmospheric  $\text{CO}_2$  content (400 ppm) and the predicted end-of-century, or beyond, atmospheric content of 1000 ppm (Fig. 3).

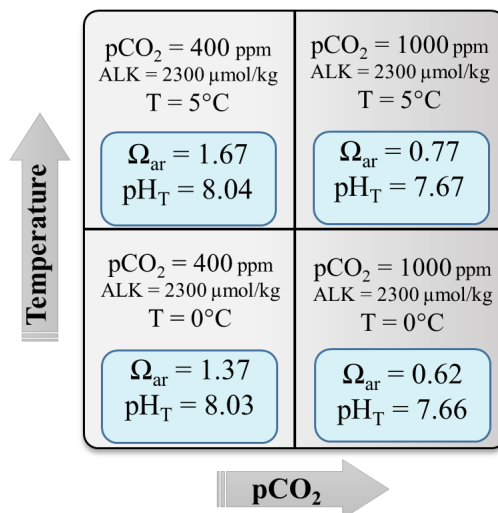


Figure 3. Effect of warming by  $5^\circ\text{C}$  and elevated seawater  $\text{CO}_2$  content (in part per million (ppm)) on pH (total scale) and the saturation state for aragonite ( $\Omega_{\text{ar}}$ ) in hypothetical Arctic seawater at surface (1 atm). The salinity (35) and alkalinity (ALK = 2300  $\mu\text{mol/kg}$ ) were kept constant. The calculations were performed using the package seacarb for R (Gattuso et al., 2016) on the basis of solubility constants of Lueker et al. (2000)).

The size of areas where surface seawater is undersaturated with respect to aragonite is growing from the poles towards lower latitudes so that the surface waters north of Iceland are predicted to be largely undersaturated with respect to aragonite and high-Mg calcites by 2100 (Orr et al. 2005, Bopp et al. 2013). Seawater  $\Omega$  decreases also with increasing pressure so that  $\Omega$  is generally highest at the ocean surface and decreases with increasing depth. Thus, undersaturated seawater at surface generally reflects undersaturated seawater throughout the water column. Indeed, the long-term monitoring of seawater carbon chemistry north of Iceland shows that  $\Omega$  is decreasing throughout the water column down to (at least) the monitoring station bottom depth of 1800 m (Ólafsson et al. 2009). The depth at which aragonite becomes undersaturated, or the aragonite saturation horizon, is at 1700 m in the Iceland Sea, and is shoaling at a rate of  $6 \text{ m y}^{-1}$  based on 20 years of observations (Jón Ólafsson, personal communication). As changes are increasingly rapid with decreasing depth, the horizons for higher saturation levels are shoaling at a faster rate, i.e.  $\Omega_{\text{aragonite}} = 1.1, 1.2$  and  $1.3$  are shoaling at a rate of approx. 8, 11 and 13 m per year (Jón Ólafsson, personal communication).



## **2.2 Contrasting ocean environments**

The pH of the global oceans has decreased by 0.1 units over the last 200 years but it is important to keep in mind that this is merely an average for global surface oceans (Sabine et al. 2004). In reality, seawater pH varies on spatial and temporal scales together with other parameters of the inorganic carbon system. This variation is driven by biotic and abiotic processes (Hofmann et al. 2011). Photosynthesis and respiration are the main biotic processes that drive change in the inorganic carbon system. Primary production of organic carbon removes CO<sub>2</sub> from seawater whereas respiration of organic carbon is a vital process which results in an addition of CO<sub>2</sub> into the seawater surrounding the organism. As aforementioned, temperature is the main driver of global variability and salinity contributes to variability in the inorganic carbon system mainly in coastal regions, in close proximity to terrestrial runoff (e.g. Waldbusser & Salisbury 2014, McGrath et al. 2016). Pressure contributes to the vertical changes in the seawater inorganic carbon system.

A varying combination of these factors result in a considerable ocean-wide variability in the inorganic carbon system (Hofmann et al. 2011). In the intertidal zone the pH range can be an order of a magnitude higher than the change expected due to anthropogenic increase in atmospheric CO<sub>2</sub> (Morris & Taylor 1983). Coastal regions generally exhibit considerable spatial and temporal environmental changes (Borges 2011) whereas the deep ocean exhibits little environmental variability compared to the coastal and shallow ocean (e.g. Olafsson et al. 2010).

This thesis describes research conducted in these three contrasting environments: the intertidal zone, coastal region and the deep ocean environment (Fig. 2).

### **2.2.1 Intertidal - Paper I & II**

The intertidal environment is unlike any other on the planet. It is the thin strip of substrate that periodically experiences life in the sea and atmosphere. On rocky shores, tidal pools are a common sight. These pools are filled with coastal seawater during high tide but act as water tubs of varying sizes when the tide retreats. When water-exchange with the coastal seawater is cut of (or re-established), the pool environment may undergo rapid changes. Weather and terrestrial runoff drive changes in temperature and salinity, and biological processes (e.g. photosynthesis, respiration and calcification) alter the seawater chemical composition (Morris & Taylor 1983). Changes to the physico-chemical environment also exert strong influences on organism physiology within the pool, including the rates of biological processes (e.g. Egilsdottir et al. 2016). The organisms that commonly inhabit tidal pools must, therefore, be well adapted to environmental fluctuations.

Calcifying coralline algae (Corallinales, Rhodophyta) are among taxa that exhibit such adaptations and are commonly observed in tidal pools globally (Nelson 2009). In fact, coralline algae diversification reveals the ability of this group to colonize a wide range of

light and temperature conditions in addition to withstand considerable environmental fluctuations over short (daily) and long (geological) time scales (Aguirre et al. 2000, McCoy & Kamenos 2014). For example, coralline algae are common in the intertidal zone but also include the deepest observed species of macroalgae (Nelson 2009).

Although taxonomically inaccurate, coralline algae are generally divided into two functional groups: articulated and crustose coralline algae (Martone & Denny 2008). Articulated (geniculate) coralline algae have calcified inter-genicula between non-calcified genicula that allow the erect algal thalli to bend with currents without breaking (Martone & Denny 2008; Fig. 4). Crustose (non-geniculate) coralline algae form crusts on rocks and other substrates, often with ‘warts’ extending from the crust and include free living forms, referred to as rodoliths (Nelson 2009). Many crustose and articulated species of coralline algae are common in tidal pools.

The susceptibility of coralline algae to ocean acidification was predicted at the time when ocean acidification research was in its infancy, due to their precipitation of the relatively soluble type of calcium carbonate, high-Mg calcite (Feely et al. 2004, Andersson et al. 2008). In 2010, when the work presented in this thesis was underway, the relatively few published studies investigating the effects of coralline algae provided evidence suggesting they were among the most vulnerable taxa to ocean acidification (Kroeker et al.



*Figure 4. Dr. Sophie Martin shows how fronds of previously separated individuals of *Ellisolandia elongata* have been cemented together as a result of calcification during the experiment described in paper II.*

2010). However, coralline algae in tidal pools may experience daily fluctuations in pH over a range on an order of a magnitude greater than the change due to the anthropogenic additions of CO<sub>2</sub> to the atmosphere (Morris & Taylor 1983, Huggett & Griffiths 1986). Seeing that the majority of the studies considered by Kroeker et al. (2010) investigated subtidal species, this evoked the following question: Are coralline algal species that commonly inhabit tidal pools less susceptible to future changes than coralline algal species that generally inhabit the subtidal environment?

The work presented in *papers I and II* was undertaken with this research question in mind and conducted at the *Station Biologique de Roscoff* in France in close collaboration with Dr. Sophie Martin. A model species that is common in tidal pools in France was chosen for the research: the articulated coralline alga *Ellisolandia elongata* (J. Ellis & Solander, (1786); revised by Hind and Saunders (2013)). Note that over the duration of the work presented in *papers I and II*, the model species went from being accepted under the taxonomic name *Corallina elongata* J. Ellis & Solander, 1786 to being considered a

synonym of *Ellisolandia elongata* (J. Ellis & Solander) K. R. Hind & G. W. Saunders, 2013, resulting in a different species name used in the papers. Nevertheless *papers I* and *II* describe rate processes in the same species, acclimated to the same or similar tidal pool environment.

The aim of *paper I* was to improve understanding of the rate of photosynthesis, respiration and calcification under varying environmental conditions *in situ* and in two seasons: summer and winter (Egilsdottir et al. 2016). Data presented in this study provide information on respiration, photosynthesis and calcification in a coralline alga inhabiting an intertidal rock pool and shows significant seasonal differences in these processes but such baseline data are often lacking from experimental studies investigating the effects of ocean acidification on biota. This study highlighted the importance of taking different light regimes into consideration when studying the response of coralline algae to environmental change. Although photosynthesis and calcification were observed to be highly related, an interesting finding was the decoupling of photosynthesis and calcification under high irradiances in summer, suggesting that a dynamic relationship exists between these metabolic processes.

The aim of *paper II* was to investigate the effect of differing  $p\text{CO}_2$  concentrations on the rates of photosynthesis, respiration and calcification in the tidal pool algae to see if it was less susceptible than previously investigated coralline algal species from subtidal environments (Egilsdottir et al. 2013, Egilsdottir et al. 2014). The experimental conditions were in accordance to those described in the guide-book *Guide to Best Practices for Ocean Acidification Research and Data Reporting* (2010), or 380, 550, 750 and 1000  $\mu\text{atm } p\text{CO}_2$ . Also, considering the relevance of the skeletal solubility, the effects of varying  $p\text{CO}_2$  conditions on the Mg/Ca ratio in their skeleton was investigated.

This study provides evidence supporting the hypothesis that coralline algae inhabiting environments where pH/ $p\text{CO}_2$  fluctuates naturally (e.g. intertidal zone and tidal pools) are likely to exhibit less negative responses to elevated  $p\text{CO}_2$ , within its natural range of variability, than those inhabiting relatively stable environments. Subsequently, this study provides support to the prediction of a greater resilience of organisms acclimated and/or adapted to highly variable pH/ $p\text{CO}_2$  environments. However, in future oceans, algae already subjected to high  $p\text{CO}_2$ /pH fluctuations will be exposed to still more extreme  $p\text{CO}_2$  scenarios (> 1000 atm) and, therefore, it cannot be excluded that they will be negatively impacted by future  $p\text{CO}_2$  levels. An interesting finding was the lower ratio of Mg/Ca in the algal skeleton under elevated  $\text{CO}_2$ , suggesting their calcite structure could become moderately less soluble under elevated  $\text{CO}_2$ .

Using the same experimental setting and general methods, Noisette et al. (2013) compared the response of three coralline alga to elevated  $p\text{CO}_2$ , including *E. elongata* from a tidal pool, crustose coralline algal species from the low intertidal zone (*Lithophyllum incrustans*) and a rodolith forming species from the subtidal environment (*Lithothamnion*

*corallioides*). The original hypothesis, that organisms naturally exposed to significant environmental variability, will be less susceptible to future pH/pCO<sub>2</sub> variations was partially supported but the results also indicated highly species-specific responses to the experimental setting. Indeed, *E. elongata* was the most resistant to elevated pCO<sub>2</sub> and may have developed adaptations to strong daily variations in pH, commonly occurring in tidal pools.

### **2.2.2 Coastal - Paper III**

Continental shelf regions are the most biogeochemically active areas of the biosphere and exhibit great temporal and spatial variability in the physico-chemical environment (Gattuso et al. 1998). Coastal regions are also highly dynamic and diverse, resulting from the extremely wide range of physical and biochemical settings in coastal regions that drive changes in the physico-chemical properties of seawater, through multiple interacting factors of biotic and abiotic origin (Waldbusser & Salisbury 2014).

A lack of comprehensive data on variability in the inorganic carbon system in coastal regions is currently an issue for two research fields. Firstly, understanding the movement of inorganic carbon among Earth's spheres requires modelling of biogeochemical systems which again requires observational data on carbon fluxes within and between spheres (Bauer et al. 2013). Secondly, to understand the implications of ocean acidification as a result of anthropogenic CO<sub>2</sub> emissions it is important to understand the coupling between biological processes and the temporal and spatial fluctuations in the inorganic carbon system (Dupont et al. 2010b, Kelly et al. 2013, Waldbusser & Salisbury 2014, Kapsenberg et al. 2015). Data for coastal regions are especially relevant as the majority of experimental studies investigating the response of marine biota to varying CO<sub>2</sub> concentrations focus on shallow water species (e.g. see studies considered in Kroeker et al. 2013).

High latitude coastal regions are typically sparsely populated and more pristine than many temperate and tropical coastal regions that often are densely populated and pressured by eutrophication or pollution, which may directly or indirectly influence the inorganic carbon system (Doney 2010). In fact, human actions leading to changes in nutrient delivery rates could have greater effect on the inorganic carbon system in eutrophicated coastal systems than the increase in atmospheric CO<sub>2</sub> (Borges & Gypens 2010). Contrastingly, the environment and biota in pristine coastal systems are more likely to be affected by increased atmospheric CO<sub>2</sub> than other anthropogenic stressors (Borges & Gypens 2010).

The aim of *paper III* was to document the spatial and temporal fluctuations in the inorganic carbon system in a high latitude coastal region and by that improve the understanding of the spatial and temporal variability experienced by marine species, particularly calcifiers (Egilsdóttir et al. in prep.).

The region chosen for the investigation was Breiðafjörður Bay, a pristine and high latitude coastal region of ecological and economical importance (Skarphedinsdottir et al. 2010). The bay is located on the southwest coast of Iceland and harbours an extensive macrophyte-based ecosystem (kelp) due to the extensive area of shallow hard substrate in the inner part of the bay (Gunnarsson 1991).

The findings of the study show that Breiðafjörður Bay is well-mixed vertically but with a slight across bay gradient, relating to terrestrial runoff in the northern most part of the bay. Seasonality in the physico-chemical environment was considerable as was the chlorophyll *a* concentration which was near-zero in winter. Temperature ranged from  $-0.8^{\circ}\text{C}$  in winter to  $14^{\circ}\text{C}$  in summer. The  $\text{pH}_T$  ranged from 8.02 to 8.25 and  $\Omega_{\text{aragonite}}$  ranged from 1.5 to 3 from winter to summer respectively. Temperature and biological processes contributed significantly and antithetically to the seasonal variation in  $p\text{CO}_2$  with surface  $p\text{CO}_2$  ranging from 212 to  $417 \mu\text{atm}$ . As the sea surface was undersaturated with respect to  $p\text{CO}_2$  except for a short time in winter, Breiðafjörður was a moderate sink of atmospheric  $\text{CO}_2$ , at a rate of  $1.8 \pm 0.2 \text{ mol C m}^{-2} \text{ y}^{-1}$ .

Based on the pristine state of the bay, ecosystem composition, limited terrestrial runoff and accessibility we suggest this coastal region is well suited for long term monitoring of ocean warming and acidification in a high-latitude coastal environment.

### **2.2.3 Deep Ocean - Paper IV**

The deep-sea, defined as that below 200 m depth, includes over 90% of benthic substrate on Earth (Kennish 2000). Contrary to some expectations in the past (e.g. see essay by Anderson & Rice 2006), there is an enormous amount of biodiversity that exists in the deep sea (Rex & Etter 2010). Accessing the deep ocean is a very costly and difficult endeavour, explaining why ecosystems therein are insufficiently studied compared with shallow water or terrestrial systems (May & Godfrey 1994, Higgs & Attrill 2015). Yet, it is now more urgent than ever to document and understand biodiversity patterns and ecology in benthic ecosystems which are currently undergoing dynamic alterations to their habitat of anthropogenic origin (Crain et al. 2008, Rogers 2015). It has been pointed out that ocean acidification is a risk to biodiversity in the North Atlantic (Gehlen et al. 2014). However, the lack of information on species occurrences, distribution and biodiversity patterns below the photic zone severely limit attempts to predict what the implication of ocean changes will be for marine biota and ecosystems in some regions of the North Atlantic.

The research presented in *paper IV* (Egilsdóttir et al. submitted) was motivated by a realization of the extent of the changes in the inorganic carbon system in the deep Nordic Seas (Olafsson et al. 2009) and in consideration of model predictions indicating the seawater will be largely undersaturated with respect to aragonite throughout the water column in by 2100 (Orr et al. 2005, Bopp et al. 2013). Benthic molluscs were chosen for

the study as calcifying molluscs are among the most susceptible marine taxa under ocean acidification (Gazeau et al. 2013, Kroeker et al. 2013, Waldbusser et al. 2015).

Fortunately, data on the distribution of gastropods and bivalves in Icelandic waters was available for this investigation from the benthic sampling project BIOICE (Benthic Invertebrates of Icelandic waters, 1991–2004). Species richness was estimated for different depth ranges and patterns of  $\alpha$ -diversity (local diversity) were estimated as the Hurlbert-Sanders expected number of species in a sample of 20 individuals  $E(S_{20})$ . The regional and depth related  $\beta$ -diversity (change in species composition) was analysed using a novel approach of partitioning  $\beta$ -diversity into two ecologically distinct components: community dissimilarity resulting from species replacement (or turnover) and dissimilarity resulting from species loss (nestedness) or gain (Baselga 2010, 2012). Nestedness was also analysed independently using the software BINMATNEST (Rodríguez-Gironés et al. 2010).

Biodiversity patterns of bivalves and gastropods differed considerably between the bathymetric gradients north and south of the GIF ridge. A clear decrease in  $\alpha$ -diversity and an increase in nestedness with depth was observed north of the GIF ridge, whereas a moderate decrease, and no decrease in  $\alpha$ -diversity was observed in bivalves and gastropods, respectively, south of the GIF ridge where turnover is the dominant  $\beta$ -diversity component.

As suggested by this and previous studies, the benthic environment below ~500 m depth north of the GIF ridge is relatively species poor compared to the North Atlantic south of the GIF ridge. As a result of limited biodiversity at high latitudes, the ecosystems are generally considered particularly vulnerable to disturbances caused by rapid environmental changes (Barry et al. 2013). However, a direct relationship between ecosystem resilience and biodiversity is not fully established and may depend on species assemblages and various ecosystem components (Oliver et al. 2015a, Oliver et al. 2015b). Nevertheless, the likelihood of a resilient species functionally replacing a co-existing susceptible species within an ecosystem is likely to increase with increasing biodiversity.

Data presented in this last paper contribute significantly to the knowledge of molluscan biodiversity and species composition along bathymetric gradients in the high latitude North Atlantic. This information is important in guiding further attempts to evaluate the degree of vulnerability in bivalve and gastropods communities in the Nordic Seas, where environmental changes relating to ocean warming and acidification are observed and predicted, and for efforts to predict the implications of these changes for deep sea benthic communities more generally.

### **3 Conclusions and perspective**

One of the major challenges facing the scientific community at present relates to the multiple environmental changes occurring now at the onset of what is referred to as the Anthropocene (Crutzen 2006, IPCC 2014). The research presented in this thesis was conducted at a time of an ongoing fruitful scientific debate about the responses of calcifying biota to ocean acidification (Gattuso & Hansson 2011, Gattuso et al. 2015). Recent evidence suggests that calcifying biota are particularly vulnerable to ocean acidification, supporting early hypothesis (Zondervan et al. 2001, Kroeker et al. 2013). However, studies conducted over the last decade have also demonstrated a lack of generality in species' responses to elevated CO<sub>2</sub>, with some species-specific responses reported in seemingly alike species (Dupont et al. 2010b), or even differing responses in populations of the same species (e.g. Parker et al. 2011).

Although a considerable amount of baseline information has been assembled on species responses to CO<sub>2</sub>, including the work on tidal pool coralline algae presented in this thesis, there is yet limited understanding of which mechanisms drive these responses. One of the key parts of information generally lacking concerns species habitats, and how their environment changes over time (Kelly & Hofmann 2013, Hofmann et al. 2014). Some of the research presented in this thesis contributes information on large scale environmental variability in species habitats (i.e. in tidal pool or coastal water environmental variability). Also lacking, albeit not the focus of the work presented in this thesis, is information on micro-environments of species (e.g. within a burrow of an infaunal species or within the diffusive boundary layer surrounding organisms) (Hurd et al. 2011, Cornwall et al. 2014). In some isolated habitats, such as the deep sea, information on large scale environmental variability is available (e.g. the Iceland Sea). However, a knowledge gap regarding the species composition and ecosystems is, as of yet, the main barrier for understanding the implications of ocean changes in these habitats.

Accordingly, the research presented in this thesis contributes information needed to understand the implications anthropogenic environmental changes, in particular ocean acidification, for calcifying biota in intertidal, coastal and deep-sea environments.





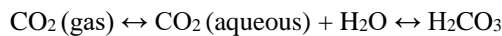
# Appendix: Overview of the inorganic carbon system in seawater

Major components of the inorganic carbon system in seawater and the mechanics of ocean acidification (based on Dickson et al. (2007) and Zeebe and Wolf-Gladrow (2001)).

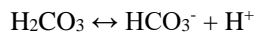
Biological processes are significant drivers of CO<sub>2</sub> changes in seawater, mainly through photosynthesis (↓[CO<sub>2</sub>]) and respiration (↑[CO<sub>2</sub>]). Anthropogenic increase in atmospheric [CO<sub>2</sub>] is now an additional driver of seawater [CO<sub>2</sub>] due to the subsequent increased adsorption of CO<sub>2</sub> in seawater on a global scale.

Note that the reactions can go in both directions (indicated by ↔) and are driven by changes in the concentration of carbon dioxide [CO<sub>2</sub>] in seawater.

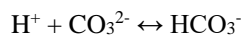
When carbon dioxide gas dissolves in seawater, it reacts with water to form carbonic acid:



Carbonic acid converts into bicarbonate (HCO<sub>3</sub><sup>-</sup>) and hydrogen ions (H<sup>+</sup>):



Hydrogen ions react with carbonate ions (CO<sub>3</sub><sup>2-</sup>) to form bicarbonate:



So, the elevated CO<sub>2</sub> in seawater results in a net increase in [H<sup>+</sup>] (decrease in pH), a net increase in [HCO<sub>3</sub><sup>-</sup>] and a net decrease in [CO<sub>3</sub><sup>2-</sup>] (Table 1).

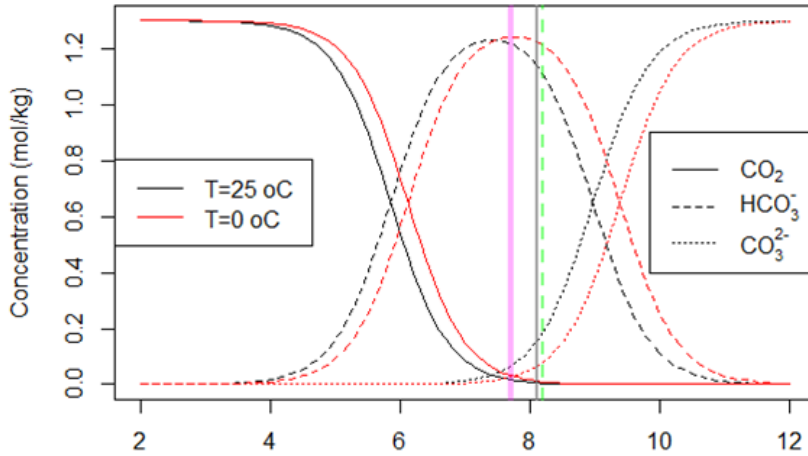


Figure 5. Bjerrum plot showing the change in the relative contribution of carbon dioxide [ $\text{CO}_2$ ], bicarbonate [ $\text{HCO}_3^-$ ] and carbonate [ $\text{CO}_3^{2-}$ ] to the dissolved inorganic carbon as a function of  $\text{pH}_T$  and the effect of two temperatures,  $0^\circ$  (red lines) and  $25^\circ$  (black lines). The green dashed line indicates an average pre-industrial open ocean  $\text{pH}$  of 8.2, the grey line represents the present day average open ocean  $\text{pH}$  of 8.1 and the pink line a predicted average open ocean  $\text{pH}$  for the end of the 21<sup>st</sup> century of 7.7. The plot was drawn using the package seacarb for R (Gattuso et al. 2016) and based on a salinity of 35, 1 atm pressure (ocean surface) and disassociation constants,  $K_1$  and  $K_2$ , given by Lueker et al. (2000).

Table 1. Parameters of the inorganic carbon system in seawater. Arrows indicate a positive ( $\uparrow$ ), negative ( $\downarrow$ ) or no ( $-$ ) concentration change when  $\text{CO}_2$  is added into the system.

$[\text{H}^+]$	Hydrogen ion concentration	$\uparrow$
<b>pH</b>	$-\log[\text{H}^+]$	$\downarrow$
<b><math>\text{CO}_2</math></b>	Carbon dioxide	$\uparrow$
<b><math>\text{HCO}_3^-</math></b>	Bicarbonate	$\uparrow$
<b><math>\text{CO}_3^{2-}</math></b>	Carbonate ion	$\downarrow$
<b>DIC</b>	Dissolved inorganic carbon	$\uparrow$
<b>TA</b>	Total alkalinity	$-$
<b><math>\Omega</math></b>	Calcium carbonate saturation state	$\downarrow$

## pH

pH is defined as the negative base-10 logarithm of the hydrogen ion concentration in moles per liter of solution ( $\text{pH} = -\log [\text{H}^+]$ ). As this is a logarithmic scale, a change in  $[\text{H}^+]$  may be perceived to be considerably less significant when expressed as pH compared to the absolute change in  $[\text{H}^+]$ . For instance, the preindustrial ocean surface had an average pH of ~8.2 but today, the average surface ocean pH is currently ~8.1 which actually translates to a 30% increase in  $[\text{H}^+]$  since the year 1750 (Sabine et al. 2004). A pH decrease from 8.2 to 7.7 as predicted by the year 2100 (Caldeira & Wickett 2005) translates to a 150% increase in  $[\text{H}^+]$ . Note, that if a solution pH decreases, it is acidifying, even though the solution pH is still above 7. Thus, the effects of  $\text{CO}_2$  on the ocean chemistry are generally referred to as ‘ocean acidification’ even though the vast bulk of the Earth’s seawater is slightly basic.

## Total Alkalinity (TA)

Seawater total alkalinity (TA (or  $A_T$ )) is commonly defined as ‘the excess base’ in seawater, or the sum of excess proton acceptors:  $\text{TA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] + [\text{HPO}_4^{2-}] + 2[\text{PO}_4^{3-}] + \text{etc.}$   $[\text{SiO}(\text{OH})_3^-] - [\text{H}^+]_F - [\text{HSO}_4^-] - [\text{HF}] - \text{etc.}$ , where brackets represent the total concentration of these constituents in a solution (Dickson et al. 2007). The order of anions reflects their contribution to seawater alkalinity but bicarbonate ions, carbonate and boron are by large the most important contributors to seawater alkalinity, and thus the other anions mentioned above are often ignored. The addition (or removal) of  $\text{CO}_2$  does not change TA since the net reaction produces the same number of equivalents of positively contributing species ( $\text{H}^+$ ) as negative contributing species ( $\text{HCO}_3^-$  and/or  $\text{CO}_3^{2-}$ ). However, the removal (or addition) of  $\text{CO}_3^{2-}$  through calcification (or dissolution) results in a decrease (or increase) in seawater TA. This mechanism gave rise to the development of the alkalinity anomaly technique (Smith & Key 1975) which was used in *papers I and II* to estimate calcification rates in a calcifying coralline alga.

## The saturation state for calcium carbonate ( $\Omega$ )

The solubility of a calcium carbonate crystal is affected by the concentration of calcium (that is abundant in seawater) and the carbonate ion concentration but the concentration of the latter decreases with increasing concentration of  $\text{CO}_2$ . So that:

$$\Omega = [\text{Ca}^{2+}] \times [\text{CO}_3^{2-}] \div K_{\text{sp}}$$

When  $\Omega = 1$ , a solution considered saturated with respect to the  $\text{CaCO}_3$  structure.

When  $\Omega > 1$ , the solution is supersaturated and the structure thermodynamically stable.

When  $\Omega < 1$ , the solution is undersaturated with respect to the  $\text{CaCO}_3$  type which results in an unstable structure that will dissolve with time.

The solubility constant ( $K_{\text{sp}}$ ) depends on the type of calcium carbonate. There are three general types of biogenic calcium carbonates produced by marine organisms: aragonite, calcite and high-Mg calcite. Aragonite (e.g. precipitated by corals and molluscs) and calcite (e.g. precipitated by crustaceans and coccolithophores) have the same chemical

composition ( $\text{CaCO}_3$ ) but have different crystal structures, which causes them to have differing physical properties (e.g. Anthony et al. 2016). Aragonite is harder (Mohs scale: 3.5-4) than calcite (Mohs scale: 3) but also ~1.5 times more soluble than calcite in seawater. Some organisms (e.g. coralline algae and echinoderms) produce calcite with a high ratio of Mg/Ca within the calcite lattice. The solubility of high Mg-calcites increases with an increasing ratio of Mg/Ca, so that the structure becomes more soluble than aragonite when the ratio is greater than ~0.12 (> 12% Mg) (Morse et al. 2006, Andersson et al. 2008).

The physical properties of seawater, temperature, salinity and pressure also influence  $\Omega$ . For example, the surface ocean  $\Omega_{\text{aragonite}}$  ranges from < 1 in the polar regions to > 4 at low latitudes.  $\Omega_{\text{aragonite}}$  also decreases with increased pressure. As a result of temperature, the aragonite saturation horizon (ASH: The depth below which  $\Omega_{\text{aragonite}} < 1$ ) is ~2 times deeper in the North Atlantic than in the Nordic Seas.

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# **Paper I**

## **Photosynthesis and calcification in the articulated coralline alga *Ellisolandia elongata* (Corallinales, Rhodophyta) from intertidal rock pools**

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Author contributions: HE & SM conducted experiments, analyzed data and wrote the manuscript. JO edited the manuscript.



# Photosynthesis and calcification in the articulated coralline alga *Ellisolandia elongata* (Corallinales, Rhodophyta) from intertidal rock pools

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Calcifying coralline algae are functionally important in many ecosystems but their existence is now threatened by global climate change. The aim of this study is to improve our understanding of coralline algal metabolic functions and their interactions by assessing the respiration, photosynthesis and calcification rates in an articulated (geniculate) coralline alga, *Ellisolandia elongata*. Algal samples selected for this case study were collected from an intertidal rock-pool on the coast of Brittany (France). Physiological rates were assessed in summer and winter by measuring the concentration of oxygen, dissolved inorganic carbon and total alkalinity fluxes at five irradiance levels and in the dark using incubation chambers.

Respiration, photosynthetic and calcification rates were strongly affected by seasonal changes. Respiration increased with temperature, being ten-fold higher in summer than in winter. Photosynthetic parameters of the photosynthesis-irradiance ( $P$ - $E$ ) curve,  $P_g^{max}$ ,  $P_n^{max}$  and  $E_k$ , were two- to three-fold higher in summer relative to winter. Photoinhibition was observed under high irradiance indicating an acclimation of *E. elongata* to low irradiance levels. Parameters of the calcification-irradiance ( $G$ - $E$ ) curve,  $G^{max}$  and  $E_k$ , were approximately two-fold higher in summer compared with winter. In summer, calcification rates were more strongly inhibited under high irradiance than photosynthetic rates, suggesting a dynamic relationship between these metabolic processes. By inhabiting intertidal rock pools, *E. elongata* exhibits tolerance to a dynamic physico-chemical environment. Information on respiration, photosynthesis and calcification rates in a calcifying coralline alga inhabiting such dynamic environments in terms of pH and temperature is important in order to better understand how ocean acidification and warming will affect coralline algae in the future.

**Key words:** calcification, *Corallina elongata*, coralline algae, *Ellisolandia elongata*, irradiance,  $P$ - $E$  curve, photosynthesis, tidal pool

## INTRODUCTION

Changes in climate and ocean acidification are underway as a result of anthropogenic increase in carbon dioxide concentrations in the atmosphere (IPCC, 2013). Investigating organism physiology in relation to environmental variability provides useful information for predicting acclimation and adaptation to future conditions (Kelly & Hofmann, 2013). Calcifying benthic algae are generally considered as highly sensitive to climate change and ocean acidification (Kroeker *et al.*, 2013), but many species are also common in habitats where pH and temperature gradients are steep. Among calcifying benthic algae, red coralline algae (Corallinales,

Rhodophyta) are the most important taxa in terms of global carbon cycling and ecosystem engineering (Nelson, 2009). They can be found over a wide latitudinal range and at all depths in the photic zone (Adey & MacIntyre, 1973; Steneck, 1986), where they are known to provide ecosystem services by sheltering high biodiversity and to favour larval recruitment and settlement (Adey, 1998). Coralline algae are also of importance with respect to the carbon and carbonate cycle in shallow coastal waters through high community calcium carbonate production (Martin *et al.*, 2007). Articulated coralline algae (also referred to as geniculate coralline algae) have non-calcified segments (genicula) between calcified segments (intergenicula), which provide flexibility to the erect algal thallus (Martone & Denny, 2008). They serve as ecosystem

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engineers by providing three-dimensional frameworks that support diverse assemblages of macrofauna (Dayton, 1972; Kelaher *et al.*, 2001; Benedetti-Cecchi, 2006; Noël *et al.*, 2009). Their unpalatable calcareous structure acts as a barrier against grazing, thus protecting diverse algal epiphyte assemblages (Pfister & Hay, 1988).

Coralline algae have been identified as among the most vulnerable calcifying marine taxa, threatened by ocean acidification and decreasing calcium carbonate saturation levels (Kroeker *et al.*, 2013). Reliable predictions of coralline algal responses to future environmental changes require knowledge of the processes involved in calcification and their interaction with other metabolic processes and environmental factors. Coralline algae precipitate calcite within the cell wall, and can also deposit calcite within intercellular spaces (Borowitzka, 1989). They precipitate high-Mg calcite (Ries, 2011) which becomes more soluble with increased incorporation of  $Mg^{2+}$  into the calcite lattice. The solubility of calcite exceeds that of aragonite when the Mg/Ca mol% is above approximately 12 (Morse *et al.*, 2006; Andersson *et al.*, 2008), with higher values often observed in calcite produced by coralline algae (Chave, 1954). An organic matrix within the cell wall is important for the calcification process, but the exact chemical and functional properties of the organic matrix are not yet fully understood (Borowitzka, 1989; Rahman & Halfar, 2014). For example, chitin was found recently to be present in a coralline alga species possibly serving an important role in nucleation, growth and orientation of the mineral crystals (Rahman & Halfar, 2014).

The equilibrium between inorganic carbon species in an aquatic system is an important environmental factor affecting the processes that underlie  $CaCO_3$  bioprecipitation (Borowitzka, 1982; Borowitzka & Larkum, 1987; Raven, 2011; Basso, 2012). For example, supersaturation of calcium carbonate is generally required for calcification to take place in algae (Borowitzka, 1989). The calcium carbonate saturation state ( $\Omega_{CaCO_3}$ ) in seawater and at the calcification site is affected by photosynthesis and respiration through the removal or addition of  $CO_2$  from or to the extracellular environment (Borowitzka, 1989). In this way, photosynthesis induces calcification, as is evident by the strong relationship between the metabolic rates of these processes in all calcifying algae (Borowitzka & Larkum, 1976; Pentecost, 1978; Borowitzka, 1989; Martin *et al.*, 2013a).

Photosynthetic rates in algae are primarily controlled by environmental factors such as irradiance, nutrient concentration, temperature and the availability of inorganic carbon as photosynthetic substrate (Hurd *et al.*, 2014). A useful method for investigating photophysiological characteristics in

algae is to look at the relationship between irradiance and photosynthesis using  $P$  (photosynthesis) versus  $E$  (irradiance) curves (also termed  $P-I$  curves) (Lüning, 1990). Photosynthetic rates are positively correlated with irradiance until the photosynthetic mechanism of the algae becomes saturated (Lüning, 1990). The irradiance needed to saturate an alga is generally high for algae acclimated or adapted to high levels of irradiance and low for algae acclimated or adapted to dim light conditions (Lüning, 1990). Under higher irradiances algae may either continue to photosynthesize at the maximum rate, or photosynthetic rates may be negatively affected above a certain irradiance level, i.e. photoinhibition occurs (Lüning, 1990). Due to the close relationship between photosynthesis and calcification, an irradiance response curve may also be produced for calcification (then termed  $G-E$  (or  $G-I$ ) curve) (Chisholm, 2000; Martin *et al.*, 2006, 2013a, 2013b). Through comparing irradiance response curves for photosynthesis and calcification in algae, a better understanding of the relationship between these metabolic processes may be achieved.

Articulated coralline algae frequently inhabit the intertidal environment, in particular tidal pools (Johansen, 1981). Tidal pools are habitats where water exchange is limited or temporally non-existent, as a result of local tidal activity (i.e. amplitude and tidal cycles) and pool locality (i.e. high or low within the intertidal zone) (Morris & Taylor, 1983). Because of biological processes and physico-chemical fluxes, the carbonate chemistry within a tidal pool may exhibit significant periodical variation, which is generally reflected by large pH fluctuations (Morris & Taylor, 1983; Huggett & Griffiths, 1986).

*Ellisolandia elongata* (previously known as *Corallina elongata*) inhabits intertidal rock pools where large fluctuations in physico-chemical variables occur diurnally (Egilsdottir *et al.*, 2013). It has been suggested that organisms inhabiting environments where large fluctuations in  $CO_2$ /pH and temperature are common have a relatively good capacity to acclimate or adapt to future ocean acidification and warming (Kelly & Hofmann, 2013; Hofmann *et al.*, 2014). Indeed, previous studies indicate that *E. elongata* has greater tolerance to ocean acidification than other coralline algae whose distribution is limited to more stable environments (Egilsdottir *et al.*, 2013; Noisette *et al.*, 2013).

Although, *E. elongata* is predicted to exhibit resilience under future conditions, there have been no studies on the relationship between photosynthesis and calcification in *E. elongata* under natural conditions. As such, there is a lack of information on the seasonal difference in the rates of these processes. Such data can, for



example, be used for predicting the response of *E. elongata* to climate change and ocean acidification. The aim of this study was to characterize the metabolic activity of photosynthesis and calcification and how these processes interact *in situ* within the articulated coralline alga *E. elongata* inhabiting tidal pools where strong physico-chemical variations occur.

## MATERIALS AND METHODS

### Collection of algae

Individuals of *Ellisolandia elongata* (J. Ellis & Solander, 1786) K. R. Hind & G. W. Saunders 2013 were collected in a shaded tidal pool in the low intertidal level on the rocky beach 'Les Amiets' in Cléder on the north coast of Brittany, France (48°41'45 N, 4°7'26 W) on 11 August 2010 (summer) and on 21 February 2013 (winter). The irradiance in photosynthetic active radiations (PAR) at the surface of the pool was measured using a flat quantum sensor (LiCor, Li-190SA, LI-COR Biosciences, USA). Irradiance was *c.* 30  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in summer and *c.* 15  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in winter under the shade of rocks overhanging the tidal pool at around midday in sunny conditions. Temperature in the pool at the time of collection of algae was 17°C in summer and 10°C in winter. The physico-chemical characteristics of the rock pool are given in Table 1. Individual algae with a frond length of 5–10 cm and free of epiphytic organisms were selected for the experiment. They were carefully separated from their substratum to obtain their encrusting base. After experiments the fresh weight (FW) of the algae was determined and converted to dry weight (DW) using the FW/DW relationship established by Egilisdottir *et al.* (2013).

### Incubation procedure and physiological measurements

*Ellisolandia elongata* were incubated under natural sunlight from 13:00 to 16:00. In summer, algae were transferred directly to incubation chambers and incubated *in situ* in a

large neighbouring pool exposed to direct sunlight. In winter the algae were transported to the Roscoff Marine Station in a thermostatic tank within 1 h of collection and incubated outside under natural sunlight in large baths filled with natural seawater pumped from 10 m depth in front of the station at ambient temperature. Temperature during incubation varied from 17 to 24°C in summer and from 6 to 9°C in winter (Table 2), being representative of changes in the pool during emersion at these seasons (Table 1). Five sets of individual algae ( $N = 5$ ) were incubated per irradiance level in 220 and 460 ml clear and black glass bottles. Incubations at all irradiance levels were performed simultaneously in each season for 1 to 3 h in summer and 2 to 4 h in winter, depending on the expected algal metabolic response. Mean DW of the sets of algae was  $0.41 \pm \text{SE } 0.03 \text{ g}$  in summer and  $0.81 \pm \text{SE } 0.05 \text{ g}$  in winter. Black plastic covers were used to assess respiration and calcification rates in the dark. Four neutral density filters were used to assess net production and calcification in algae simultaneously exposed to five different mean irradiance (PAR) levels (I1, I2, I3, I4, and I5; Table 2) of 91, 158, 235, 614 and 1118  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in summer and 30, 75, 135, 290 and 500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in winter, with the highest irradiances corresponding to natural full light. Irradiance was measured with a Li-Cor quantum sensor (LiCor Li-190SA, LI-COR Biosciences, Nebraska). Reference incubations without algae were included for full light and dark conditions to assess and correct for metabolism by any organisms in the seawater. Oxygen fluxes were estimated by measuring the oxygen concentration at the beginning and end of each incubation with an oxygen probe (Oxymeter HQ40D, Hach Lange, Ltd portable LDO™, Loveland, Colorado). pH was measured using a pH meter (HQ40D, Hach Lange, Ltd portable LDO™, Loveland, Colorado) calibrated on the total scale ( $\text{pH}_T$ , expressed on the total hydrogen ion concentration scale) using Tris/HCl and 2-aminopyridine/HCl buffer solutions with a salinity of 35 (Dickson *et al.*, 2007). For measurements of total alkalinity ( $A_T$ ), seawater samples were filtered through 0.7  $\mu\text{m}$  Whatman GF/F filters into 100 ml glass bottles, immediately poisoned with mercuric chloride and stored in a cool dark place pending analyses.  $A_T$  was determined potentiometrically using an automatic titrator (Titroline alpha, Schott SI Analytics, Mainz, Germany) calibrated on the National

**Table 1.** Tidal pool seawater temperature and carbonate chemistry parameters.

Treatment	Temperature (°C)	$\text{pH}_T$	$A_T$ (mmol $\text{kg}^{-1}$ )	$\text{pCO}_2$ ( $\mu\text{atm}$ )	DIC (mmol $\text{kg}^{-1}$ )	$\text{CO}_3^{2-}$ (mmol $\text{kg}^{-1}$ )	$\text{HCO}_3^-$ (mmol $\text{kg}^{-1}$ )	$\text{CO}_2$ (mmol $\text{kg}^{-1}$ )	$\Omega_{\text{aragonite}}$	$\Omega_{\text{calcite}}$
Summer Day (Light)	16.7–19.0	8.15–8.61	2.290	71–300	1.650–2.004	0.201–0.434	1.214–1.792	0.002–0.011	3.1–6.7	4.8–10.4
Summer Night (Dark)	15.0–15.1	7.68–7.82	2.290	721–1030	2.168–2.217	0.075–0.101	2.040–2.104	0.027–0.038	1.2–1.5	1.8–2.4
Winter Day (Light)	10.0–10.3	8.00–8.15	2.340	304–454	2.102–2.172	0.127–0.169	1.920–2.025	0.013–0.020	1.9–2.6	3.0–4.0
Winter Night (Dark)	8.4–9.3	7.87–7.92	2.340	562–632	2.216–2.234	0.090–0.100	2.090–2.114	0.026–0.029	1.4–1.5	2.2–2.4

The pH on total scale ( $\text{pH}_T$ ) and total alkalinity ( $A_T$ ) were measured. Other parameters of the carbonate chemistry were calculated as described in the Materials and Methods section. Measurements were performed in the tidal pool where *Ellisolandia elongata* were collected in summer (see Egilisdottir *et al.*, 2013) and in a near-by tidal pool of similar characteristics in winter, as the original tidal pool had been eradicated due to a storm. Values are presented as a range of measurements conducted at different locations inside the pool one hour after the low tide at 17:30, 24 July 2012 (daylight) and at 06:00, 25 June 2012 (night) in summer, and at 15:00, 2 February 2014 (daylight) and at 08:00, 10 February 2014 (night) in winter. Only one measurement per season was performed for  $A_T$ .

**Table 2.** Range (minimum–maximum) of temperature and carbonate chemistry parameters within incubation chambers during incubations.

Irradiance level	Dark	I1	I2	I3	I4	I5
<b>Summer</b>						
Temperature	17.1–23.1	17.1–23.1	17.1–23.6	17.1–22.1	17.1–23.4	17.1–21.8
pH <sub>T</sub>	8.07–8.17	8.17–8.30	8.17–8.36	8.17–8.34	8.17–8.30	8.17–8.39
A <sub>T</sub> (mmol kg <sup>-1</sup> )	2.276–2.293	2.238–2.290	2.240–2.290	2.248–2.290	2.254–2.290	2.267–2.292
pCO <sub>2</sub> (µatm)	280–371	184–280	151–280	166–280	185–280	142–280
DIC (mmol kg <sup>-1</sup> )	1.966–1.992	1.800–1.986	1.752–1.986	1.802–1.986	1.812–1.986	1.773–1.986
CO <sub>3</sub> <sup>2-</sup> (mmol kg <sup>-1</sup> )	0.207–0.220	0.213–0.300	0.213–0.332	0.213–0.310	0.213–0.307	0.213–0.337
HCO <sub>3</sub> <sup>-</sup> (mmol kg <sup>-1</sup> )	1.736–1.769	1.494–1.764	1.416–1.764	1.486–1.764	1.503–1.764	1.432–1.764
CO <sub>2</sub> (mmol kg <sup>-1</sup> )	0.010–0.011	0.005–0.010	0.004–0.010	0.005–0.010	0.005–0.010	0.004–0.010
Ω <sub>aragonite</sub>	3.3–3.5	3.3–4.7	3.3–5.2	3.3–4.9	3.3–4.8	3.3–5.3
Ω <sub>calcite</sub>	5.0–5.3	5.1–7.2	5.1–8.0	5.1–7.4	5.1–7.4	5.1–8.1
<b>Winter</b>						
Temperature	5.8–8.8	7.1–8.8	7.1–8.0	7.1–8.1	7.1–8.2	7.1–8.2
pH <sub>T</sub>	8.10–8.11	8.10–8.33	8.10–8.41	8.10–8.42	8.10–8.37	8.10–8.35
A <sub>T</sub> (mmol kg <sup>-1</sup> )	2.380–2.394	2.335–2.383	2.322–2.383	2.330–2.383	2.331–2.383	2.340–2.383
pCO <sub>2</sub> (µatm)	342–362	186–352	148–352	145–352	167–352	178–352
DIC (mmol kg <sup>-1</sup> )	2.190–2.205	2.027–2.190	1.961–2.190	1.962–2.190	1.993–2.190	2.016–2.190
CO <sub>3</sub> <sup>2-</sup> (mmol kg <sup>-1</sup> )	0.137–0.148	0.141–0.222	0.141–0.247	0.141–0.252	0.141–0.232	0.141–0.224
HCO <sub>3</sub> <sup>-</sup> (mmol kg <sup>-1</sup> )	2.028–2.050	1.796–2.033	1.707–2.033	1.703–2.033	1.753–2.033	1.784–2.033
CO <sub>2</sub> (mmol kg <sup>-1</sup> )	0.016–0.017	0.009–0.017	0.007–0.017	0.007–0.017	0.008–0.017	0.008–0.017
Ω <sub>aragonite</sub>	2.1–2.2	2.1–3.4	2.1–3.7	2.1–3.8	2.1–3.5	2.1–3.4
Ω <sub>calcite</sub>	3.3–3.5	3.4–5.3	3.4–5.9	3.4–6.0	3.4–5.5	3.4–5.3

Mean irradiance levels (in µmol photons m<sup>-2</sup> s<sup>-1</sup>), in summer: I1 = 91, I2 = 158, I3 = 235, I4 = 614, I5 = 1118, and in winter: I1 = 30, I2 = 75, I3 = 135, I4 = 290, I5 = 500.

The pH on total scale (pH<sub>T</sub>) and total alkalinity (A<sub>T</sub>) were measured. Other parameters of the carbonate chemistry were calculated as described in the Materials and Methods section.

Bureau of Standards scale and calculated using a Gran function applied to pH values ranging from 3.5 to 3.0 (Dickson *et al.*, 2007). Measurements of A<sub>T</sub> were corrected by comparison with standard reference material provided by Andrew G. Dickson (Batch 111). Other parameters of the carbonate chemistry were calculated from pH<sub>T</sub>, A<sub>T</sub>, temperature and salinity (35) using the software CO2SYS, EXCEL Macro version 2.1 (originally designed by Lewis & Wallace, 1998). Calculations were based on a set of constants K1 and K2 from Mehrbach *et al.* (1973), refit by Dickson and Millero (1987) (Tables 1 and 2).

### Data treatment and statistical analysis

Primary production was estimated directly from oxygen fluxes. Net production (P<sub>n</sub>) and respiration (R) rates (µmol O<sub>2</sub> g<sup>-1</sup> DW h<sup>-1</sup>) were calculated as follows:

$$P_n \text{ or } R(\text{O}_2) = \left( \frac{\Delta \text{O}_2}{\Delta t \times V} \right) / DW \quad (1)$$

where ΔO<sub>2</sub> is the change in dissolved oxygen (µmol l<sup>-1</sup>), Δt is the incubation time (h), DW is the dry weight of the algae and V is the volume of the incubation chamber (l).

The variations of dissolved inorganic carbon (DIC) in the incubations integrate both organic carbon metabolism (photosynthesis and respiration) and inorganic carbon metabolism (calcification and dissolution) (Zeebe & Wolf-Gladrow, 2001). Calcification rates were estimated using the alkalinity anomaly technique (Smith & Key, 1975; Chisholm & Gattuso, 1991) which is based on the

relationship: Ca<sup>2+</sup> + 2HCO<sub>3</sub><sup>-</sup> → CaCO<sub>3</sub> + CO<sub>2</sub> + H<sub>2</sub>O, for which total alkalinity decreases by 2 equivalents for each mol of CaCO<sub>3</sub> precipitated.

Calcification rates (G, µmol CaCO<sub>3</sub> g<sup>-1</sup> DW h<sup>-1</sup>) was calculated as follows:

$$G = - \left( \frac{\Delta A_T}{2 \times \Delta t \times V} \right) / DW \quad (2)$$

where ΔA<sub>T</sub> is the change in total alkalinity (µeq l<sup>-1</sup>).

P<sub>n</sub> and R expressed in terms of carbon (µmol CO<sub>2</sub> g<sup>-1</sup> DW h<sup>-1</sup>) were calculated by the difference between the initial and final DIC concentrations (calculated by using the software CO2SYS) as follows:

$$P_n \text{ or } R(\text{CO}_2) = - \left( \frac{\Delta \text{DIC} - (0.5 \times \Delta A_T)}{\Delta t \times V} \right) / DW \quad (3)$$

where ΔDIC is the change in dissolved inorganic carbon (mmol l<sup>-1</sup>).

The relationship between irradiance (E, µmol photon m<sup>-2</sup> s<sup>-1</sup>) and P<sub>n</sub> (or G) was established using P<sub>n</sub> (or G) versus E curves following the function of Platt *et al.* (1980) modified by the addition of a respiration (R) or dark calcification term (G<sub>d</sub>, µmol CaCO<sub>3</sub> g<sup>-1</sup> DW h<sup>-1</sup>):

$$P_n = P_s \times \left( 1 - e^{-\alpha \times \frac{E}{E_s}} \right) \times e^{-\beta \times \frac{E}{E_s}} + R \quad (4)$$

$$G = G_s \times \left(1 - e^{-\alpha \times \frac{E}{E_k}}\right) \times e^{-\beta \times \frac{E}{E_k}} + G_d \quad (5)$$

where  $P_s$  and  $G_s$  are scaling parameters defined as the maximum rate of photosynthesis or calcification respectively in the absence of photoinhibition (or decrease in calcification rate under high irradiance;  $\mu\text{mol O}_2$ , DIC or  $\text{CaCO}_3 \text{ g}^{-1} \text{ h}^{-1}$ ),  $\alpha$  is the initial slope of the relationship ( $\mu\text{mol g}^{-1} \text{ h}^{-1} (\mu\text{mol m}^{-2} \text{ s}^{-1})^{-1}$ ),  $\beta$  is the photoinhibition coefficient ( $\mu\text{mol g}^{-1} \text{ h}^{-1} (\mu\text{mol m}^{-2} \text{ s}^{-1})^{-1}$ ). In the absence of evident photoinhibition, the  $\beta$  value was set to zero.

The deduced maximal rate of gross photosynthesis (or calcification) at light saturation,  $P_g^{\text{max}}$  (or  $G_g^{\text{max}}$ ), is calculated as:

$$P_g^{\text{max}} \left(\text{or } G_g^{\text{max}}\right) = P_s \left(\text{or } G_s\right) \times \left(\frac{\alpha}{\alpha + \beta}\right) \times \left(\frac{\beta}{\alpha + \beta}\right)^{\frac{\beta}{\alpha}} \quad (6)$$

The maximal rate of net production (or calcification),  $P_{\text{max}}^{\text{net}}$  (or  $G^{\text{max}}$ ) can be estimated as:

$$P_n^{\text{max}} = P_g^{\text{max}} + R \quad (7)$$

$$G^{\text{max}} = G_g^{\text{max}} + G_d \quad (8)$$

The saturating irradiance ( $E_k$ , index of light adaptation) is expressed as:

$$E_k = \frac{P_g^{\text{max}}}{\alpha} \text{ or } \frac{G_g^{\text{max}}}{\alpha} \quad (9)$$

The compensation irradiance ( $E_c$ ) where the rate of photosynthesis equals the rate of respiration is expressed as:

$$E_c = \frac{R}{\alpha} \quad (10)$$

$P_n$ - and  $G$ - $E$  curves were fitted to the data using the statistical software STATGRAPHICS *Plus, version 5.0 for Windows* (StatPoint Technologies Inc., 2013), establishing five individual curves for  $\text{O}_2$  flux,  $\text{CO}_2$  flux and calcification per season. The statistical software *R 2.15.0* (The R Foundation for Statistical Computing, 2013) was used when examining differences between seasons. Normality of data was estimated via the Shapiro–Wilk normality test. The Welch two sample t-test was used to identify differences between seasons where data was normally distributed or where log or square-root transformation of data yielded normal distribution. When normality was not achieved, a Wilcoxon Rank Sum test was used. Results are expressed as means  $\pm$  standard error (SE).

## RESULTS

### Respiration and photosynthesis

The mean respiration rate ( $R$ ) was higher in summer than in winter ( $\text{O}_2$  fluxes:  $t = -6.63$ ,  $P < 0.01$ ;  $\text{CO}_2$  fluxes:  $t = 3.97$ ,  $P < 0.05$ ; Table 3). In both seasons, primary production measured under various

**Table 3.** Parameters of the light response curves for *Ellisolandia elongata* net primary production (in  $\text{O}_2$  and  $\text{CO}_2$  fluxes) and calcification in winter and in summer.

	Primary production						Calcification					
	in $\text{O}_2$ fluxes			in $\text{CO}_2$ fluxes			in $\text{CO}_2$ fluxes			in $\text{CaCO}_3$ fluxes		
	Summer	Winter	$P$	Summer	Winter	$P$	Summer	Winter	$P$	Summer	Winter	$P$
$R$ or $G_d$ ( $\mu\text{mol g}^{-1} \text{ DW h}^{-1}$ )	$-12.01 \pm 1.67$	$-0.92 \pm 0.11$	** (t)	$16.90 \pm 4.14$	$0.44 \pm 0.22$	* (t)	$1.35 \pm 0.51$	$-0.24 \pm 0.10$	* (t)	$1.35 \pm 0.51$	$-0.24 \pm 0.10$	* (t)
$P_n^{\text{max}}$ or $G^{\text{max}}$ ( $\mu\text{mol g}^{-1} \text{ DW h}^{-1}$ )	$62.2 \pm 5.5$	$20.3 \pm 1.3$	** (w)	$-69.1 \pm 4.9$	$-20.4 \pm 1.6$	** (w)	$5.0 \pm 1.5$	$3.2 \pm 0.1$	** (w)	$5.0 \pm 1.5$	$3.2 \pm 0.1$	ns
$P_n^{\text{max}}$ or $G^{\text{max}}$ ( $\mu\text{mol g}^{-1} \text{ DW h}^{-1}$ )	$50.2 \pm 4.7$	$19.4 \pm 1.2$	** (w)	$-52.2 \pm 3.6$	$-20.0 \pm 1.7$	** (w)	$6.3 \pm 1.0$	$2.9 \pm 0.2$	** (w)	$6.3 \pm 1.0$	$2.9 \pm 0.2$	* (t)
$E_k$ ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ )	$82.6 \pm 13.2$	$30.5 \pm 5.7$	* (t)	$89.7 \pm 19.4$	$29.3 \pm 7.8$	* (t)	$65.7 \pm 11.6$	$31.9 \pm 6.9$	* (t)	$65.7 \pm 11.6$	$31.9 \pm 6.9$	* (t)
$E_c$ ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ )	$15.6 \pm 3.0$	$1.4 \pm 0.3$	** (t)	$19.2 \pm 5.6$	$0.6 \pm 0.3$	* (t)		$1.7 \pm 1.1$			$1.7 \pm 1.1$	
$\alpha$	$0.87 \pm 0.19$	$0.74 \pm 0.12$	ns	$0.95 \pm 0.13$	$0.94 \pm 0.29$	ns	$0.08 \pm 0.02$	$0.11 \pm 0.02$	ns	$0.08 \pm 0.02$	$0.11 \pm 0.02$	ns
$\beta$	$0.013 \pm 0.009$	$0.003 \pm 0.002$	ns	$0.010 \pm 0.010$	$0.002 \pm 0.001$		$0.015 \pm 0.065$	$0.002 \pm 0.001$		$0.015 \pm 0.065$	$0.002 \pm 0.001$	ns

Metabolic rates are expressed in  $\mu\text{mol g}^{-1} \text{ dry weight h}^{-1}$  and irradiance in  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ .  $P_n^{\text{max}}$  (or  $G^{\text{max}}$ ) is the maximum gross production (or calcification) rate,  $P_n^{\text{max}}$  (or  $G^{\text{max}}$ ) is maximum net production (or calcification) rate,  $E_k$  is the saturating irradiance,  $E_c$  is the compensation irradiance,  $R$  (and  $G_d$ ) is the rate of respiration (or calcification in the dark),  $\alpha$  is the initial slope of the curve and  $\beta$  is the photoinhibition coefficient which was set to 0 in the case of no photoinhibition.

Values are expressed as mean  $\pm$  SE ( $n = 5$ ) with estimates of statistical differences ( $P$  values) from Welch two sample t-tests (t) and Wilcoxon rank sum tests (w). \*\*\*  $P < .001$ ; \*\*  $P < .01$ ; \*  $P < .05$ ; ns  $P > .05$ ; • no statistical comparison.

irradiance levels showed a strong relationship with irradiance in terms of both oxygen production ( $r^2 = 0.88\text{--}0.98$  in summer;  $r^2 = 0.78\text{--}0.99$  in winter) and net CO<sub>2</sub> uptake ( $r^2 = 0.87\text{--}0.98$  in summer;  $r^2 = 0.87\text{--}0.98$  in winter; Fig. 1). Photoinhibition was recorded for primary production at irradiance levels above *c.* 400  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in summer and a negligible photoinhibition above *c.* 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  was observed in winter (Table 3; Fig. 1). In summer, maximum gross O<sub>2</sub> production and CO<sub>2</sub> uptake at light saturation ( $P_g^{max}$ ) were about three times those recorded for winter (O<sub>2</sub> fluxes: W=25,  $P < 0.01$ ; CO<sub>2</sub> fluxes: W=25,  $P < 0.01$ ; Table 2). In winter the maximum net production ( $P_n^{max}$ ) was about 40% that of summer (O<sub>2</sub> fluxes: W=25,  $P < 0.01$ ; CO<sub>2</sub> fluxes: W=25,  $P < 0.01$ ; Table 3). The saturating irradiances ( $E_k$ ) for primary production were about three times higher in summer, relative to winter (O<sub>2</sub> fluxes:  $t = 5.42$ ,  $P < 0.05$ ; CO<sub>2</sub> fluxes:  $t = 5.26$ ,  $P < 0.05$ ; Table 3) and the compensation irradiances ( $E_c$ ) were

higher in summer than in winter (O<sub>2</sub> fluxes:  $t = 4.66$ ,  $P < 0.01$ ; CO<sub>2</sub> fluxes:  $t = 3.32$ ,  $P < 0.05$ ; Table 3). Coefficient  $\alpha$  (the initial slope of the curve) did not differ between seasons for both O<sub>2</sub> and CO<sub>2</sub> fluxes. Coefficient  $\beta$  (photoinhibition coefficient) did not differ between seasons for O<sub>2</sub> fluxes. No statistical comparison between seasons was conducted for  $\beta$  considering CO<sub>2</sub> fluxes, since photoinhibition was not indicated by all five *P-E* curves calculated for each season. The net production rates under 30  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and 15  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (*in situ* midday irradiance in summer and winter respectively) were estimated from the *P-E* curves to  $8.4 \pm 3.1 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$  and  $-5.6 \pm 4.5 \mu\text{mol C g}^{-1} \text{ DW h}^{-1}$  in summer and  $7.4 \pm 1.0 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$  and  $-11.3 \pm 1.5 \mu\text{mol C g}^{-1} \text{ DW h}^{-1}$  in winter. Gross production ( $P_g = P_n + R$ ) was thus estimated to  $20.4 \pm 3.1 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$  and  $-22.5 \pm 1.9 \mu\text{mol C g}^{-1} \text{ DW h}^{-1}$  in summer and  $8.4 \pm 1.0 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$  and  $-11.8 \pm 1.6 \mu\text{mol C g}^{-1} \text{ DW h}^{-1}$  in winter.

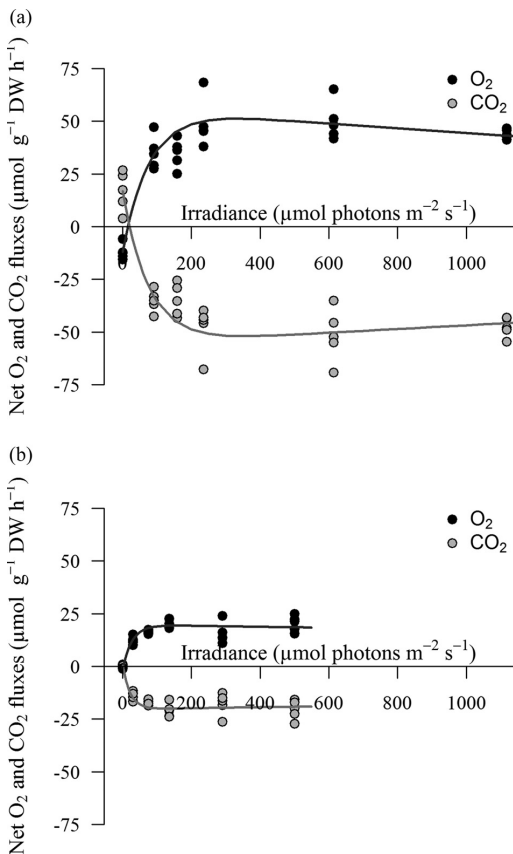


Fig. 1. The relationship between irradiance and *Ellisolandia elongata* net primary production (in O<sub>2</sub> and CO<sub>2</sub> fluxes) in (a) summer and (b) winter.

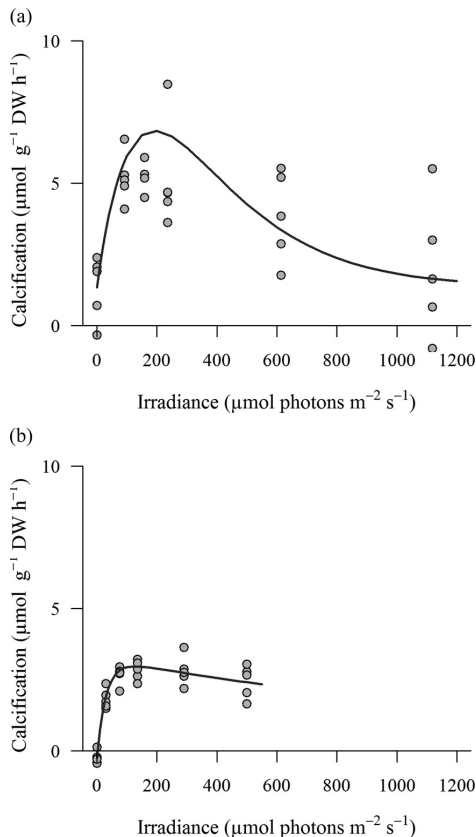
### Calcification

Calcification rates in the dark ( $G_d$ ) differed between seasons ( $t = 3.07$ ,  $P < 0.05$ ; Table 3).  $G_d$  was positive in summer but negative in winter, indicating net dissolution of calcium carbonate. A strong relationship was observed between irradiance and calcification in summer ( $r^2 = 0.72\text{--}1.00$ ; Fig. 2a) and winter ( $r^2 = 0.93\text{--}0.99$ ; Fig. 2b). At high irradiances in summer the relationship between irradiance and calcification was negligible with a high variance observed for calcification at high irradiances (Fig. 2a). Calcification was inhibited at irradiances higher than *c.* 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in both summer and winter. Maximum net calcification ( $G_n^{max}$ ) differed between seasons ( $t = 3.07$ ,  $P < 0.05$ ; Table 3) and was about two times higher in summer relative to winter.  $E_k$  was approximately two times higher in summer than in winter ( $t = 2.50$ ,  $P < 0.005$ ; Table 3). Since calcification in the dark was positive in summer, an estimate for  $E_c$  in summer was unavailable and consequently a statistical comparison between seasons was irrelevant. For calcification, coefficients  $\alpha$  and  $\beta$  did not differ between seasons (Table 3). Calcification at midday *in situ* irradiance estimated from the *G-E* curves was  $3.3 \pm 0.3 \mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ DW h}^{-1}$  in summer at 30  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $1.1 \pm \text{SE } 0.1 \mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ DW h}^{-1}$  in winter at 15  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ .

## DISCUSSION

### Respiration

The respiration rates observed here varied seasonally in terms of both O<sub>2</sub> and CO<sub>2</sub> fluxes, being more than



**Fig. 2.** The relationship between irradiance and calcification rates in *Ellisolandia elongata* in (a) summer and (b) winter.

10-fold higher in summer in comparison with winter. It is well known that respiration in seaweeds is temperature-dependent (Hurd *et al.*, 2014). An increase in respiration rate with elevated temperature has been reported for several species of coralline algae until the temperature exceeds their thermal tolerance limit (see Martin *et al.*, 2013b for a review). For example, the respiration rate in the temperate crustose coralline alga *Lithophyllum yessoense* increased by ten-fold, from 3 to 29  $\mu\text{g O}_2 \text{ cm}^{-2} \text{ h}^{-1}$  between 5 and 25°C (Ichiki *et al.*, 2001). Changes in respiration rates observed between winter and summer in *E. elongata* are high in comparison with the three-fold increase measured in the temperate unattached coralline alga (maerl) *Lithothamnion corallioides* between 10°C (winter) and 16.5°C (summer) (Martin *et al.*, 2006) or in the temperate crustose coralline alga *Lithophyllum cabiochae* between 13°C (winter) and 22°C (summer) (Martin *et al.*, 2013b). The large differences observed between winter and summer in *E. elongata* are mainly due to the high respiration rates recorded in summer. A three- to four-fold lower respiration rate was

recorded for this species at a temperature range of 13 to 17°C in a Mediterranean subtidal environment (3  $\mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ; El Haïkali *et al.*, 2004), and at a constant temperature of 18°C in a laboratory (4  $\mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ; Egilsdottir *et al.*, 2013). Temperature was considerably higher in summer in the rock pool at the time of measurement, reaching 23°C at the end of the incubation and explaining the high summer respiration rates in *E. elongata* observed in this study. Higher respiration rates in summer in comparison to winter may also reflect high rates of primary production shortly before dark measurements took place, as light-enhanced dark respiration has been observed in plants and algae (Raghavendra *et al.*, 1994; Martin *et al.*, 2013a). The large seasonal difference observed for respiration could be attributed in part to the low temperature during winter measurements (reaching below 6°C). In winter, the algae were carefully transported to a different location and incubated in water-baths. Therefore, it is conceivable that different handling and transportation of algae prior to incubation contributed seasonal differences in respiration and other metabolic activity.

Although changes between day and night respiration rates occur in subtidal coralline algae at more stable temperatures (Martin *et al.*, 2013a), our results suggest that respiration in coralline algae may vary strongly with temperature changes in tidal pools, even on a very short time scale. Such variations may in turn have consequences for the whole metabolism of algae and in particular for the balance between respiration and photosynthesis.

### Photosynthesis

The photosynthesis of *E. elongata* was significantly influenced by season. The gross production,  $P_g$ , under ambient irradiance was two- to three-fold higher in summer than in winter. These seasonal fluctuations of  $P_g$  are related to changes in both temperature and irradiance, which are the fundamental parameters in the control of algal photosynthesis (Hurd *et al.*, 2014). Photosynthetic characteristics of the  $P-E$  curves reflect this, displaying three-fold higher values of  $P_g^{max}$  in summer compared with winter. A similar seasonal influence of temperature and irradiance on photosynthesis has previously been reported in other temperate coralline algal species such as *L. corallioides* or *L. cabiochae*, which exhibited values of  $P_g^{max}$  two- to three-fold higher in summer compared with winter (Martin *et al.*, 2006, 2013b). A strong seasonal difference was observed in the photosynthetic parameters  $E_k$  and  $E_c$ , with both being considerably lower in winter, reflecting a greater degree of low-light acclimation in winter than in summer.  $E_k$

was two- to three-fold higher in summer compared with winter, in agreement with  $E_k$  previously reported for low light acclimated coralline algae, in the range of 7–32  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Kühl *et al.*, 2001; Payri *et al.*, 2001; Roberts *et al.*, 2002; Martin *et al.*, 2013a).  $E_c$  was more than 10-fold higher in summer than in winter, the summer value is consistent with that previously reported for *E. elongata* acclimated to low irradiance ( $E_c = 20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; Algarra *et al.*, 1991). Moreover,  $E_c$  in winter is similar to that reported for other coralline algae living in dim light conditions such as *L. cabiochae* in winter under very low irradiance ( $< 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; Martin *et al.* 2013b). Such low  $E_c$  reflects the ability of *E. elongata* to make a net gain from photosynthesis at very low light levels.

Photoinhibition was observed at high irradiances, as has previously been reported in low light acclimated algae (Kain, 1987; Sagert *et al.*, 1997; Kühl *et al.*, 2001; Roberts *et al.*, 2002; Martin *et al.*, 2013b). Recently, photoinhibition was also observed in *E. elongata* under rapid light curves (RLCs) (Korbee *et al.*, 2014) giving further evidence for *E. elongata* being a ‘shade plant’, which Häder *et al.* (2003) postulated was characteristic of all geniculate coralline algal species. Coralline algae have been reported to exhibit dynamic photoinhibition strategies which probably enable the algae to tolerate high irradiance levels rather than undergoing photodamage (Burdett *et al.*, 2014). For example, Burdett *et al.* (2014) reported a varying reflectance of the thallus of crustose coralline algae at different times of the day and varying intracellular concentrations of the antioxidant compound DMSP (dimethylsulphoniopropionate).

Intertidal rock pools typically exhibit large diel fluctuations in temperature and irradiance levels, both of which affect photosynthesis in algae. Temperature directly affects the biochemistry at the algal cellular level. For example, temperature may influence the activity of the enzyme carbonic anhydrase, active transport of  $\text{CO}_2$  and  $\text{HCO}_3^-$  and diffusion rates, which can affect photosynthesis through the supply of substrate to carbon fixation pathways (Hurd *et al.*, 2014). In this study, temperature exhibited similar variability at all irradiance levels for each season, but it is likely to contribute to the difference in  $P$ - $E$  curves between seasons.

Irradiance affects seawater in tidal pools indirectly through regulation of photosynthesis. As observed in this study, a positive relationship exists between photosynthetic rates and irradiance, up to near the saturating irradiance  $E_k$  (Lüning, 1990). In this way, irradiance influences parameters of seawater chemistry in tidal pools, such as carbonate chemistry and nutrient concentrations (Morris & Taylor, 1983; Hurd *et al.*, 2014). Contrary to respiration, algal photosynthesis removes  $\text{CO}_2$  from seawater and subsequently leads to an elevation in pH and a decrease in

total inorganic carbon concentrations (Hurd *et al.*, 2014). High pH has been reported to negatively affect photosynthesis in some macroalgal species (Borowitzka, 1981; Middelboe & Hansen, 2007). For example, Björk *et al.* (2004) found certain algal species to be absent in tidal pools where high pH values and low inorganic carbon concentration occurred temporarily. The effect of pH on photosynthesis in *E. elongata* has only been investigated for a pH range of 7.7 to 8.1, with no significant effects observed (Egilisdottir *et al.*, 2013; Noisette *et al.*, 2013). Like most macroalgae, coralline algae have  $\text{CO}_2$  concentrating mechanisms (CCMs) that allow the algae to utilize low concentrations of inorganic carbon (Borowitzka, 1981; Gao *et al.*, 1993). These mechanisms are diverse in their exact functionality but can, for example, promote active transport of inorganic carbon across membranes and/or may facilitate the conversion of  $\text{HCO}_3^-$  to  $\text{CO}_2$  for photosynthetic metabolism (Raven *et al.*, 2012). Effective CCMs explain why some algae, including many species of coralline algae, are commonly found in rock pools where pH is frequently elevated and inorganic carbon concentrations often low (Raven, 2011).

#### Calcification

Although calcification in articulated coralline algal communities may have the potential to significantly influence regional carbon balance (Bensoussan & Gattuso, 2007), very few data are available on calcification for rates in articulated coralline algae *in situ* (El Haïkali *et al.*, 2004; Bensoussan & Gattuso, 2007; Fisher & Martone, 2014), and none of these studies considered the seasonal response of this process.

Irradiance had a positive effect on calcification both in winter and in summer. These findings are in agreement with previous studies revealing calcification in coralline algae to be greater in the light than in the dark (Borowitzka, 1981; Gao *et al.*, 1993).

In winter,  $G_d$  was below zero indicating a net dissolution of calcium carbonate. Dissolution of calcium carbonate at night, in winter, has previously been observed within a shallow infralittoral ecosystem dominated by *E. elongata* in the Mediterranean (Bensoussan & Gattuso, 2007). Borowitzka (1981) suggested a positive relationship between  $\Omega_{\text{CaCO}_3}$  of the surrounding seawater and calcification rates in coralline algae in the dark. In this study, the seawater within the incubation chambers was supersaturated with respect to aragonite throughout all incubations and, based on calculations by Ries (2011), is unlikely to have become undersaturated with respect to the high-Mg calcite produced by the algae (Mg/Ca mol %  $\approx 17$ –19; Egilisdottir *et al.* 2013). However, the chemical properties of seawater in the micro-environments within the diffusion boundary layer differ from the surrounding seawater, as a result of algal

metabolism (De Beer & Larkum, 2001; Roberts *et al.*, 2002; Cornwall *et al.*, 2014). Thus, it is conceivable that the dissolution observed in the dark in winter is indicative of undersaturation of the high-Mg calcite within the diffusion boundary layer as a result of respiration.  $G_d$  in the summer was significantly different from  $G_d$  in the winter but was about two times lower than previously described for *E. elongata*, 2.5  $\mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ DW h}^{-1}$  (El Haikali *et al.*, 2004). However, El Haikali *et al.* (2004) attributed this relatively high calcification rate in the dark to 'residual light metabolism', which has also been suggested for high calcification rates in the dark measured in other coralline algae (Chisholm, 2000; Martin *et al.*, 2006). Similarly, the seasonal difference in  $G_d$  observed in this study may result from different rates of 'residual light metabolism', affected by temperature and irradiance prior to, and during incubations and/or reflecting different  $\Omega_{\text{CaCO}_3}$  between seasons.

The net calcification rate  $G_n$ , estimated for ambient irradiance around midday was three-fold higher in summer relative to winter and  $G^{max}$  was two-fold higher in summer relative to winter.  $G^{max}$  in summer was consistent with the mean calcification of *E. elongata* (9  $\mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ DW h}^{-1}$ ) measured under higher irradiances in the Mediterranean (El Haikali *et al.*, 2004).

In agreement with previous studies (see Borowitzka, 1989 and references therein), a relationship between irradiance and calcification was observed both in summer and in winter. Borowitzka and Larkum (1976) suggested that photosynthetic rates and calcification rates were directly related in calcifying algae and an association between them has also been reported in other studies (Pentecost, 1978; Martin *et al.*, 2006, 2013a, 2013b). However, above *c.* 200  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  in summer, calcification rates indicate a stronger inhibition than observed for photosynthetic rates. As with photosynthetic rates, inhibition of calcification processes under high irradiances probably reflects a local acclimation of *E. elongata* to a shaded environment. Calcification inhibition under high irradiances (up to 800–1000  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) was not observed for 'light-acclimated' tropical coralline algae such as *Hydrolithon onkodese*, *Neogoniolithon brassica-florida*, *Hydrolithon reinboldii* and *Neogoniolithon conicum* (Chisholm, 2000) or for the temperate species *L. corallioides* living in shallow waters (1–8 m depth) (Martin *et al.*, 2006). However, inhibition of calcification was reported under high irradiance levels for *L. cabiochae* living in dim-light conditions ( $\leq 35 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ; Martin *et al.*, 2013b).

Photosynthesis may promote calcification in two major ways: by elevation of  $\Omega_{\text{CaCO}_3}$  and by production of organic tissue or compounds. Photosynthesis (and respiration) regulates pH and

$\Omega_{\text{CaCO}_3}$  within the diffusion boundary layer and at the calcification site via uptake (and production) of  $\text{CO}_2$  (Smith & Roth, 1979; Gao *et al.*, 1993; Hurd *et al.*, 2011). As reviewed by Borowitzka (1989), a certain degree of  $\Omega_{\text{CaCO}_3}$  is required to induce nucleation and growth of the algal calcium carbonate crystals.  $\Omega_{\text{CaCO}_3}$  may also be relevant for structure, morphology and size of the skeleton. Nucleation and calcification can also be induced by photosynthesis via production of a fibrous organic matrix in the cell walls that is required for forming calcite crystals (Borowitzka, 1989). In coralline algae the organic matrix produced also affects the crystal polymorph, i.e. induces the formation of calcite instead of aragonite (Borowitzka, 1989). The enzyme carbonic anhydrase may be involved in the interplay between photosynthesis and calcification, as originally suggested by Digby (1977) and later by Hofmann *et al.* (2012) and Martin *et al.* (2013b), by catalysing the conversion of  $\text{CO}_2$  into  $\text{HCO}_3^-$  and then  $\text{CO}_3^{2-}$ . Indeed, the presence of carbonic anhydrase has been reported in *E. elongata* (Mercado *et al.*, 1997). However, carbonic anhydrase is also used in photosynthesis to convert  $\text{HCO}_3^-$  to  $\text{CO}_2$ , which could then lower  $\Omega_{\text{CaCO}_3}$ . Therefore, it is conceivable that calcification may be reduced at the same time as high photosynthetic rates are observed.

Results presented here for *E. elongata* suggest a direct relationship between photosynthetic production and calcification. However this relationship was found to be limited to low irradiance levels, since calcification and photosynthetic processes seem to evolve differently under high irradiances. In summer, calcification rates varied greatly at high irradiances. This could reflect different acclimations to irradiance in the selected algae, as a result of algae inhabiting distinct localities in the rock pool in terms of irradiance regimes (Häder *et al.*, 1997). Anatomical differences between algae may also explain variable responses. For example, Borowitzka (1979) demonstrated that the uppermost segments calcified at a much higher rate than those below it and that they also had a much higher rate of carbon fixation into organic material than the lower segments. In addition, Borowitzka and Vesik (1979) observed more chloroplasts in young segments compared with older segments. Furthermore, it is conceivable that the equation used to convert wet mass of algae into dry mass may have contributed to the variability observed in the data.

Calcification rates in coralline algae are affected by temperature and irradiance, which both exhibit strong diurnal fluctuations in intertidal rock pools. Temperature is positively correlated with  $\Omega_{\text{CaCO}_3}$ , potentially explaining how elevated temperature may promote calcification. This is perhaps especially relevant for calcification in the dark, when the absence of photosynthetic metabolism increases

the relative importance of abiotic environmental factors. Temperature also directly affects diffusion rates and metabolic processes that may play a role in calcification (Hurd *et al.*, 2014). For example, temperature affects carbonic anhydrase activity and active transport of  $\text{HCO}_3^-$  and  $\text{CO}_2$ , both of which are also important for photosynthesis (Hurd *et al.*, 2014). As discussed above, much of the observed response in calcification may be directly associated with the photosynthesis and respiration of the algae themselves. In addition, the biological metabolism of all the species residing in a tidal pool can influence the chemical properties of the seawater and subsequently affect physiological processes in coral-line algae inhabiting the pool.

Since calcifying algae in intertidal rock pools experience large fluctuations in the physico-chemical environment, it has been suggested that they have physiological advantages as ocean acidification and warming advances (Egilsdottir *et al.*, 2013). However, ocean acidification and global warming may affect organisms synergistically in the future. The species investigated in this study inhabited a low-lit tidal pool and respiration increased significantly over the temperature range observed in the study. Thus, the minimum pH and  $\Omega_{\text{CaCO}_3}$  in rock pools might be lowered in the future as a result of increased temperatures leading to elevated respiration rates, but this effect may be somewhat offset due to the positive correlation between temperature and  $\Omega_{\text{CaCO}_3}$ . Coralline algae inhabiting low light environments may be especially at risk from a rise in temperature, since higher irradiance levels could be required to accomplish a sustainable net photosynthetic production in the algae.

## Conclusions

Data presented in this study provide information on respiration, photosynthesis and calcification in a coralline alga inhabiting an intertidal rock pool and the seasonal differences in these processes. This information is relevant for developing an understanding of how climate change will affect coralline algae in the future. This study also highlights the importance of taking different light regimes into consideration when studying the response of coralline algae to environmental change. Certainly, a dynamic relationship exists between the metabolic processes involved in photosynthesis and calcification and thus, care must be taken when assuming a direct relationship between the rates of these processes.

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## DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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## AUTHOR CONTRIBUTIONS

H. Egilsdottir: field experiments, drafting and editing manuscript; J. Olafsson: editing manuscript; S. Martin: field experiments, drafting and editing manuscript.

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## **Paper II**

# **Effects of $p\text{CO}_2$ on physiology and skeletal mineralogy in a tidal pool coralline alga *Corallina elongata***

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Author contributions: HE & SM conducted field work, analyzed data and wrote the manuscript. LN conducted field work and edited the manuscript. JO edited the manuscript.



# Effects of $p\text{CO}_2$ on physiology and skeletal mineralogy in a tidal pool coralline alga *Corallina elongata*

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**Abstract** Marine organisms inhabiting environments where  $p\text{CO}_2/\text{pH}$  varies naturally are suggested to be relatively resilient to future ocean acidification. To test this hypothesis, the effect of elevated  $p\text{CO}_2$  was investigated in the articulated coralline red alga *Corallina elongata* from an intertidal rock pool on the north coast of Brittany (France), where  $p\text{CO}_2$  naturally varied daily between 70 and 1000  $\mu\text{atm}$ . Metabolism was measured on algae in the laboratory after they had been grown for 3 weeks at  $p\text{CO}_2$  concentrations of 380, 550, 750 and 1000  $\mu\text{atm}$ . Net and gross primary production, respiration and calcification rates

were assessed by measurements of oxygen and total alkalinity fluxes using incubation chambers in the light and dark. Calcite mol % Mg/Ca ( $m\text{Mg}/\text{Ca}$ ) was analysed in the tips, branches and basal parts of the fronds, as well as in new skeletal structures produced by the algae in the different  $p\text{CO}_2$  treatments. Respiration, gross primary production and calcification in light and dark were not significantly affected by increased  $p\text{CO}_2$ . Algae grown under elevated  $p\text{CO}_2$  (550, 750 and 1000  $\mu\text{atm}$ ) formed fewer new structures and produced calcite with a lower  $m\text{Mg}/\text{Ca}$  ratio relative to those grown under 380  $\mu\text{atm}$ . This study supports the assumption that *C. elongata* from a tidal pool, where  $p\text{CO}_2$  fluctuates over diel and seasonal cycles, is relatively robust to elevated  $p\text{CO}_2$  compared to other recently investigated coralline algae.

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## Introduction

Red calcareous coralline algae (Corallinales, Rhodophyta) are abundant and widespread in most benthic coastal areas. They thrive from the polar oceans to the tropics, within all depths of the photic zone and in almost every habitat type (Adey and Macintyre 1973; Steneck 1986). Coralline algae play critical biological and ecological roles in shallow waters where they develop (Foster 2001). They act as cement and contribute to consolidate coral reefs and reduce their erosion (Jokiel et al. 2008). Moreover, they favour larval recruitment and settlement of numerous invertebrates (Adey 1998) and shelter high biodiversity (Nelson 2009). Coralline algae are of particular importance with respect to the carbon and carbonate cycle in shallow coastal waters through high community photosynthesis and respiration (Martin et al. 2005, 2006, 2007) and through high calcium carbonate production and dissolution (Barrón et al. 2006;

Martin et al. 2007). Coralline algae precipitate calcite in extracellular spaces bound by cell walls, and calcification appears to be driven by internal and external control (Borowitzka 1981; Hurd et al. 2011; Raven and Hurd 2012). They are major producers of high-magnesium calcite (Mg calcite), with mole percentage (mol %)  $\text{MgCO}_3$  reaching up to 30 % in the tropics, but decreasing at higher latitudes and with lower temperatures (Chave 1954; Andersson et al. 2008). The solubility of high-Mg calcite increases with mol %  $\text{MgCO}_3$  augmentation and is the most soluble form of biogenic  $\text{CaCO}_3$  at mol %  $\text{MgCO}_3$  higher than 12 % (Morse et al. 2006; Andersson et al. 2008). Coralline algae are thus potentially vulnerable to ocean acidification due to the solubility of their skeleton.

In recent years, there has been a considerable addition to the literature on the impact of ocean acidification on coralline algae. Highly reduced coverage or absence of coralline algae has effectively been reported close to naturally acidified shallow-water  $\text{CO}_2$  vents (Hall-Spencer et al. 2008; Martin et al. 2008; Porzio et al. 2011). Ocean acidification has been reported to negatively affect early recruitment (Agegian 1985; Kuffner et al. 2008) and to inhibit spore production and growth (Cumani et al. 2010). Furthermore, growth rate has been shown to be negatively affected by elevated  $p\text{CO}_2$  (Agegian 1985; Jokiel et al. 2008; Hofmann et al. 2011; Ragazzola et al. 2012). Coralline algae are of particular interest to investigate as they conduct two physiological processes (photosynthesis and calcification) that use dissolved inorganic carbon ( $\text{DIC}$ :  $\text{CO}_2$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ) as substrate. The interplay between these processes makes it particularly difficult to foresee how elevated  $p\text{CO}_2$  will affect algae in the future. Many studies suggest negative effects of  $p\text{CO}_2$  on calcification (Gao et al. 1993; Anthony et al. 2008; Semesi et al. 2009; Gao and Zheng 2010; Büdenbender et al. 2011) and on photosynthesis (Anthony et al. 2008; Gao and Zheng 2010; Hofmann et al. 2011). However, moderately elevated  $p\text{CO}_2$  has been reported to increase calcification rates (Smith and Roth 1979; Ries et al. 2009) and Semesi et al. (2009) reported an increase in photosynthetic rates due to elevated  $p\text{CO}_2$ .

It has been suggested that organisms presently surviving in highly variable environments are likely to be more robust to ocean acidification (Moulin et al. 2011; Raven 2011). The ability of organisms to tolerate significant pH/ $p\text{CO}_2$  fluctuations may be a result of adaptation (a genetic trait shared by the population) and/or acclimation (owing to phenotypic plasticity of the individual). The effect of ocean acidification on organisms acclimated to different pH/ $p\text{CO}_2$  environments has recently been under investigation. Moulin et al. (2011) compared the effect of pH on the progeny of the sea urchin *Paracentrotus lividus* collected from two distinct tide pools on the same shore and found

that the offspring of urchins from the tide pool with greater  $p\text{CO}_2$  flux showed better resistance to acidification. Similarly, cold-water coral *Lophelia pertusa* was able to acclimate to moderately elevated  $p\text{CO}_2$  over a 6-month period (Form and Riebesell 2012). Martin and Gattuso (2009) investigated the effect of elevated  $p\text{CO}_2$  on the coralline alga *Lithophyllum cabiochae* over 1 year. They reported reduced calcification under elevated  $p\text{CO}_2$  after 1 month in the experimental conditions but observed no effects after 1 year of acclimatization. To date, most studies investigating the effects of ocean acidification on coralline algae have been short-term and overlooked potentially naturally acclimated algae from environments where pH/ $p\text{CO}_2$  varies.

Articulated (i.e. geniculate) coralline algae frequently inhabit the intertidal environment and tidal pools where large pH variations are common (Morris and Taylor 1983; Huggett and Griffiths 1986). Anthropogenic elevation of atmospheric  $p\text{CO}_2$  is predicted to decrease ocean pH by 0.3–0.4 units by the end of this century (Haugan and Drange 1996; Caldeira and Wickett 2003), but diurnal variation in tidal pools often exceeds this variation by an order of magnitude, as a result of photosynthesis and respiration (Truchot and Duhamel-Jouve 1980; Morris and Taylor 1983; Poole and Raven 1997).

We hypothesize that coralline algae from tidal pools will be less affected by elevated  $p\text{CO}_2$  than algae collected from more stable environments. To test this, we investigated the effects of various  $p\text{CO}_2$  levels on primary production, respiration, calcification and skeletal mol % Mg/Ca (mMg/Ca) in the articulated coralline alga *Corallina elongata* from an intertidal rock pool.

## Materials and methods

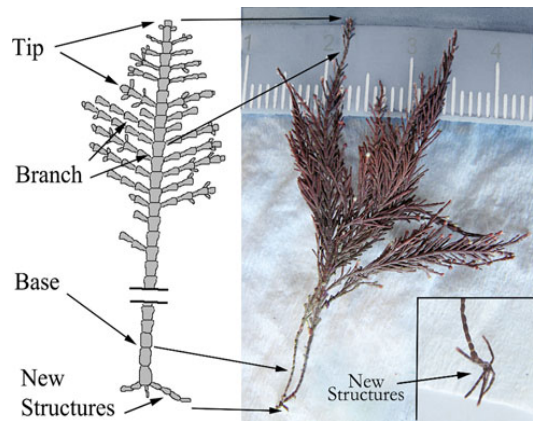
### Biological material

Specimens of *Corallina elongata* Ellis and Solander, 1786, were collected in a shaded tidal pool on the intertidal rocky shore of “Les Amiets”, Cléder, France on the north coast of Brittany (48°41'45 N, 4°7'26 W) on July 27, 2010. The irradiance of photosynthetic active radiations (PAR) at the surface of the pool was measured using a flat quantum sensor (LiCor, Li-190SA, LI-COR Biosciences, USA). It was ca. 30  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  at midday under sunny conditions. The  $p\text{CO}_2$  concentration in the rock pool varied locally according to the depth of the pool and the presence of other macroalgae. At low tide, it ranged from 70 to 300  $\mu\text{atm}$  in the light to 700–1000  $\mu\text{atm}$  in the dark (Table 1). Algal fronds with length of 6–10 cm and free of epiphytic organisms (e.g. Fig. 1) were selected for the experiment and carefully separated from their substrate to

**Table 1** Parameters of the carbonate chemistry and temperature in the rock pool where *Corallina elongata* were collected

	Temperature (°C)	pH <sub>T</sub>	AT (mmol kg <sup>-1</sup> )	pCO <sub>2</sub> (µatm)	DIC (mmol kg <sup>-1</sup> )	CO <sub>3</sub> <sup>2-</sup> (mmol kg <sup>-1</sup> )	HCO <sub>3</sub> <sup>-</sup> (mmol kg <sup>-1</sup> )	CO <sub>2</sub> (mmol kg <sup>-1</sup> )	Ω <sub>aragonite</sub>	Ω <sub>calcite</sub>
Light (day)	16.7–19.0	8.15–8.61	2.290	70–299	1.650–2.004	0.202–0.433	1.214–1.792	0.002–0.011	3.10–6.71	4.80–10.36
Dark (night)	15.0–15.1	7.68–7.82	2.290	720–1030	2.168–2.217	0.075–0.101	2.040–2.104	0.027–0.038	1.15–1.55	1.79–2.41

The pH on total scale (pH<sub>T</sub>) and total alkalinity (AT) were measured while other parameters of the carbonate chemistry were calculated from pH<sub>T</sub>, AT, temperature and salinity (35). Values are presented as a range of measurements conducted at different locations inside the pool 1 h after the low tide at 17:30 24-07-2012 (daylight) and at 06:00 25-06-2012 (night). Only one measurement was performed for AT.



**Fig. 1** Schema and picture of a *Corallina elongata* frond. Tips, branches, basal parts and new structures growing from the base of the algae were sampled for mMg/Ca analyses

obtain their encrusting base. They were transferred directly to the *Roscoff Marine Station* laboratory mesocosms in a cool box and subsequently randomly assigned to twelve 10-L aquaria, approximately 25 individual fronds per aquarium. To acclimatize the algae to the experimental conditions, they were maintained in controlled conditions of temperature and  $p\text{CO}_2$  for 3 weeks prior to the metabolic measurements. Samples for chlorophyll and analysis of mMg/Ca were collected on 30 August 2010, after 1 month in the experimental conditions.

The fresh weight (FW) of fronds used in the experiments was determined with a precision of 1 mg. FW was obtained by tap drying and weighing the algae and was converted to dry weight (DW) using the linear relationship  $\text{DW} = 0.6545 \times \text{FW} + 0.0400$  ( $R^2 = 0.92$ ;  $N = 22$ ). This relationship was established from FW and DW measured on 22 individual fronds grouped together from all treatments. DW was measured after 48 h of desiccation in an oven at 60 °C.

#### Experimental set-up

The experimental set-up consisted of four  $p\text{CO}_2$  conditions: 380, 550, 750 and 1000 µatm. The  $p\text{CO}_2$  concentrations chosen for this study were within the range naturally occurring in situ and are associated with the Intergovernmental Panel on Climate Change (IPCC) models on trajectories of atmospheric carbon emissions to the year 2100, that is, 380 ppm as ambient and 550, 750 and 1000 ppm as predicted by the end of the century (Solomon et al. 2007). There were three replicate aquaria per treatment, and  $p\text{CO}_2$  was gradually increased over 4 days from introduction to the aquaria. Seawater (salinity of 35) was pumped in at the front of the *Roscoff Marine Station*. It was delivered to the

aquaria at a rate of  $100 \text{ ml min}^{-1}$  (a renewal rate of  $60 \% \text{ h}^{-1}$ ) from four 100 L tanks where  $p\text{CO}_2$  was adjusted by bubbling with  $\text{CO}_2$ -free air or pure  $\text{CO}_2$  gas. Seawater pH (on the total scale,  $\text{pH}_T$ ) was adjusted to the desired level, that is,  $\text{pH}_T$  of 8.10, 7.94, 7.81 and 7.71, corresponding to the desired level of  $p\text{CO}_2$  (380, 550, 750 and  $1000 \mu\text{atm}$ ). The pH was controlled in each 100 L tank within  $\pm 0.05$  pH unit using a continuous pH-stat feedback system (IKS Aquastar, Karlsbad, Germany) that regulated the addition of  $\text{CO}_2$ -free air or pure  $\text{CO}_2$  into the tanks. The pH values of the pH-stat system were adjusted from daily measurements of  $\text{pH}_T$  in the aquaria using a pH-meter (HQ40D, Hach Lange, Ltd portable LDO<sup>TM</sup>, Loveland, USA) calibrated on the total scale using Tris/HCl and 2-aminopyridine/HCl buffer solutions with a salinity of 35 (Dickson et al. 2007). For measurements of total alkalinity (AT), 100 ml seawater samples were filtered through  $0.7 \mu\text{m}$  Whatman GF/F filters, immediately poisoned with mercuric chloride and stored in a cool dark place pending analyses. AT was determined potentiometrically using an automatic titrator (Titroline alpha, Schott SI Analytics, Germany) calibrated on the National Bureau of Standards scale. AT was calculated using a Gran function applied to pH values ranging from 3.5 to 3.0 (Dickson et al. 2007) and corrected by comparison with standard reference material provided by Andrew G. Dickson (CRM Batch 92). Parameters of the carbonate chemistry were determined from  $\text{pH}_T$ , temperature, AT and salinity using *CO2Sys EXCEL Macro, version 1.02*. Calculations were based on a set of constants *K1* and *K2* from Mehrbach et al. 1973, refit by Dickson and Millero 1987 (Table 2). Irradiance above the aquaria was adjusted to  $30 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , corresponding to the midday summer irradiance in the tidal pool at the collection site. The light source consisted of 39 W fluorescent tubes (JBL Solar Ultra Marin Day, JBL Aquaria, Nelson, New Zealand) placed above the aquaria, and the photoperiod was adjusted to 14/10 (Light/Dark, h). Temperature was adjusted to  $18 \text{ }^\circ\text{C}$  in the water bath surrounding the aquaria, corresponding to that in the field (Table 1), and controlled using a feedback system (IKS Aquastar, Karlsbad, Germany) connected to 150 W submersible heaters. The aquaria were cleaned weekly to control growth of other algae.

### Physiological measurements

Incubation experiments were carried out in the light (ambient irradiance of  $30 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) and dark on the 18th and 19th of August 2010, respectively. Three algal fronds from each 10 L aquaria were placed in a 200 ml plexiglas chambers and incubated over a period of 2–3 h. Photosynthetic and respiratory rates were quantified from measurements of dissolved oxygen concentration

**Table 2** Parameters of the carbonate chemistry and temperature in the experimental treatments

$p\text{CO}_2$ treatment ( $\mu\text{atm}$ )	Temperature ( $^\circ\text{C}$ )	$\text{pH}_T$	AT ( $\text{mmol kg}^{-1}$ )	$p\text{CO}_2$ ( $\mu\text{atm}$ )	DIC ( $\text{mmol kg}^{-1}$ )	$\text{CO}_3^{2-}$ ( $\text{mmol kg}^{-1}$ )	$\text{HCO}_3^-$ ( $\text{mmol kg}^{-1}$ )	$\text{CO}_2$ ( $\text{mmol kg}^{-1}$ )	$\Omega_{\text{aragonite}}$	$\Omega_{\text{calcite}}$
380	$18.6 \pm 0.1$	$8.09 \pm 0.01$	$2.351 \pm 0.004$	$362 \pm 70$	$2.072 \pm 0.004$	$0.199 \pm 0.002$	$1.861 \pm 0.055$	$0.012 \pm 0.000$	$3.08 \pm 0.04$	$4.74 \pm 0.07$
550	$18.5 \pm 0.1$	$7.94 \pm 0.01$	$2.349 \pm 0.003$	$547 \pm 12$	$2.150 \pm 0.004$	$0.149 \pm 0.003$	$1.983 \pm 0.007$	$0.018 \pm 0.000$	$2.31 \pm 0.04$	$3.57 \pm 0.06$
750	$18.4 \pm 0.1$	$7.81 \pm 0.01$	$2.350 \pm 0.001$	$770 \pm 13$	$2.209 \pm 0.003$	$0.114 \pm 0.002$	$2.069 \pm 0.005$	$0.026 \pm 0.000$	$1.77 \pm 0.03$	$2.73 \pm 0.04$
1000	$18.4 \pm 0.1$	$7.72 \pm 0.01$	$2.348 \pm 0.003$	$977 \pm 19$	$2.245 \pm 0.003$	$0.095 \pm 0.002$	$2.117 \pm 0.004$	$0.033 \pm 0.001$	$1.46 \pm 0.03$	$2.26 \pm 0.04$

Temperature, pH on total scale ( $\text{pH}_T$ ) and total alkalinity (AT) were measured as described in text

Other parameters of the carbonate chemistry were calculated from pH<sub>T</sub>, AT ( $2.350 \pm 0.003 \text{ mmol kg}^{-1}$ ), temperature and salinity (35)

Values are presented as mean  $\pm$  SE,  $N = 60$



( $O_2$ ,  $\mu\text{mol L}^{-1}$ ) at the beginning and the end of the incubations with a non-invasive fibre-optic oxygen meter (FIBOX 3, PreSens, Germany). Net production (NP) and respiration (R) rates ( $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) were calculated as follows:

$$\text{NP (or R)} (O_2) = \left( \frac{\Delta O_2}{\Delta t \times V} \right) / DW \quad (1)$$

where  $\Delta O_2$  is the change in dissolved oxygen ( $\mu\text{mol L}^{-1}$ ),  $\Delta t$  is the incubation time (h),  $DW$  is the dry weight of the algae and  $V$  is the volume of the incubation chamber (L). Control incubations without algae were carried out to correct for any biological activity in the seawater.

Gross production (GP) was calculated as the sum of |NP| and |R|.

Calcification was estimated using the alkalinity anomaly technique (Smith and Key 1975; Chisholm and Gattuso 1991) which is based on the relationship:  $\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O}$ , for which total alkalinity decreases by 2 equivalents for each mol of  $\text{CaCO}_3$  precipitated. Calcification rate (G,  $\mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ h}^{-1}$ ) was calculated as follows:

$$G = -1 \times \left( \frac{\Delta AT}{2 \times \Delta t \times V} \right) / DW \quad (2)$$

where  $\Delta AT$  is the change in total alkalinity ( $\mu\text{eq L}^{-1}$ ).

#### Chl *a* analyses

Chlorophyll *a* content of thalli was measured in all individual fronds used in the experiment. A branch of each frond was removed, weighed and immediately frozen at  $-20^\circ\text{C}$  pending analyses. They were ground in 10 ml 90 % acetone with a cold mortar pestle on an ice bath under dark conditions. The extract was poured into 15-ml centrifuge tubes and placed in the dark at  $4^\circ\text{C}$  overnight. Samples were then centrifuged for 20 min at 4,000 rpm. Total Chl *a* concentration in the supernatant was determined according to the method of Jeffrey and Humphrey (1975), using a spectrophotometer (Helios Gamma, Thermo Electron Corporation, England). Two successive extractions were necessary for a complete Chl *a* extraction.

#### mMg/Ca analyses

The mole % ratio of Mg/Ca (mMg/Ca) in the seawater used in the experiment was analysed. Tips, branches (below the tips), basal parts and newly formed structures at the basal part of the fronds (Fig. 1) were sampled for mMg/Ca analysis. Algal samples (9 per treatment) were cleaned with distilled water, dried at room temperature at the *Roscoff Marine Station* and transported to the *Marine Research Institute*, Iceland, where skeletal tips, branches and basal

parts were sampled from each frond (Fig. 1). Small samples ( $<0.01 \text{ g}$ ) of skeletal material or seawater were placed in 20 ml polyethylene vials which had been cleaned by soaking for 3 days in 5 %  $\text{HNO}_3$  and then washed with distilled water and dried. To dissolve the calcium carbonates, 1 ml of 65 %  $\text{HNO}_3$  suprapure acid (Merck, Germany) was added to the samples and left to dissolve for 14 h, after which 4 ml of deionised purified water (Milli-Q purification system, Millipore, USA) was added to the sample. The mMg/Ca ratios were analysed at the *University of Iceland Institute of Earth Sciences*, with an inductively coupled plasma atomic emission spectrometer (ICP-AES Spectro Ciros<sup>TM</sup>, Germany). The ICP analysis was calibrated with mixtures of NIST-traceable single-element solutions (Spex Industries Inc. NJ, USA).

#### Data analysis

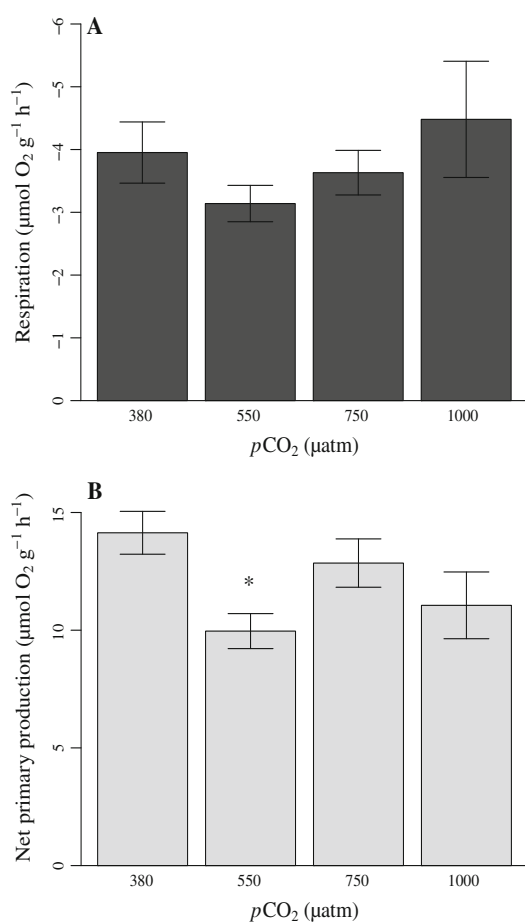
The effect of  $p\text{CO}_2$  on respiration, net and gross production, and light and dark calcification was investigated using a nested one-way analysis of variance (ANOVA) with 3 replicate aquaria nested within each  $p\text{CO}_2$  treatment and three algae within each aquaria (9 algae per treatment). Mauchly's sphericity test was applied to investigate assumptions of equality of variance, and Dagnelie's test (based on the Shapiro-Wilk test) was applied to investigate assumptions of normality, prior to analysis. When data were not normally distributed, a logarithmic or square-root transformation was performed. In cases of significant differences between treatments, a post hoc test (Student–Newman–Keuls rejective multiple test procedure) was applied to explore differences between treatments. To compare mMg/Ca in calcite formed during the experiment, data from all elevated  $p\text{CO}_2$  treatments were pooled ( $N = 9$ ) and compared with data from the 380  $\mu\text{atm}$  ( $N = 7$ ) using a Welch's two-sample *t* test. Analysis was completed using the statistical package R 2.12.0 (©The R Foundation for Statistical Computing). Results are presented as mean  $\pm$  SE.

## Results

The biomass of the individual algal fronds used in the experiment was not significantly different among treatments (ANOVA,  $F_{(3, 8)} = 3.3112$ ,  $P = 0.078$ ). Mean weight of algal fronds was  $0.45 \text{ g DW} \pm 0.03$ . Chlorophyll *a* content in thalli did not differ significantly between treatments (ANOVA,  $F_{(3, 8)} = 0.9700$ ,  $P = 0.453$ ) and averaged  $422 \pm 14 \mu\text{g g}^{-1} \text{ DW}$ .

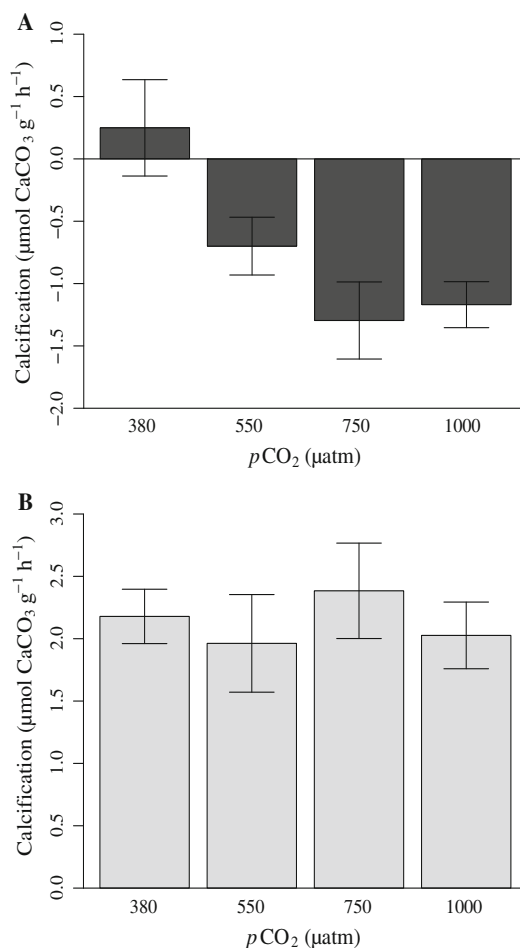
Respiration rate did not differ significantly between treatments (ANOVA,  $F_{(3, 8)} = 0.6024$ ,  $P = 0.632$ ; Fig. 2a) and averaged  $-3.80 \pm 0.29 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ . Net

primary production differed significantly between treatments (ANOVA,  $F_{(3, 8)} = 5.6908$ ,  $P = 0.022$ ; Fig. 2b). A multiple comparison test (SNK,  $P = 0.041$ ) showed that net primary production was significantly lower at 550  $\mu\text{atm } p\text{CO}_2$  ( $9.96 \pm 0.74 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ) when compared to 380  $\mu\text{atm } p\text{CO}_2$  ( $14.14 \pm 0.91 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ) while there was no significant difference with other treatments. Gross production did not differ between treatments (ANOVA,  $F_{(3, 8)} = 3.7057$ ,  $P = 0.061$ ). It averaged  $15.80 \pm 0.68 \mu\text{mol O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ . Dark calcification did not differ significantly between treatments (ANOVA,  $F_{(3, 8)} = 2.9814$ ,  $P = 0.096$ ; Fig. 3a). Mean dark calcification was negative ( $-0.73 \pm 0.17 \mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ DW h}^{-1}$ ) indicating dissolution. Calcification in the light did not differ significantly between treatments (ANOVA,  $F_{(3, 8)} = 0.2410$ ,  $P = 0.866$ ; Fig. 3b). It averaged  $2.14 \pm 0.16 \mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ DW h}^{-1}$ .

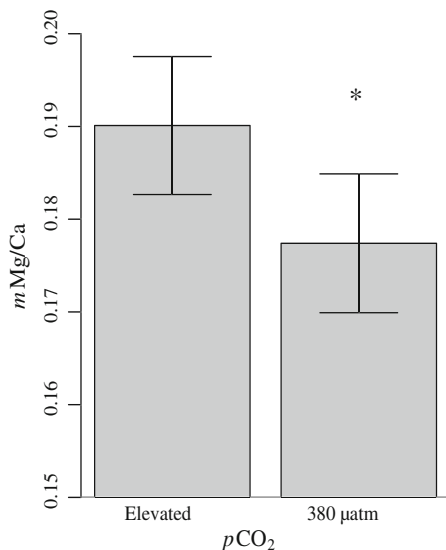


**Fig. 2** *Corallina elongata* (a) dark respiration and (b) net primary production rates under ambient irradiance ( $30 \mu\text{mol m}^{-2} \text{ h}^{-1}$ ) for each  $p\text{CO}_2$  treatment. Data are presented as mean  $\pm$  SE ( $N = 9$ )

The experimental seawater  $\text{mMg}/\text{Ca}$  ratio was  $5.21 \pm 0.02$ . Skeletal  $\text{mMg}/\text{Ca}$  was significantly higher in tips compared to branches in all treatments (Student  $t$  tests,  $p < 0.05$ ; Table 3). Skeletal  $\text{mMg}/\text{Ca}$  did not differ between treatments in tips (ANOVA,  $F_{(3, 8)} = 1.955$ ,  $P = 0.199$ ; mean  $0.185 \pm 0.001$ ), branches (ANOVA,  $F_{(3, 8)} = 0.6826$ ,  $P = 0.587$ ; mean  $0.176 \pm 0.002$ ) or basal parts (ANOVA,  $F_{(3, 8)} = 2.1266$ ,  $P = 0.175$ ; mean  $0.181 \pm 0.001$ ). The number of newly calcified structures (Fig. 1) produced by the algae during the acclimation period was unbalanced between treatments ( $N = 7, 2, 4$  and 3 at  $p\text{CO}_2$  of 380, 550, 750 and 1000  $\mu\text{atm}$ , respectively). Thus, a total of 9 out of 27 algae grown at elevated  $p\text{CO}_2$  (i.e. 550, 750 and 1000  $\mu\text{atm}$ ) formed new structures at the base, compared to 7 out of 9 algae at 380  $\mu\text{atm}$ . New structures formed by algae at elevated  $p\text{CO}_2$  had a



**Fig. 3** *Corallina elongata* calcification rates in the (a) dark and (b) light under ambient irradiance ( $30 \mu\text{mol m}^{-2} \text{ h}^{-1}$ ) for each  $p\text{CO}_2$  treatment. Data are presented as mean  $\pm$  SE ( $N = 9$ )



**Fig. 4** Skeletal *mMg/Ca* in new structures formed by *Corallina elongata* in the control treatment ( $p\text{CO}_2$  of 380  $\mu\text{atm}$ ;  $N = 7$ ) and in the elevated  $p\text{CO}_2$  treatments (550, 750 and 1000  $\mu\text{atm}$ ;  $N = 9$ )

significantly lower *mMg/Ca* ratio ( $0.177 \pm 0.002$ ) compared to algae at 380  $\mu\text{atm}$  ( $0.190 \pm 0.003$ ; *t* test,  $p < 0.001$ ; Fig. 4).

## Discussion

The results from this study support the assumption that *C. elongata* from a tidal pool, where  $p\text{CO}_2$  fluctuates over diel and seasonal cycles, is relatively robust to elevated  $p\text{CO}_2$  compared to other recently investigated coralline algae. Gross primary production, which refers to the total energy fixed by photosynthesis, was not affected by  $p\text{CO}_2$ . Nonetheless, it is worth noting that algae grown at 550  $\mu\text{atm}$  exhibited a lower net primary production rate than those grown at 380  $\mu\text{atm}$ , but the observed difference was modest and production rates observed at 750 and 1000  $\mu\text{atm}$  were similar to that observed at 380  $\mu\text{atm}$ . Macroalgal species exhibit mixed responses to elevated  $p\text{CO}_2$  in terms of primary production (see review by Hurd

et al. 2009). Israel and Hophy (2002) reported no effect of increased  $p\text{CO}_2$  on growth and photosynthesis in 12 non-calcifying marine macroalgae, with the exception of *Hypnea musciformis* which exhibited negative growth under elevated  $p\text{CO}_2$ . Various responses of primary production have been reported in coralline algae. Anthony et al. (2008) observed a strong negative effect on net productivity in the tropical crustose coralline alga, *Porolithon onkodes*, whereas increased photosynthetic rate was reported for the tropical rhodolith forming genus *Hydrolithon* sp. (Semesi et al. 2009) and the articulated coralline alga *Amphiroa anceps* (Borowitzka 1981). The majority of coralline algae have carbon-concentrating mechanisms (CCMs), that is, they actively transport inorganic carbon across one or more cell membranes and/or externally convert bicarbonate ions to carbon dioxide to sustain photosynthesis (Raven et al. 2012). The presence of CCMs mean that photosynthesis is carbon saturated at current concentrations, and thus, a lack of photosynthetic response in coralline algae is to be expected in most species. The absence of photosynthetic response of *C. elongata* to  $p\text{CO}_2$  observed in the present study was consistent with the uniformity of chlorophyll *a* content in thalli between treatments, but changes may occur in other photosynthetic pigments such as phycobiliproteins (Gao and Zheng 2010) that were not analysed in this study.

Respiration occurs both in the dark and light and is a vital process for growth and survival of algae and plays a critical role in modulating their carbon balance. *C. elongata* dark respiration was not affected by elevated  $p\text{CO}_2$ . This is in agreement with previous studies addressing the effect of elevated  $p\text{CO}_2$  on both soft macroalgae (Zou et al. 2011a; Zou et al. 2011b) and coralline algae (Semesi et al. 2009). The lack of respiration response to elevated  $p\text{CO}_2$  in the present study for *C. elongata* can be explained by the lack of response of photosynthesis and the subsequent absence of change in the supply of respiratory substrates.

In contrast with most coralline algae examined to date (Gao et al. 1993; Anthony et al. 2008; Semesi et al. 2009; Gao and Zheng 2010; Büdenbender et al. 2011; Hofmann et al. 2011) calcification rates in *C. elongata* were not affected by elevated  $p\text{CO}_2$ . Nonetheless, some studies are in agreement with the results presented here, reporting no

**Table 3** Calcite *mMg/Ca* ratio in *Corallina elongata* skeletal parts for each  $p\text{CO}_2$  treatment

Algal part	380 $\mu\text{atm}$	550 $\mu\text{atm}$	750 $\mu\text{atm}$	1000 $\mu\text{atm}$
Tips	$0.186 \pm 0.002$	$0.189 \pm 0.001$	$0.183 \pm 0.003$	$0.183 \pm 0.001$
Branches	$0.179 \pm 0.002$	$0.178 \pm 0.002$	$0.173 \pm 0.005$	$0.174 \pm 0.003$
Basal parts	$0.182 \pm 0.003$	$0.180 \pm 0.002$	$0.177 \pm 0.003$	$0.184 \pm 0.003$
New structures	$0.190 \pm 0.003$	$0.178 \pm 0.007$	$0.175 \pm 0.005$	$0.180 \pm 0.001$

Values are presented as mean  $\pm$  SE.  $N = 9$  per treatment for tips, branches and basal parts  
 $N = 7, 2, 4$  and 3 for new structures at  $p\text{CO}_2$  of 380, 550, 750 and 1000  $\mu\text{atm}$  respectively

change in calcification rates under elevated  $p\text{CO}_2$  alone (Martin and Gattuso 2009) or a parabolic response with an increase in calcification rates under moderately increased  $p\text{CO}_2$  (Smith and Roth 1979; Ries et al. 2009). This variance in responses may be related to the regulation of pH (and consequently carbonate saturation) at the site of calcification, especially in the light with an increase of pH via photosynthesis. The resilience of *C. elongata* calcification in high  $p\text{CO}_2$  conditions may be explained by acclimation to fluctuating concentrations of  $p\text{CO}_2$  in the intertidal rock pool, especially in the dark where  $p\text{CO}_2$  can naturally reach 1000  $\mu\text{atm}$ . Although calcification rates in the dark did not differ significantly between treatments, it can be noted that net dissolution ( $\text{CaCO}_3$  dissolution >  $\text{CaCO}_3$  formation) was recorded only under elevated  $p\text{CO}_2$  of 550, 750 and 1000  $\mu\text{atm}$ . Net dissolution of algal carbonate structure in the dark at elevated  $p\text{CO}_2$  has been reported in other coralline algal species such as in *Corallina pilulifera* grown at 1600  $\mu\text{atm}$   $\text{CO}_2$  (Gao et al. 1993) and in *Lithothamnion glaciale* at 700  $\mu\text{atm}$  (Büdenbender et al. 2011). Calcification in coralline algae occurs within the cell wall and is affected both by the surrounding seawater chemistry and driven by internal and external controls. In the dark, calcification may be negatively affected by the decrease in internal and external pH due to respiration, while in the light the internal and external pH increases due to the removal of  $\text{CO}_2$  via photosynthesis favour calcification (Borowitzka 1981; Raven 2011).

The apparent vulnerability of calcifying coralline algae to ocean acidification has been attributed to their formation of high-Mg calcite structures that are particularly soluble compared to other biogenic carbonates. Calcite  $m\text{Mg}/\text{Ca}$  in algal tips, branches and basal parts did not differ between  $p\text{CO}_2$  treatments. Branches and basal parts were fully formed prior to being grown for 1 month at various  $p\text{CO}_2$  explaining the lack of treatment effect. Although tips exhibit no significant effect of treatment on  $m\text{Mg}/\text{Ca}$ , a slightly lower ratio was observed at 1000 and 750  $\mu\text{atm}$  compared to 550 and 380  $\mu\text{atm}$   $p\text{CO}_2$ . It is possible that tips of algae analysed in this study contained calcite produced prior as well as during the experiment, explaining the very slight but insignificant effect of  $p\text{CO}_2$ . In all treatments, tips had a significantly higher  $m\text{Mg}/\text{Ca}$  ratio than branches. It has been suggested that coralline algae are unable to regulate Mg incorporation in their calcite crystal lattice (Mucci and Morse 1983; Ries 2006). Temperature (Chave 1954; Ries 2006; Kamenos et al. 2008), growth (Kolesar 1978), dissolution (Müller et al. 1972; Hover et al. 2001) and calcium carbonate saturation (Agegian 1985; Ries 2011) are all known to affect  $m\text{Mg}/\text{Ca}$  in calcifying algae. It is conceivable that the observed differences in  $m\text{Mg}/\text{Ca}$  between skeletal parts may effectively result from different environmental parameters in the rock pool at the time of

calcification. Seawater  $m\text{Mg}/\text{Ca}$  is also known to affect skeletal  $m\text{Mg}/\text{Ca}$  in algae but predominantly only on geological time scales (Ries 2006). The  $m\text{Mg}/\text{Ca}$  in the experimental seawater in this study was 5.2, corresponding to the ratio recognized in present day seawater (Hardie 1996).

Although tips, branches and basal parts of algae did not grow sufficiently for any effects of  $p\text{CO}_2$  to be observed during the course of 30 days in the experimental treatments, several algae formed new calcified structures at the base. These structures were observed and sampled from 7 out of 9 algae at 380  $\mu\text{atm}$  but only 2, 4 and 3 out of 9 algae at 550, 750 and 1000  $\mu\text{atm}$   $p\text{CO}_2$ . Algae cultured at 550, 750 and 1000  $\mu\text{atm}$  had a significantly lower  $m\text{Mg}/\text{Ca}$  in new skeletal structures than those cultured at 380  $\mu\text{atm}$ . These findings are in agreement with previous studies where low calcium carbonate saturation negatively affected the Mg incorporation of the calcifying coralline algae *Neogoniolithon* sp. (Ries et al. 2009) and *Porolithon gardineri* (Agegian 1985). It is not clear if such mineralogical plasticity will confer resilience of coralline algae to elevated  $p\text{CO}_2$  due to less solubility of their skeleton (Ries 2011). Also, it is questionable whether the decrease in Mg incorporation will be sufficient to aid algae in their future survival, especially if ocean warming simultaneously increases Mg incorporation. Moreover, Ragazzola et al. (2012) observed elevated  $p\text{CO}_2$  to weaken the skeletal structure in the coralline algae *Lithothamnion glaciale*, demonstrating that information on  $m\text{Mg}/\text{Ca}$  ratio in the skeletal calcite may not be sufficient to define the robustness of the structure to elevated  $p\text{CO}_2$ .

The ability of coralline algae to acclimate to future levels of  $p\text{CO}_2$  has not been extensively studied to date. The need for such studies was underlined by Martin and Gattuso (2009) as calcification of *Lithophyllum cabiochae* was reduced after being treated with elevated  $p\text{CO}_2$  for 1 month, but no effect of elevated  $p\text{CO}_2$  was observed after 1 year. In addition, long-term acclimation to  $p\text{CO}_2$  has been shown to reduce negative effects on calcification on the cold-water coral *Lophelia pertusa* (Form and Riebesell 2012) and limit negative effects on adults and progeny of sea urchins (Moulin et al. 2011; Dupont et al. 2012). Sites with naturally elevated  $p\text{CO}_2$  such as  $\text{CO}_2$  vents have provided opportunities to study acclimation in coralline algae and other species (Hall-Spencer et al. 2008; Martin et al. 2008; Porzio et al. 2011). Porzio et al. (2011) observed a reduced abundance of calcifying algal species at naturally acidified  $\text{CO}_2$  vent sites, but surprisingly, one coralline algal species was more abundant at mean pH 7.8 (957  $\mu\text{atm}$   $p\text{CO}_2$ ) than at a mean pH 8.1 (334  $\mu\text{atm}$   $p\text{CO}_2$ ), possibly reflecting different tolerance levels of coralline algal species. Coralline algae that frequently inhabit sites where  $p\text{CO}_2$  is naturally elevated over short or long periods of time (such as intertidal rock pools and sites near  $\text{CO}_2$

vents) may already have adapted to elevated  $p\text{CO}_2$  levels since they are able to acclimate to these environments. Indeed, elevated  $p\text{CO}_2$  had little effect on *C. elongata* collected from a rock pool where  $p\text{CO}_2$  naturally reached 1000  $\mu\text{atm}$  during the night.

Here, we tested only the effects of elevated  $p\text{CO}_2$  but ignore potential synergistic effects with other environmental parameters such as temperature. Although, it is conceivable that the algae under investigation here were also acclimated to the strong seasonal and diel temperature variations in the rock pool, but this needs to be investigated further. Moreover, coralline algae inhabit the intertidal and subtidal environment where space is limited and competition for space is ample, and thus, slightly reduced fitness may have considerable ecological consequences for a species. Porzio et al. (2011) examined the abundance of all algal species along a natural pH gradient (see above) and found *C. elongata* (along with most other calcifying algae) most abundant at pH 8.1 (334  $\mu\text{atm } p\text{CO}_2$ ), greatly reduced at pH 7.8 (957  $\mu\text{atm } p\text{CO}_2$ ) and absent at pH 6.7 (20,812  $\mu\text{atm } p\text{CO}_2$ ). In the same study, the abundance of fleshy algae was considerably less affected by elevated  $p\text{CO}_2$ .

This study has given evidence supporting the assumption that coralline algae inhabiting environments where pH/ $p\text{CO}_2$  fluctuates naturally (e.g. intertidal zone and tidal pools) are likely to exhibit less negative responses to elevated  $p\text{CO}_2$  within its natural range of variability than those inhabiting relatively stable environments. Subsequently, this study has provided support to the hypothesis that predicts a greater resilience of organisms acclimated and/or adapted to highly variable pH/ $p\text{CO}_2$  environments of future ocean acidification. However, in future oceans, algae already subjected to high  $p\text{CO}_2$ /pH fluctuations will be exposed to still more extreme  $p\text{CO}_2$  scenarios (>1000  $\mu\text{atm}$ ) and, therefore, it cannot be excluded that they will be negatively impacted by future  $p\text{CO}_2$  levels.

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## Erratum to: Effects of $p\text{CO}_2$ on physiology and skeletal mineralogy in a tidal pool coralline alga *Corallina elongata*

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Unfortunately, Fig. 4 was incorrectly published in both online and print version of the article. The corrected figure is given below.

The online version of the original article can be found under  
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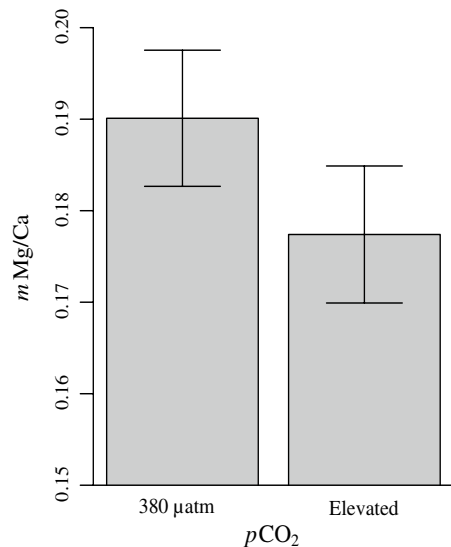
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**Fig. 4** Skeletal  $m\text{Mg}/\text{Ca}$  in new structures formed by *Corallina elongata* in the control treatment ( $p\text{CO}_2$  of 380  $\mu\text{atm}$ ;  $N = 7$ ) and in the elevated  $p\text{CO}_2$  treatments (550, 750 and 1,000  $\mu\text{atm}$ ;  $N = 9$ )





## **Paper III**

# **Seasonal variability in inorganic carbon chemistry in a pristine shallow sub-Arctic fjord in Iceland**

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Author contributions: HE measured inorganic carbon data and wrote the manuscript. SRO, analysed nutrients and edited the manuscript. JO contributed to data analysis and edited the manuscript. EBO managed field work, measured chlorophyll and edited the manuscript.



# Seasonal variability in inorganic carbon chemistry in a pristine shallow sub-Arctic fjord in Iceland

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## Abstract

Information on temporal and spatial variability in seawater properties within contrasting coastal regions is important for understanding ecological and biogeochemical processes in near-shore ecosystems and the implications of environmental changes.

This study describes the spatial and seasonal variability of the seawater environment in Breiðafjörður bay, Iceland, a pristine sub-Arctic coastal region that includes extensive areas of benthic substrate, dominated by macrophytes. On 19 surveys of five sampling stations throughout a year (April 2010 – May 2011), information was collected on physical (temperature and salinity) and chemical (nutrients,  $p\text{CO}_2$  and dissolved inorganic carbon (DIC)) properties of the seawater, along with information on chlorophyll  $a$  concentration.

Our findings suggest that Breiðafjörður is relatively well-mixed vertically but has a slight gradient across the bay, relating to terrestrial runoff in the northern most part. Density profiles suggest the clockwise coastal currents transport seawater into the bay in the south with water export mainly in the northern part of the bay. There was significant seasonality observed in temperature, nutrients, chlorophyll  $a$  and the inorganic carbon system. Temperatures ranged from  $-0.8^\circ\text{C}$  in winter to  $14^\circ\text{C}$  in summer. Nitrate was depleted in summer but in winter chlorophyll  $a$  concentration indicates no, or negligible, phytoplankton biomass. Surface seawater  $\text{pH}_T$  (pH on total scale) ranged from 8.02 to 8.27 and  $\Omega_{\text{aragonite}}$  ranged from 1.5 to 3, from winter to summer respectively. Breiðafjörður was a net sink of atmospheric  $\text{CO}_2$  at a rate of  $1.8 \pm 0.2 \text{ mol C m}^{-2} \text{ y}^{-1}$  with surface  $p\text{CO}_2$  ranging from 212 to 417  $\mu\text{atm}$  from summer to winter. Biological utilization of  $\text{CO}_2$  in summer and low temperatures in winter resulted in surface seawater being undersaturated with respect to the atmosphere throughout the year, except for a short period in winter when seawater and atmosphere  $p\text{CO}_2$  were near equilibrium.

As a result of the pristine state of the bay, its ecosystem composition and limited terrestrial runoff, we suggest this bay is well suited for long term monitoring of ocean warming and

acidification in a high-latitude coastal environment and for studying the biological implications of these changes in a macrophyte dominated coastal region.

**Keywords:**

Coastal, hydrography, seasonality, carbon chemistry, sub-Arctic, Arctic, Breiðafjörður, Iceland, CO<sub>2</sub> air-sea flux

## Introduction

In recent decades interest has grown regarding the chemistry of inorganic carbon in seawater, which can largely be explained by the need to understand the coupling between the atmosphere and oceans, and the fate of anthropogenic carbon dioxide (CO<sub>2</sub>) in the Earth systems (Borges 2011, Le Quéré et al. 2014). Coastal regions are shallow ocean environments that extend from the intertidal zone to the continental shelf edge (~ 200 m depth) (Kennish 2000). Despite representing only 7% of the ocean surface they play a significant role in the cycling of carbon and have been estimated to absorb roughly 0.3 Pg C yr<sup>-1</sup> (Chen & Borges 2009) which corresponds to 21% of the total contribution of the open ocean, 1.4 Pg C yr<sup>-1</sup> (Takahashi et al. 2009). However, these estimates are subject to considerable uncertainties due to the dynamic nature of coastal regions and the lack of data from contrasting regions collected on spatial and temporal scales appropriate for reliable flux estimations (Borges 2011). This regional diversity results from the extremely wide range of physical and biochemical settings in coastal regions that drive changes in the physico-chemical properties of seawater, through multiple interacting factors of biotic and abiotic origin such as terrestrial fresh water drainage (Dai & Trenberth 2002, Fransson et al. 2015), varying nutrient input (Borges & Gypens 2010), coastal bathymetry (e.g. Kelly 1985, Rodrigues & Lorenzetti 2001), regional biodiversity (Borges et al. 2005), and seasonality (McClanahan 1988, Borges & Frankignoulle 1999, Delille et al. 2000). Coastal regions are also among the most biogeochemically active areas of the biosphere as a result of the extensive input of organic matter and nutrients from terrestrial sources along with their shallow bathymetry (Gattuso et al. 1998, Borges 2011). On that account, it has been estimated that 15% to 30% of the oceanic primary production and about 80% of oceanic organic matter burial occurs in the coastal regions, and ~50% of the oceanic CaCO<sub>3</sub> deposition (Gattuso et al. 1998).

The lack of comprehensive data on variability in the inorganic carbon system in coastal regions is currently an issue for two research fields. Firstly, understanding the movement of inorganic carbon among Earth's spheres requires modelling of biogeochemical systems, which again requires observational data on carbon fluxes within and between spheres (Bauer et al. 2013). Secondly, to understand the implications of ocean acidification as a result of anthropogenic CO<sub>2</sub> emissions, it is important to understand the coupling between biological processes and the temporal and spatial fluctuations in the inorganic carbon system (Dupont et al. 2010, Kelly et al. 2013, Waldbusser & Salisbury 2014, Kapsenberg et al. 2015). Data for coastal regions are especially relevant as the majority of experimental studies investigating the response of marine biota to varying CO<sub>2</sub> concentrations focus on shallow water species (e.g. see studies considered in Kroeker et al. 2013).

High latitude regions experience some of the highest rates of environmental changes resulting from the anthropogenic addition of CO<sub>2</sub> into the atmosphere, both in terms of ocean warming and acidification (IPCC 2014). High latitude ecosystems are also considered vulnerable to rapid environmental changes with the effects of climate change already documented in the marine realm (Wassmann et al. 2011, Barry et al. 2013). On top of that, coastal regions in high latitudes are generally relatively pristine as a result of these regions being sparsely populated but much of the available data on seasonal fluxes in coastal carbon chemistry is from densely populated temperate regions in the North Atlantic (Doney 2010, Borges 2011). Compared to tropical ocean regions there is strong seasonality in the biogeochemical environment at

temperate and higher latitudes (Lutz et al. 2007) which needs to be considered when quantifying annual carbon fluxes and to understand the full ranges of environmental conditions organisms are exposed to in their habitat at different life stages (Hofmann et al. 2010).

Iceland is a sub-Arctic island and that is a part of a topographic boundary that separates the Nordic Seas from the sub-polar North Atlantic Ocean. Long-term open ocean data on inorganic carbon chemistry in the Iceland Sea north of Iceland and the Irminger Sea south-west of Iceland reveal relatively rapid decreases in surface water pH and calcium carbonate saturation states ( $\Omega$ ) as a result of anthropogenic CO<sub>2</sub> emissions (Olafsson et al. 2009, Bates et al. 2014). The Arctic surface waters north of Iceland are a particularly strong sink for CO<sub>2</sub> (Takahashi et al. 2009). However, the seasonal fluxes of inorganic carbon in Icelandic coastal regions and the sea-air CO<sub>2</sub> fluxes have not been investigated to date.

Breiðafjörður is a large (~ 6000 km<sup>2</sup>) and open bay in the western part of Iceland. The bay is approximately 50 km wide and 125 km long and has a maximum depth > 200 m and is characterized by an extended area with a bottom depth shallower than 30 meters. An interesting feature of the bay is the inclusion of half of the Iceland's coastline due to the large number (> 3000) of island, islets and reefs in the inner part of the bay, and extensive areas of hard substrates (Ingólfsson 2006).

The hydrography of the bay has not been studied intensively but is likely to be under comparable influences as Faxa Bay that lies directly south of Breiðafjörður and has been more studied in terms of hydrography (Stefánsson 1968, Stefánsson & Gudmundsson 1978). Both bays have relatively wide openings, allowing for water-exchange with the shelf waters. The shelf waters southwest and west of Iceland include the Irminger current that flows northward carrying warm Atlantic Water (Huthnance 2010) and a geostrophic coastal current that flows clockwise around Iceland (Ólafsson et al. 2008). The Faxa Bay hydrography is strongly driven by spatial density differences, the prevailing wind regime and to a lesser extent by tidal currents (Stefánsson & Gudmundsson 1978). The tidal amplitude in the Breiðafjörður region is the greatest in Iceland, reaching about 4 m at average spring tides (slightly greater than in Faxa Bay) (Icelandic Coast Guard, Hydrographic Department – database) Although the hydrography in Breiðafjörður is likely to be driven by similar factors as the Faxa Bay hydrography, it could be more greatly influenced by the large tidal amplitude due to the shallow bathymetry in the inner part of the bay, resulting in stronger mixing by tidal currents.

The coastal waters south and southwest of Iceland are generally less saline than the Atlantic waters off shore of Iceland due to the mixing with fresh runoff water from the south- and southwest coast (Ólafsson 1985). The added annual mean fresh water drainage into the bay is  $\sim 200 \pm 50 \text{ m}^3 \text{ s}^{-1}$  and mostly concentrated in the northern part of the bay (Icelandic Meteorological Office - database). The catchment area surrounding the bay is sparsely populated resulting in a limited anthropogenic fingerprint compared to many coastal regions around the globe (Ólafsson 1986, Skarphedinsdottir et al. 2010).

The coastal regions south and southwest of Iceland are productive and important for recruitment of commercially utilized fish stocks, including cod and haddock (Ólafsson 1985). The onset of spring blooms of phytoplankton in Faxa bay is around late March but the phytoplankton growth can vary annually depending on the interaction between terrestrial runoff and the wind regime (Thordardottir 1986). The extensive areas of hard substrate in the inner part of Breiðafjörður also harbours a vast macrophyte-based ecosystem, including the

kelps *Laminaria hyperborea* and *L. digitata* (Gunnarsson 1991). The bay also harbours various fish, bird and invertebrate species, with the latter most group including calcifying species that are considered susceptible to low calcium carbonate saturation ( $\Omega$ ), such as maerl-forming coralline algae and molluscs (Marteinsdottir et al. 2000, Solmundsson et al. 2003, Ingólfsson 2006, Jonasson et al. 2007, Kristjánsson et al. 2013).

The aim of this study is to document the spatial and temporal fluctuations in the inorganic carbon system in a high latitude coastal region and by that improve understanding of the biological implications of the continuing absorption of anthropogenic CO<sub>2</sub> in coastal regions. The finding presented in the study allows for a description of the hydrographic setting within Breiðafjörður Bay that not only adds to the existing information on coastal environmental fluctuations but is also of value for future research on the ecology of the bay and for developing an understanding of the implications of anthropogenic changes in this and other comparable regions (Kapsenberg et al. 2015).

## Material and methods

### *Station locations*

Data was collected on the physical properties of seawater (temperature and salinity) throughout the water column at five sampling stations along a north-south transect within Breiðafjörður Bay, parallel to the bay opening (Fig. 1). Data on inorganic carbon chemistry, nutrients and chlorophyll *a* were collected from the surface at all stations and from the water column at the deepest sampling station (70 m) for estimating vertical variability in the bay (Fig. 1).

### *Sample collection*

Data on temperature, salinity, nutrient concentrations, chlorophyll *a* concentration, partial pressure of CO<sub>2</sub> ( $p\text{CO}_2$ ) and dissolved inorganic carbon (DIC) were collected on 19 cruises over the time period April 2010 to May 2011 (Table 1). During one additional cruise (06.12.10) the data was collected on the same parameters, except for data on  $p\text{CO}_2$  and DIC (Table 1). Continuous measurements of temperature (accuracy  $\pm 0.15$  °C), salinity (accuracy  $\pm 1.0\%$ ) and depth (accuracy  $\pm 0.12$  m), of the water column were obtained using a CTD (YSI 6000, YSI Incorporated, USA) connected to a hand held data logger by a cable which was lowered through the water column at every station. Due to salinity sensor malfunctions, the salinity was measured in discrete samples collected on the cruise dates 23.04.10, 05.05.10, and 11.06.10 at the Marine and Freshwater Research Institute in Iceland. Salinity measurements were also considered erroneous on the cruise date 17.04.10 so for calculating parameters of the inorganic carbon chemistry, salinities for the cruise conducted on 23.04.10 were used. Discrete water samples were collected using a 5 L Niskin bottle from a depth of 1 meter (surface) at all five sampling stations and also from 10, 20, 30, and 70 m at station 2. Sub-samples were collected for laboratory based analysis of  $p\text{CO}_2$ , DIC, nitrate (nitrate + nitrite), phosphate and silicate concentrations from surface and the four additional depths at station 2. Phytoplankton biomass was estimated through the proxy chlorophyll *a*. Sub-samples for

analysis of chlorophyll *a* were collected from surface at all sampling stations and from 10 and 20 m depth at station 2. Samples for the determination of inorganic nutrient concentrations were collected in 250 mL soft low density polyethylene bottles washed with dilute hydrochloric acid prior to each cruise and frozen pending analysis using a Chemlab three channel autoanalyzer (Grasshoff 1970). Water samples for analysing  $p\text{CO}_2$  were collected into 500 mL screw capped Pyrex bottles, poisoned using  $\text{HgCl}_2$  and stored for a maximum of 3 weeks pending analysis using a gas chromatograph, calibrated with three air- $\text{CO}_2$  standards. Samples for the determination of DIC were collected into 250 mL tightly sealed glass bottles, poisoned using  $\text{HgCl}_2$  and stored for a maximum of 2 months pending coulometric determinations using an Coulometrics Model CM-5011 instrument. Analysis of  $p\text{CO}_2$ , DIC and inorganic nutrients were carried out at the Marine Research Institute in Iceland using the methods and quality control described in more detail by Olafsson et al. (2010). Samples for analysis of chlorophyll *a* concentration were collected into 1 L bottles and stored on ice in coolers until the samples were filtered in the lab. A sub sample (500-750 mL) was then filtered under dim light through a  $0.5 \mu\text{m}$  GF/F filter (Whatman®), blotted dry and stored frozen at  $-80^\circ\text{C}$ . Chlorophyll *a* extraction was conducted using 3.0 mL 100% acetone to soak the filters prior to sonication before the samples were stored at  $-20^\circ\text{C}$  over night. Quantification of chlorophyll *a* concentrations was conducted using a spectrophotometer (Beckman Coulter DU 730, Beckman Coulter, USA).

#### *Meteorological data*

Data were obtained from the Icelandic Meteorological Office database from two automatic weather stations recording wind at 10 minutes intervals. One station, at Stykkishólmur, ( $65.07^\circ\text{N}$ ,  $22.73^\circ\text{W}$ ), the other at a more exposed location, Gufuskálar, ( $64.90^\circ\text{N}$ ,  $23.93^\circ\text{W}$ ) (Fig. 1). The latter station may better represent the offshore stations but Stykkishólmur the near shore stations.

#### *Sea surface temperature records*

To examine inter-annual sea surface temperature (SST) variability in Breiðafjörður in relation to the data presented in this study, records were obtained from the Marine Research Institute, Reykjavik, on continuous temperature monitoring at Flatey Island (in close proximity to station 2) for the period 2005 -2015.

#### *Calculation of inorganic carbon parameters*

Total alkalinity (TA), total hydrogen ion pH ( $\text{pH}_T$ ) and saturation states for aragonite and calcite ( $\Omega_{\text{aragonite}}$  and  $\Omega_{\text{calcite}}$  respectively) were computed from observed  $p\text{CO}_2$ , DIC, temperature, salinity, pressure, phosphate and silicate concentrations using the *seacarb* package developed by Gattuso et al. (2016) for the software *R*. Parameters of the inorganic carbon chemistry were calculated on the basis of the carbonic acid dissociation constants of Lueker et al. (2000) and with revised boron-chlorinity ratio given by Lee et al. (2010).



### *Drivers of pCO<sub>2</sub> variability and CO<sub>2</sub> air-sea flux*

Takahashi et al. (2002) presented a method for decoupling the effects of temperature and what was referred to as the ‘net biology effect’ on seasonal pCO<sub>2</sub> fluxes (in μatm) in seawater. The ‘net biology effect’ includes all biogeochemical processes acting on net CO<sub>2</sub> utilization, air-sea CO<sub>2</sub> exchange and changes in DIC by advection and freshwater discharges. This method was applied to data from all 5 depths at station 2 collected on 17 cruises from 17.04.10 through 25.03.11. The contribution of the ‘net biology effect’ to changes in pCO<sub>2</sub> was estimated through removing the temperature effect by normalizing the values for pCO<sub>2</sub> to a constant temperature of 5.2°C, which is the mean annual surface temperature at the station (determined through polynomial regression of temperature as a function of day in year):

$$pCO_{2B} = pCO_2(T_{\text{mean}}) = pCO_2 \cdot \exp(0.0423 \cdot (T_{\text{mean}} - T_{\text{obs}})) ,$$

where  $T_{\text{mean}}$  is the mean annual temperature (5.2°C),  $T_{\text{obs}}$  is the observed or *in situ* temperature and  $0.0423^\circ\text{C}^{-1}$  is a constant describing the temperature effect on pCO<sub>2</sub> for isochemical seawater ( $\delta \ln pCO_2 / \delta T$ ) as determined by Takahashi et al. (1993) for North Atlantic surface water. The effect of temperature changes on pCO<sub>2</sub> was computed by perturbing the mean annual pCO<sub>2</sub> of 320 μatm (determined through polynomial regression of pCO<sub>2</sub> as a function of day of year) with the difference between mean and observed temperature through the following equation:

$$pCO_{2T} = pCO_2(T_{\text{obs}}) = (\text{mean annual } pCO_2) \times \exp(0.0423 \cdot (T_{\text{obs}} - T_{\text{mean}}))$$

The ‘net biology effect’ on pCO<sub>2</sub> can be represented by the seasonal amplitude of pCO<sub>2</sub> values corrected to the mean annual temperature:

$$B = (\Delta pCO_2)_{\text{bio}} = (pCO_2 \text{ at } T_{\text{mean}})_{\text{max}} - (pCO_2 \text{ at } T_{\text{mean}})_{\text{min}} ,$$

where  $B$  is the ‘net biological effect’ and the subscripts ‘max’ and ‘min’ indicate seasonal maximum and minimum values.

The effect of temperature changes on the mean annual pCO<sub>2</sub> value is represented by the seasonal amplitude of pCO<sub>2</sub> at  $T_{\text{obs}}$  values computed using the Eq. (2) above:

$$T = (\Delta pCO_2)_{\text{temp}} = (pCO_2 \text{ at } T_{\text{obs}})_{\text{max}} - (pCO_2 \text{ at } T_{\text{obs}})_{\text{min}} ,$$

where  $T$  describes the effect of temperature on mean annual pCO<sub>2</sub> value. The relative importance of the ‘net biological effect’ and temperature effect can be expressed by the ratio,  $T/B$  or the difference between  $T$  and  $B$  ( $T - B$ ). The effect of temperature changes on pCO<sub>2</sub> are considered to exceed the ‘net biological effect’ where  $T/B$  is greater than 1 or  $T - B$  positive but the opposite applies when  $T/B$  is  $< 1$  and  $T - B$  is negative.

The flux of CO<sub>2</sub> between the atmosphere and the surface was estimated following the method described by (Takahashi et al. 2009). The revised Wanninkhof 1992 relationship between gas exchange and wind speed was used (Wanninkhof 1992, 2014). Atmospheric pCO<sub>2</sub> at the ocean surface where humidity is 100% was estimated:

$$(pCO_2)_{\text{air}} = XCO_2(P_{\text{baro}} - P_{\text{sw}}) ,$$

where  $P_{\text{baro}}$  is the barometric pressure at the sea surface (Stykkishólmur, meteorological station, obtained from the Icelandic Meteorological Office - database) and  $P_{\text{sw}}$  is the water vapour pressure at the temperature and salinity observed at each station on the section.

Globalview data product (CO<sub>2</sub>-ICE) on atmospheric  $p\text{CO}_2$  from Stórhöfði, Iceland (GLOBALVIEW-CO<sub>2</sub> & Cooperative Global Atmospheric Data Integration Project. 2013 2013), was used with the station data to calculate the air-sea partial pressure difference:

$$\Delta p\text{CO}_2 = [(p\text{CO}_2)_{\text{sw}}] - [(p\text{CO}_2)_{\text{air}}]$$

The net sea-air CO<sub>2</sub> flux ( $F$ ) is estimated by the following equation:

$$F = k \alpha \Delta p\text{CO}_2 = \text{Tr} \Delta p\text{CO}_2 ,$$

where  $k$  is the CO<sub>2</sub> transfer velocity,  $\alpha$  is the solubility of CO<sub>2</sub> in seawater and  $\text{Tr}$  is the sea-air gas transfer coefficient. The transfer velocity,  $k$  was calculated based on 30 day running means of the quadratic wind speed for the periods between cruises and the average CO<sub>2</sub> solubility in the same period so that:

$$k \text{ (cm h}^{-1}\text{)} = 0.251 (U_{10})^2 (\text{Sc}/660)^{-1/2} ,$$

where  $U_{10}$  is the wind speed and  $\text{Sc}$  is Schmidt number (Wanninkhof 2014).

The reported CO<sub>2</sub> flux rates were based on calculations for 17 time periods between observations April 2010 to April 2011. The wind-speed data used to calculate flux of CO<sub>2</sub> between the atmosphere and ocean surface was from two meteorological stations, Gufuskálar and Stykkishólmur (Fig. 1) but the observed wind-speed at the former was generally higher than observed at the latter, yielding differing CO<sub>2</sub> flux rates. An average of the two wind-speed data sets may best represent the conditions over the north-south transect but flux rates were also calculated separately for each wind-speed data (Table 2).

Data analysis was performed using *R* (R Core Team 2014) and *Ocean Data View* (Schlitzer 2016).

## Results

### *Surface*

The monthly average surface temperatures recorded at Flatey differed by an average of 3.2°C ( $\pm 0.6$ ) per month over the years 2005 through 2015 (Fig. 2). It is notable that 2010, May to October, were relatively warm months compared to the five previous and five subsequent years (Fig. 2).

General trends in the physico-chemical environment at surface were comparable at all stations. However, relating to the closest proximity to a source of terrestrial runoff, station 1 exhibited deviations from a general trend.

Along the sampling transect, surface temperature ranged from -0.8°C at station 1 in March 2011 to 14°C recorded in July and August (Fig. 3; Table 1). The temperature did not vary spatially across the bay with the exception of the winter months (December to April) when a general trend of increasing temperature from north to south was observed (Fig. 3). Salinity ranged from 32.7 to 34.7 at surface throughout the sampling period, with notably lowest salinity recorded at the northern most part of the sampling transect (Fig. 3). Salinity decreased gradually from November 2010 to May 2011 at all stations (Fig. 3).

The seasonal changes in nutrients were large. Nitrate concentration was near zero from June through August 2010 with a general trend of decreasing concentration from north to south in winter, relating to salinity. Phosphate concentrations were low but not fully depleted in summer and exhibited limited spatial variability. Silicate was nearly depleted in early June and late July but a higher concentration was observed at station 1 in mid-winter than at the other sampling stations (Fig. 3). Chlorophyll *a* concentrations varied throughout the spring and the summer but were notably low in spring and early summer at station 1 compared to the other stations (Fig. 3). The maximum observed chlorophyll *a* concentration was 4-6  $\mu\text{g L}^{-1}$  but concentrations were consistently low at all stations from early November 2010 through March of 2011 (Fig. 3), indicative of limited or no primary production in the plankton environment.

The surface concentrations of inorganic carbon parameters varied seasonally and similarly at the five sampling stations, except for a moderate deviation from a general trend observed at station 1 (Fig.4). DIC ranged from 1980 to 2161  $\mu\text{mol kg}^{-1}$  and  $p\text{CO}_2$  ranged from 212 in early summer, to a maximum of 417  $\mu\text{atm}$  observed in winter (Fig. 4). The surface  $p\text{CO}_2$  only exceeded the atmospheric  $p\text{CO}_2$  on one sampling date in autumn (05.11.10).  $\text{pH}_T$  was lowest in winter, with a minimum of 8.02 and highest in early summer, 8.27 (Fig. 4). Total alkalinity ranged from 2242 to 2336  $\mu\text{mol kg}^{-1}$  over the sampling period (Fig. 4). Surface waters were supersaturated ( $\Omega > 1$ ) with respect to aragonite and calcite throughout the sampling period but  $\Omega_{\text{aragonite}}$  and  $\Omega_{\text{calcite}}$  reached a maximum of 3.2 and 5.0 respectively in summer but were lowest in late winter or 1.3 and 2.1 respectively (Fig. 4).

#### *Depth profiles*

Depth profiles at station 2 indicate that the bay is relatively well mixed vertically but generally exhibits a moderate increase in salinity from surface to the station bottom depth of 70 m (Fig. 5). Temperature did not vary with depth but in July and August, temperature was moderately higher at surface compared to other depths (Fig. 5). Chlorophyll *a* concentrations were generally comparable between 1, 10 and 20 m. Density stratification of the water column was limited in winter but was greater in summer when the lowest density was generally observed at surface in the northern part of the bay, and the greatest density observed at a bottom depth of 50-60 m at stations 3 and 4 (Fig. 6). Throughout the sampling period, the measured chemical properties of the seawater (nutrients,  $p\text{CO}_2$  and DIC) exhibited negligible or moderate variability from surface to a bottom depth of 70 m (Fig. 5). Despite negligible changes in  $p\text{CO}_2$  and DIC, the calculated values for  $\text{pH}_T$  and  $\Omega$  decreased with increasing depth, due to the thermodynamic effects of pressure (Fig. 5).

#### *Drivers of $p\text{CO}_2$ variability and $\text{CO}_2$ air-sea flux*

Temperature and the 'net biological effect' contributed significantly and antithetically to seasonal variation in  $p\text{CO}_2$  at station 2 but the latter effect drove a greater proportion of the  $p\text{CO}_2$  variations over the year, as indicated by  $T/B = 0.65$  and  $T-B = -103$  (Fig. 7).

Carbon flux between the atmosphere and ocean shows a north-south trend with highest flux at station 1, reflecting significant  $\Delta p\text{CO}_2$  variability along the transect (Fig. 4, Table 2). The annual flux rates are presented as averages of all the stations and based on quadratic wind

speed at the exposed, Gufuskálar, and less exposed, Stykkishólmur, meteorological stations. On a yearly basis, the mean CO<sub>2</sub> flux from the atmosphere to the sea is estimated to be  $1.80 \pm 0.16 \text{ mol C m}^{-2} \text{ y}^{-1}$  (Table 2). Clearly, the winds have greater influence on the overall CO<sub>2</sub> flux than the spatial trend. The daily CO<sub>2</sub> sink rate was greatest in spring  $\sim 15 \text{ mmol m}^{-2} \text{ d}^{-1}$  but for a short period in the autumn, the atmosphere and seawater  $p\text{CO}_2$  were near equilibrium, resulting in negligible flux rates (Fig. 8). The flux of CO<sub>2</sub> from the atmosphere to the sea was primarily driven by biological activity in summer but by the seawater temperature change in winter (Fig. 7).

## Discussion

The seasonal changes in the physico-chemical environment in Breiðafjörður Bay were considerable as is characteristic for a high latitude environments where seasonality in irradiance is extreme (Lutz et al. 2007).

Accordingly, there was negligible photosynthetic activity and phytoplankton biomass in the pelagic environment in winter (November to March), based on the very low chlorophyll *a* concentrations ( $< 1.0 \mu\text{g/L}$ ) observed throughout the high winter months. Based on chlorophyll *a* and evolution in nutrient concentrations, the onset of the phytoplankton bloom occurred in March, which agrees with previous observations for Faxa Bay, directly south of Breiðafjörður Bay (Thordardottir 1986). The full utilization of nitrate throughout the summer suggests the primary production is nitrate limited during this period, relating not only to new production in phytoplankton, but also to the extensive macrophyte beds where *Laminaria hyperborea* and *L. digitata* biomass may reach 9 and 14 kg m<sup>-2</sup> respectively (Gunnarsson 1991). The relative contribution of the phytoplankton and benthic algae to the observed variability in nutrients and inorganic carbon in Breiðafjörður Bay is, however, unknown. Nevertheless, a complete utilization of silicate in mid-June and late July presumably reflects the timing of rapid growth of diatom species in the planktonic community (Paasche 1973).

A considerable proportion of the seasonal variability in the inorganic carbon system in Breiðafjörður was driven by biological activity. The  $p\text{CO}_2$  variability over the year was largely driven by the ‘net biological effect’ but to a lesser extent by the change in temperature as indicated by the  $T/B < 1$ . The  $T/B$  ratio was 0.65 which is similar to that previously reported from the Southern Bight of the North Sea of 0.74 (Schiettecatte et al. 2007). As aforementioned, the ‘net biological effect’ includes not only the effects of respiration and photosynthesis on  $p\text{CO}_2$  but could also be affected by air-sea CO<sub>2</sub> exchange and changes in dissolved inorganic carbon by advection and freshwater discharges (Takahashi et al. 2002). Although seawater advection could have contributed to the ‘net biological effect’ (in particular to the increased ‘net biological effect’ in autumn and winter), it is unlikely to be significantly influenced by salinity variations at station 2 (for which the  $T/B$  was calculated) or air-sea  $p\text{CO}_2$  exchange which is a relatively slow process.

Over the year, the surface seawater was undersaturated with respect to atmospheric  $p\text{CO}_2$ , mainly due to drawdown of CO<sub>2</sub> by primary production in summer but as a result of low

temperatures in winter. Accordingly, Breiðafjörður is a sink for CO<sub>2</sub> on a yearly basis, which is consistent with observations from previous studies on the coastal region and continental margins in the sub-Arctic and Arctic (e.g. Fransson et al. 2001, Bates 2006) and high latitude coastal regions in the Southern Oceans (e.g. Bates et al. 1998, Carrillo & Karl 1999, Arrigo et al. 2008). The estimated annual sink rate of CO<sub>2</sub> in Breiðafjörður (1.8 mol C m<sup>-2</sup> y<sup>-1</sup>) also supports previously estimated air-sea flux rates for continental shelves in subpolar NE Atlantic of 1.6 mol C m<sup>-2</sup> y<sup>-1</sup>, despite the latter estimate being based on limited data (Chen & Borges 2009). As Breiðafjörður is an open and well mixed bay with limited fresh water input, it is not surprising that its seawater characteristics, including flux rates, relate more to the continental shelf than semi-closed coastal systems or near-shore systems (e.g. estuaries) where total annual CO<sub>2</sub> flux is typically from sea to atmosphere (Gattuso et al. 1998, Chen & Borges 2009).

It is of interest to compare the findings reported here to that from the North Sea, which is among the best studied ocean regions in terms of changes in the inorganic carbon system on annual and seasonal time scales, due to the some similarities of the findings presented here to that from the North Sea (Schiettecatte et al. 2007, Chen & Borges 2009). Most notably, given the contrasting properties of the regions, the annual CO<sub>2</sub> sink rates reported here for Breiðafjörður are comparable to estimates for the North Sea, 1.5-2.2 C m<sup>-2</sup> d<sup>-1</sup> (Bozec et al. 2005). The seasonal variation in daily CO<sub>2</sub> sink rates estimated for Breiðafjörður (from 0-1 mmol C m<sup>-2</sup> d<sup>-1</sup> in winter to 15 mmol C m<sup>-2</sup> d<sup>-1</sup> in spring) are also similar to that estimated for the North Sea of -1 C m<sup>-2</sup> d<sup>-1</sup> in autumn (flux from sea to atmosphere) to 14 C m<sup>-2</sup> d<sup>-1</sup> in spring (Frankignoulle & Borges 2001). The reason why annual and seasonal CO<sub>2</sub> flux rates are similar between Breiðafjörður bay and the North Sea could be attributed to the added nutrient delivery to the latter region as a result of anthropogenic activity, promoting phytoplankton growth beyond what would be expected in a pristine environment (Reid et al. 1990). Moreover, the location of the North Sea at lower latitude (56°N) allows for longer periods of irradiance levels that are sufficient to sustain photosynthetic activity (Joint & Pomroy 1993). These arguments are supported by findings indicating that the inter-annual variations in pCO<sub>2</sub> in the Southern Bight of the North Sea are strongly driven by nutrient availability and the amplitude and timing of biological activity (Schiettecatte et al. 2007). Furthermore, Borges and Gypens (2010) showed that eutrophication within the North Sea has resulted in increased autotrophy and subsequently, increased drawdown of CO<sub>2</sub> in the region. They also noted that changes in nutrient delivery to coastal zones may mitigate (or exacerbate in some cases) ocean acidification due to the increase in atmospheric CO<sub>2</sub> (Borges & Gypens 2010). This suggests that ocean acidification as a result of elevated atmospheric CO<sub>2</sub> content, could pose a greater threat to pristine coastal regions at high latitudes, including Breiðafjörður, compared to more eutrophicated coastal regions at temperate and tropical latitudes (Doney 2010).

The DIC varied in relation to pCO<sub>2</sub>, with the observed lowest concentrations in summer, reflecting mostly the transformation of inorganic carbon to organic carbon through autotrophic activity whereas the total alkalinity varied mainly in relation to salinity.

The seasonal pH amplitude in Breiðafjörður was about 0.25 units which translates to a ~70% change in [H<sup>+</sup>] over the year. This range is greater than the pH range reported for fjords in the western part of Norway where pH ranged from ~8.05 to 8.2 (i.e. ~40% change in [H<sup>+</sup>]) (Omar et al. 2016). The timing of the maximum observed pH is comparable between the western part of Norway and Breiðafjörður, relating to the phytoplankton spring bloom (Omar et al. 2016).

The biological relevance of  $\Omega$  is restricted to calcifying organisms. The  $\Omega_{\text{aragonite}}$  range of  $\sim 1.5$  in winter to  $\sim 3$  in summer is similar to the  $\Omega_{\text{aragonite}}$  range of 1.6 to 2.5 reported from winter to summer in Kongsjorden in Svalbard (Fransson et al. 2016) and  $\sim 1.3$  to  $\sim 3$  reported for western Norwegian fjords (Omar et al. 2016). The effect of  $\Omega$  is likely to be species-specific and dependent on the type of calcium carbonate the calcifying organism produces and (Langer et al. 2006, Kroeker et al. 2013). Molluscs are considered a particularly vulnerable taxa (Kroeker et al. 2013) but calcifying species typically produce shells from aragonite often together with calcite (Kobayashi & Akai 2013, Togo & Suzuki 2013). For example, an  $\Omega_{\text{aragonite}}$  of 1.5 likely presents a critical threshold for the pteropod *Limacina helicina*, which is commonly observed in Kongsfjorden, Svalbard (Bednaršek & Ohman 2015). Benthic mollusc species have also been reported to be susceptible to  $\Omega_{\text{aragonite}}$ , in particular early life stages, that may exhibit  $\Omega_{\text{aragonite}}$  tipping points below which larval shell formation fails (Waldbusser et al. 2015).

Abundant species of calcifying molluscs in Breiðafjörður Bay include the commercially harvested Icelandic scallop (*Chlamys islandica*) and European whelk (*Buccinum undatum*) along with non-commercially fished species such as the long lived *Arctica islandica* (Pálsson et al. 2014). Many other species of calcifying molluscs also make up a significant proportion of food items in the diet of the Icelandic common eider population in Breiðafjörður (*Somateria mollissima*) (Kristjánsson et al. 2013). Echinoderms and red coralline algae are also considered vulnerable groups to low  $\Omega$  as they produce high-Mg calcites which are more soluble than aragonite, when the ratio between magnesium and calcium exceeds  $\sim 0.12$  (Morse et al. 2006, Andersson et al. 2008). Analysis of the Mg/Ca ratio in the asteroid *Asterias rubens* collected in Breiðafjörður Bay was above 0.12 but the ratio was lower for echinoids collected in the bay, or  $\sim 0.07$  in calcite produced by both *Echinus esculentus* and *Strongylocentrotus droebachiensis* (Lebrato et al. 2013).

The implications of seasonal changes in  $\Omega$  for calcifying biota are not well known; in particular, those of  $\Omega$  in winter (Waldbusser & Salisbury 2014).

In fact, the seasonal range in pH and  $\Omega$  in macrophyte-dominated coastal regions can be similar or even less than that observed over diel scales, resulting from an increase in pH during the day due to photosynthesis but a decrease in pH during the night, due to respiration (Kapsenberg & Hofmann 2016). The daily variability in pH was not investigated here but is likely to be less intense in summer and winter compared with that observed at lower latitudes, as a result of the long and short photoperiods in summer and winter respectively, in high latitudes (Krause-Jensen et al. 2016). Indeed, Krause-Jensen et al. (2016) showed that balanced light and dark cycles lead to diurnal oscillations in pH, whereas long photoperiods ( $>21$  h) can lead to a continuous increase in pH in a high latitude kelp-dominated environment. Based on experimental evidence they also suggested that future increases in  $\text{CO}_2$  uptake from the atmosphere will have positive effect on kelp growth and extend existing kelp regions at high latitudes through the increase in inorganic carbon as photosynthetic substrate, and that this will subsequently benefit calcifying species in these regions (Krause-Jensen et al. 2016). However, high latitudes environments also experience very low irradiance levels in winter that significantly limit photosynthetic production, as evidence suggests in this study, effectively removing the potential for macroalgae to elevate  $\Omega$  in their surrounding environment and potentially resulting decreased  $\Omega$  under future atmospheric  $\text{CO}_2$  levels in winter. Thus, a better understanding of the implication of future changes in coastal regions requires information on the environmental variability over short (hourly) to long (yearly) time scales, along with

knowledge of species life histories, in particular the occurrence (on temporal and spatial scales) of vulnerable life stages (Waldbusser & Salisbury 2014).

The data presented here for seasonal changes in the physico-chemical environment are likely to represent general seasonal variability in Breiðafjörður Bay. The mean monthly surface temperature in Flatey of 3.2°C suggests that the amplitude of seasonal changes may vary moderately between years. However, Breiðafjörður Bay is unlikely to be influenced by large scale climate oscillations or variable river runoff which generally characterize coastal regions where significant inter-annual variability in seawater composition and air-sea CO<sub>2</sub> fluxes have been reported (Borges & Frankignoulle 1999, Friederich et al. 2002, Ianson & Allen 2002, Borges et al. 2008).

The coastal regions in the sub-Arctic North Atlantic are changing as a result of anthropogenic CO<sub>2</sub> emissions with warming and increasing number of extreme hot days observed (Lima & Wetthey 2012). It is well understood that temperature variations strongly affect organism biochemistry including photosynthesis of macroalgae and respiration rates in both autotrophic and heterotrophic organisms (Davison 1991, Gillooly et al. 2001). Experimental and observational studies indicate that non-calcifying macrophytes are already affected negatively in some regions at lower latitudes but at higher latitudes elevated  $p\text{CO}_2$  and temperature could positively affect growth and result in a distribution expansion of some benthic macroalgal species, including kelp (Koch et al. 2013, Brodie et al. 2014, Krause-Jensen et al. 2016, Sunday et al. 2016).

Breiðafjörður is a pristine bay that exhibits moderate inter-annual variability, is ecologically and economically important, is subjected to limited terrestrial runoff, and harbours an extensive macrophyte-based ecosystem and calcifying taxa considered vulnerable to ocean acidification and low  $\Omega$ . On that basis, we suggest that Breiðafjörður Bay could be considered a model coastal region for studying the synergistic effect of ocean acidification and ocean warming in macrophyte dominated high latitude coastal regions.

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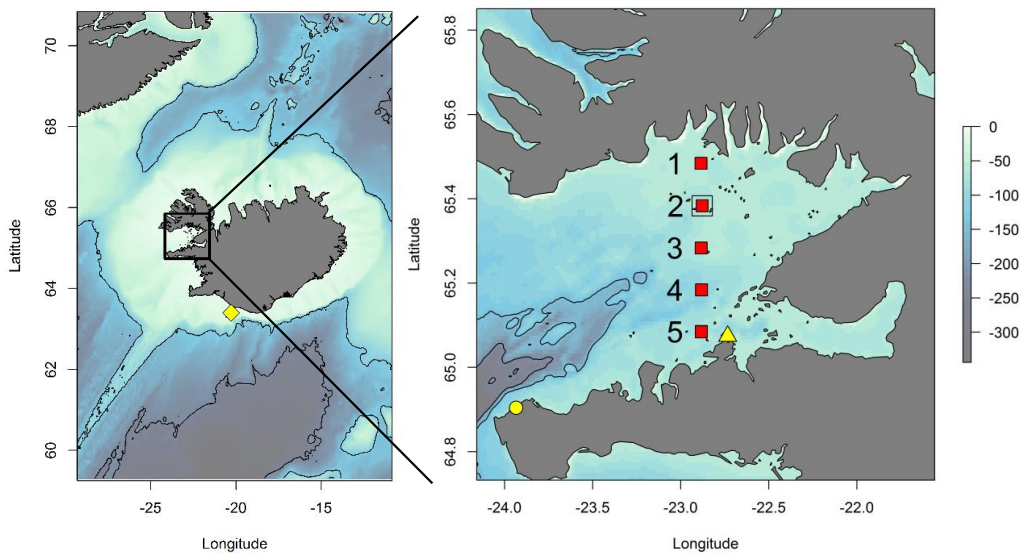


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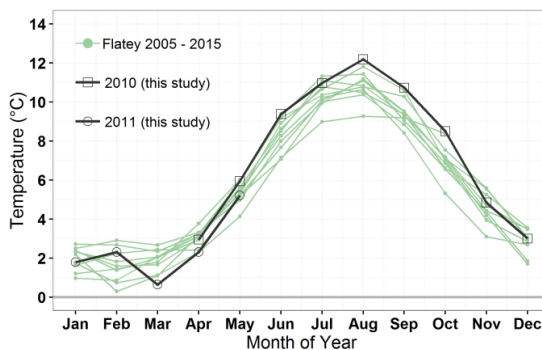
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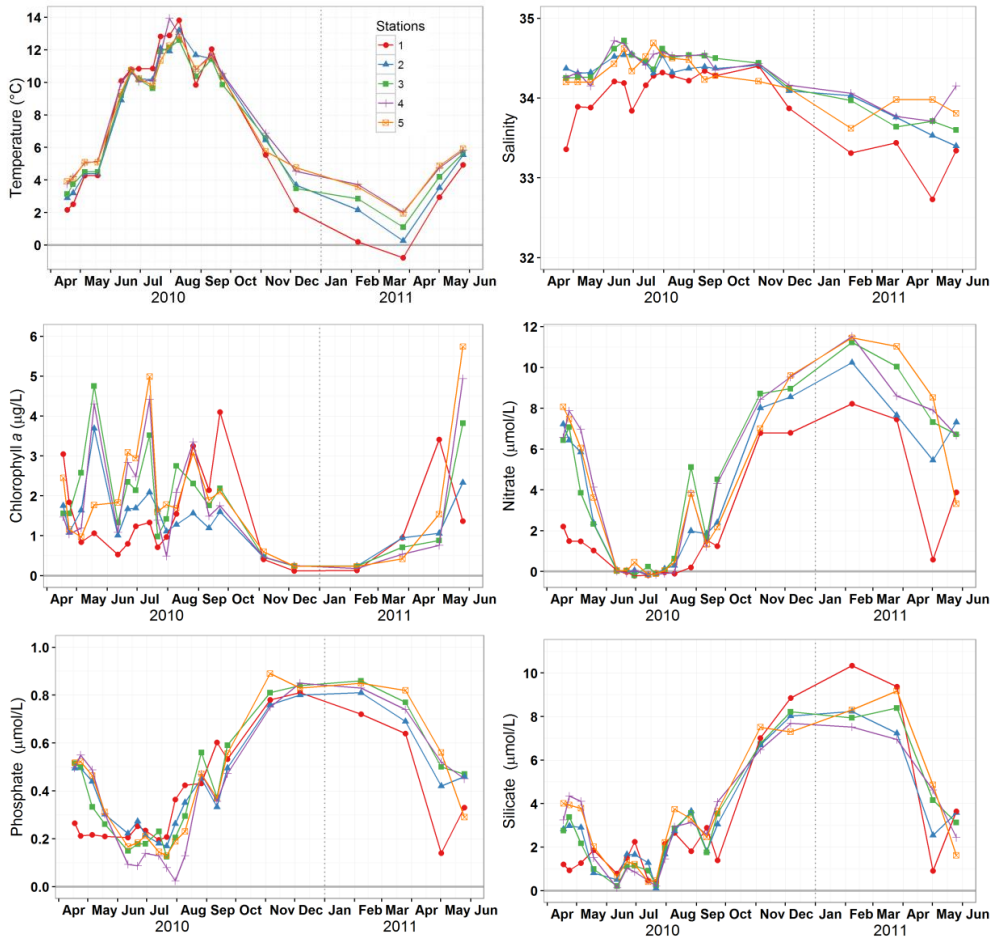
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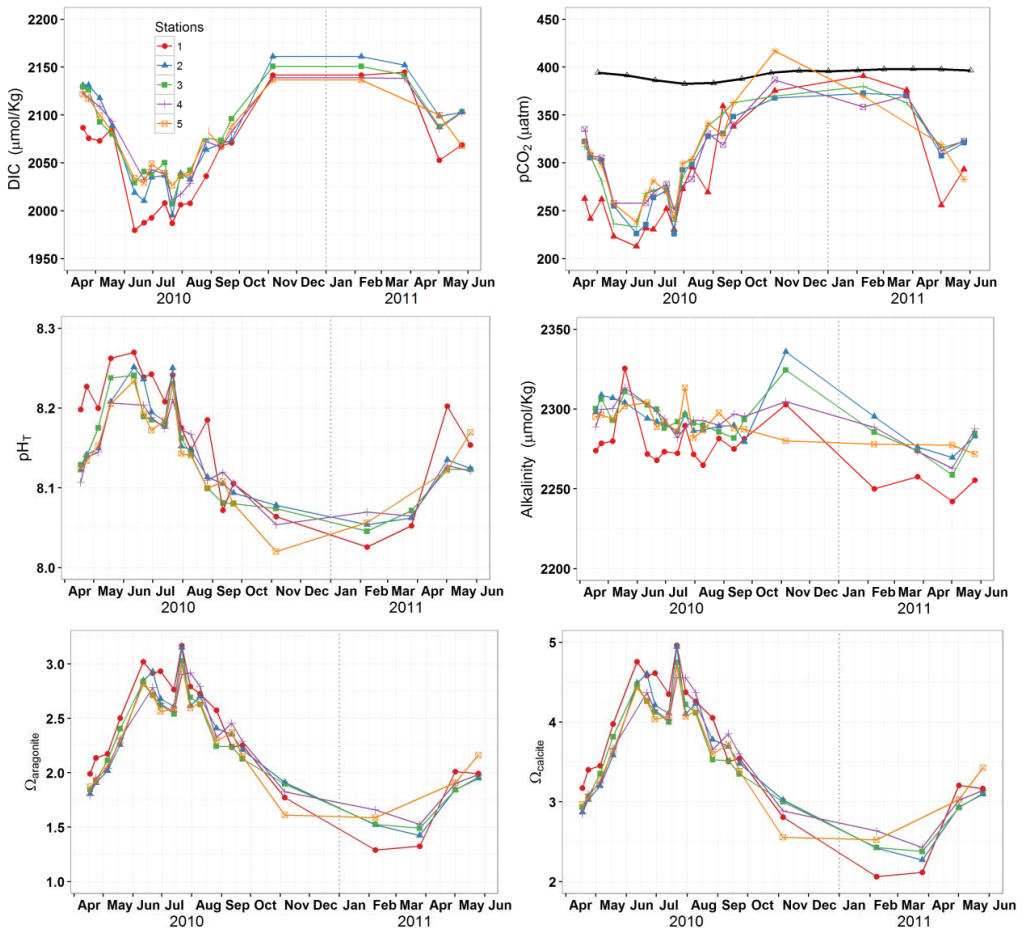
**Figure 1.** The geographical location of Breiðafjörður Bay in Iceland (left) and stations within the bay (right). Seawater chemistry samples were collected from surface at all five stations (red squares). At station 2 (encompassing square) samples were also taken from four additional depths down to the bottom depth of 70 meters. Flatey Island is located in close proximity to station 2. Contour lines represent 1000 m depth intervals (left) and 100 m depth intervals (right). Meteorological data for calculating CO<sub>2</sub> air-sea flux came from two stations, Gufuskálar (yellow point) and Stykkishólmur (yellow triangle). Data on atmospheric CO<sub>2</sub> was obtained from Stórhöfði meteorological station, Globalview CO<sub>2</sub>-ICE (yellow diamond).



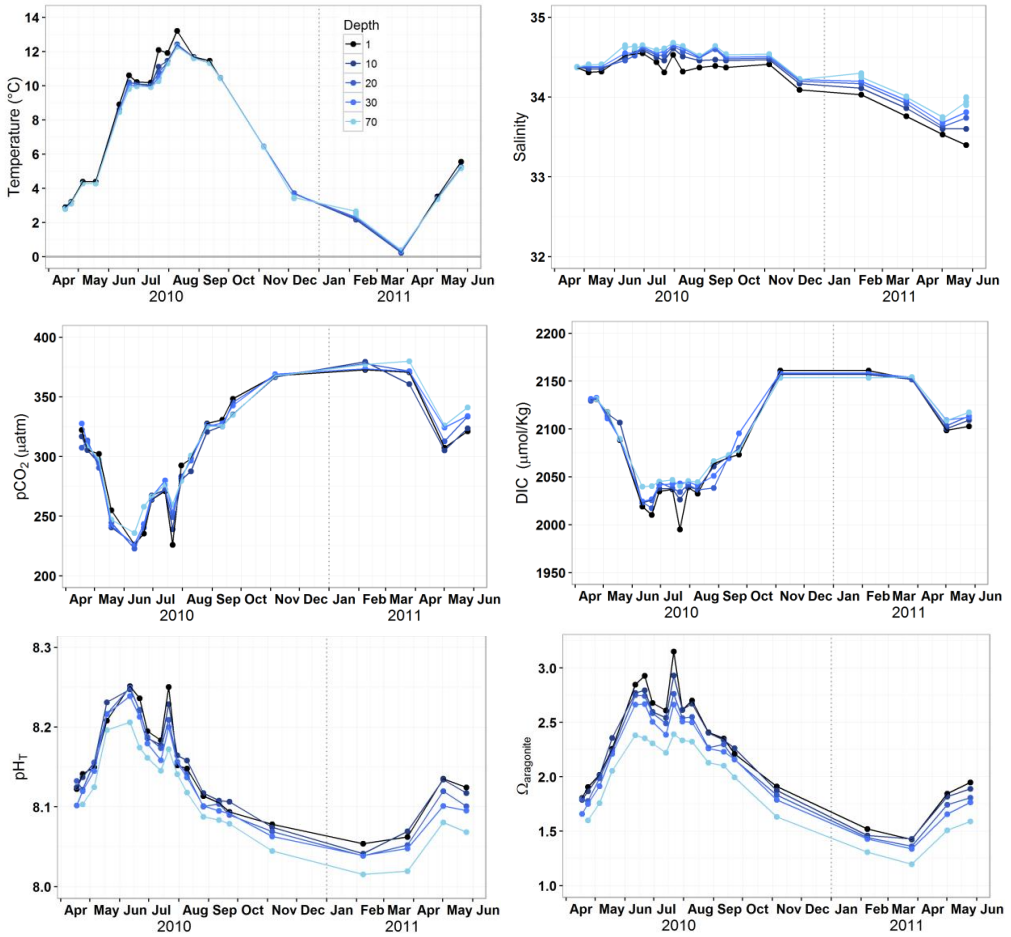
**Figure 2.** Mean monthly sea surface temperatures from 2005 through 2015 at Flatey Island (Fig.1). The period of this study is shown as grey lines with squares (2010) and circles (2011).



**Figure 3.** Surface layer temperature and salinity properties in Breiðafjörður Bay from April 2010 through May 2011 along with nutrient and chlorophyll-*a* concentrations.

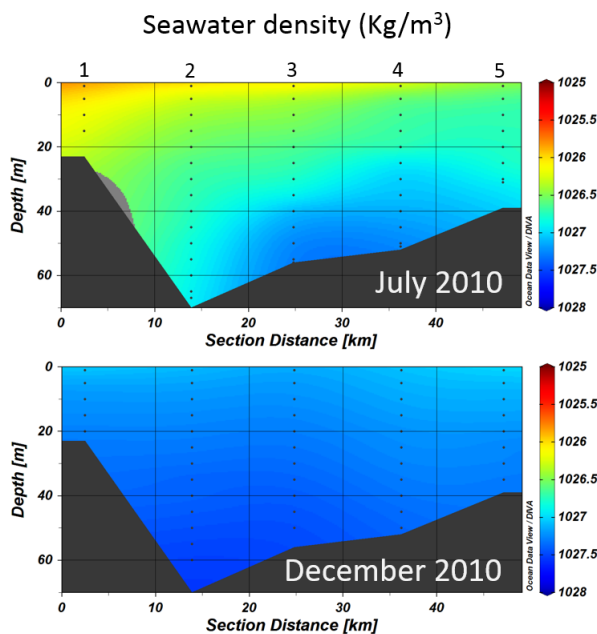


**Figure 4.** Surface layer inorganic carbon chemistry parameters in Breiðafjörður Bay from April 2010 through May 2011. Dissolved inorganic carbon (DIC) and partial pressure of  $\text{CO}_2$  ( $p\text{CO}_2$ ) were measured. Other parameters were calculated. Monthly atmospheric  $p\text{CO}_2$   $\mu\text{atm}$  from Stórhöfði meteorological station (Globalview  $\text{CO}_2\text{-ICE}$ ; black trace and triangles) is shown in comparison to seawater  $p\text{CO}_2$ .

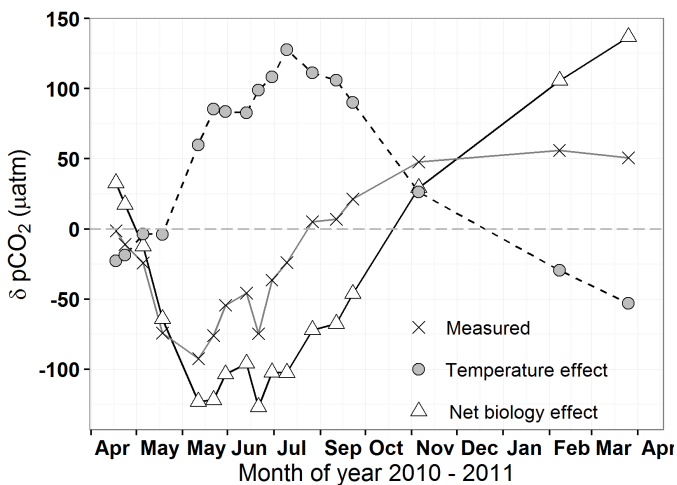


**Figure 5.** Changes in temperature, salinity, partial pressure of CO<sub>2</sub> ( $p\text{CO}_2$ ), dissolved inorganic carbon (DIC) from surface to a bottom depth of 70 m in Breiðafjörður Bay from April 2010 through May 2011. pH on total scale ( $\text{pH}_T$ ) and saturation state for aragonite ( $\Omega_{\text{aragonite}}$ ) were calculated.

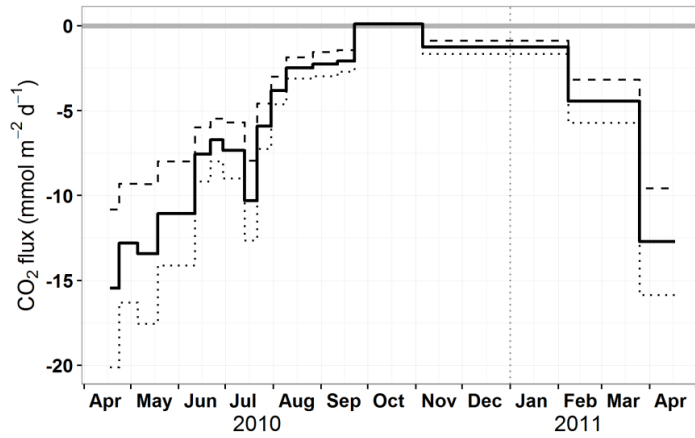




**Figure 6.** Seawater density on north-south sections across Breiðafjörður Bay from station 1 to 5 in summer (July) and winter (December) 2010. The bathymetry reflects station bottom depths.



**Figure 7.** The effect of temperature ( $pCO_{2T}$ ; grey circles and dashed line) and the 'net biological effect' ( $pCO_{2B}$ ; white triangles and black line) on the observed changes in surface  $pCO_2$ , presented as deviations from the mean  $pCO_2$  ( $y = 320$ ), over a year.



**Figure 8.** Daily CO<sub>2</sub> air-sea flux rates averaged for stations 1 to 5 in Breiðafjörður Bay. Negative values indicate flux from the atmosphere to the ocean. Dashed and dotted lines show CO<sub>2</sub> flux based on wind speed data from Gufuskálar and Stykkishólmur respectively.

**Table 1.** Cruise dates and observed surface seawater properties in Breidafjörður Bay as averages  $\pm$  standard deviations for five stations on the north-south transect, Fig.1.

Year	Cruise	Date	Temperature (°C)	Salinity	Nitrate ( $\mu\text{mol L}^{-1}$ )	Phosphate ( $\mu\text{mol L}^{-1}$ )	Silicate ( $\mu\text{mol L}^{-1}$ )	pCO <sub>2</sub> ( $\mu\text{atm}$ )	DIC ( $\mu\text{mol kg}^{-1}$ )
2010	1	17.04.10	3.2 $\pm$ 0.7	*nd <sup>1</sup>	6.1 $\pm$ 2.3	0.46 $\pm$ 0.11	2.8 $\pm$ 1.0	312 $\pm$ 28	2118 $\pm$ 18
	2	23.04.10	3.5 $\pm$ 0.7	34.09 $\pm$ 0.41	6.1 $\pm$ 2.6	0.46 $\pm$ 0.14	3.1 $\pm$ 1.3	294 $\pm$ 30	2114 $\pm$ 22
	3	05.05.10	4.7 $\pm$ 0.4	34.20 $\pm$ 0.18	4.8 $\pm$ 2.2	0.39 $\pm$ 0.11	2.8 $\pm$ 1.2	290 $\pm$ 18	2098 $\pm$ 17
	4	18.05.10	4.7 $\pm$ 0.4	34.16 $\pm$ 0.17	2.7 $\pm$ 1.2	0.28 $\pm$ 0.04	1.4 $\pm$ 0.5	246 $\pm$ 16	2086 $\pm$ 5
	5	11.06.10	9.5 $\pm$ 0.5	34.50 $\pm$ 0.19	0.0 $\pm$ 0.0	0.17 $\pm$ 0.05	0.5 $\pm$ 0.3	228 $\pm$ 11	2015 $\pm$ 25
	6	21.06.10	10.7 $\pm$ 0.1	34.55 $\pm$ 0.21	0.0 $\pm$ 0.0	0.20 $\pm$ 0.07	1.3 $\pm$ 0.2	252 $\pm$ 17	2020 $\pm$ 21
	7	29.06.10	10.3 $\pm$ 0.3	34.36 $\pm$ 0.31	0.1 $\pm$ 0.2	0.20 $\pm$ 0.04	1.4 $\pm$ 0.5	263 $\pm$ 19	2031 $\pm$ 22
	8	13.07.10	10.1 $\pm$ 0.5	34.40 $\pm$ 0.14	0.1 $\pm$ 0.1	0.18 $\pm$ 0.04	0.7 $\pm$ 0.4	270 $\pm$ 10	2035 $\pm$ 16
	9	21.07.10	12.0 $\pm$ 0.5	34.44 $\pm$ 0.18	0 $\pm$ 0	0.14 $\pm$ 0.05	0.3 $\pm$ 0.1	237 $\pm$ 10	2005 $\pm$ 15
	10	30.07.10	12.6 $\pm$ 0.8	34.52 $\pm$ 0.12	0.1 $\pm$ 0.1	0.21 $\pm$ 0.12	1.9 $\pm$ 0.3	285 $\pm$ 11	2027 $\pm$ v14
	11	09.08.10	13.1 $\pm$ 0.5	34.43 $\pm$ 0.12	0.3 $\pm$ 0.3	0.29 $\pm$ 0.11	3.0 $\pm$ 0.4	297 $\pm$ 8	2030 $\pm$ 13
	12	26.08.10	10.7 $\pm$ 0.7	34.43 $\pm$ 0.13	3.0 $\pm$ 1.9	0.48 $\pm$ 0.05	3.1 $\pm$ 0.8	322 $\pm$ 30	2066 $\pm$ 18
	13	11.09.10	11.6 $\pm$ 0.3	34.41 $\pm$ 0.13	1.5 $\pm$ 0.3	0.41 $\pm$ 0.02	2.1 $\pm$ 0.5	338 $\pm$ 17	2069 $\pm$ 3
	14	22.09.10	10.3 $\pm$ 0.3	34.36 $\pm$ 0.09	2.9 $\pm$ 1.4	0.53 $\pm$ 0.05	3.1 $\pm$ 1.0	350 $\pm$ 12	2082 $\pm$ 10
	15	05.11.10	6.2 $\pm$ 0.6	34.38 $\pm$ 0.10	7.8 $\pm$ 0.9	0.80 $\pm$ 0.06	6.9 $\pm$ 0.4	383 $\pm$ 20	2146 $\pm$ 10
	16	06.12.10	3.7 $\pm$ 1.0	34.07 $\pm$ 0.12	8.7 $\pm$ 1.1	0.83 $\pm$ 0.02	8.0 $\pm$ 0.6	nd	nd
2011	1	07.02.11	2.5 $\pm$ 1.4	33.80 $\pm$ 0.32	10.5 $\pm$ 1.4	0.81 $\pm$ 0.06	8.5 $\pm$ 1.1	374 $\pm$ 12	2146 $\pm$ 10
	2	25.03.11	0.9 $\pm$ 1.2	33.72 $\pm$ 0.20	9.0 $\pm$ 1.5	0.73 $\pm$ 0.07	8.2 $\pm$ 1.1	370 $\pm$ 5	2144 $\pm$ 6
	3	01.05.11	4.1 $\pm$ 0.8	33.53 $\pm$ 0.48	6.0 $\pm$ 3.2	0.43 $\pm$ 0.17	3.4 $\pm$ 1.7	302 $\pm$ 26	2085 $\pm$ 19
	4	25.05.11	5.6 $\pm$ 0.4	33.66 $\pm$ 0.33	5.6 $\pm$ 1.8	0.40 $\pm$ 0.08	2.9 $\pm$ 0.9	309 $\pm$ 19	2089 $\pm$ 19

\*nd<sup>1</sup>: Due to sensor malfunctions the salinity data from 23.04.10 was used for calculating carbon chemistry parameters.

\*nd<sup>2</sup>: No samples collected.

**Table 2.** CO<sub>2</sub> air-sea flux based on  $\Delta p\text{CO}_2$  and wind speed data from two meteorological stations: Gufuskálar, an exposed coastal site with relatively high winds and Stykkishólmur a more sheltered coastal site with lower winds.

Station	Stykkishólmur wind CO <sub>2</sub> flux (mol C m <sup>-2</sup> y <sup>-1</sup> )	Gufuskálar wind CO <sub>2</sub> flux (mol C m <sup>-2</sup> y <sup>-1</sup> )	Average CO <sub>2</sub> flux (mol C m <sup>-2</sup> y <sup>-1</sup> )
1	-1.46	-2.48	-1.97
2	-1.36	-2.35	-1.86
3	-1.27	-2.19	-1.73
4	-1.38	-2.40	-1.89
5	-1.15	-1.99	-1.57
Average for stations:	-1.32 ± 0.12	-2.28 ± 0.20	<b>-1.80 ± 0.16</b>

## **Paper IV**

# **Diversity of bivalves and gastropods (Mollusca) in the sub-Arctic North Atlantic: Comparing patterns of alpha- and beta- diversity to the north and south of the Greenland-Iceland-Faroe ridge**

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Author contributions: HE analyzed data and wrote the manuscript, NM analyzed data and edited the manuscript, GG edited the manuscript.



# Diversity of bivalves and gastropods (Mollusca) in the sub-Arctic North Atlantic: comparing patterns of alpha- and beta-diversity to the north and south of the Greenland-Iceland-Faroe ridge

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## Abstract

We describe and compare the diversity of bivalves and gastropods along the bathymetric gradient north and south of the Greenland-Iceland-Faroe (GIF) topographic ridge, which separates the Nordic Seas from the bulk of the North Atlantic. Patterns of  $\alpha$ -diversity were estimated as  $E(S_{20})$ . Regional and depth related  $\beta$ -diversity was analysed and the additive contribution of turnover (species replacement) and nestedness (species loss/gain) to  $\beta$ -diversity calculated using a novel approach.

Despite a significant number of shared species, the diversity patterns varied between the regions and moderately between bivalves and gastropods. North of the GIF ridge, species loss with increasing depth resulted in a predominant decrease in bivalve and gastropod  $\alpha$ -diversity between 300 and 2000 m depth. Accordingly, species assemblages in the lower bathyal zone were partly nested subsets of the assemblages in the shallower bathyal zone. Nestedness resultant dissimilarity contributed to a greater proportion of  $\beta$ -diversity in bivalves north of the GIF ridge compared to gastropods.

South of the GIF ridge,  $\alpha$ -diversity decreased with depth in bivalves, with no clear trend in gastropods. This finding contradicts the recognized increase in  $\alpha$ -diversity in the bathyal zone in the North Atlantic basin, perhaps due to the particular oceanographic regime directly south of the GIF ridge. Species turnover dominated  $\beta$ -diversity south of the GIF ridge.

This study sheds new light on deep sea diversity patterns in the high latitude North Atlantic and provides baseline data on species occurrences, for future explorations of the impacts of ocean warming and acidification on benthic molluscs throughout the region.

**Key words:** Alpha diversity, beta diversity, nestedness, deep sea, sub-Arctic, Iceland

## Introduction

The benthic realm harbours a remarkable proportion of the ocean's biodiversity (Snelgrove 1999). Marine invertebrates that reside on (epifaunal) or in (infaunal) benthic sediments make up a large fraction of this diversity and provide key ecosystem services, for example through provision of structural habitat and through bioturbation (Snelgrove 1999, Queirós et al. 2013). The vast majority (91%) of marine benthic habitat lies below 200 m depth where darkness prevails and autotrophic activity is negligible (Kennish 2000). Accessing the deep ocean is a very costly and logistically challenging endeavour, explaining why ecosystems in the deep sea remain insufficiently studied compared with shallow water or terrestrial systems (May & Godfrey 1994, Higgs & Attrill 2015). It is now more urgent than ever to document and to understand biodiversity patterns and ecological processes in deep-sea benthic ecosystems, as these systems, typified by relative environmental stability (Seibel & Walsh 2003, Hofmann et al. 2011), limited food availability (Lutz et al. 2007) and low temperatures (which limit rate processes) (Childress 1995) may be particularly sensitive to natural and human-induced environmental changes that are occurring now at unprecedented rates (Crain et al. 2008, Gehlen et al. 2014, Rogers 2015).

Since the early work of Hessler and Sanders (1967), much has been learned about general patterns of benthic species biodiversity in the deep ocean and how it changes along bathymetric gradients (Rex & Etter 2010). Alpha ( $\alpha$ ) diversity describes local diversity, for example within a single sample or site. Regional and depth related changes in  $\alpha$ -diversity were the focus of the majority of early studies so that spatial trends are now considered reasonably well understood for several parts of the North Atlantic (Rex & Etter 2010). In general, the  $\alpha$ -diversity of macrofauna in the North Atlantic, excluding the Nordic Seas, exhibit a unimodal diversity pattern: being relatively low in shallower waters, increasing towards the lower bathyal or upper abyssal zones and decreasing towards the deeper abyss (Rex & Etter 2010). Patterns of benthic diversity in the Nordic Seas is strikingly different from that described in the North Atlantic. The  $\alpha$ -diversity in the Nordic Seas and the Arctic Ocean is remarkably low in lower bathyal and abyssal depths (Bett 2001) although the diversity on the shelves is not considered particularly low (Piepenburg et al. 2011). Consequently, there is a steep decline in  $\alpha$ -diversity within the bathyal depth range.

Beta ( $\beta$ ) diversity describes changes in community composition within a region or along a gradient (Whittaker 1960, Whittaker 1972). Increased sampling effort and data availability has facilitated research on  $\beta$ -diversity in the ocean, with results interpreted almost exclusively as spatial replacement of species along depth or horizontal gradients (McClain & Rex 2015). However,  $\beta$ -diversity can also be affected by species loss (or gain) resulting in smaller communities forming ordered subsets of the species composition of larger communities, a pattern also referred to as 'nestedness' (Ulrich & Gotelli 2007, Almeida-Neto et al. 2008). The specific ecological significance of either species replacement or nestedness has driven multiple efforts intended to mathematically quantify the contribution of these components to  $\beta$ -diversity (McClain & Rex 2015). Recently, studies of deep sea diversity have used a method presented by Baselga (2010, 2012) to partition  $\beta$ -diversity into two additive components: nestedness resultant dissimilarity and dissimilarity resulting from species turnover, allowing for new insight into deep sea diversity patterns (Wagstaff et al. 2014, Stuart et al. 2016). For example, Brault et al. (2013b) concluded that abyssal neogastropod assemblages are likely supported



through source-sink dynamics, partly based on a significant increase in nestedness with depth. Although not a commonly used method in research of deep sea diversity, due to the challenge of standardized sampling of macrofauna, a regional comparison approach for  $\alpha$ - and  $\beta$ -diversity patterns can also help identify the processes that drive deep sea diversity patterns (e.g. Brault et al. 2013a).

The Mollusca is a species rich and diverse phyla whose species are among the more conspicuous and biodiverse invertebrate macrofauna (Alongi 1990, Linse et al. 2006). Within the Mollusca phylum, gastropods and bivalves are the most species rich classes (WoRMS 2015); both groups playing crucial roles as keystone species (Paine 1969) or ecosystem engineers in shallow waters ecosystems as a result of their feeding mechanisms or calcification traits (e.g. Hall-Spencer & Moore 2000, Gutiérrez et al. 2003). Calcifying molluscs are considered particularly susceptible to ocean acidification and the concurrent decrease in the saturation calcium carbonate ( $\Omega$ ) (Gazeau et al. 2013).  $\Omega$  also decreases with depth (Millero 2007), yet disentangling the effects of  $\Omega$  and other environmental parameters that relate to depth, such as food availability and sediment characteristics, is challenging and not the focus of this study. However, to understand biodiversity patterns of calcifying molluscs is a first step towards understanding the implication of ocean acidification and the decrease in  $\Omega$  (Urban et al. 2016). The need for baseline data is especially urgent in the Nordic Seas, due to the naturally low calcium carbonate saturation state of seawater which is decreasing at an alarming rate in both surface and deep waters (Olafsson et al. 2009, Skogen et al. 2014).

The Greenland-Iceland-Faroe (GIF) ridge is a topographic barrier that separates two relatively deep ocean basins: the Nordic Sea basin to the north and the bulk of the North Atlantic basin to the south. While the maximum sill depth of the Greenland-Iceland ridge and the Iceland-Faroe Island ridge is 620 m and 480 m respectively, the seafloor reaches depths exceeding 3000 m south and north of the GIF ridge (Jakobsson 2002). The biogeographic boundaries at the GIF ridge coincides with a transition between colder and warmer water masses so that Iceland coincides with the Arctic Front (Hansen & Meincke 1979). Thus, the island is considered to occupy a key position in the North Atlantic in terms of biogeographical species ranges (Briggs 1970, Dahlgren et al. 2000). Comparison of species diversity, including hyperbenthic amphipods (Weisshappel & Svavarsson 1998, Weisshappel 2000), benthic isopods (Svavarsson et al. 1993, Svavarsson 1997) and foraminifera (Gudmundsson 1998) have previously been conducted between benthic habitats directly north and south of the GIF ridge. However, a comparison of mollusc species diversity patterns in the regions has not been conducted to date.

The aim of this study was to increase understanding of biodiversity patterns in the high latitude North Atlantic by comparing  $\alpha$ - and  $\beta$ -diversity patterns in molluscs along the bathymetric gradient north and south of the GIF ridge. To achieve this goal we use a comparative approach and species data on bivalves and gastropods collected during the sampling program BIOICE (Benthic Invertebrates of Icelandic waters).

By adding to the general understanding of biodiversity patterns in the Nordic Seas and North Atlantic south of the GIF ridge, our results provide an important baseline for future work aimed at quantifying ecosystem responses to rapid environmental changes taking place in the region (Urban et al. 2016) – information vital for shaping conservation strategies that minimize the detrimental consequences of such changes (Cavanagh et al. 2016).

## Materials and methods

### *Study regions*

The study regions encompass the Icelandic shelf and slope within the Iceland Sea to the north of the GIF ridge and the Icelandic shelf and slope south of the GIF ridge (Fig. 1). These regions share topographic similarities as they both include the Icelandic shelf and slope and parts of the Mid-Atlantic Ridge but the broad separation by the GIF ridge results in regional hydrographic differences (Stefánsson 1962). The Iceland Sea is a part of the Nordic Seas along with the Norwegian Sea and Greenland Sea (Jakobsson 2002). The hydrographic properties of the Iceland Sea are generally described as Arctic Intermediate Water overlying Arctic Deep Water (Stefánsson 1962, Swift et al. 1980). These waters are characterized by sub-zero temperatures throughout the water column in winter and a shallow (~200 m) mixed layer in the summer, in which temperatures exceed 0°C (Olafsson 2003, Olafsson et al. 2009). The oceanographic environment south of the ridge reflects a dynamic interaction between water masses with different physical properties, with temperatures ranging from 2°C to > 10°C (Hansen & Østerhus 2000, Malmberg & Valdimarsson 2003). Ocean warming and ocean acidification have been observed at the surface in both the Iceland and the Irminger Sea (southwest of Iceland) but changes in deeper waters resulting from anthropogenic activity are particularly apparent down to at least 1800 m in the Iceland Sea over the last two decades (Olafsson et al. 2009, Olafsson et al. 2010).

### *Biological data*

Benthic samples were collected between 20 and 3020 m depth during the years 1991 to 2004 of the BIOICE program (Gudmundsson 1998). Four types of towed sampling gears were used in the program, depending on bottom type, but the present study is limited to a modified RP sled (Rothlisberg & Percy 1977, Brattegard & Fosså 1991) and a Sneli sled (Sneli 1998) since these gears were most frequently deployed and sampled similar species assemblages. Species records and their taxonomic classification were manually examined and edited where necessary to correspond to entries in the database ‘World Register of Marine Species’ (WoRMS 2015) and synonyms standardized to a single name. Information on the sample data considered for the study, details on all species recorded, number of samples they were recorded in and their observed depth range in each study region are provided in a supplemental document (Suppl. document 1).

### *Analytical approach*

Sample data collected using a modified RP sled and Sneli sled was pooled for analysis of biodiversity as the sampled species assemblages were similar between the gear types (see suppl. doc. 1).

The number of unobserved species, in addition to the observed species richness, was estimated based on the singletons and doubletons in species matrices (Chao 1987) along with a standard

error (Chiu et al. 2014). Estimations were carried out for each region and separately for four 500 m depth ranges, between surface and 2000 m depth, below which species data was considered insufficient for the richness estimation.

Due to the qualitative nature of the sled-samples,  $\alpha$ -diversity was estimated using the Sanders-Hurlbert expected number of species (Sanders 1968, Hurlbert 1971) in a sample normalized to 20 individuals ( $E(S_{20})$ ), excluding sample data where fewer individuals were collected. Accordingly, the number of samples available for the analysis were reduced to a total of 252 and 250 for bivalves north and south of the GIF ridge respectively, and a total of 146 and 197 samples for gastropods within the same regions respectively. To investigate how  $E(S_{20})$  varied with depth in each region, samples were averaged into 100 m bins and a linear least squares regression used to identify trends in  $E(S_{20})$  as a function of depth between 300 to 2000 m. A locally weighted regression, or *loess* smoother, was applied to the full depth range to visualize general trends (Cleveland 1979, Cleveland & Devlin 1988).

To investigate the relationship of depth and region with species assemblages of bivalves and gastropods, a 2 dimensional NMDS analysis on presence – absence species matrices was conducted, excluding species occurring in less than 5% of samples in each region. The NMDS analysis was based on Sørensen (Bray-Curtis) sample dissimilarity (Sørensen 1948). The contribution of depth and region to MDS1 (primary NMDS axis) and MDS2 (secondary NMDS axis) were analysed using a least squares linear regression and a Student t-test respectively.

Optimal methods for analysis of  $\beta$ -diversity along environmental gradients require data collected using standardized sampling methods. Although qualitative sled sampling gear was employed in the BIOICE program, sampling sleds were typically towed for a standardized time period of 20 minutes. Nevertheless, we recognize the issues associated with the lack of fully standardized sampling gear and resulting from the non-random and unsystematic spatial distribution of sample localities in the BIOICE program, especially the relatively limited sampling effort at depths below 1500 m. To address these issues, sample data were aggregated into 100 m depth bins, and bins with fewer than 100 and 50 individual bivalves and gastropods respectively, in either or both regions, were discarded from analysis of  $\beta$ -diversity. Data were converted to presence-absence (binary) species matrices. Analysis was restricted to an upper depth limit of 300 m to exclude the shallow water communities on the Iceland shelf in the analysis of  $\beta$ -diversity. As a result, the analysis was restricted to all 14 depth bins within the range of 300 – 1600 m for bivalves but 13 depth bins for gastropods due to the exclusion of the 1200 m depth bin. To investigate  $\beta$ -diversity dynamics within regions, including the contribution of nestedness to  $\beta$ -diversity, we use two independent methods.

Baselga (2010, 2012) describes how  $\beta$ -diversity can be mathematically separated into two components:  $\beta$ -diversity resulting from species replacement between sites which is commonly referred to as species turnover, and the part of dissimilarity that can be attributed to the effect of species loss (or gain) or species nestedness. The calculations of  $\beta$ -diversity and the relative contribution of turnover and nestedness are based on Sørensen (Bray-Curtis) dissimilarity (Sørensen 1948). Multiple site dissimilarity measures (indicated by capital letters) were generated and gave a single score for  $\beta$ -diversity ( $\beta_{SØR}$ ) and the relative contribution of species turnover ( $\beta_{SIM}$ ) and nestedness ( $\beta_{SNE}$ ) to dissimilarity in each region. A comparison between multiple site dissimilarity scores can only be made between regions with equal sample sizes (Baselga 2012). However, a repeated sampling of the dissimilarity scores for a subset of the

original data frame yields average metrics with standard errors that can be compared between regions with different sample sizes (Baselga 2012). Although the depth bins were standardized between regions, we report multiple site dissimilarity scores based on 100 sampling repetitions to increase comparability of the results. Through pairwise comparisons of samples (indicated by lower case letters) we also assessed if  $\beta$ -diversity metrics, referred to as  $\beta_{sor}$ ,  $\beta_{sim}$  and  $\beta_{sne}$ , varied depending on sample depth separations. The distribution of all three metric scores were visualized using Kernel density plots. The relationship between pair-wise dissimilarity scores and sample separation depth was explored through least squares linear regression, but recognizing that pairwise comparisons inflate the degrees of freedom, a regression was only seen as significant where the resulting  $p$ -value for the slope was equal to, or below 0.001.

The direction of nestedness along the bathymetric gradient was explored using the software BINMATNEST (Rodríguez-Gironés & Santamaría 2006, Rodríguez-Gironés et al. 2010). The ‘temperature’ of the species presence – absence matrix which is defined as the sum of ‘surprises’ in an arranged matrix (ranging from 0° to 100°) was determined reflecting a fully nested matrix to nested pattern respectively. The analysis also ranks the depth bins on the basis of nestedness which can be used to inform of the direction of nestedness along an environmental gradient (Stuart et al. 2016). To test if significant nestedness occurred in the species matrices and to rank depth bins on the basis of nestedness, a computation of 1000 null matrices was performed and the species matrices tested for randomness using the recommended choice of null model in BINMATNEST (model 3) (Rodríguez-Gironés & Santamaría 2006).

With the exception of the BINMATNEST software, all data analysis was performed using R (R Core Team 2014), and the *vegan* (Oksanen et al. 2015) and *betapart* packages for R (Baselga & Orme 2012).

## Results

### *Species richness and $\alpha$ -diversity*

Although bivalves were generally more abundant in samples, gastropods were roughly twice as species rich as bivalves in both regions. The recorded depth distribution of bivalve and gastropod species based on sample data collected in the BIOICE program suggests a more rapid loss in species richness with increasing depth north of the GIF ridge compared to that south of the GIF ridge (Fig. 2 & 3). However, these figures also highlight the limited data from the lower bathyal and abyssal depths (> 1500 m), which certainly results in an underestimate of maximum depth distribution for at least some species of bivalves and gastropods.

The total observed bivalve species richness was 85 north of the GIF ridge and 114 in south of the GIF ridge, with 71 species shared between both regions. Based on the analysis of undetected species richness, the total estimated bivalve species richness was  $95 \pm 8$  and  $137 \pm 15$  north and south of the GIF ridge respectively. A total bivalve species richness of  $7 \pm 1$  was estimated for the depth range 1501-2000 m north of the GIF ridge compared to a total of  $34 \pm 6$  south of the GIF ridge (Fig. 4). The observed gastropod species richness was 173 and 189 north and south of the GIF ridge respectively, with 133 species shared between regions.

Estimated total gastropod species richness was  $201 \pm 12$  and  $266 \pm 33$  north and south of the GIF ridge. Gastropod species estimated within the depth range 1501-2000 m was  $19 \pm 11$  and  $73 \pm 9$  north and south of the GIF ridge, respectively (Fig. 4).

Analysis of  $E(S_{20})$  for bivalves and gastropods north of the GIF ridge indicates a unimodal relationship with depth from the Iceland shelf to the lower bathyal depths, reaching a maximum of  $\sim 5$  species between 100 and 600 m (Fig. 5). In the same region a linear regression model of  $E(S_{20})$  fitted within the 300 - 2000 m depth range presents a steep decrease in both bivalve and gastropod  $\alpha$ -diversity along the bathymetric gradient (Table 1). South of the GIF ridge a similar unimodal relationship between  $\alpha$ -diversity and depth was observed in bivalves and a modest decrease within the 300 - 2000 m depth range. No clear trend in gastropod  $E(S_{20})$  was identified south of the GIF ridge (Fig. 5).

### *Species communities*

Depth influenced species community composition more strongly than region in both bivalves and gastropod, but the relationships between these factors and species composition was stronger for bivalves compared to gastropods (Fig. 6). The MDS1 score was strongly correlated with the logarithm of depth in bivalves (Pearson's product-moment correlation:  $r = 0.84$ ,  $t = 33$ ,  $df = 469$ ,  $p < 0.001$ ) and the MDS2 differed between the study regions (t-test:  $t = -26$ ,  $df = 458$ ,  $p < 0.001$ ). The MDS1 score for gastropod assemblages was also significantly correlated with the logarithm of depth although not as strongly as in bivalves (Pearson's product-moment correlation:  $r = 0.50$ ,  $t = -12$ ,  $df = 406$ ,  $p < 0.001$ ) and the MDS2 also differed by region (t-test:  $t = 11$ ,  $df = 405$ ,  $p < 0.001$ ).

Analysis of the  $\beta$ -diversity dynamics in bivalve and gastropod species assemblages between 300-1600 m suggests that although the overall  $\beta$ -diversity is comparable between the bathymetric gradient north and south of the GIF ridge, the relative contribution of nestedness and turnover to dissimilarity differs between the regions.

In bivalves,  $\beta$ -diversity was higher north of the GIF ridge compared to that south of the GIF ridge (t-test on pairwise scores for  $\beta_{\text{SOR}}$ :  $t = 40.5$ ,  $df = 189$ ,  $p < 0.001$ ; Fig. 7). Multiple site dissimilarities for the region north of the GIF ridge indicate a similar contribution of nestedness ( $\beta_{\text{SNE}}$ ) and turnover ( $\beta_{\text{SIM}}$ ) to the overall  $\beta$ -diversity ( $\beta_{\text{SOR}}$ ) in the region with  $\beta_{\text{SOR}} = 0.76 \pm 0.02$ ,  $\beta_{\text{SIM}} = 0.39 \pm 0.02$  and  $\beta_{\text{SNE}} = 0.37 \pm 0.03$ . Nestedness contributed to a lesser degree to the regional dissimilarity south of the GIF ridge where  $\beta_{\text{SOR}} = 0.74 \pm 0.01$ ,  $\beta_{\text{SIM}} = 0.58 \pm 0.03$  and  $\beta_{\text{SNE}} = 0.17 \pm 0.03$ . Pair-wise scores suggest that species assemblage differences increase with increasing depth separation in both regions, i.e.  $\beta_{\text{SOR}}$  was positively related to depth separation. The increase in  $\beta_{\text{SOR}}$  reflect an higher  $\beta_{\text{SNE}}$  scores with increasing depth separation north of the GIF ridge, whereas species turnover contributes to a greater extent to the change in species assemblages south of the GIF ridge (Fig. 7).

In gastropods,  $\beta$ -diversity was higher north of the GIF ridge compared to that south of the GIF ridge (t-test on pairwise scores for  $\beta_{\text{SOR}}$ :  $t = 47.8$ ,  $df = 163$ ,  $p < 0.001$ ; Fig. 7). Multiple site metrics for the region north of the GIF ridge,  $\beta_{\text{SOR}} = 0.81 \pm 0.01$ ,  $\beta_{\text{SIM}} = 0.60 \pm 0.02$  and  $\beta_{\text{SNE}} = 0.21 \pm 0.02$  suggest a lesser contribution of nestedness to  $\beta$ -diversity in gastropods compared to bivalves. Multiple site metrics gastropod assemblages in the region south of the GIF ridge were  $\beta_{\text{SOR}} = 0.75 \pm 0.01$ ,  $\beta_{\text{SIM}} = 0.64 \pm 0.02$  and  $\beta_{\text{SNE}} = 0.11 \pm 0.02$ . Results of pair-wise

comparisons for gastropods indicate an increase in  $\beta$ -diversity ( $\beta_{\text{sor}}$ ) with increasing depth separation in both regions. An increase in  $\beta_{\text{sne}}$  with increasing depth separation was observed north of the GIF ridge in gastropods, similar to the trend observed in bivalves. Contrary to that observed in bivalves there was also a statistically significant increase in  $\beta_{\text{sne}}$  with depth in gastropods south of the GIF ridge, but the slope of the relationship was modest. Regression models for gastropods did not detect a trend in  $\beta_{\text{sim}}$  with increasing depth separation for either region.

The BINMATNEST test rejected the null hypothesis of no nestedness in bivalve and gastropod species matrices in both regions. A correlation of the rank order of nestedness for each depth bin also indicated a significant linear increase in nestedness with increasing depth (Fig. 8). The relationship was relatively strong north of the GIF ridge but relatively weak south of the GIF ridge in both bivalves and gastropods.

## Discussion

### *Species richness and $\alpha$ -diversity*

We found that species richness on the Icelandic shelf north and south of the GIF ridge was similar. This result supports the conclusion of Piepenburg et al. (2011) that benthic species richness on the shelves (above ~ 200 m) within the Arctic Seas is not particularly low compared to species richness at lower latitudes, as has been previously suggested based on limited sample data. However, we observed a pronounced decrease in  $\alpha$ -diversity with increasing depth between 300 and 2000 meter depth north of the GIF ridge, in both bivalves and gastropods. Notably, species richness estimates indicate an order of a magnitude decrease from the 501-1000 m depth range to the 1501-2000 m depth range in bivalves. These findings are in accordance with a decrease in  $\alpha$ -diversity with increasing depth that has previously been described in other benthic taxa in the Nordic Seas, including isopods (Svavarsson 1997), amphipods (Stephensen 1940, Weisshappel & Svavarsson 1998), polychaetes (Narayanaswamy et al. 2005, Oug et al. 2016), foraminifera (Mackensen et al. 1985, Gudmundsson 2002) and the overall macrofauna (Bett 2001). In fact, studies on benthic diversity in the deep Nordic Sea basin have consistently described low species richness compared to deep zones in other seas and ocean basins, including the North Atlantic (Bouchet & Warén 1979, Dahl 1979, Rex et al. 2000). The  $\alpha$ -diversity trends presented here are also similar to that described for the Arctic Ocean as a whole (Bodil et al. 2011).

The  $\alpha$ -diversity patterns observed south of the GIF ridge, i.e. a decrease in  $E(S_{20})$  in bivalves along the bathymetric gradient and a lack of trend in gastropod  $E(S_{20})$ , contrasts with previously described diversity patterns directly south of the GIF ridge and in the broader North Atlantic (Rex & Etter 2010). For example, Svavarsson (1997) reported an significant increase in isopod  $E(S_{200})$  directly south of the GIF ridge from 200 m to 1500 m, but it is important to note that isopod diversity trends are known to deviate from general trends in other macrofauna as the order includes many species specifically adapted to the deep-sea (Hessler et al. 1979, Rex & Etter 2010). In the broader North Atlantic, Bett (2001) and Narayanaswamy et al. (2005) also reported an increase in overall macrofauna and polychaete  $E(S_n)$  between 200 and 2000 m depth in the Rockall Trough, south of the Wyville-Thomson ridge. Also, Brault et al.

(2013a) described an increase in  $E(S_{20})$  towards 2000 m depth in bivalves in the western and eastern North Atlantic. The explanation for the observed deviation from the general trend directly south of the GIF ridge is unknown and requires further comprehensive data on the environmental setting of the region. It is however possible, that the oceanographic regime directly south of the GIF ridge might affect biodiversity patterns (Gudmundsson 1998), in particular, the relatively brisk current below  $\sim 1500$  m depth, carrying North Atlantic Deep Water (NADW) mixed with cool overflow water from the Nordic Seas westward along the Icelandic slope (Dickson et al. 1990, Logemann et al. 2013). Current regimes can also impact upon sediment properties through resuspension of fine sediments, with sediment particle size recognized as an important driver of benthic diversity (Etter & Grassle 1992, Gray 2002). A numerical modelling study of sediments indicated that fine grained sediments or clay did not increase with increasing depth south of the GIF ridge as was observed north of the GIF ridge (Ostmann et al. 2014). However, due to the likely patchy sediment environments along the bathymetric gradient (Gray 1981) and few benthic sampling localities (11) included in the study (Ostmann et al. 2014), the importance of sediment composition in driving diversity trends south of the GIF ridge remains uncertain.

### *Species communities*

General trends in bivalve and gastropod  $\alpha$ - and  $\beta$ -diversity were similar north and south of the GIF ridge, but there were some marked differences between the groups. A difference is not unexpected as bivalves and gastropods have evolved independently for nearly 500 million years and have different habitat requirements and feeding mechanisms that should be considered (Ponder & Lindberg 2008). Most bivalves have low mobility, are often infaunal and are generally suspension or deposit feeders (Roy et al. 2000). Gastropods, on the other hand, are richer in species than bivalves, are functionally more diverse, operating as predators, grazers, omnivores, deposit feeders or carnivores, and are largely epifaunal (Hughes 1986).

This considered, it is possible that the greater phylogenetic- and functional diversity of gastropods, could account for the larger unexplained variance in overall gastropod assemblages as compared to bivalve assemblages (Fig. 6). Despite these differences, a similar pattern was observed in bivalves and gastropods, i.e. that species assemblages varied more as a function of water depth than region, indicating that water depth, or specifically the environmental changes associated with water depth, are a significant driver of  $\beta$ -diversity in both regions. This is in agreement with McClain et al. (2012), who described depth to be a better predictor of deep-sea bivalve  $\beta$ -diversity than geographical separation.

Our exploration of the BIOICE data also found that a significant number of species were shared between regions to the north and south of the GIF ridge, despite environmental differences.

This, in conjunction with the rapid decrease in  $\alpha$ -diversity along the bathymetric gradient may, at least in part, reflect the geological history of the North Atlantic (Barry et al. 2013). During the last glacial maximum, ice sheets covered the Nordic Seas, limiting surface primary production and the subsequent downward flux of organic matter to benthos. The benthic environment changed dramatically when the ice retreated  $\sim 14$  thousand years ago resulting in a significant increase in primary production at the surface and changes in water circulation

(Smith et al. 1997, Müller et al. 2009). While Pleistocene glaciations likely eradicated much of the shelf and deep sea fauna in the Arctic, other shelf fauna found refuge in the deep sea and is considered the ancestral fauna of some of today's Arctic deep sea fauna (Nesis 1984). Thus, a large proportion of the fauna on the shelves of the Nordic Seas may have been introduced from the North Atlantic within the Holocene.

Although the geological history of the North Atlantic is likely to play a significant role in shaping the species composition in the Nordic Seas, recent studies provide evidence supporting previous hypothesis indicating that energy dynamics, including chemical energy (i.e. food availability) and kinetic energy (i.e. temperature) are the most important drivers of both  $\alpha$ - and  $\beta$ -diversity patterns in the benthic marine environment (Tittensor et al. 2010, Yasuhara & Danovaro 2016). Temperature is a particularly important driver at temperatures below 5°C (Yasuhara & Danovaro 2016) and thus the transition from ~ 2-3° south of the GIF ridge to a ~ -1°C north of the ridge is likely to present many species with an physiological barrier. Food availability and temperature are not mutually exclusive drivers but can influence species performance synergistically (e.g. Brockington & Clarke 2001). The ocean surface north and south of the GIF ridge exhibit significant seasonality in surface primary production, but there is notably shorter phytoplankton blooms in the Iceland Sea, north of the GIF ridge (Pálsson et al. 2012, McGinty et al. 2016). Therefore, the benthic community in the deep Nordic Seas may be largely composed of species who have successfully adapted to the extreme energy constraints in the region.

The majority of the bivalve and gastropod species sampled north of the GIF ridge were recorded at depths shallower than 500 m, with a few species showing an extended distribution throughout the bathyal range (Fig. 2). No gastropods species were observed in the few samples collected below 2000 m. However, the epibenthic pectinid bivalve *Hyalopecten frigidus* (the species with deepest distribution in Fig. 2 - north of the GIF ridge) was observed in the majority of samples collected below 2000 m, suggesting that it is well adapted to the energy constraints of the deep Nordic Sea basin.

Based on Baselga's (2010) partitioning and the BINMATNEST rank order for bivalve and gastropod assemblages north of the GIF ridge, increasing community dissimilarity is largely due to the increasing nestedness, or species loss with depth, and is related to the concurrent decrease in  $\alpha$ -diversity. Brault et al. (2013b) also reported lowered  $\alpha$ -diversity, coupled with increasing nestedness from bathyal to abyssal depths in neogastropods. They argued that this pattern provides evidence of source-sink dynamics, i.e. that the abyssal populations are not self-sustaining due to the poor habitat quality at greater depths and require introduction of individuals from shallower habitats (Rex et al. 2005, Brault et al. 2013b). Similarly, the increase in nestedness north of the GIF ridge in concert with the decreasing  $\alpha$ -diversity could indicate that at least some populations of bivalves and gastropods are not self-sustainable in the deeper part of their observed depth ranges, but instead require introduction from a 'source'-population at shallower depths where food is more abundant and calcium carbonate saturation states ( $\Omega$ ) higher. For example, bivalve molluscs have been shown to require species specific  $\Omega$  for successful larval development (Waldbusser et al. 2015). However, as pointed out by Brault et al. (2013b) the diversity trends we observed do not provide sufficient evidence in themselves to establish the existence of source-sink dynamics in a meta-population. This would require information the genetic structure on the 'source' and 'sink' populations (e.g. Manier & Arnold 2005).



The contribution of species turnover to  $\beta$ -diversity was considerably greater south of the GIF ridge and nestedness resultant dissimilarity less significant. Nevertheless, the BINMATNEST analysis of nestedness indicated a significant increase in nestedness with depth south of the GIF ridge, albeit the correlation was relatively weak compared to that north of the GIF ridge. This was an unexpected finding as species turnover was expected to dominate  $\beta$ -diversity to a greater extent in this region, but likely relates to the aforementioned unexpected decrease in  $\alpha$ -diversity with increasing depth observed south of the GIF ridge.

### *Conclusions and recommendations*

Biodiversity patterns of bivalves and gastropods differed considerably between the bathymetric gradients north and south of the GIF ridge. A clear decrease in  $\alpha$ -diversity and an increase in nestedness with depth was observed north of the GIF ridge, whereas a moderate decrease, and no decrease in  $\alpha$ -diversity was observed in bivalves and gastropods, respectively, south of the GIF ridge where species turnover is the dominant  $\beta$ -diversity component.

Data presented here contribute significantly to the knowledge of molluscan biodiversity and species composition along bathymetric gradients in the high latitude North Atlantic. This information is important in guiding future attempts to evaluate the degree of vulnerability in bivalve and gastropods communities in the Nordic Seas where environmental change related to ocean warming and acidification is observed and predicted (Olafsson et al. 2009, Bopp et al. 2013) and for efforts to predict the implications of these changes for deep sea benthic communities more generally (Widdicombe & Spicer 2008, Urban et al. 2016).

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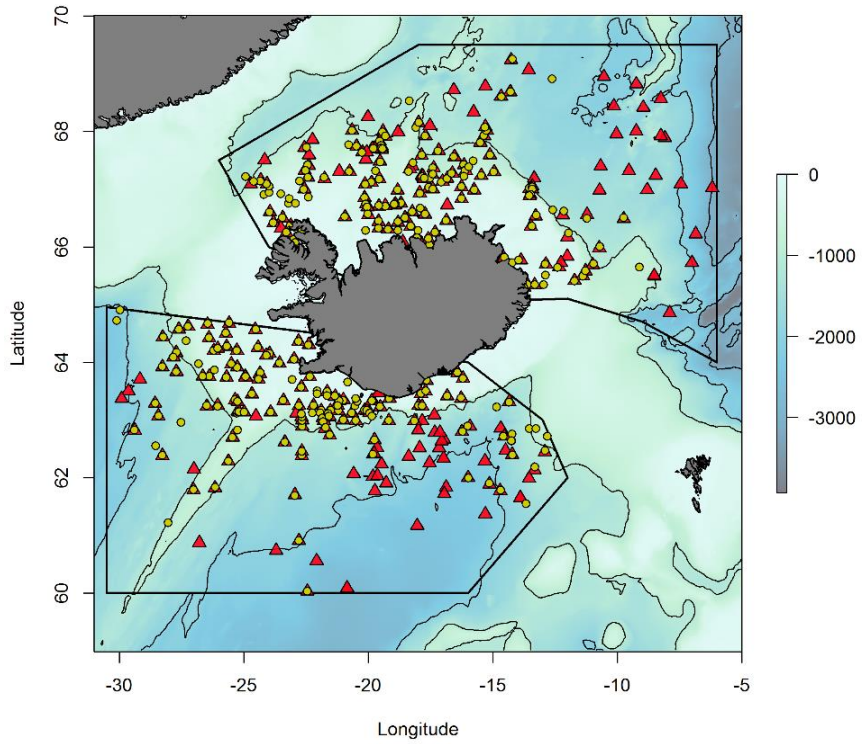
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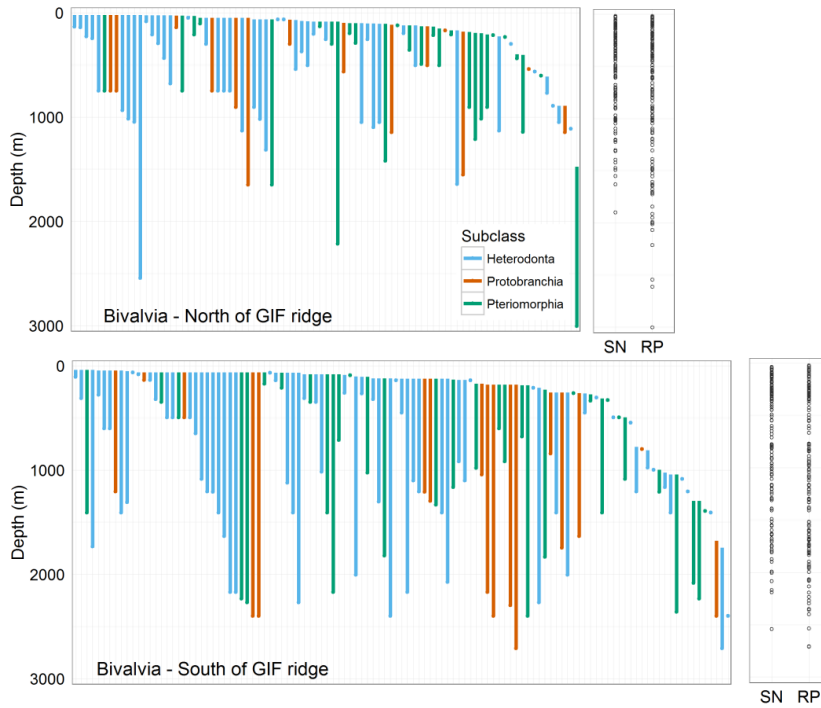
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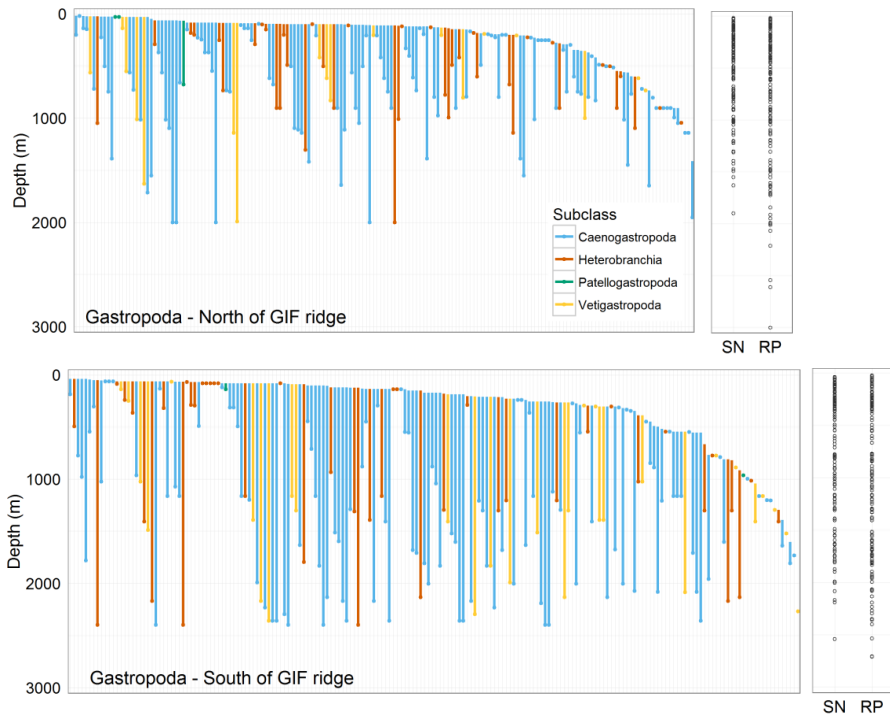


**Fig. 1.** The study regions north and south of the Greenland-Iceland-Faroe ridge and geographic location of BIOICE samples included in the study. Red triangles and yellow circles are localities of benthic samples collected through towing a modified RP sledge and Sneli sledge respectively. Contour lines are placed at 1000 meter depth intervals.

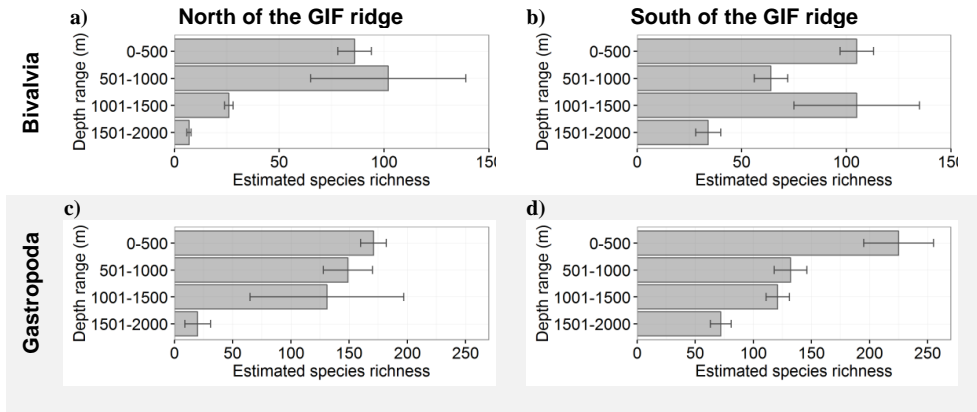


**Fig. 2.** The observed depth ranges of bivalve species in the Iceland Sea basin north of the GIF ridge (top) and along the bathymetric gradient south of the GIF ridge (bottom). Each vertical bar represents the depth range of one species. On the right are the depths of individual samples collected in the regions as part of the BIOICE program, separated by sled gear with ‘SN’ and ‘RP’ representing Sneli sled and RP sled respectively.

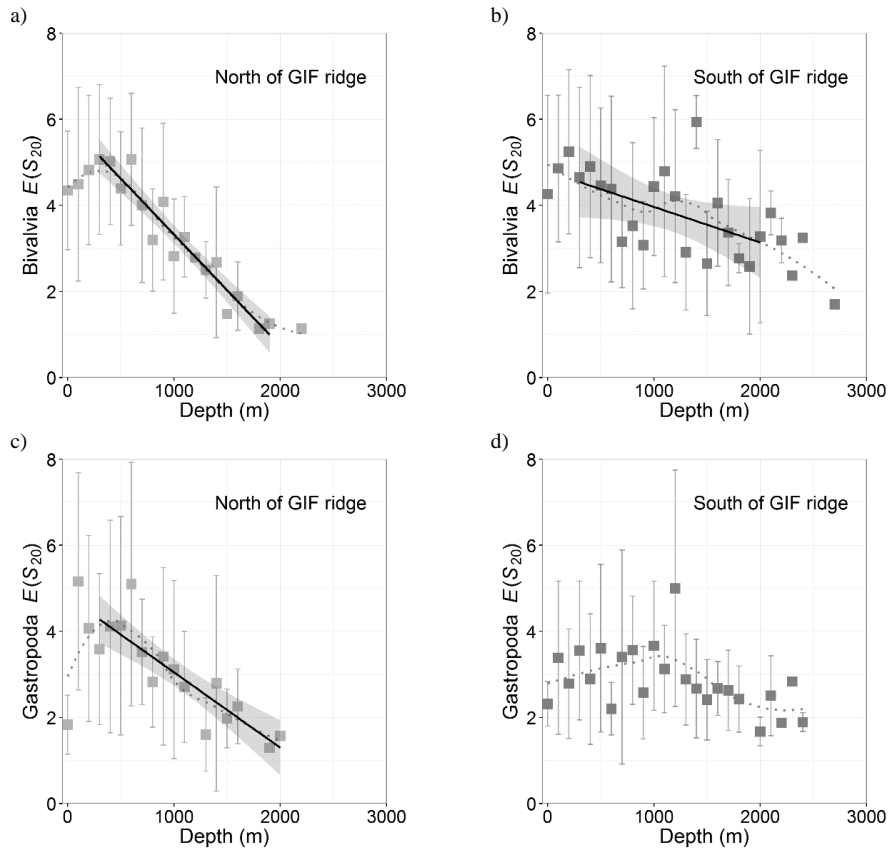




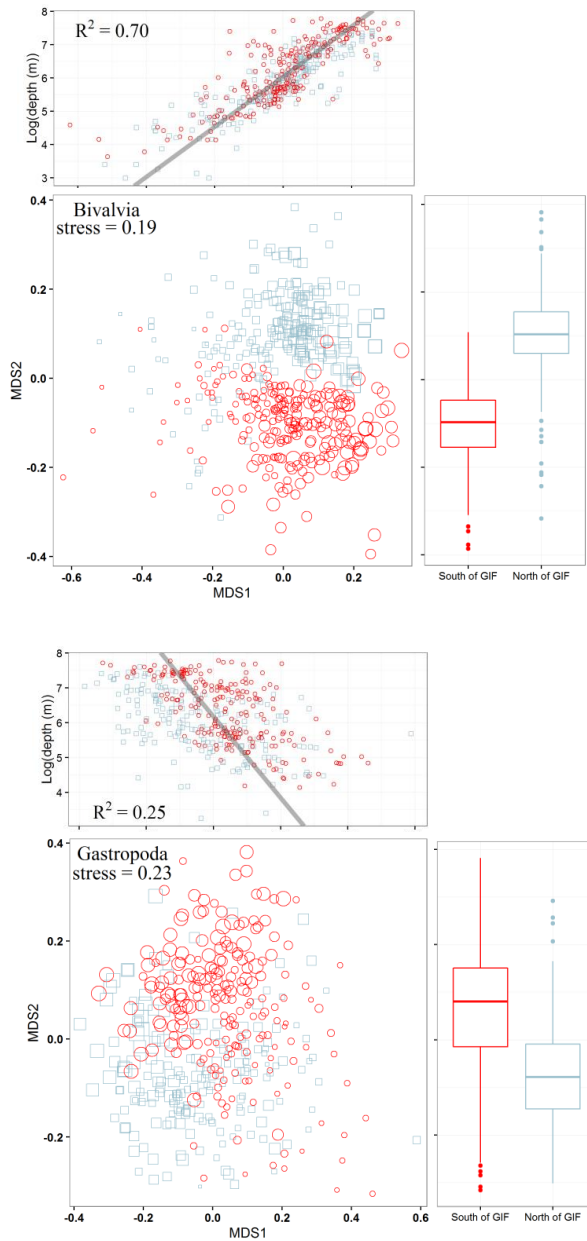
**Fig. 3.** The observed depth ranges of gastropod species in the Iceland Sea basin north of the GIF ridge (top) and along the bathymetric gradient south of the GIF ridge (bottom). Each vertical bar represents the depth range of one species. On the right are the depths of individual samples collected in the regions as part of the BIOICE program, separated by sled gear with ‘SN’ and ‘RP’ representing Sneli sled and RP sled respectively.



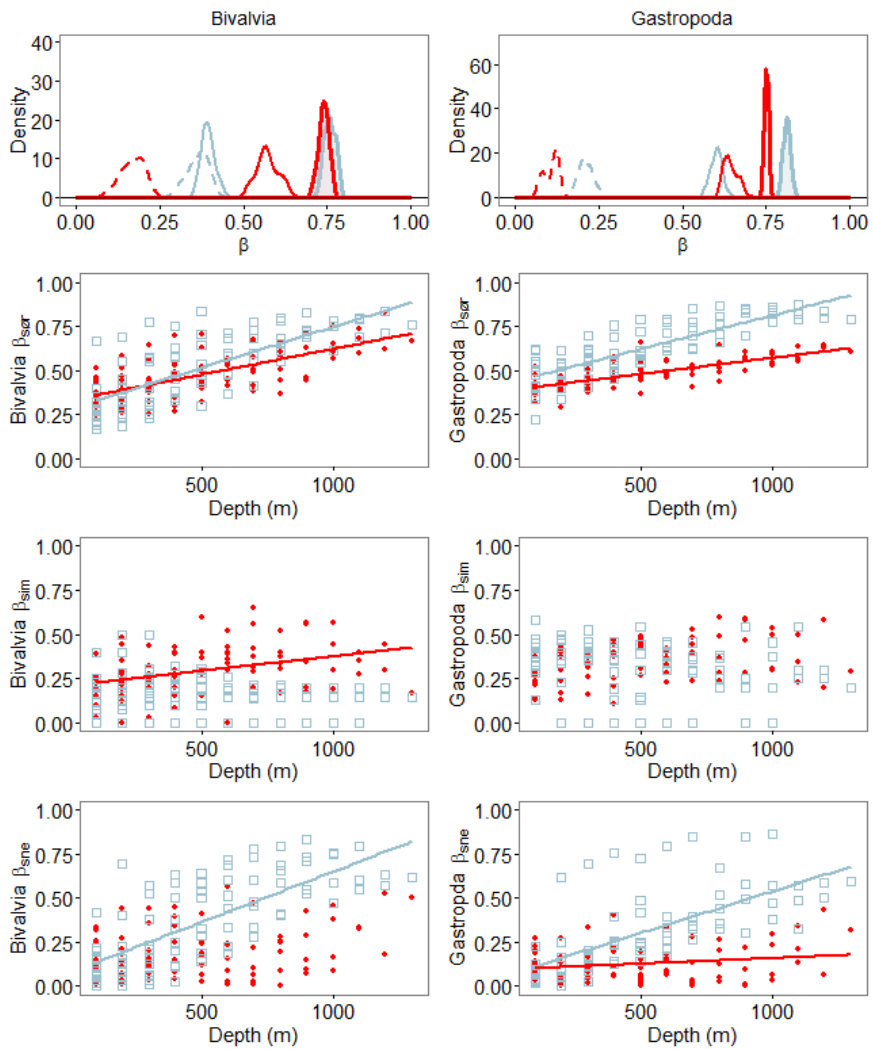
**Fig. 4.** Estimated species richness within 500 m depth intervals based on (Chao 1987) and error bars represent standard errors of the estimates based on Chiu et al. (2014) for a) bivalves north of the GIF ridge and b) bivalves south of the GIF ridge, c) gastropods north of the GIF ridge and d) gastropods south of the GIF ridge.



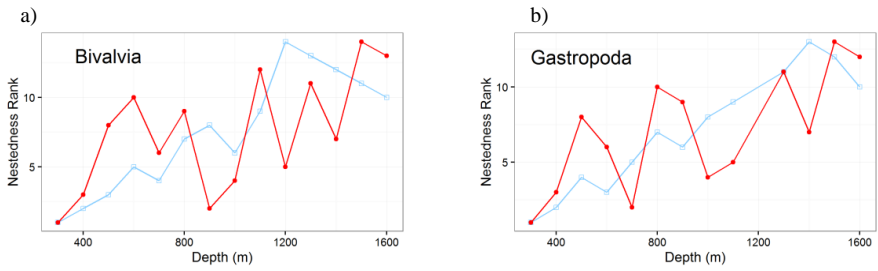
**Fig. 5.** Hurlbert-Sanders expected number of species in a sample of 20 individuals,  $E(S_{20})$ , for a) bivalves north of the GIF ridge, b) bivalves south of the GIF ridge, c) gastropods north of the GIF ridge and d) gastropods south of the GIF ridge. Rarefied samples were averaged into 100 m depth bins with error bars representing standard deviations but where only one sample was available for rarefaction there is no error bar shown. Black lines and shaded region are fitted linear regression models and standard error respectively. Dotted line represents a *loess* smoother for identifying trends throughout the depth range.



**Fig. 6.** Two-dimensional non-metric multidimensional scaling (NMSD) plots for bivalve and gastropod assemblages. For each class a central plot shows the results of the analysis with sample data representing the Iceland Sea basin north of the GIF ridge (pale blue squares) and the region south of the GIF ridge (red circles). The point size relates to the logarithm of sample depth. On top is a correlation of the MDS1 scores and the logarithm of depth which was significant in bivalves and gastropods and to the right a boxplot shows the significant regional difference for MDS2 scores in both groups.



**Fig. 7.** Pair-wise dissimilarity of bivalve (left) and gastropod (right) assemblages as a function of depth separation among depth bins in the Iceland Sea north of the GIF ridge (pale blue square) and south of the GIF ridge (red points). Pairwise overall  $\beta$ -diversity, or  $\beta_{ser}$  is partitioned into  $\beta_{sim}$  or dissimilarity resulting from species replacement and  $\beta_{sne}$  or dissimilarity resulting from nestedness or species loss (or gain). Regression lines indicate where least square linear regression resulted in a  $p < 0.001$  for the slope. Top graphs are Kernel density plots showing the distribution of  $\beta_{ser}$  (line and shaded area below line),  $\beta_{sim}$  (line and white area below) and  $\beta_{sne}$  (broken line).



**Fig. 8.** The rank order of nestedness calculated using the BINMATNEST software for a) bivalves and b) gastropods, north (pale blue squares and line) and south (red dots and line) of the GIF ridge.

**Table 1.** Results from least square linear regression models of  $E(S_{20})$  as a function of depth between 300 and 2000 m. Coefficients are based on the equation  $y = a + b d$ , where  $a$  is the intercept and  $b$  is the slope which was statistically evaluated, and ‘d’ is depth in meters. There was not a statistically significant linear relations in Gastropoda south of the GIF ridge.

Class	Region	$a$	$b$	Std. Error	t-value	$R^2$	$p$ -value
Bivalvia	North of GIF	5.92	-0.00259	0.00020	-12.84	0.92	***
	South of GIF	4.79	-0.00083	0.00039	-2.18	0.22	*
Gastropoda	North of GIF	4.80	-0.00175	0.00028	-6.34	0.76	***
	South of GIF				ns		ns

‘\*\*\*’ represents  $p < 0.001$ , ‘\*\*’ represents for  $p < 0.01$ , ‘\*’ represents  $p < 0.05$  and ns represents  $p > 0.05$ .

**Table 2.** Results from linear least squares regression models of Baselga’s (2010, 2012) pairwise  $\beta$ -diversity metrics as a function of depth separation between 300 and 1600 m. Coefficients are based on the equation  $y = a + b ds$  where  $a$  is the intercept and  $b$  is the slope which was statistically evaluated and ‘ds’ stands for depth separation.

Class	Region	Metric	$a$	$b$	Std. Error	t-value	$R^2$	$p$ -value
Bivalvia	North of GIF	$\beta_{sor}$	0.28	0.00046	0.00004	10.9	0.57	***
		$\beta_{sim}$						ns
		$\beta_{sne}$	0.08	0.00057	0.00006	10.1	0.53	***
	South of GIF	$\beta_{sor}$	0.33	0.00029	0.00003	8.9	0.47	***
		$\beta_{sim}$	0.21	0.00017	0.00004	4.0	0.15	***
		$\beta_{sne}$						ns
Gastropoda	North of GIF	$\beta_{sor}$	0.43	0.00038	0.00003	12.5	0.67	***
		$\beta_{sim}$						ns
		$\beta_{sne}$	0.07	0.00047	0.00006	7.9	0.45	***
	South of GIF	$\beta_{sor}$	0.39	0.00018	0.00002	9.8	0.56	***
		$\beta_{sim}$						ns
		$\beta_{sne}$						ns

‘\*\*\*’ represents  $p < 0.001$ , ns represents  $p > 0.001$





## Final words

Natural sciences are fundamental for the advancement of human societies in the future, so they may prosper in piece with each other and nature.

When reflecting on his working life, the natural scientist Charles Darwin wrote:

*“Whenever I have found out that I have blundered, or that my work has been imperfect, and when I have been contemptuously criticized, and even when I have been overpraised, so that I felt mortified, it has been my greatest comfort to say hundreds of times to myself that ‘I have worked as hard and as well as I could, and no man can do more than this.’ I remember when in Good Success Bay, in Tierra del Fuego, thinking that I could not employ my life better than in adding a little to Natural Sciences. This I have done to the best of my abilities, and critics may say what they like, but they cannot destroy this conviction.”*

(in *Life and Letters of Charles Darwin*, 1887)

These humble words serve to remind of the honor and privilege of getting to add a little to the natural sciences, regardless of how meager the contribution may be in comparison to that made by Charles Darwin.

