The effect of ocean acidification on plant-animal interactions in a *Posidonia oceanica* meadow

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The effect of ocean acidification on plant- animal interactions in a *Posidonia oceanica* meadow

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Dedicated to my family for all of your support- thank you!

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"How inappropriate to call this planet Earth, when it is quite clearly Ocean"

~Arthur C. Clarke~

Abstract

Ocean acidification may be one of the greatest challenges facing society over the next 50-100 years. In the Mediterranean Sea, meadows of the seagrass *Posidonia oceanica* play a pivotal role in coastal systems, providing shelter and food for a highly diversified plant and animal community, and nursery for several commercial species of fish and bivalves. This thesis aims to provide a broad overview of the effect of acidification on *P. oceanica*, and its associated community, through observation of seagrass meadows associated with subtidal CO_2 vents off the Castello Aragonese, Ischia, Italy. Control (mean pH 8.1) and acidified (mean pH 7.8) stations were situated in seagrass meadows on both the north and south side of the Castello Aragonese. Research was concentrated into three main objectives; to investigate the effect of acidification on 1) plant structure and function, 2) invertebrate community dynamics, and 3) macroherbivore grazing pressure. Results suggested that *P. oceanica* and its associated community should be robust to levels of acidification projected for the end of this century. Seagrass density, invertebrate abundance, and *P. oceanica* grazing pressure all increased in response to acidification. Although a slight decrease in invertebrate diversity was observed in acidified stations, species richness was maintained. Increased grazing by the fish *Salpa salpa* was most highly correlated to a decrease in epiphytic algal cover and C:N content of the leaves, and an increase seagrass density. Whilst fish may select leaves with low algal cover and C:N content, previous research suggests that increased shoot density is a response to high grazing pressure. The abundance of many groups of invertebrate taxa was positively correlated to shoot density, whilst decapods decreased during months of reduced canopy height, suggesting changes in *P. oceanica* condition variables, in response to acidification, may lead to a cascade of indirect effects which have positive or negative influences on the abundance of the associated invertebrate community. One of the most striking findings of this thesis was the ability of calcifying species to persevere, and even flourish in acidified conditions. This may be due to the complex nature of the seagrass, and high levels of photosynthesis, leading to local maintenance of pH microclimates. Secondly, the high degree of pH variability that naturally occurs in these shallow water habitats may result in organisms that are be better equipped to withstand pH variation. This research suggests that highly productive, non-calcifying, biogenic habitats, such as seagrass beds, may provide a refuge from OA, and highlights the importance of their conservation.

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First and foremost, I would like to thank my supervisors: Dr Maria Cristina Buia, Dr Maria Cristina Gambi and Prof David Paterson for their advice and support throughout this project. The two Cristina's for their priceless knowledge on the local flora and fauna, and support both below and above water, without which this project would not have been possible, and Dave for your constructive criticism, and comments, which have hugely helped with the write-up of this thesis.

Carrying out my PhD research at the Stazione Zoologica Anton Dohrn in Ischia, Italy has been an amazing experience, because of the opportunity to study the unique CO_2 vents present in Ischia, but also due to the welcome and assistance of the laboratory staff at Villa Dohrn. I would like to thank Captain Vincenzo Rando, Bruno Iacono and Maurizio Lorenti for their assistance with fieldwork. The extremely time consuming task of taxonomical identification of invertebrates and placement into feeding guilds was performed by staff at Villa Dorhn: Maria Beatrice Scipione for amphipods, Francesco Paulo Patti for molluscs, Maria Cristina Gambi for polychaetes, Maurizio Lorenti for tanaids and isopods, and Valerio Zupo for decapods. The opportunity to be able to evaluate invertebrate communities at lower taxonomical levels is really appreciated. I wish to thank Vincenzo Saggiomo and his staff at the Management and Ecology of Coastal Areas for assistance with nutrient and elemental analysis. Many thanks to Sara Fioretti for her assistance with sorting invertebrate samples, you were a great help, not only in your work, but in providing companionship during the long hours staring down a microscope. Thanks to Piero Calosi at the University of Plymouth for the use of the TA meter, it is much appreciated.

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1. Introduction

1.1 Introduction

Acidification of the oceans may be one of the greatest challenges facing society over the next 50-100 years. The role of the ocean in supporting goods and services cannot be underestimated. In order to provide policy relevant science, we must understand how acidification will affect the marine environment at the ecosystem level. This thesis aims to provide a holistic overview of the responses of the most productive species of seagrass in the Mediterranean, *Posidonia oceanica*, and its associated community, to natural acidification observed at CO_2 vents off the island of Ischia, Italy.

1.2 Ocean chemistry

The release of anthropogenic greenhouse gases into the atmosphere has increased steadily since the industrial revolution. Greenhouse gases are trace gases such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (NO). These gases are of concern as they alter the energy budget of the earth by changing the net balance of incoming solar radiation and outgoing infrared radiation between the troposphere and the stratosphere. This change is called radiative forcing. Greenhouse gases reduce the amount of energy leaving the atmosphere, leading to a warming effect. Evidence that our planet is warming is now unequivocal (IPCC 2007; European Environment Agency 2012). Other climatic effects from the increase of these gases include; increased intensity of tropical cyclones (Krishnamurti et al. 1998) and increased frequency of El Niño like conditions (Timmermann et al. 1999), alongside conditions of greater precipitation or drought (IPCC 2007). Climate change can be defined as 'a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods' (UNFCCC 1992).

Of the greenhouse gases released, CO_2 is of the greatest concern, due to the large quantities released (IPCC 2007). Anthropogenic CO_2 release primarily comes from two sources: 1) emissions from fossil-fuel combustion and industrial processes and 2) the CO_2 flux from land-use changes (particularly forest clearing) (Raupach et al. 2007). Emissions from fossil-fuel combustion and industrial processes are of most concern as this produces more than five times the quantity of CO_2 released from land-use changes (7.9 GtC y^{-1} versus 1.5 GtC y^{-1}), and emissions are accelerating rapidly (Raupach and Canadell 2007). The concentration of CO_2 in the atmosphere has risen from 280ppm in preindustrial times to a level of 390ppm in 2010, an increase of approximately 40% (Fig 1.1). In 2013 levels of 400ppm were measured at Mauna Loa volcano for the first time in recorder history (Showstack 2013). This level is expected to increase to over 750ppm by the end of the century (IPCC 2001a; Raven et al. 2005)

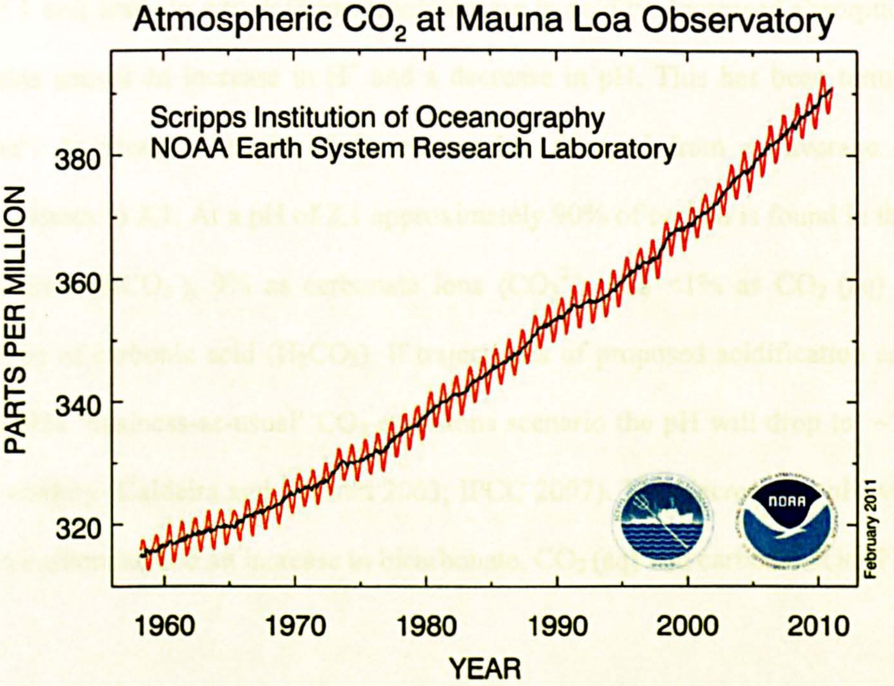


Figure 1.1 Mean monthly atmospheric CO_2 levels found at the Mauna Loa Laboratory, Hawaii. The red curve signifies monthly averages, whilst the black curve represents seasonally corrected data (NOAA 2011).

The release of CO_2 into the atmosphere does not only affect the earth's climate, but has consequences for the future of the world's oceans. Our seas have currently absorbed over 500 billion tons of CO_2 since preindustrial times, which equates to over one third of anthropogenic carbon emissions since the industrial revolution (Sabine and Feely 2007). The rate of absorption is expected to increase significantly in the future (Riebesell et al. 2007), and this increase will drastically change ocean chemistry. When CO_2 is dissolved in seawater, rapid and reversible reactions cause it to exist in four forms; CO_2 (aq), carbonic acid (H_2CO_3), bicarbonate (+ HCO_3^-), and carbonate (CO_3^{2-}):

$$CO_2 + H_2O = H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + H^+$$

The ratio of these forms is dependent on the pH of the seawater. pH is measured on a logarithmic scale and determined by the quantity of H^+ ions present ($pH = -\log(H^+)$), hence a decrease of 1 unit leads to a tenfold increase/decrease in acidity. Increased absorption of CO_2 by the oceans causes an increase in H^+ and a decrease in pH. This has been termed 'ocean acidification'. At present the pH of the oceans has dropped from an average of 8.2 of preindustrial times to 8.1. At a pH of 8.1 approximately 90% of carbon is found in the form of bicarbonate ions (HCO_3^-), 9% as carbonate ions (CO_3^{2-}), and <1% as CO_2 (aq) and trace concentrations of carbonic acid (H_2CO_3). If trajectories of proposed acidification are correct, under the IS92a 'business-as-usual' CO_2 emissions scenario the pH will drop to ~7.8 by the end of this century (Caldeira and Wickett 2003; IPCC 2007). This decrease in pH will cause a reduction in carbonate, and an increase in bicarbonate, CO_2 (aq) and carbonic acid (Fig. 1.2).

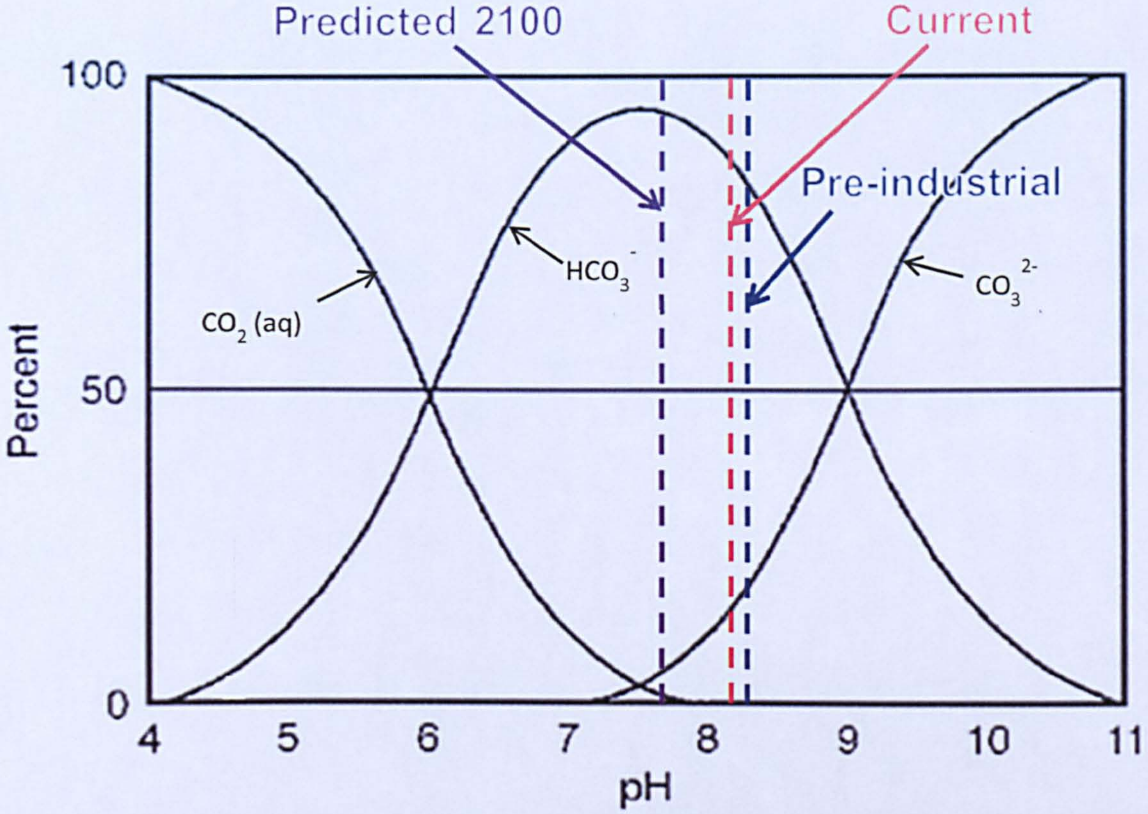
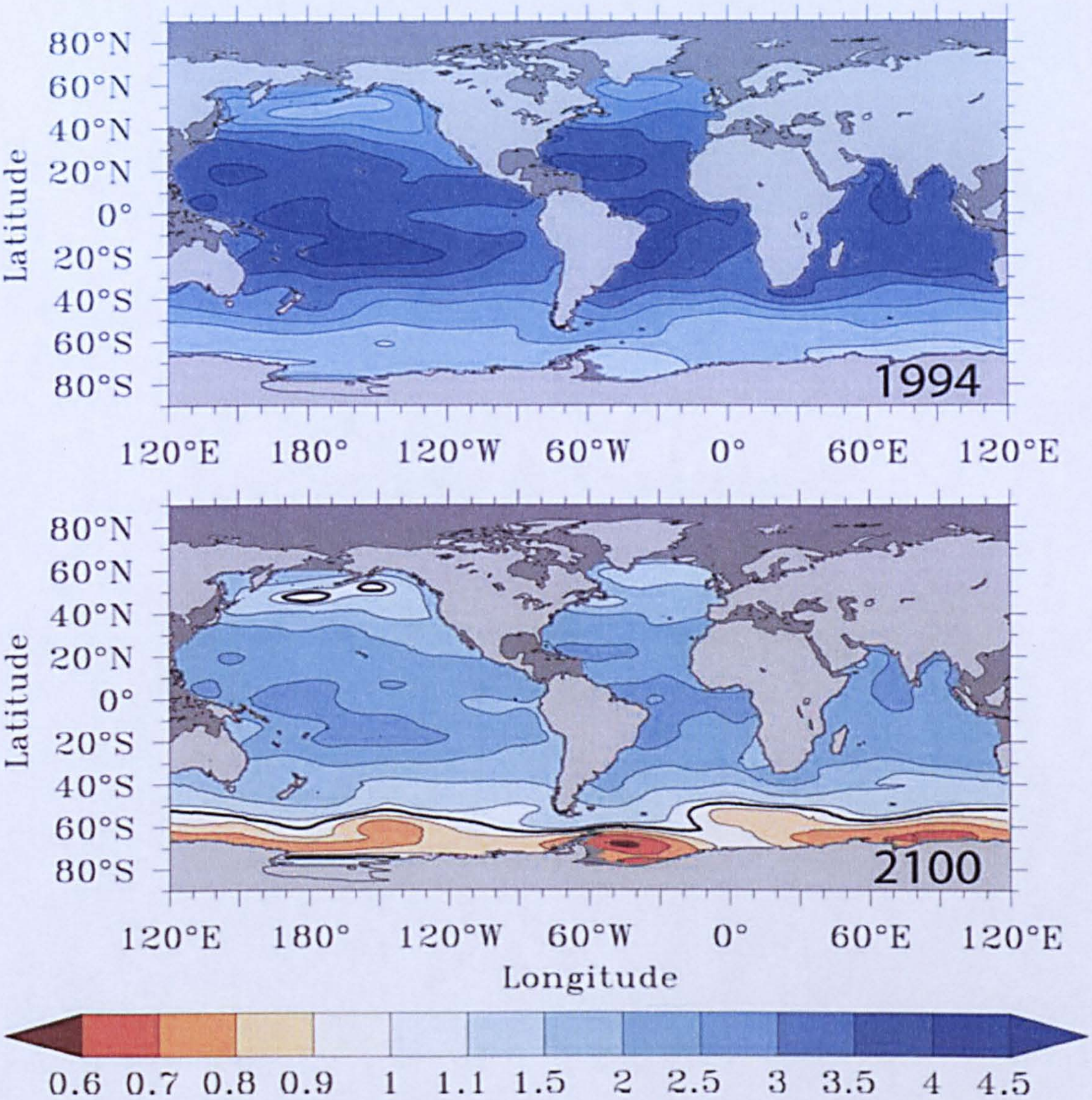


Figure 1.2 Changes in concentrations of ionic states of CO_2 in relation to a pH gradient.

This change will not only affect the acidity of the oceans but will affect the $CaCO_3$ saturation state. Generally marine organisms secrete two different type of $CaCO_3$: calcite and aragonite. The saturation state of these minerals is determined by their themodynamic potential to form or dissolve (Atkinson & Cuet 2008). Saturation states are dependent on the concentrations of carbonate and calcium ions, divided by the stoichiometric solubility product (K_{sp}). K_{sp} is the product of the concentrations of those ions (Ca_2^+ and CO_3^{2-}) when the mineral is at equilibrium (neither forming nor dissolving) at the given temperature, salinity, and pressure.

$\Omega = [Ca^{2+}][CO_3^{2-}]/K_{sp}$

When $\Omega > 1.0$, the formation of the mineral is thermodynamically favorable; when $\Omega < 1.0$, dissolution of the mineral is favorable (Atkinson & Cuet 2008; Fabry et al. 2008). The saturation states of seawater are primary governed by variations in the ratio of CO_3^{2-} to the solubility product, therefore increased absoption of anthropogenic CO_2 leads to strong changes in calcite and aragonite saturation states (Feely et al. 2004). Ω shows a positive correlation with salinity (Key et al. 2004). This may also be altered by the more intensified rainfall and storm variability occurring through climate change, which will alter salinity of coastal areas during these events (IPCC 2007), leading to a further decrease in Ω. Under the IS92a 'business-as-usual' scenario concentrations of CO_3^{2-} (and therefore the saturation state of calcite and aragonite) is expected to decrease by 47- 48% (Fig. 1.3). Carbonic acid (H_2CO_3), which is currently only found in trace amounts and is corrosive to organisms, will increase by 178% (Fabry et al. 2008). These changes in carbonate chemistry will not only impact organisms but may lead to changes in biogeochemical cycling in the oceans (Doney et al. 2009a). The extent of changes in pH and Ω will vary locally, although the extent of these changes for the Mediterranean is generally unknown. Currently calcite and aragonite saturation states are generally quite high, particularly in shallow waters (Fig. 1.4)



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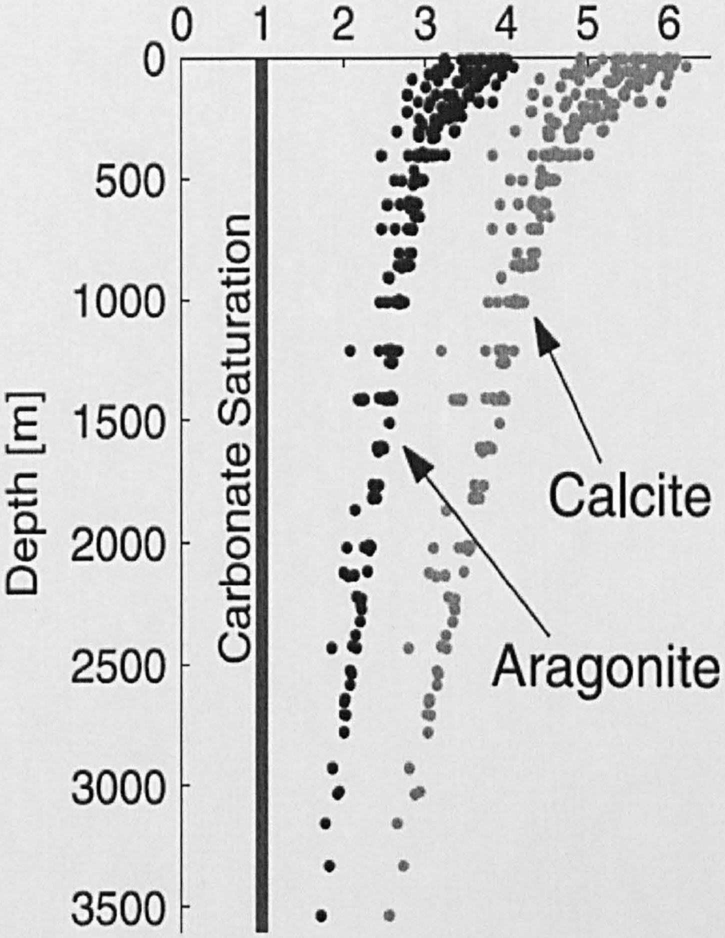
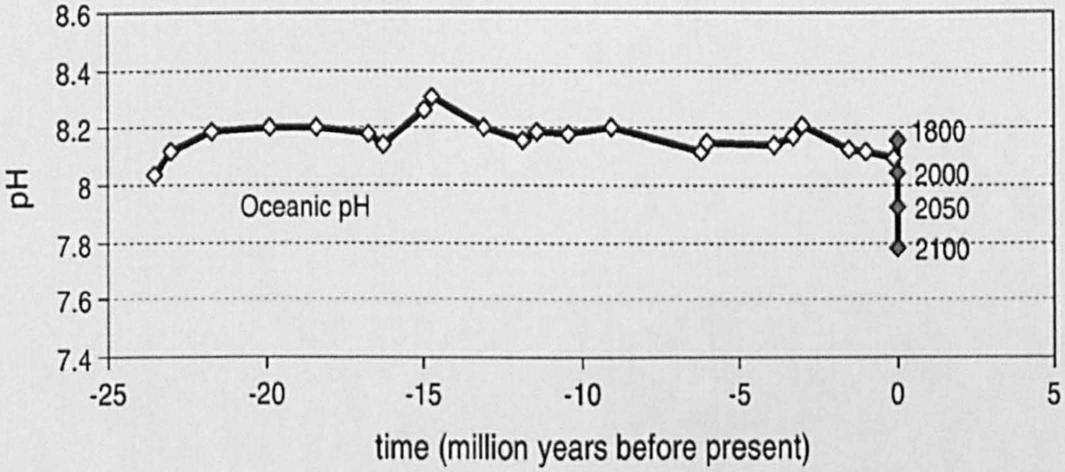


Figure 1.4 Vertical profiles of calcite and aragonite saturation states in the Mediterranean Sea (from Schneider et al. 2007)

In the past 20 million years ocean surface water pH has not dropped below pH 8 (Fig 1.5), although organisms have experienced low pH in the past. Surface water pH may have dropped to as low as 7.4 - 7.5 during the Cretaceous and Jurassic period and yet many calcifying plankton originated and thrived during this time (Fig. 1.6) (Ridgwell and Schmidt 2010). A reason for this may be that during these periods the calcite and aragonite saturation of the oceans are thought to have remained high due to the large input of Ca^+ from dissolution and weathering, leading to high alkalinity (Ridgwell 2005), and a carbonate system much different from expected future conditions (Ridgwell and Schmidt 2010). Much of the present day concern over acidification relates to the speed at which the pH is changing, ocean chemistry is changing over 100 times faster than natural fluxes, which occurred slowly through geological timescales (Siegenthaler et al. 2005).



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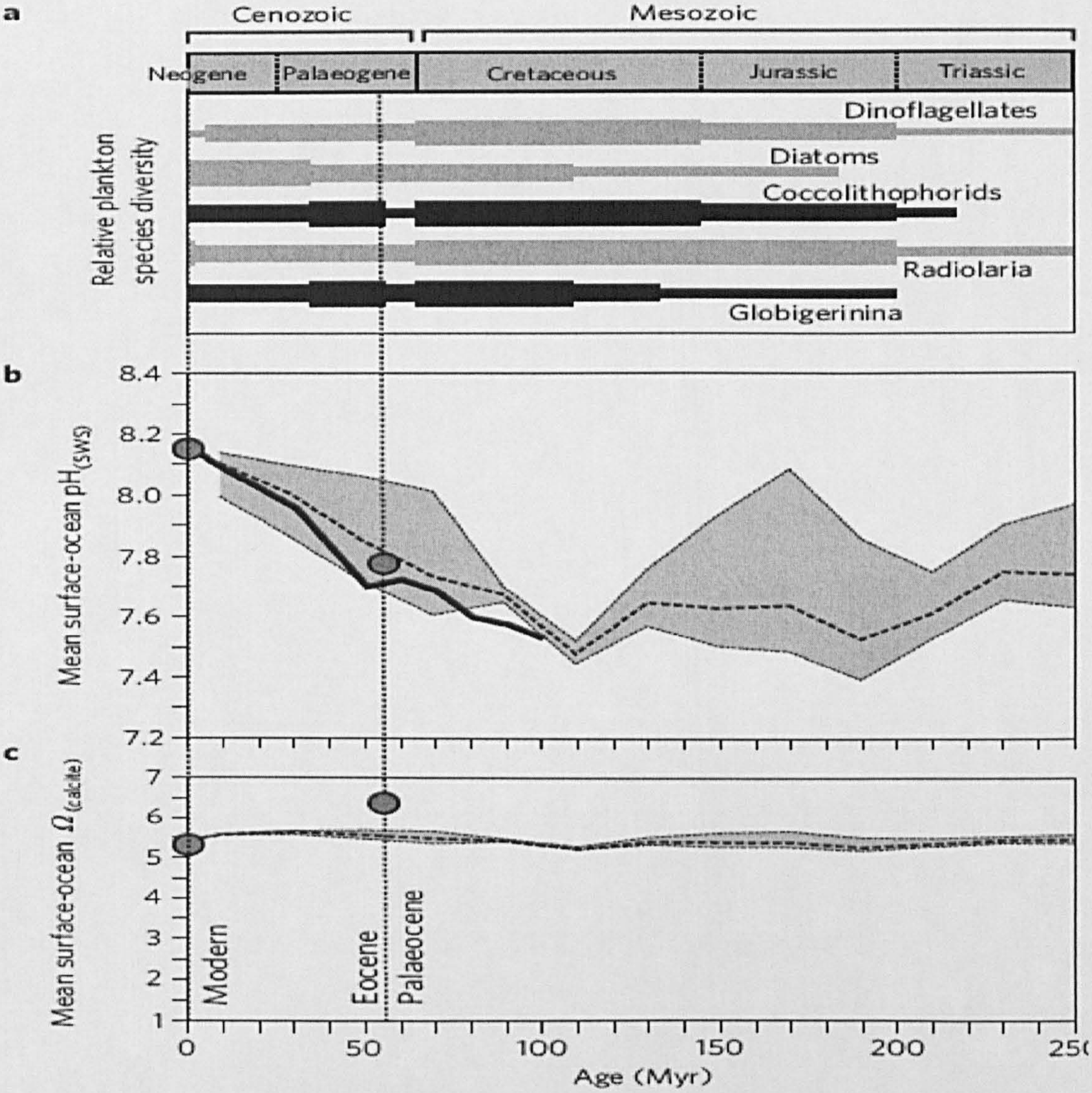


Figure 1.6 Surface oceans over geological timescales a) planktonic diversity on major taxa: black = calcifying, grey = non-calcifying (from Martin 1995). b) mean surface-ocean pH; dashed black line and grey represent mean model reconstruction, maximum and minimum (from Ridgwell 2005), solid black line from Tyrell and Zeebe (2004); circles represent Earth-system model reconstructions by Ridgwell and Schmidt (2010). c) surface-ocean calcite saturation state; dashed line and grey represent mean model reconstruction and uncertainties in pCO_2 respectively (Ridgwell 2005); circles Earth-system model reconstructions by Ridgwell and Schmidt (Ridgwell and Schmidt 2010); dashed vertical line represents the Palaeocene-Eocene boundary (Ridgwell and Schmidt 2010). Figure taken from Ridgwell and Schmidt (2010).

1.2 Current knowledge of the effects of ocean acidification

If anthropogenic CO_2 emissions continue to increase, there is now little doubt that marine organisms will be impacted (Fabry et al. 2008), which could lead to an overall reduction in biodiversity (Hall-Spencer et al. 2008; Fabricius et al. 2011). Research indicates that species responses to a reduction in pH will be species specific. For example, positive adaptations that may enable the survival of some calcifying organisms have been shown, whilst other species appear less able to adapt (Sunday et al. 2011). Effects of OA are not always deleterious, but may be advantageous such as: increased growth in calcifying organisms (e.g. the sea star *Crossaster papposus*, see Dupont et al. 2010b), or enhanced biological carbon fixation in phytoplankton (Riebesell et al. 2007). Due to such variability in results, it has been difficult to predict the effect of OA on ocean ecosystems.

Calcification is widespread in marine organisms (Brennan et al. 2004), and is a process particularly susceptible to future changes in ocean carbonate chemistry (Orr et al. 2005; Fabry et al. 2008). Hence, a large number of studies have focused on calcification (e.g. Gazeau et al. 2007; Brennand et al. 2010; Gutowska et al. 2010), although more recently the requirement to understand the effect of OA on non-calcifying organisms has been recognized (e.g. Mercado et al. 1999; Connell and Russell 2010; Suggett et al. 2012).

Calcification responses of organisms have been extremely varied (Ries et al. 2009; Hikami et al. 2011), suggesting different sensitivities to low pH. Some species show reduced calcification rates (Maier et al. 2009; Ries et al. 2010), whilst other species appear to increase their calcification rates under low pH (McDonald et al. 2009; Gutowska et al. 2010). Gene expression analysis has revealed similar variability. For example calcification genes are downregulated in the larvae of the sea-urchin *Strongylocentrotus purpuratus* (Stumpp et al. 2011), up-regulated in the larvae of the sea-urchin *Paracentrotus lividus* (Martin et al. 2011), and did not change in different life stages of the red abalone *Haliotis rufescens* (Zippay and Hofmann 2010). Variability in response has even been obtained between similar studies of the same species, e.g. the coccolithophore *Emiliania huxleyi* (Riebesell et al. 2000; Feng et al. 2008; Iglesias-Rodriguez et al. 2008). It is uncertain whether variation in these results is context dependent, due to strain specific variation, or whether culture procedures; e.g. temperature, nutrient limitation, UV radiation, exposure time, culture density, acclimation time or other factors were responsible.

Calcification is not the only process that will be affected by OA, other physiological indices such as growth, survival, metabolism, and development may be affected (Fabry et al. 2008), particularly through disruption to the extracellular acid-base balance (Widdicombe and Spicer 2008). Research shows similar variation in species responses to these other physiological indices as they do to calcification, although early life history stages may be more susceptible (Fig. 1.7). For example, under low pH larval growth was reduced in a number of sea urchin species (Clark et al. 2009), whilst in the sea star *Crossaster papposus* larval growth increased (Dupont et al. 2010b). A species lifestyle is likely to be important in determining whether they may tolerate low pH conditions (Widdicombe and Spicer 2008), and the difference in growth response between these echinoderms is thought to be due to the lecithotropic nature of the *C. papposus* larvae. The supply of nutrition by the egg yolk of *C. papposus* may confer an advantage to this species under low pH scenarios (Dupont et al. 2010b). Many different life-history strategies or growth strategies may enable organisms to withstand low pH. For example organisms that have tissues or organic layers which cover their shells may be less susceptible to OA, as these layers may protect against corrosive seawater (Lombardi et al. 2011a; Rodolfo-Metalpa et al. 2011).

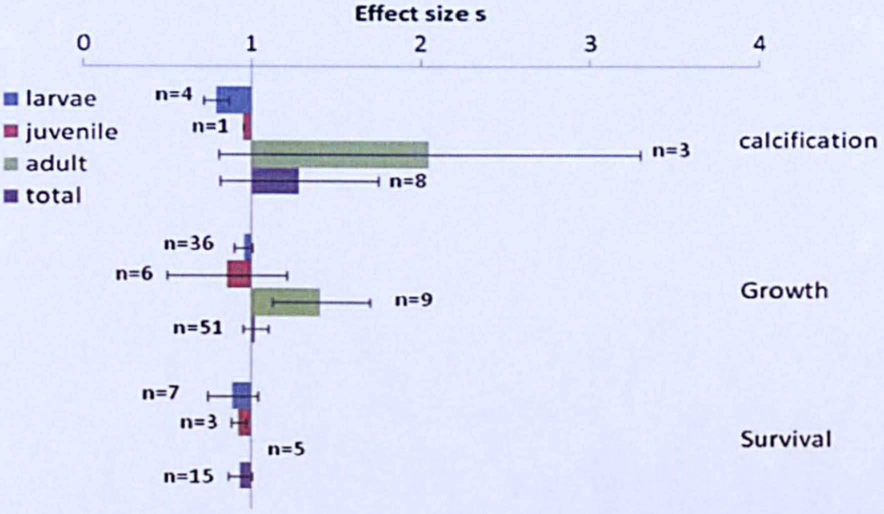


Figure 1.7 Meta-analysis of mean effect size (± SE) on the calcification, growth and survival of different life history stages of echinoderms exposed to ocean acidification (Dupont et al. 2010a).

The ability of a species to regulate the pH of extracellular fluids under a decrease in seawater pH, has been implicated as a key factor in OA response (Melzner et al. 2009; Ries et al. 2009). Those that can't regulate internal pH will conform to external conditions, leading to acidosis and impacting pH-dependent processes. Those that can regulate internal pH may be able to reduce these impacts (Melzner et al. 2009), although this regulation may come at an energetic cost (Portner et al. 2004). Examples of this include; the brittlestar *Amphiura filiformis*, where calcification was maintained but with great muscle loss (Wood et al, 2008), and larvae of the Atlantic cod *Gadus morhua*, where increased growth was at the cost of organ development and health (Frommel et al. 2011).

Many researchers have tried to synthesize the effects of OA through meta-analytic techniques (Dupont et al. 2010a; Hendriks et al. 2010; Kroeker et al. 2010) and review (Fabry et al. 2008; Doney et al. 2009b), although a consensus on the biological impact of OA has not been reached (e.g. Dupont et al. 2010a; Hendriks and Duarte 2010). Most authors suggest that there will be widespread alterations to marine ecosystems (Fabry et al. 2008; Kroeker et al. 2010), although the ability of species to adapt to future conditions is unknown (Doney et al. 2009b).

In respect to proposed future acidification, species abilities to adapt or tolerate these changes will be important in determining survival success, but accurate predictions must take into consideration a complex array of both biotic and environmental interactions (Russell et al. 2012). Whilst OA will occur simultaneously with changes in other physical variables such as temperature, salinity, and oxygen, the interaction between species will change as well. Food availability will change through altered composition of primary producers (e.g. Kuffner et al. 2008; Wootton et al. 2008), loss of nutritional value of the prey species (e.g. Rossoll et al. 2012), or decline of important prey species (e.g. Lischka et al. 2011), whilst predator-prey interactions may change through loss of sensory capabilities (e.g. Dixson et al. 2010; Ferrari et al. 2011a), changes in metabolism (e.g. Gooding et al. 2009) or reductions in shell defense (e.g. Bibby et al. 2007; Comeau et al. 2010). Changes in competitive interactions through the differential responses of species in the same trophic guild will also be important for determining future OA scenarios (e.g. Jokiel et al. 2008; Cigliano et al. 2010). Deep sea vents provide an excellent example of how fitness can be a relative concept, dependant on the biotic interactions present. For example, bivalves found at deep sea vents with low $p\mathrm{CO}_2$ (pH 5.36-7.29) show a sharp reduction in calcification. Although shell defences are greatly reduced, this species continues to thrive due to the absence of predators (Tunnicliffe et al. 2009). A species ability to simply tolerate low pH may not be enough, as other organisms that can adapt and thrive in low pH conditions will have the competitive advantage. Marine organisms will therefore face the test of coping with multiple challenges at all levels.

1.3 Background to research

As results show that OA will have numerous and highly varied consequences for life in the oceans, no method of OA research can completely fill our research requirements, and a multitude of scales, from single-species perturbation experiments to multi-species mesocosm and *in situ* observational studies should be used to investigate OA at the ecosystem level.

1.3.1 Experimental studies

Although perturbation experiments are the key approach, and a valuable tool in investigating the effects of OA, they do not mimic the complexity of the oceans. Short-term perturbation experiments can be useful for understanding the physiological processes which are affected by lowered pH such as calcification, acid-base balance and metabolic rates, alongside examining the success of different life history stages in terms of growth, survival and reproduction. These experiments are generally undertaken on single species in highly controlled environments with manipulation of only a single variable (Widdicombe et al. 2010), although experiments examining multiple stressors (e.g. Brennand et al. 2010), predator-prey relationships (Ferrari et al. 2011b) or even both (Landes and Zimmer 2012) are developing. Extrapolating these results to future acidification scenarios is fraught with difficulty, because (1) of uncertainty in future changes in biotic interactions (Russell et al. 2012), and (2) these experiments may hide positive adaptations (Doney et al. 2009b), or negative impacts (Dupont et al. 2010c) that may occur during chronic acidification.

Mesocosm studies can reduce these uncertainties (Godbold et al. 2009), as they enable observations on the impacts of ocean acidification in more natural settings with multiple species over several trophic levels and include surrounding and indirect effects while allowing for controlled manipulation (e.g. Grossart et al. 2006; Egge et al. 2009). These studies are important, particularly in understanding the impact on planktonic communities, but lack the ability to incorporate higher trophic levels (Riebesell et al. 2010).

1.3.2 Observational studies

An alternative method for OA research is the study of habitats which are characterized by low pH, such as CO_2 vent sites (e.g. Italy) and areas of acidic upwelling (e.g. west coast of America; Feely et al. 2008). Exploiting natural pH gradients in space or time can help unravel the ecosystem level effects of OA by seeing how species, communities and ecosystems react to a decrease in pH in a natural environment (Barry et al. 2010). Ecological research at CO_2 vent sites in Italy (e.g. Hall-Spencer et al. 2008; Suggett et al. 2012), Papua New Guinea (e.g. Fabricius et al. 2011) and Mexico (Crook et al. 2012) has flourished over recent years.

The island of Ischia, (Gulf of Naples, Italy) has a long history of volcanism and gas venting in shallow submarine sites (Tedesco 1996). The CO_2 vents of the Castello Aragonese, Ischia have been present for more than 20 years, although their magnitude has increased over recent years (MC Buia, pers comm.), and allow for observation of the long-term consequences of chronic exposure to low pH on species abundance, diversity and community structure (Hall-Spencer et al. 2008). When using *in situ* observational measurements confounding factors such as temperature, nutrients, exposure and light must be taken into consideration (Barry et al.

2010). One major confounding factor when using CO_2 vents can be the release of heat and sulphides into the water column alongside CO_2. Sulphides are toxic to most organisms (Powell and Somero 1986), and will significantly confound experimentation. Vents found at the Castello Aragonese are unusual, as they do not produce heat or spew out toxic compounds. Vent gas consists of 90.1 - 95.3% CO_2, 3.2 - 6.6% N_2, 0.6 - 0.8% O_2, 0.08 - 0.1% Ar and 0.2 - 0.8% CH_4 (Hall-Spencer et al. 2008).

Vents are found at the north and south side of the Castello Aragonese, and have been used as natural analogs of OA for many studies. Much of the previous research has focused on the rocky sublittoral (e.g. Kroeker et al. 2011b; Porzio et al. 2011), where CO_2 bubbles released by the vents are most abundant, although bubbles are also released into the seagrass habitat (Fig. 1.8). The CO_2 gradient running parallel to the rocky shore has been documented (Kroeker et al. 2011b).

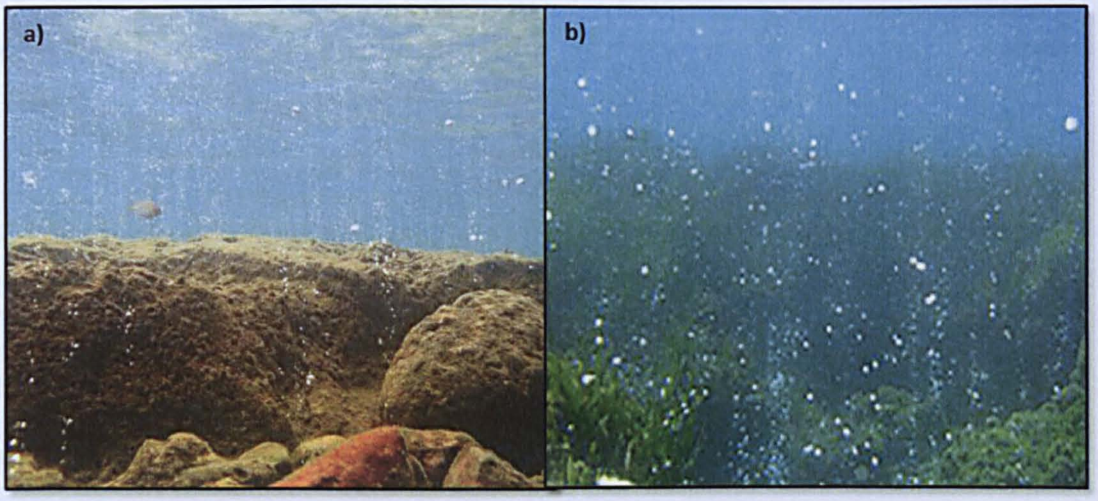


Figure 1.8 CO_2 bubbles released into a) the rocky subtidal and b) *Posidonia oceanica* habitat at the Castello Aragonese.

1.3.4 Current research

These vents can provide important information on the long-term effects of OA on benthic assemblages and ecosystems, incorporating ecosystem processes such as production, competition, and predation (Hall-Spencer et al. 2008). Research at the CO_2 vents of Ischia, Italy began in 2007 when Hall-Spencer et al. (2008) documented the decline of calcifying taxa such as sea-urchins and coralline algae along a pH gradient. Barnacles and gastropods appeared to be more resistant to pH, and abundances only declined in extremely low pH zones. Non-calcifying taxa such as the seagrass *Posidonia oceanica* and some species of non-calcifying algae all appeared resistant and even flourished under reduced pH conditions. Research undertaken at these vents has been significant, a summary of which can is presented (Table 1.1).

Table 1.1 Summary of research from the CO_2 vents at the Castello Aragonese, Ischia, Italy. Findings have been placed in columns dependent on whether results showed a decrease (<), remained the same (=) or increased (>).

<	=	>
Benthic foraminifera (Dias et al. 2010) Invertebrate settlement (Cigliano et al. 2010; Ricevuto et al. 2012) Macroalgal diversity (Porzio et al. 2011) Coraline algal cover (Hall-Spencer et al. 2008; Martin et al. 2008; Porzio et al. 2011; Kroeker et al. 2013b) Sea urchin abundance (Hall-Spencer et al. 2008) Total epiphytic $CaCO_3$ (Martin et al. 2008) Diversity of benthic invertebrates **(Kroeker et al. 2011b)** Biomass of benthic invertebrates **(Kroeker et al. 2011b)**	Bryozoan calcification rates (Rodolfo-Metalpa et al. 2010) Bryozoan cover (Martin et al. 2008) Hydrozoan cover (Martin et al. 2008) *P.oceanica* production (Hall-Spencer et al. 2008)	Bryozoan calcification rates (Rodolfo-Metalpa et al. 2010) Skeletal/shell dissolution (Hall-Spencer et al. 2008; Lombardi et al. 2011c) Dissolution of coralline algae (Hall-Spencer et al. 2008; Martin and Gattuso 2009) *P.oceanica* shoot density (Hall-Spencer et al. 2008) Bacterial diversity (Meron et al. 2013)

The most notable impact reported has been the decline in diversity observed along the pH gradient (Cigliano et al. 2010; Dias et al. 2010; Kroeker et al. 2011b; Porzio et al. 2011). Some calcareous taxa appear to be particularly affected by acidified conditions. Martin et al. (2008) found that there was a severe decline in coralline algal cover and $CaCO_3$ biomass along a decreasing pH gradient, whilst Dias et al. (2010) found that the abundance and diversity of sediment foraminifera assemblages decreased; changing from a calcareous dominated assemblage to an assemblage with only non-calcareous agglutinated taxa present. At the intermediate pH station the number of species decreased from 23 to 6, leading to a 75% loss of calcareous individuals and showing that pH levels of 7.8 will have severe impacts for foraminifera communities in the future. Kroeker et al. (2011b) observed that many calcifying invertebrate species were still present in zones with a mean pH of 7.8 but absent in zones with a mean pH < 7.3, suggesting that invertebrates show some degree of tolerance to pH values predicted for the end of this century. Under extremely low pH conditions there was a severe decrease in the abundance of gastropods and decapods, whilst taxa such as polychaetes, amphipods, and tanaids appeared resistant to even extremely low pH values. Community richness, evenness, and biomass decreased, whilst invertebrate abundance remained the same, suggesting that density compensation occurs through the proliferation of taxa tolerant to low pH. These results suggest that, whilst calcifying sedentary taxa such as coralline algae and foraminifera may be severely affected, at mean pH 7.8 invertebrate taxa will not show significant community level effects, but that there may be a threshold pH in which a sudden ecological shift (tipping point) is observed (Kroeker et al. 2011b).

Cigliano et al. (2010) investigated invertebrate settlement along a pH gradient using artificial collectors. Abundance of individuals did not differ between pH zones, consistent with the findings of Kroeker et al. (2011b), although in contrast, diversity and evenness decreased in both the low and extremely low pH site. This was due to the decrease in settlement of calcifying organisms, and further research revealed that this pattern was maintained over time (Ricevuto et al. 2012). Studies have shown that the juvenile stage of invertebrates may be less tolerant to low pH (Dupont et al. 2010a), which may lead to the differences observed between the studies. These studies took place in the rocky reef habitat surrounding the Castello, so the effects of acidification on the benthic invertebrate population within the *Posidonia oceanica* meadows are still unknown.

Considerable effort has been focused on studying bryozoans at the CO_2 vents, as this taxa, although calcifying, appear tolerant to low pH (Martin et al. 2008). Rodolfo-Metalpa et al. (2010) observed that although the bryozoan, *Myriapora truncata,* maintained net calcification rates under acidified conditions, calcification was impeded during periods of high seawater temperatures. Lombardi et al. (2011c) discovered that although *M. truncata* maintained calcification under acidified conditions, their skeletons contained lower levels of Mg. This suggests that the outer levels (with higher Mg concentrations) were being corroded. Both studies observed that dead colonies suffered high levels of dissolution in acidified waters, suggesting that organic tissues provide a protective role against acidification. Further research found that new zooids were not produced in acidified sites but that the organic cuticle surrounding the skeleton increased in thickness (Lombardi et al. 2011a). The bryozoan, *Schizoporella errata,* is able to survive under low pH conditions, although investigation showed a decrease in protective zooids and an increase in feeding zooids (Lombardi et al. 2011b). These results suggest bryozoans may be able to withstand future ocean conditions, but that there may be an alteration in energy allocation, energetic cost or fitness associated with this.

Rodolfo-Metalpa et al. (2011) used transplantation experiments along a pH gradient to show that molluscs and corals may continue to calcify and grow in low pH. This research revealed that species which have external organic layers such as the periostracum in the mussel, *Mytilus galloprovincialis*, and the organic tissue which completely covers the skeleton of the coral, *Cladocora caespitose*, may protect these organisms against dissolution. Organisms with exposed skeletons; such as the limpet, *Patella caerulea*, and the coral, *Balanophyllia europaea*, tolerated low pH by increasing net calcification rates to counteract shell/skeletal dissolution. Similar to the observations for the bryozoan, *Myriapora truncata* (Rodolfo-Metalpa et al. 2010), this study found that these species ability to calcify under low pH was disrupted during periods of high water temperatures. This suggests that although some species may be able to tolerate ocean acidification, the combined stress of ocean warming may lead to a collapse in these populations.

Seagrasses and non-calcifying species of algae are expected to benefit from OA, due to the increased availability of $CO_2(aq)$, increasing photosynthesis and growth rates (Koch et al. 2013). Hall-Spencer et al. (2008) documented the increase in seagrass density and non-calcareous algal cover along a pH gradient. Porzio et al. (2011) found a simplification in macroalgal community composition in the rocky sublittoral occurred along a pH gradient. The number of species decreased by 5% in at pH 7.8 and by 72% in pH 6.7, leading to a community characterized by only a few species. Although there was a reduction in the number of calcifying species, the cover of some calcifying species was greater at pH 7.8.

Kroeker et al. (2013b) deployed settlement tiles along the pH gradient and analysed the cover of different functional groups of algae for 14 months. They found that the settlement and cover of coralline algae did not differ between control and low (pH 7.8) zones for the initial period of deployment, but after 6 months there was significantly lower cover of calcifying algae at low pH, whilst the cover of turf algae greatly increases. Kroeker et al. (2013b) concluded that whilst in ambient pH conditions there is a balance between the competitive dynamics of calcareous and turf algae, when pH decreases it leads to an increased competitive ability of the turf algae which overgrows and outcompetes calcareous species. This study highlights the importance of being able to include species-interactions in OA research. A second consideration is that whilst turf algae increased in the studies by Kroeker et al. (2013b), and Porzio et al. (2013), a decrease in turf algae was observed in the study by Porzio et al. (2011). Although these studies took place at the Castello Aragonese, observations in the former studies were made during spring and summer, whilst the latter were made in October. This suggests that although some species of turf algae (such as ephemeral species present in spring and summer) may be resilient or respond favourably to OA, other species may not. This highlights the important of sampling at different times of the year and, where possible, the advantage of identifying taxa down to greater resolution.

1.4 Preliminary investigation

Since the degree of acidification in the seagrass beds was not certain, a preliminary study of the seawater pH at different stations was conducted in March 2011. Three stations at the north side and three stations at the south side of the Castello Aragonese were chosen as potential *Posidonia oceanica* study sites. These sites were in the seagrass but adjacent to the rocky reef sites used in other studies (Hall-Spencer et al. 2008; Kroeker et al. 2011b; Porzio et al. 2013);

two of each in areas where CO_2 vents were active and a third station away from the vents, to be used as a control. Control stations were named N1 and S1, whilst proposed acidified stations were names S2, S3, N2, and N3 (Fig. 1.9).

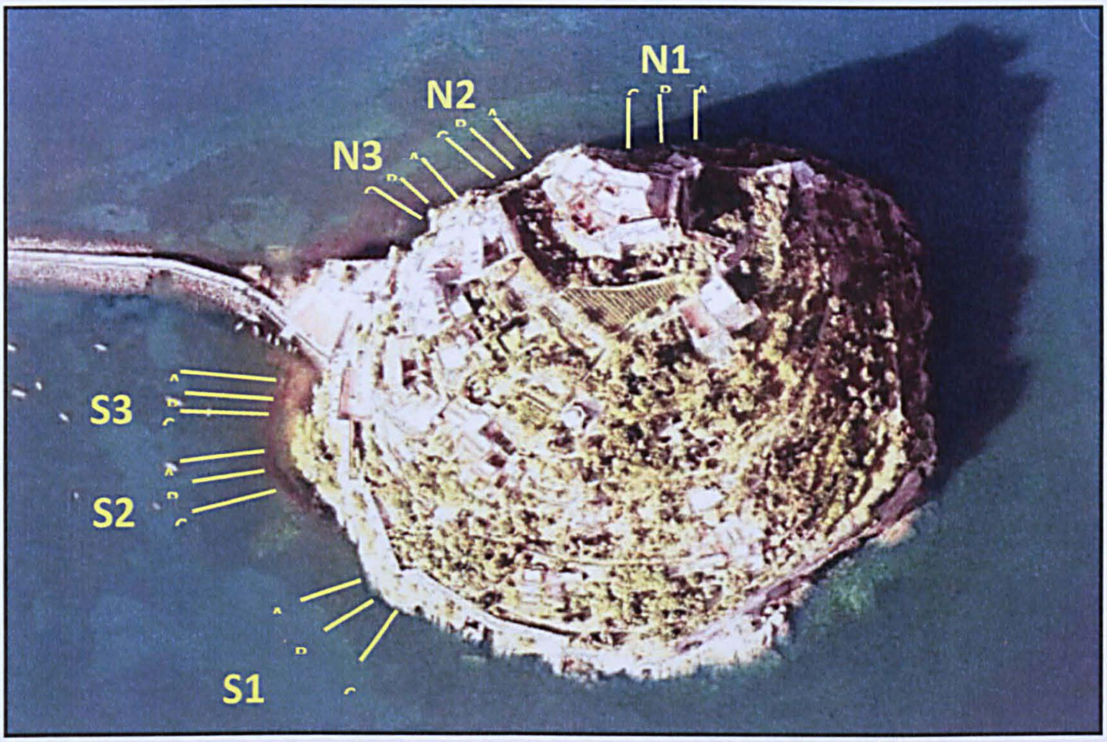


Figure 1.9 Position of transects lines along which pH samples were taken, for each station, around the Castello Aragonese.

Three transects were laid out for each site, 6 m apart and extending from the shore until approximately 20 m into the seagrass (Fig. 1.8). The rocky subtidal extends from the shore to the start of the seagrass (brown sections: Fig. 1.9). Seawater samples were collected at the beginning of the transect, 40 cm into the start of the seagrass and then each metre until the end of the transect. Depths ranged from approximately 1.5 m at the beginning of the transect to 4 m at the end. It must be taken into consideration that pH at the CO_2 vents is variable (Kroeker et al. 2011b), and hydrodynamic forces such as water advection and wave action causes a dilution effect in acidified areas and an increase in pH (S. Garrard, pers. obs.). For this reason all transect measurements were carried out on calm days in April 2011 to ensure an accurate picture of acidification during periods of low mixing. These transects showed that *P. oceanica* stations N1 and S1 on both the north and south side of the Castello Aragonese were not affected by the CO_2 vents and maintained a pH of 8.1 - 8.2. N3 and S2 showed the greatest degree of acidification (mean pH of 7.5 and 7.6 respectively) (Fig. 1.10).

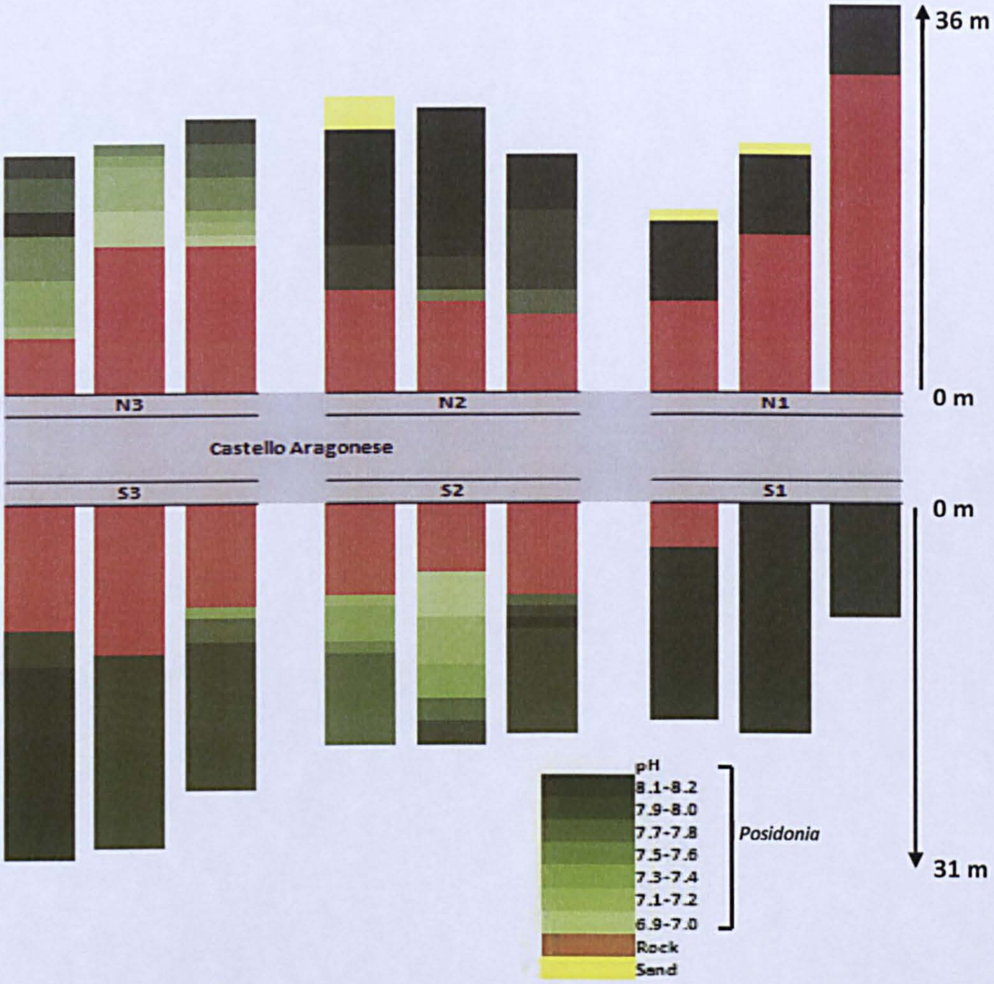


Figure 1.10 Representation of the pH gradient in the seagrass along the transects of each station (N1, N2, N3, S1, S2, and S3) around the Castello Aragonese. Green areas are where seagrass occurred, whilst brown equals rocky sublittoral and yellow equals sand. Each block (🟩) represents pH at each meter from the shore.

From these results it was determined that stations N3 and S2 were suitable acidified stations, and N1 and S1 were suitable control sites. Stations N2 and S3 were excluded from further research for this PhD, as they only showed a trend towards marginal acidification. Once acidified and control sites were determined, these areas were marked by buoys to ensure that studies were consistently carried out in the same areas each time. A further tertiary control site was established at Lacco Ameno, a site with similar *Posidonia oceanica* meadow characteristics (Zupo et al. 2006), that is 6 km from (and unaffected by) submarine CO_2 vents (Tedesco 1996). This site was selected to provide a comparison with the Castello Aragonese control sites to provide background on the spatial variability of seagrass structural parameters. Each site had an area of approximately $60m^2$ and was located at a depth of 2.5 – 3.5 m.

1.5 Research aims and objectives

Under the threat of ocean acidification, species will have three possible responses: to tolerate or adapt to a reduction in pH, or become locally extinct. Research suggests that a number of species will be lost as the oceans acidify (Hall-Spencer et al. 2008; Fabricius et al. 2011). Species loss could cause a loss in community stability and ecosystem function (Lehman and Tilman 2000; Worm et al. 2006), although if functionally similar species proliferate, this may buffer this impact (Tilman 1996). Species compensation dynamics may be particularly relevant for fast growing, species rich groups (Vinebrooke et al. 2003).

Positive adaptations than enable the survival of calcifying organisms have been shown at the CO_2 vents of Ischia (Rodolfo-Metalpa et al. 2011), whilst other species appear unable to adapt (Lombardi et al. 2011c). Changes in species composition may not be solely based upon species' ability to tolerate or adapt to environmental conditions. Indirect effects, such as changes in food availability, competition or predation, will be important in structuring community composition, as will the combined effects of multiple stressors (e.g temperature and OA).

Posidonia oceanica is an extremely important ecosystem within the Mediterranean coastal zone, covering large areas of the shallow subtidal and providing an important habitat for many species of algae, invertebrates and fish (Mazzella et al. 1992). Very little is known of how this system will respond to OA, as it is not possible to experimentally acidify a whole *Posidonia oceanica* system. The subtidal CO_2 vents around the Castello Aragonese provide a model system for investigating this. Recent research has shown increased *P. oceanica* shoot density at acidified stations (Hall-Spencer et al. 2008), and the change in functional groups of seagrass epiphytes along a pH gradient, has been documented (Martin et al. 2008), but knowledge on other aspects of how a *P. oceanica* system responds to acidification is lacking. This PhD research will provide a broad overview of how plant structure and function, the invertebrate community and macroherbivore grazing pressure respond to acidified conditions. This research will help towards providing a more comprehensive understanding of the effect of OA on a *P. oceanica* system. This response will be addressed through a number of different objectives:

1. To assess the natural variation in the flora and fauna associated with *Posidonia oceanica* through analysis of previous research.
2. To investigate how nutrient availability and *P. oceanica* function change under a decrease in pH.

3. To assess changes in invertebrate community dynamics between acidified and control conditions in a *P. oceanica* meadow
4. To compare the response of benthic invertebrates to acidification in two different habitats: *P. oceanica* and the rocky subtidal.
5. To investigate whether the dynamics of herbivory on *P. oceanica* differ under low pH conditions.

Each of the field investigations (objectives 2, 3 and 5) will be carried out over a temporal timescale of months to determine how results fluctuate at different times of the year, as this has not been considered in previous research (e.g. Hall-Spencer et al. 2008; Cigliano et al. 2010; Kroeker et al. 2011b).

2. The floral and faunal associated communities of *Posidonia oceanica*: over 40 years of research

2.1 Introduction

Seagrasses are marine phanerograms, of which there are approximately 60 species (representing <0.02% of the angiosperm flora) (Hemminga and Duarte 2000). Of the higher plants, they are the only group that have developed the capabilities to survive a completely submerged marine existence (Den Hartog 1970). Extensive beds can be found along the coastlines of all continents, other than Antarctica (Hemminga and Duarte 2000). They have sometimes been termed 'ecosystem engineers' (Jones et al. 1997; Thomas et al. 2000), as they physically and biologically influence ecosystem qualities by altering the local hydrodynamic properties of an area, and providing space and resources for a multitude of organisms (Mazzella et al. 1993; Brun et al. 2009), including many commercially important species of fish and invertebrates (Beck et al. 2001; Del Pilar Ruso and Bayle-Sempere 2006). Seagrasses represent some of the most productive and diverse marine habitats (Guinotte and Fabry 2008). Seagrass swards show extremely high rates of primary production, which can be attributed to the epiphytic flora, plankton and sediment microbes, as well as the seagrass itself (Moncreiff et al. 1992).

Macrofaunal diversity and abundance is generally greater in seagrass meadows than adjacent unvegetated habitat (Orth et al. 1984 and references therein; Ansari et al. 1991; Mattila et al. 1999). Several hypotheses have been proposed to explain the increase in density and abundance of fauna in seagrass beds; the increased complexity of habitat provides increased niche space for organisms (Heck and Wetstone 1977; Ansari et al. 1991; Mazzella et al. 1992), seagrass canopy provides organisms protection from predation (Heck and Wetstone 1977; Orth et al. 1984), alteration to local hydrodynamics promotes larval settlement (Dirnberger 1993), and the increased abundance of food items available attracts organisms into the meadows to feed (Hemminga and Duarte 2000). The importance of seagrasses as structuring agents is supported by studies that have shown invertebrate abundance is positively correlated with seagrass above ground biomass (Heck and Wetstone 1977; Stoner 1980; Mattila et al. 1999; Attrill et al. 2000).

Alongside enhancing marine biodiversity, primary and secondary production, seagrass habitats are also important for a number of other beneficial ecosystem processes:

- Sediment stabilization through binding and modification of the sediment (Fonseca 1989), and a reduction of flow and wave velocity, (Fonseca and Fisher 1986; Gambi et al. 1990; Fonseca and Cahalan 1992), thereby reducing coastal erosion.
- Improvement of water quality through dissipation of kinetic energy (Short and Short 1984), and acquisition of nutrients from the water column (Hemminga et al. 1991; Hemminga and Duarte 2000). Removal of nutrients from the water column increases light penetration by reducing their availability for phytoplankton growth (Hemminga and Duarte 2000), whilst reduced kinetic energy causes enhanced particle deposition (Gacia et al. 2003). The combination of these processes reduces turbidity and improves water quality.
- Important role in global carbon and nutrient cycling. Although seagrasses are only responsible for a small proportion of oceanic net primary production (<1%), a large quantity of their biomass ends up in the sediments as refractory detritus, forming biogenic concretions (Hemminga and Duarte 2000; Buia et al. 2004). This detritus can remain in the sediments for thousands of years, acting as a sink for biogenic elements

(Mateo et al. 1997). It has been estimated that seagrasses are responsible for the storage of 15% of organic carbon in the oceans (Duarte and Chiscano 1999).

- Production of marine resources. Seagrass beds are an important habitat for many juvenile stages of commercially important species of offshore fish and invertebrates (Watson et al. 1993; Connolly 1994; Beck et al. 2001), alongside supporting many subsistence fisheries (Unsworth et al. 2010).

Posidonia oceanica is the most dominant species of seagrass in the Mediterranean, estimated to cover an area of 25,000-50,000 km^2 or 1-2% of the Mediterranean seafloor (Bethoux and Copinmontegut 1986; Pasqualini et al. 1998). Studies spanning over 4 decades have revealed the high diversity of floral and faunal communities associated with this species (e.g. Van der Ben 1971; Novak 1982b; Gambi et al. 1992; Francour 1997; Bologna and Heck 1999; Guidetti 2000a; Balata et al. 2008). More recently studies have focused on how anthropogenic activities impact these communities (e.g. Dimech et al. 2002; Bongiorni et al. 2005; Ruiz et al. 2009; Ben Brahim et al. 2010; Terlizzi et al. 2010).

But in order for us to understand the impacts that anthropogenic activities or climate change have on these communities, it is important to understand the natural variability found in undisturbed meadows. A major objective of older marine ecological research was to taxonomically describe the species that occur in different habitats; therefore a wealth of information on the composition of *P. oceanica*-associated communities is available. One of the major drawbacks is that much of this research has been left behind in the digital era. Having been published in print and never transferred into electronic format, it is consigned to archives and at risk of being forgotten. In terms of research on *P. oceanica*, a further drawback is that considerable amounts were published in local journals in the researcher's native language. Other considerations with interpreting the results of studies on *P. oceanica* associated communities is that research methodology has changed over the past few decades and the variance of mean values are not cited in much of the primary literature of the 1970s and 1980s (e.g. Eugene 1979; Buia et al. 1985a; Chessa et al. 1989; Mazzella et al. 1989). This means that statistical comparisons between data sets cannot be made, leaving out any possibility of meta-analysis.

This does not imply that older research should be disregarded. In fact, quite the opposite. With the recent growth in population and tourism in coastal regions of the Mediterranean, and the increased coastal urban sprawl to accommodate this, anthropogenic impacts have had substantial effects on coastal environments and very few pristine habitats remain (European Environment Agency 1999). In this sense, historic research is likely to give a more accurate picture of the associated communities in less disturbed meadows.

Here we present the findings, taken from multiple studies of *P. oceanica* and its associated communities, using data and results from studies spanning more than four decades. Where studies incorporated comparisons of disturbed and control meadows, data was only extracted from results from control meadows. In order to build a picture of how seagrass architecture and the floral and faunal communities vary with depth we have compiled a table of data from numerous studies. Where numerical data was not presented in the primary literature, values were mined from graphs using Data Thief III (v. 1.6).

2.2 The *Posidonia oceanica* habitat

Posidonia oceanica (L.) Delile is the most important species of seagrass found in the Mediterranean basin, and forms some of the most productive meadows globally (Pergent-Martini et al. 1994). This is due to its dominance, complexity and ability to form extensive, continuous meadows (Buia et al. 2004). It can be found growing from the surface down to depths of over 40 m, although this depth limit is only found for particularly clear waters (Augier and Boudouresque 1979). In areas where water transparency is reduced, the depth limit of *P. oceanica* may be 10-15 m (Bodoy et al. 1982; Avril et al. 1984). It forms dense mono-specific meadows, which harbour a wide variety of associated plants and animals (Mazzella et al. 1992), and is a 'climax' community, which shows low levels of resilience to anthropogenic impacts (Buia and Mazzella 2000).

P. oceanica is the longest lived species of marine phanerogams (Duarte 1991; Duarte et al. 1999), and its shoots are able to live for at least 30 years (Marba et al. 1996). It is characterized by a large number of shoots, high above ground biomass and a large leaf area index (LAI) (Guidetti et al. 2002). Sexual reproduction is of low importance in *P. oceanica*, as flowering is highly irregular and often has a low success rate (Piazzi et al. 1999). The growth of meadows is typically by vegetative propagation. This may partially explain the low genetic diversity found in these meadows (Raniello and Procaccini 2002). The newest leaves are found in the middle of the shoot and oldest leaves on the outside. This species shows intrinsic seasonal growth, with highest growth rates during winter and spring (Ott 1979), and a drastic decrease in growth during the summer months (Drew 1978). Leaf length is determined by four main factors; growth, erosion, microbial decomposition and herbivory (Wittman et al. 1981; Casola et al. 1987; Alcoverro et al. 1997). Highest biomass of the prairie is found during early summer (Buia et al. 1992). New leaves sprout from the middle of the shoot, so the oldest leaves are found on the outside, whilst the newest leaves are found on the inside (Fig. 2.1). Leaves are ranked from the newest to the oldest (e.g leaf rank 1 is a juvenile leaf, whilst rank 5 would be an adult leaf).

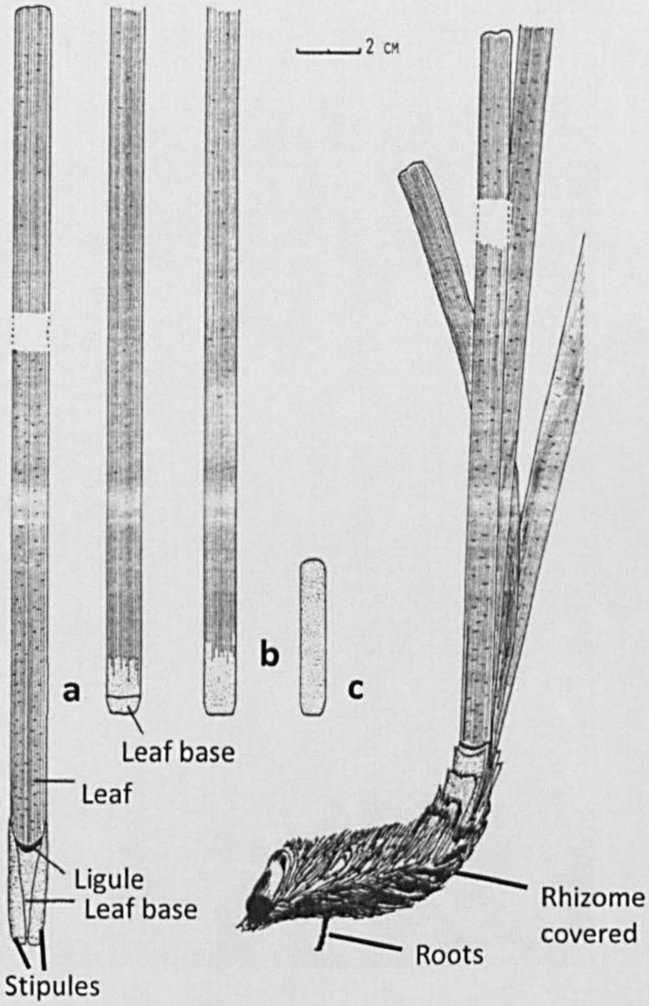


Figure 2.1 Detailed image of a *Posidonia oceanica* shoot, with rhizome covered by scales (old sheaths). Newest leaves are found at the middle of the shoot with oldest leaves on the outside. a) adult leaf, b) intermediate leaf, c) juvenile leaf. (Image from: Buia et al. 2004)

Above ground production positively corresponds with increasing density (Alcoverro et al. 1995b). In dense beds biomass has been found to reach 3000-3500g DW m^2 (Ott 1980; Libes et al. 1982), a value greater than that of mangrove forests and far greater than phytoplankton productivity (Thorhaug 1981). Above and below ground standing crop is higher than in other marine phanerogams (Mazzella et al. 1992). Large below ground standing crop is due to its unique ability to form biogenic 'mattes'. The 'matte' is formed by the continuous burial of rhizomes, roots and sheaths to form a course-fibred structure which can be centuries to millennia old (Mateo et al. 1997). This structure is composed of living and dead tissue mixed with sediment, which can be several metres thick (Romero et al. 1994).

Seagrass architecture is important in structuring the communities found in seagrass meadows, and depth is an important factor. Leaf area index (LAI) decreases with depth (Mazzella and Jorg 1984), as does shoot density (Battiato et al. 1982b) (Table 2.1). Differentiation in physical factors (such as light, water movement, exposure to erosion, siltation) between shallow and deep stands are important in structuring the characteristics of *P. oceanica* beds and their associated biological communities (den Hartog 2003). Hydrodynamic conditions strongly influence meadow characteristics such as the number of leaves per shoot and mean leaf length (Pessani et al. 1987).

Table 2.1 Comparison of values of measured parameters of structural architecture and floral and faunal communities in unimpacted, shallow ($\leq$10m), intermediate (11-20m) and deep (21m+) *Posidonia oceanica* systems. Numbers in brackets show the number of studies included.

Parameter	**Maximum and Minimum Values**			**References**
	Shallow	Intermediate	Deep	
Mean shoot density (no./m^2)	408 – 1025 $^{(7)}$	78 – 535 $^{(15)}$	63 – 420 $^{(9)}$	(Giraud et al. 1979; Bay 1984; Pirc 1984; Buia et al. 1985b; Pessani et al. 1987; Pergent 1990; Pergent and Pergentmartini 1990; Pergent and Pergentmartini 1991; Alcoverro et al. 1995a; Barbieri et al. 1995; Mostafa and Halim 1995; Pergent et al. 1997; Torricelli and Peirano 1997; Tsirika et al. 2007)
Mean leaf area index (LAI)	3.27 – 19.8 $^{(7)}$	2.20 – 21.6 $^{(9)}$	0.48 – 11.26 $^{(6)}$	(Giraud et al. 1979; Bay 1984; Pirc 1984; Buia et al. 1985b; Pessani et al. 1987; Bianchi C.N 1989; Borg and Schembri 1995; Mostafa and Halim 1995; Blundo 1999; Balestri et al. 2004; Tsirika et al. 2007)
Mean epiphyte biomass (g/dw/sh)	0.789 – 0.011 $^{(5)}$	0.967 – 0.019 $^{(2)}$	-----	(Thelin and Bedhomme 1983; Romero 1988; Alcoverro et al. 1997; Balata et al. 2008; Kruzic 2008)
No. macroalgal epiphytic species	10 - 40$^{(7)}$	6 - 48 $^{(5)}$	1 - 50 $^{(3)}$	(Battiato et al. 1982a; Cinelli et al. 1984; Buia et al. 1985b; Mazzella et al. 1989; Orlando and Bressan 1998; Blundo 1999; Kruzic 2008)
% coverage of macroalgae	5 – 59 $^{(6)}$	0.8 – 22 $^{(5)}$	0.3 – 16.5 $^{(1)}$	(Battiato et al. 1982a; Cinelli et al. 1984; Buia et al. 1985b; Mazzella et al. 1989; Tsirika et al. 2007; Kruzic 2008)
Abundance vagile fauna (hand net method)	54-5150 $^{(4)}$	200-2180 $^{(3)}$	224-1641$^{(3)}$	(Chessa et al. 1989; Mazzella et al. 1989; Gambi et al. 1992; Scipione et al. 1996)
No. species vagile fauna (all methods)	13-70$^{(6)}$	35-131$^{(6)}$	44-132$^{(4)}$	(Chessa et al. 1989; Mazzella et al. 1989; Gambi et al. 1992; Sanchez-Jerez and Ramos Espla 1996; Scipione et al. 1996; Borg et al. 2006; Apostolaki et al. 2007; Borg et al. 2010)

Meadows can be broadly divided into two sub-compartments; the leaf canopy and the root-rhizome layer, which have distinct associated assemblages (Bianchi et al. 1989; Vasapollo 2009), although many researchers suggest that these sub-compartments should be considered as a single system (Mazzella et al. 1992; Buia et al. 2004). Rather than dividing the meadow into sub-compartments, this review will focus on the three main categories of communities associated with *P. oceanica*: 1) macroalgal and sessile animal epiphytes found on the leaves and rhizomes of the plant, 2) vagile fauna which are associated with the leaf canopy or rhizomes, and 3) large, mobile, epibenthic species which forage within the seagrass.

2.3 Macroalgal and sessile epiphytes

Epiphytes can be found on the leaves and rhizomes of seagrass. These two compartments of the plant differ in terms of the habitat that they provide for epiphytic assemblages. The leaf canopy is a smooth, exposed and short-lived substratum for epiphytes, whilst the rhizomes have a rough surface with microcavities and provide a more permanent habitat (Pansini and Pronzato 1985). The leaf canopy shows a seasonal succession of the epiphytic community and maximum biomass is achieved between spring and late summer (Thelin and Bedhomme 1983; Romero 1988). In comparison rhizome epiphytes generally show little variation among seasons (Piazzi et al. 2002), although Cocito et al. (2012) found that Bryozoa diversity increased during warm water periods, using a different approach to assess the rhizome epiphytic community: the use of seagrass artificial rhizomes (mimics). The dynamic aspect of the canopy leads to a characteristic community, whilst rhizome epiphytes consist of many species which are common to the rocky substratum of the infra-littoral plain (Chimenz 1989).

P. oceanica has a diverse epiphytic community, which primarily consists of photosynthetic communities of cyanobacteria, diatoms, and crustose, ephemeral or filamentous algae (Novak 1984; Prado et al. 2008a). Primary production from epiphytes can exceed production of the seagrass itself (Libes 1986), and this production increases during the summer months with a sharp increase in ephemeral algae (Prado et al. 2008a). Many species of invertebrates also form part of the epiphytic community on *P. oceanica* leaves, principally coming from the classes Hydrozoa and Bryozoa (Eugene 1978; Giovannetti et al. 2010). These algae and invertebrate communities play a central role in the foodweb of seagrass ecosystems (Dauby 1989). They also increase the structural complexity of the seagrass habitat (Heijs 1987). The long life span of the shoots is central to the highly diverse community of epiphytes which settle on them (Alcoverro et al. 1997).

Macroalgal epiphytes found on the leaves of *Posidonia* can be divided into three categories, dependent on their morphological features: calcareous, soft encrusting and erect algae (Mazzella et al. 1989). Published literature on species of epiphytic macroalgae found on the shoots of *P. oceanica* revealed that species composition varies vastly between meadows. From a total of 12 studies, 296 species of macroalgal epiphytes were identified, and species names checked against AlgaeBase (Guiry and Guiry 2011). Of these, 96 were found in 25% or more of the studies, and only 27 were found in 50% or more (Table 2.2). This shows the extent of macroalgal variability between meadows. Species which were common to all studies include the calcareous algae *Hydrolithon farinosum* and *Pneophyllum fragile*, and the soft encrusting algae *Ascociclus orbiculare,* and erect algae *Giraudia sphacelarioides,* whilst the erect algae *Dictyota dichotoma*, *Antithamnion cruciatum*, *Sphacelaria cirrosa, Stylonema alsidii,* and *Erythrotrichia carnea* were common to most studies. The Rhodophyceae were represented by the highest number of species (187 or 63%), whilst the Phaeophyceae had the second highest (69 or 23%), and the Chlorophyceae represented by the least number of species (40 or 14%). The number of species varied significantly between studies, although % coverage generally decreased with depth (Table 2.1).

The sessile epifaunal community is less well studied than the macroalgal component of the epiphytic community (Casola et al. 1987), and many studies have combined the floral and faunal compartments to investigate total biomass or percentage coverage (e.g. Romero 1988; Balata et al. 2010). Other studies have kept these compartments separate (e.g. Prado et al. 2007a; Giovannetti et al. 2010), although community composition is unavailable. Some studies deal only with specific fractions of the community such as hydroids or bryozoans (e.g. Boero 1981b; Pansini and Pronzato 1985; Kocak 2002). After macroalgae, Bryozoa show the greatest leaf coverage (Eugene 1978; Dalla Via et al. 1998; Piazzi et al. 2004; Pardi et al. 2006), and are also the most diverse group, followed by Hydrozoa (Prado et al. 2007a). Other groups present include Foraminifera, Cnidaria, Tunicata, Porifera, and Annelida, although Porifera are only found on the rhizomes (Pansini and Pronzato 1985; Balata et al. 2007), as the canopy is perhaps a tougher environment to colonize (Pansini and Pronzato 1985). Some species can be found exclusively on the shoots of *P. oceanica* such as the bryozoan *Electra posidonidae* or the hydroids *Sertularia perpusilla*, *Campanularia asymmetrica* and *Aglaophoenia harpago* (Boero 1981a; Matricardi et al. 1991; Vasapollo 2009).

Table 2.2 List of most common epiphytic macroalgal species found in 12 studies (>25%) (Van der Ben 1971; Panayotidis and Boudouresque 1981; Cinelli et al. 1984; Buia et al. 1985b; Soto Moreno 1992; Blundo 1999; Balata et al. 2007; Piazzi et al. 2007; Prado et al. 2007a; Jacquemart and Demoulin 2008; Kruzic 2008; Giovannetti et al. 2010). Species highlighted in light grey were present in 50% or more of the studies.

Chlorophyta

Acrochaete viridis (Reinke) R.Nielsen

Chaetomorpha aerea (Dillwyn) Kützing

Cladophora dalmatica Kützing

Cladophora sp.

Phaeophila dendroides (P.L.Crouan & H.M.Crouan) Batters

Pringsheimiella scutata (Reinke) Marchewianka

Phaeophyta

Asperococcus bullosus J.V.Lamouroux

Asperococcus sp.

Cladosiphon cylindricus (Sauvageau) Kylin

Cladosiphon irregularis (Sauvageau) Kylin

Dictyota dichotoma (Hudson) J. V. Lamouroux

Dictyota linearis (C. Agardh) Greville

Ectocarpus siliculosus (Dillwyn) Lyngbye

Ectocarpus sp.

Feldmannia irregularis (Kützing) G.Hamel

Feldmannia paradoxa (Montagne) G.Hamel

Giraudia sphacelarioides Derbès & Solier

Halopteris filicina (Grateloup) Kützing

Halopteris scoparia (Linnaeus) Sauvageau

Kuckuckia spinosa (Kützing) Kornmann

Myriactula gracilis van der Ben

Myriactula stellulata (Harvey) Levring

Myrionema orbicularis (J.Agardh) Kjellman

Myrionema strangulans Greville

Sphacelaria cirrosa (Roth) C. Agardh

Sphacelaria fusca (Hudson) S.F.Gray

Sphacelaria tribuloides Meneghini

Sphacelaria sp.

Rhodophyta

Acrochaetium secundatum (Lyngbye) Nägeli

Acrochaetium sp.

Acrosorium ciliolatum (Harvey) Kylin

Aglaothamnion cordatum (Børgesen) Feldmann-Mazoyer

Aglaothamnion tenuissimum (Bonnemaison) Feldmann-Mazoyer

Aglaothamnion tripinnatum (C.Agardh) Feldmann-Mazoyer

Anotrichium barbatum (C.Agardh) Nägeli

Antithamnion cruciatum (C.Agardh) Nägeli

Antithamnion heterocladum Funk

Antithamnion tenuissimum (Hauck) Schiffner

Antithamnion sp.

Apoglossum ruscifolium (Turner) J.Agardh

Asparagopsis armata Harvey

Callithamnion corymbosum (Smith) Lyngbye

Ceramium ciliatum (J.Ellis) Ducluzeau

Ceramium codii (H.Richards) Mazoyer

Ceramium comptum Børgesen

Ceramium deslongchampsii Chauvin ex Duby

Ceramium diaphanum (Lightfoot) Roth

Ceramium siliquosum var. *lophophorum* (Feldman-Mazoyer) Serio

Ceramium tenuissimum (Roth) Areschoug

Champia parvula (C.Agardh) Harvey

Chondria capillaris (Hudson) M.J.Wynne

Chondria mairei G.Feldmann

Colaconema daviesii (Dillwyn) Stegenga

Crouania attenuata (C.Agardh) J.Agardh

Dasya corymbifera J.Agardh

Dasya hutchinsiae Harvey

Dasya ocellata (Grateloup) Harvey

Dasya rigidula (Kützing) Ardissone

Erythrotrichia carnea (Dillwyn) J.Agardh

Eupogodon planus (C.Agardh) Kützing

Gayliella flaccida (Harvey ex Kützing) T.O.Cho & L.J.McIvor

Herposiphonia secunda (C.Agardh) Ambronn

Herposiphonia secunda f. *tenella* (C.Agardh) M.J.Wynne

Heterosiphonia crispella (C.Agardh) M.J.Wynne

Hydrolithon boreale (Foslie) Y.M.Chamberlain

Hydrolithon cruciatum (Bressan) Y.M.Chamberlain

Hydrolithon farinosum (J.V.Lamouroux) D.Penrose & Y.M.Chamberlain

Hypoglossum hypoglossoides (Stackhouse) F.S.Collins & Hervey

Jania rubens (Linnaeus) J.V.Lamouroux

Laurencia obtusa (Hudson) J.V.Lamouroux	*Pterothamnion crispum* (Ducluzeau) Nägeli
Laurencia sp.	*Pterothamnion plumula* (J.Ellis) Nägeli
Lejolisia mediterranea Bornet	*Ptilothamnion pluma* (Dillwyn) Thuret
Lithophyllum pustulatum (J.V.Lamouroux) Foslie	*Rhodophyllis divaricata* (Stackhouse) Papenfuss
Lomentaria chylocladiella Funk	*Spermothamnion flabellatum* Bornet
Monosporus pedicellatus (Smith) Solier	*Spermothamnion flabellatum f. disporum* Feldmann-Mazoyer
Nitophyllum punctatum (Stackhouse) Greville	*Spermothamnion johannis* Feldmann-Mazoyer
Pneophyllum fragile Kützing	*Spermothamnion repens* (Dillwyn) Rosenvinge
Polysiphonia furcellata (C.Agardh) Harvey	*Spyridia filamentosa* (Wulfen) Harvey
Polysiphonia scopulorum Harvey	*Stylonema alsidii* (Zanardini) K.M.Drew
Polysiphonia subulifera (C.Agardh) Harvey	*Stylonema cornu-cervi* Reinsch
Polysiphonia sp.	*Womersleyella setacea* (Hollenberg) R.E.Norris

Much research has gone into determining what causes variation in the epiphytic communities of *P. oceanica*. There is a succession of micro- and macroflora along the leaf blade from basal to apical tissue (Mazzella and Russo 1989). Basal (young) tissue is primarily colonized by pioneer species such as bacteria and diatoms from the genus *Cocconeis*, whilst an increase in calcareous and soft encrusting algae and a greater diversity of diatoms are found in older portions of the leaf (Mazzella and Russo 1989; Giovannetti et al. 2010). Diatoms are made up almost exclusively of the pennate form (Mazzella 1983; Novak 1984). Sessile invertebrates and erect algae are primarily found on older parts of the leaves, where there is higher light and water movement (Mazzella and Russo 1989). This sequential colonization and increase in biomass continues up to a maximum of 200 days (Cebrian et al. 1999). Bryozoans show a graduated distribution along the leaf and tend to reach maximum biomass on the central sections of the shoots (Casola et al. 1987; Cebrian et al. 1999). Casola et al. (1987) found that different species of bryozoa dominated along the leaf length; *Fenestrulina johannae* is a basal species, *Electra posidoniae* a central species, and *Aetea truncata* an apical species.

Although this general pattern is often found, epiphytic community composition varies significantly between meadows, depths and seasons (Piazzi and Cinelli 2000; Vanderklift and Lavery 2000; Martínez-Crego et al. 2010), due to differing environmental factors such as water movement, light availability and temperature (Van der Ben 1971; Chessa et al. 1982; Vanderklift and Lavery 2000). Lower numbers of macroalgal species are found at exposed sites, which may be due to increased exposure to wave action and increased leaf shedding, which would influence algal succession and cover (Buia et al. 1985b). Canopy parameters such as shoot density can influence local environmental factors such as light availability and water movement, which in turn affect epiphytic community composition (Tsirika et al. 2007). Epiphytic macroalgal biomass and coverage decreases with depth (Table 2.1) (Cinelli et al. 1984; Mazzella and Ott 1984; Mazzella et al. 1989), and epifaunal biomass can reach over 50% of epiphyte biomass at deeper depths (Lepoint et al. 1999). It is thought that grazing and competition are the main factors governing algal species composition of shallow meadows, whilst other factors such as environmental status may be more prominent in deep meadows (Martínez-Crego et al. 2010).

Maximum epiphytic biomass is reached during late summer in shallow beds (Tsirika et al. 2007), although this is delayed by 1-2 months in deep beds, which may be related to the thermocline (Mazzella and Ott 1984). Seasonal variation in epiphytes is more pronounced in shallow and intermediate beds than deeper beds, where the increase is negligible (Mazzella and Ott 1984). Some studies have shown that there is a drop in epiphytic biomass between spring and autumn (Thelin and Bedhomme 1983; Mazzella and Ott 1984; Romero 1988), although this decrease does not occur at all depths (Mazzella and Ott 1984; Romero 1988), or in all meadows (Alcoverro et al. 1997). *P. oceanica* production is at a minimum during the summer months (Alcoverro et al. 1995b), and grazing pressure can exceed production (Tomas et al. 2005c), which would explain this reduction in epiphytic biomass. Epiphytic bacterial and diatom abundance also shows seasonal changes with an increase of an order of magnitude between April and September (Novak 1982a).

The Rhodophyceae are the dominant epiflora throughout all seasons (Blundo 1999), and Corallinaceae the most abundant family (Mazzella et al. 1989). Filamentous and crustose forms are abundant during summer, whilst crustose forms dominate in winter (Van der Ben 1971; Tsirika et al. 2007). The crustose red algae *Hydrolithon farinosum* and *Pneophyllum fragile* can be found encrusting the leaves of *P. oceanica* at all depths and at all times of the year (Jacquemart and Demoulin 2008). Brown algae such as *Giraudia sphacelarioides* and red algae such as *Acrochaetium daviesii* and *Antithamnion cruciatum* are abundant during the summer months (Pardi et al. 2006; Tsirika et al. 2007; Jacquemart and Demoulin 2008).

2.4 Vagile fauna of the canopy and rhizomes

A high diversity of motile macroinvertebrates can be found associated with *Posidonia oceanica* meadows, ranging from large organisms such as echinoids and cephalopods down to much smaller taxa such as cumaceans, copepods and mysids. When examining the community composition of associated macrofauna, the majority of studies focus on the fraction termed 'vagile fauna' (e.g. Chessa et al. 1989; Mazzella et al. 1989; Gambi et al. 1992). Vagile fauna can be classified as a group of animals with reduced mobility, which are associated with the canopy and rhizomes of the seagrass. A high diversity of species from taxonomic groups such as decapod crustaceans, peracarid crustaceans, gastropods, bivalves, polychaetes and echinoderms can be found associated with *P. oceanica* (Gambi et al. 1992). Many of these species show a positive response to an increase in epiphytic biomass (Bologna and Heck 1999), suggesting that epiphytes may be an important source of food for these organisms (Fig. 2.2).

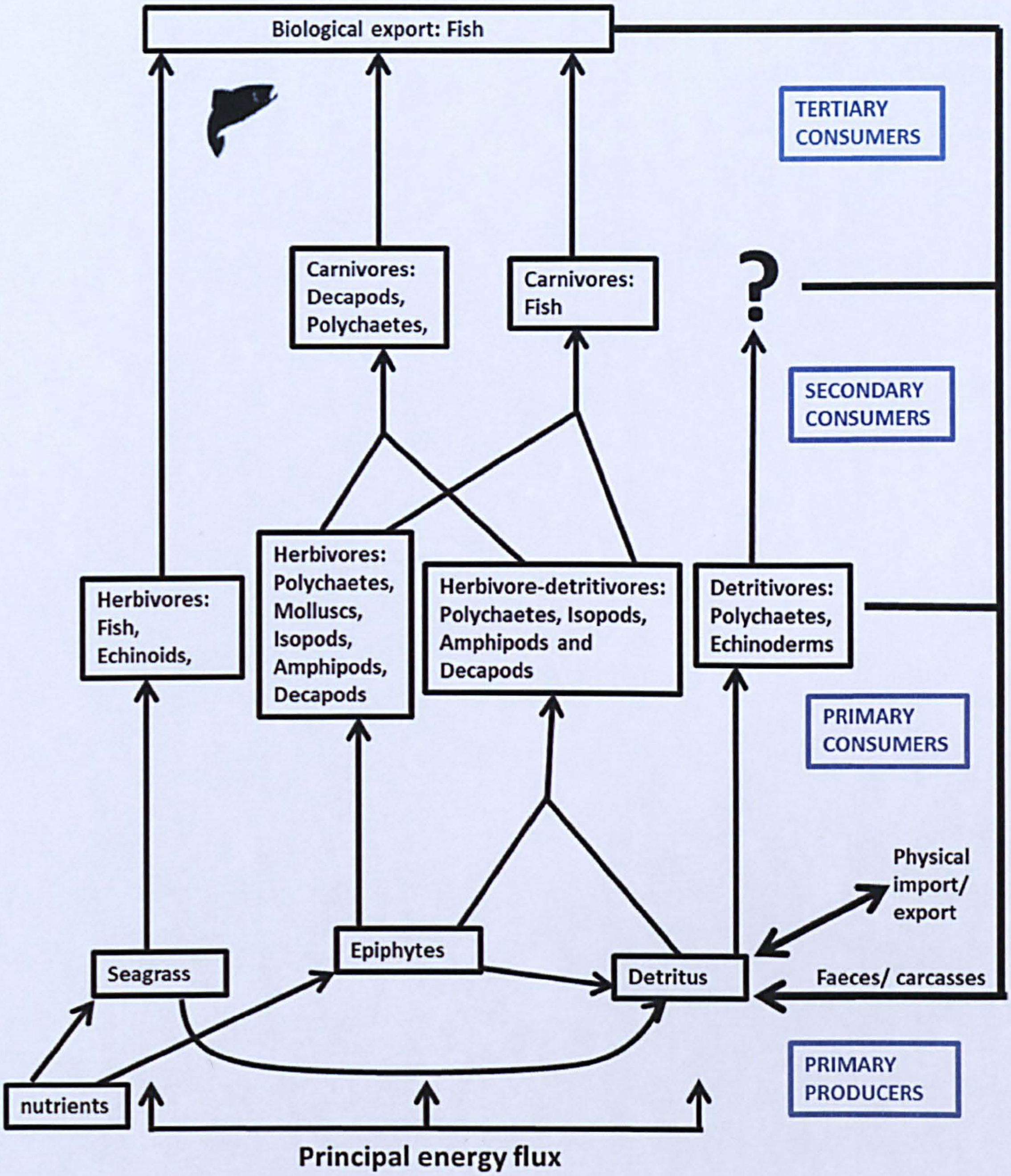


Figure 2.2 Illustration of the trophic energy flux in a *Posidonia oceanica* system (modified from Mazzella and Zupo 1995).

The diversity and abundance of vagile fauna associated with *P. oceanica* is greater than those associated with *Cymnodocea nodosa* and other seagrasses common to the Mediterranean (Buia et al. 2000; Como et al. 2008). This is thought to be due to the structural architecture of *P. oceanica* and its associated epiphytes, which gives rise to a greater niche availability for these animals than other Mediterranean seagrass species (Scipione et al. 1996). Vagile fauna can generally be divided into three categories: those that crawl (molluscs and annelids), those that have suckers (echinoderms) and those that move by using jointed appendages (crustacea) (Gadeu 1967).

Community composition varies along a depth gradient (Chessa et al. 1989), with a strong zonation of superficial (0-4 m), intermediate (6-12 m) and deep (15-30 m) communities (Scipione et al. 1982). This has been shown for molluscs (Russo 1991), hermit crabs (Zupo et al. 1985), polychaetes (Gambi et al. 1992) and isopods (Lorenti and Fresi 1982) and although temporal and spatial variation is important, this pattern of zonation remains unchanged (Russo 1984; Russo 1985). Idato et al. (1983) found that mollusc abundance is greatest at intermediate depths, whilst overall diversity was greatest in deeper prairies. Species may show preference for different depths, for example the shrimp *Hippolyte inermis* (Zupo 1994) and the gastropod *Rissoa italiensis* (Gambi et al. 1992) are mainly found in shallow beds, and the whilst the gastropod *Bittium latreillii* is most abundant, and exclusively shows recruitment, in deeper stands (Russo et al. 2002). Some species do not appear to show any preference and are represented at all depths, such as the hermit crab *Cestopagarus timidus* (Zupo et al. 1985). The increased diversity of motile macroinvertebrates at deeper stations is probably due to the more stable environment (Chessa et al. 1989; Mazzella et al. 1989). The abundance and diversity of vagile fauna found in *P. oceanica* at shallow, intermediate and deep depths varies considerably between studies (Table 2.1), suggesting that variation between meadows is high. The greatest number of individuals is generally found at shallow depths (Mazzella et al. 1989), although this appears to be dependent upon the season of sampling (Chessa et al. 1989). It must be noted that some very shallow stations (1m) exhibit low abundances of vagile fauna (Mazzella et al. 1989).

Trophic structure also changes with depth and herbivores are most abundant in shallow stations, whilst carnivores are found in their greatest numbers in deep stations, although at all depths organisms that feed either wholly or partly on micro- or macroalgae dominate (Gambi et al. 1992). Some herbivorous fauna are selective feeders, such as the polychaete *Platynereis dumerilii*, which shows preference for erect micro- and macroalgae (Gambi et al. 2000), whilst other species such as the isopod *Idotea balthica* have a more omnivorous diet, feeding on algae, seagrass, detritus and crustaceans (Sturaro et al. 2010). Meiofaunal assemblages provide an important trophic link between primary producers and higher trophic levels (Danovaro et al. 2002). Detritus is an important food source for many invertebrates such as polychaetes, isopods, amphipods and decapods (Buia et al. 2004).

Community structure of different taxonomic groups shows high variation, with different species dominating in different meadows (Russo et al. 1991; Gambi et al. 1995; Scipione 1998). Although this variation may be partially explained by differences in sampling methodology (Mazzella et al. 1992), it is clear that there is a huge spatial variation in the composition of vagile fauna. This spatial variability is more prominent in shallow stations (Gambi et al. 1995), and is thought to be primarily due to local and regional variations in water movement and sedimentation (Idato 1983; Russo et al. 1984). As an example of this variation, some studies have found polychaetes are abundant within *Posidonia oceanica* beds (e.g. Harriague et al. 2006; Como et al. 2008), whilst others have found their abundance to be negligible (e.g. Gambi et al. 1992; Sanchez-Jerez et al. 1999a). Seasonal variation in species composition is also prominent (Gambi et al. 1992), with common taxa such as amphipods and gastropods reaching their highest abundances between late autumn and winter (Gambi et al. 1992; Scipione et al. 1996). Sanchez-Jerez et al. (1999a) suggest that increased abundance of vagile fauna during late autumn and winter may be because of the decrease in the abundance of fish; therefore predation is lowest during this time.

Many species of mobile invertebrates undergo diel vertical migrations within the *Posidonia* system. Studies have shown that species diversity in the canopy increases at night, although results as to which species are variable. Russo et al. (1983) found that the abundance and diversity of molluscs (and particularly gastropods) increased at night, whilst Sanchez-Jerez et al. (1999b) found that many taxa (including decapods, polychaetes and amphipods) increased in abundance at night but gastropod abundance remained the same diurnally. Russo et al. (1983) found that the nocturnal increase in molluscs was most prominent at a deep site (25 m), whilst Sanchez-Jerez et al. (1999b) only conducted their study at a single depth (12 m). This conflict of results may be due to differences in the depths where the study took place and shows the strong influence that depth, and the environmental factors associated with this (water movement, light and sedimentation), may have on community composition. This pattern of diel vertical migration may be in response to predation pressure from predatory fish which are present during daylight hours (Sanchez-Jerez et al. 1999b).

Another functional group of organisms (some species of polychaetes and isopods) bore into the sheaths (remains of leaves found along the rhizome) of *P. oceanica*. Although they are not strictly vagile fauna, due to their burrowing nature, they will be included in this section. They are detrital feeders, primarily feeding on the dead tissue of the sheaths (Guidetti et al. 1997; Guidetti 2000b; Gambi 2002), although some larger individuals have also been observed feeding on live tissue (Guidetti 2000b; Gambi 2002). Density of borers can be highly variable between meadows and is thought to be due to shoot density or sheath toughness (Gambi et al. 2005). The isopod borer *Limnoria mazzellae* is mainly found in shallow stands and selects younger sheaths, whilst polychaete borers are found in shallow and deep stands but select older sheaths (Guidetti et al. 1997; Gambi et al. 2005).

One of the major problems with compiling a review of studies of vagile fauna found in *P. oceanica* is the wide variety of sampling methods used, such as hand-towed nets (Russo et al. 1985), airlift samplers (Terlizzi and Russo 1996), grab-samplers (Como et al. 2007) or corers (Borg et al. 2002). Methods of sampling communities are rarely perfect and each of these techniques has advantages and drawbacks in terms of their ability to quantify communities, efficiency of collection, reproducibility and destructiveness. For example, hand-towed nets will primarily collect animals associated with the leaf canopy, whilst airlift samplers may show greater efficiency for collecting fauna associated with the rhizomes (Terlizzi and Russo 1996). Thus, as a consequence, two studies, identical in all other factors other than sampling method may produce very different results.

2.5 Macrobenthos

This section will deal with larger organisms found in *Posidonia oceanica* habitats such as echinoderms, cephalopods and fish. Very few studies have carried out an evaluation of the temporal, spatial and depth distribution of cephalopods. Cephalopods tend to be transient species which travel between habitats, and their ecology and distribution is probably best described through fisheries science. Species of cuttlefish (e.g. *Sepia officinalis*: Guerra 1985) or octopus (e.g. *Octopus vulgaris*: Forcada et al. 2009) are known to forage in *P. oceanica* beds but little is understood of their distribution, their effect on the *P. oceanica* food web, or the importance of this habitat for them.

Several echinoderm species from the classes Asteroidea, Ophiuroidea, and Echinoidea are found in *P. oceanica* beds (Koukouras et al. 2007). Of these, the most studied is the sea urchin *Paracentrotus lividus*, as it is an important herbivore which consumes *P. oceanica* (Boudouresque and Verlaque 2001), and is commercially harvested for its gonads (Palacin et al. 1998). Other species of echinoderms found in *P. oceanica* include the sea urchin *Psammechinus microtuberculatus*, the star fish *Echinaster sepositus*, the brittle star *Ophiura albida* and the holothurian *Holothuria tubulosa* (Amon and Herndl 1991; Beqiraj et al. 2008). Very little is known of these species spatial and temporal distribution, although observations have shown that *H. tubulosa* shows a progressive downward migration: small individuals are found in shallow meadows, whilst larger individuals are found in deep meadows (Coulon and Jangoux 1993).

P. lividus is characteristic of *P. oceanica* habitats in the Mediterranean, and can be found in densities of between 0-18 individuals m^2 (Kirkman and Young 1981; Palacin et al. 1998; Tomas et al. 2004; Prado et al. 2008b). These animals change their feeding habits through their life history, feeding primarily on algae when they are juveniles and increasing their intake of *Posidonia* as they mature (Traer 1979). Maximum consumption rates occur during the winter months (Nedelec et al. 1982; Tomas et al. 2005c). The fish *Sarpa salpa* is another important consumer of *P. oceanica*. In contrast to *P. lividus*, maximum consumption rates of *P. oceanica* for this fish occur in summer months, when grazing can exceed primary production in shallow waters (Tomas et al. 2005c). During the winter months, mainly juvenile fish remain in the shallow beds, whilst the adults migrate to deeper habitats (Francour 1997; Peirano et al. 2001).

P. lividus and *S. salpa* are the primary consumers of *P. oceanica* (Buia et al. 2004) and very few other species feed on it directly as it is nutritionally poor, with a high C:N ratio, due to the high quantity of structural carbohydrates and low protein content (Lawrence et al. 1989). Most grazing pressure is exerted in beds <10m depth (Tomas et al. 2005c). In a study of the grazing pressure on *P. oceanica* during summer, *S. salpa* and *P. lividus* were responsible for approximately 70% and 30% of grazing respectively (Cebrian et al. 1996), although these figures can be reversed during the winter months (Peirano et al. 2001). These grazers are an important component of the seagrass community as they primarily feed on highly epiphytised leaves, increasing light availability to the seagrass and preventing epiphytic overgrowth of the plant (Van Montfrans et al. 1984). Although these animals can exert extremely high grazing pressure, which can exceed leaf primary production (Prado et al. 2008b), they do not appear to compete (Tomas et al. 2005a). This may be due to resource partitioning, allowing for co-existence. Alongside seasonal differences in feeding habits, *P. lividus* tends to feed on the lower sections of leaves, and *S. salpa* on the apical portions (Pinna et al. 2009). A high degree of spatial variability in herbivore pressure is also apparent (Cebrian et al. 1996), which has been shown to be important in structuring the spatial variation in epiphytic assemblages found on *P. oceanica* (Prado et al. 2007a).

Posidonia oceanica provides food and refuge to a number of fish species (Deudero et al. 2008), and is an important habitat for juveniles (Guidetti 2000a; Kalogirou et al. 2010). Although *S. salpa* is the focus of much literature, many different species of fish can be found foraging in *P. oceanica* beds. Unlike tropical seagrass beds, where many species show ontogenic migrations (Aguilar-Perera and Appeldoorn 2007), the majority of species are found in *P. oceanica* throughout their life-cycle (Bell 1982). This is not to say that they do not move between adjacent habitats. The fish assemblages of rocky-algal reefs are very similar to those found in *Posidonia* meadows (Guidetti 2000a), suggesting high connectivity between these two habitats. Fish larvae settlement within seagrass beds is patchy and larvae do not appear to select for seagass density or height (Bell et al. 1987), although they will generally redistribute to stands of increased density and height post-settlement (Bell and Westoby 1986b).

Valle et al. (2001) found that diversity and abundance of fish species found in *P. oceanica* increases during the night. This is comparable to data from tropical seagrass beds (Unsworth et al. 2008), and may suggest that some species of fish may reside in alternative day-time habitats and move into the *Posidonia* beds to forage during the night. A further possibility is that species that feed at night hide within the canopy during the daytime (Del Pilar Ruso and Bayle-Sempere 2006). This may prevent detection by some sampling methods. As community structure appears to vary diurnally, it is suggested that where possible, future studies should include diurnal sampling.

The most diverse fish families associated with the seagrass are Labridae and Sparidae (Valle et al. 2001; Fernández et al. 2005; Kalogirou et al. 2010), and these often have the highest abundance (Francour 1997). *S. salpa* is a shoaling species and during summer months can account for up to 70% of biomass found in shallow beds (Francour 1997). Other abundant species are: *Chromis chromis*, *Coris julis*, *Mullus surmuletu*s, *Diplodus* sp., *Symphodus* sp. and *Gobius* sp. (Guidetti 2000a; Fernández et al. 2005; Moranta et al. 2006; Deudero et al. 2008; Kalogirou et al. 2010). Although *S. salpa* is a herbivore, the majority of fish found in *Posidonia* are carnivores, primarily feeding on the invertebrates found within the canopy and rhizomes (Fig. 2.1; Francour 1997).

Fish assemblage structure shows a high degree of seasonality and abundance and diversity of fish generally peaks in the summer months (Francour 1997; Deudero et al. 2008). This increase is thought to be primarily due to the recruitment cycle and reproductive aggregations (Deudero et al. 2008), as most recruitment occurs between July and September (Garcia-Rubies and Macpherson 1995). Some species of fish such as the two-banded seabream, *Diplodus vulgaris*, appear to increase in abundance during the winter months (Forcada et al. 2009), which is probably because recruitment in this species occurs during this time (Hammoud and Saad 2007). Species from the family Sparidae are unusual in that recruitment occurs at different times of the year (Garcia-Rubies and Macpherson 1995). Long-lived species such as *Serranus cabrilla* show lower seasonal variations in density than short-lived species such as *Symphodus ocellatus* (Deudero et al. 2008). Differences in assemblage structure can also be seen at differing depths although these differences appear to vary between studies. Fernández et al. (2005) found that diversity and biomass of fish is greatest in deeper meadows, whilst Francour (1997) found diversity greatest in sheltered, shallow beds, suggesting depth effects differ between meadows. Fish species such as the sea-bass *Dicentrarchus labrax* and the saupe *Sarpa salpa* show preference for shallow meadows (Francour 1997).

A study of fish assemblages in the Eastern Mediterranean found a total of 88 species (Kalogirou et al. 2010). This is much higher than previous studies carried out in the western Mediterranean where between 22 and 53 species were found (Harmelin-Vivien and Francour 1992; Sanchez-Jerez and Ramos Espla 1996; Guidetti 2000a; Valle et al. 2001; Fernández et al. 2005; Moranta et al. 2006; Deudero et al. 2008). This may be partially explained by the migration of subtropical and tropical non-indigenous species through the Suez Canal (Golani et al. 2007; Kalogirou et al. 2010). This may cause noticeable differences in fish population dynamics between the east and the west of the Mediterranean.

It is not possible to make a comparison between studies of the number of fish found in *P. oceanica* beds as many different methods for fish enumeration have been employed. Studies can be undertaken by underwater visual census transect (UVC) (e.g. Francour 1997; Fernández et al. 2005), timed UVC (e.g. Garcia-Rubies and Macpherson 1995; Letourneur et al. 2003), beam trawl (e.g. Deudero et al. 2008), seine nets (e.g. Kalogirou et al. 2010), or trammel nets (e.g. Forcada et al. 2009). Many studies either show only the abundance of particular species (e.g. Fernández et al. 2005), only include abundant species (e.g. Francour 1997), or measured fish on an abundance scale (e.g. Guidetti 2000a; Letourneur et al. 2003). For example Guidetti (2000a) found that the mean abundance of fish in a shallow meadow (6.5-8m) was 223 per 40m^{-2} (5,575 per 1,000m^{-2}), whilst Moranta et al. (2006) found an average of 157 per 1000m^{-2} for three surveyed sites between 15-25m depth. These studies were both undertaken in summer, and although some of this difference may be depth related, the method of sampling may be critical. Guidetti (2000) surveyed a 40m transect using UVC and an abundance scale, whilst Moranta et al. (2006) surveyed larger areas using a beam trawl and counted absolute abundance. Trawling has been shown to identify a greater number of species, and UVC a greater number of individuals (Harmelin-Vivien and Francour 1992).

2.6 Conclusions

This review aimed to understand the structure of *P. oceanica*- associated communities in areas with no/ low levels of anthropogenic disturbance, to attempt to characterize the natural variability found in this system. Findings from this research suggest that natural variation in species numbers and abundances can make the task of quantifying changes in relation to anthropogenic disturbance extremely problematic. For example the number of species of epiphytic macroalgae found in deep meadows (> 21m) ranged from 1 to 50, whilst the abundance of vagile fauna caught by the hand new method in shallow meadows

(≤ 10 m) ranged from 54 to 5,150, and the abundance of fish ranged from 157 to 5,575 per 1000 m^2. Some trends in changes in depth could be observed. Seagrass density and LAI generally decreased with depth, as did the % coverage of macroalgae, whilst the number of species of vagile fauna increased with depth.

In a comparison of twelve studies, 296 species of epiphytic macroalgae were found on the leaves of P. oceanica, although only 27 species occurred in 50% or more of the studies. 66% of species belonged to the Rhodophyceae, 23% to the Phaeophyceae and only 14% to the Chorophyceae. Crustose coralline algae from the genus' *Hydrolithon* and *Pneophyllum* were common to all studies in all seasons, and crustose algae was more abundant that soft encrusting or erect epiphytic macroalgae.

The abundance of vagile fauna is generally greatest in shallow stations (≤ 10 m), whilst the number of species tends to increase with depth. Herbivore numbers are greatest at shallow stations, whilst carnivores are greatest in deeper stations. Whilst epiphytic microalgae, macroalgae and detritus are important food sources for many primary consumers, *P. oceanica* is only eaten by a small number of species, primarily the fish *Sarpa salpa* and the sea urchin *Paracentrotus lividus*. *S. salpa* can make up to 70% of fish biomass in shallow beds in the summer months (Francour 1997), although many different species of fish are found in this system (studies of species richness ranged identification of between 22 to 88 species). Species diversity is often greater in the Eastern than the Western Mediterranean, although this is likely due to the migration of alien species through the Suez Canal (Kalogirou et al. 2010), and therefore due to anthropogenic disturbance, rather than natural variation.

Posidonia oceanica is an important structural and trophic resource which supports an abundance of associated flora and fauna (Mazzella et al. 1992). This review indicates that although negative interactions which provide regulatory functions, such as competition and predation, are present, many facilitative interactions can be observed in *P. oceanica* beds. One of the major facilitative roles is the structural habitat provision provided by *Posidonia* to its associated floral and faunal communities, providing ecological niches for a diverse assemblage of organisms. In return the consumption of plant epiphytes by mesograzers has been shown to enhance seagrass leaf survival (Jernakoff and Nielsen 1997).

Studies show that there is a huge variation in the seagrass-associated flora and fauna between depths, seasons and meadows but that *P. oceanica* supports a community which is both functionally and biologically diverse. All associated communities (epiphytic, macroinvertebrates and fish) show a strong depth zonation, although it is hard to tease out the effects of depth solely, as effects are confounded by the decrease in seagrass density (Fernández et al. 2005). Algal epiphytes decrease with depth, due to light limitation and this reduces the number of herbivores accordingly.

This review provides a synthesis of many decades of research, much of which can only be found in print. It provides an overview of communities associated with undisturbed *Posidonia oceanica* meadows, selecting only results of studies where no anthropogenic stressors were mentioned. *P. oceanica* is declining at an alarming rate in the Mediterranean (Bianchi and Morri 2000), leading to the loss of critical habitat for its associated flora and fauna (Hughes et al. 2009). This review indicates the value of this habitat in terms of the biodiversity found there, leading to high levels of both primary and secondary production, and therefore the importance of seagrass meadow conservation.

3. Ocean acidification affects nutrient availability and plant function in a temperate seagrass meadow

3.1 Introduction

Anthropogenic release of CO_2 (primarily from burning fossil fuels) has increased global atmospheric concentrations from 280ppm to 390ppm since pre-industrial times, and levels could rise to over 750ppm by the end of this century (IPCC 2001b). This change in atmospheric levels of CO_2 is driving changes in ocean chemistry. Most notably, increased absorption of CO_2 in seawater leads to increased H^+ concentration and a subsequent reduction in pH (Doney et al. 2009b). Seawater is expected to decrease by approximately 0.3-0.5 pH units to approximately pH 7.8 by the end of this century (Caldeira and Wickett 2005). This is a potential threat to marine organisms and has been termed ocean acidification (OA) in the literature (Doney et al. 2009b).

Over the past few years, research into OA has grown exponentially (Kroeker et al. 2010), with particular focus on calcifying species, due to their vulnerability to changes in carbonate chemistry (Fabry et al. 2008). Results of this research shows that there is likely to be significant intra- and interspecific variability in species abilities to tolerate or adapt to OA (Garrard et al. 2013), which may be related, not only to phylogeny, but also to life strategy such as feeding habits, distribution and behaviour (Widdicombe and Spicer 2008). Many authors have voiced their concern regarding the potential loss of biodiversity that may arise from gradual OA (e.g. Hall-Spencer et al. 2008; Fabricius et al. 2011; Hale et al. 2011), and impacts may be particularly severe in calcifying habitats such as coral reefs (Hoegh-Guldberg et al. 2007).

Seagrasses form extensive beds that represent extremely productive and biologically diverse systems (Guinotte and Fabry 2008) and provide important ecosystem services such as supporting fisheries, sediment stabilization, nutrient cycling and carbon sequestration (Waycott et al. 2009). Of the seagrass species, *Posidonia oceanica* is one of the longest lived (Duarte 1991; Marba et al. 1996) and forms dense, monospecific meadows which are some of the most globally-productive marine meadows (Pergent-Martini et al. 1994). *P. oceanica* is endemic, and the most abundant species of seagrass in the Mediterranean, occupying approximately 25% of coastal waters down to a depth of 45m (Pasqualini et al. 1998). It supports a high abundance of flora and fauna (e.g. Van der Ben 1971; Gambi et al. 1992; Francour 1997) and the ecosystem formed is considered one of the climax communities of the Mediterranean infralittoral zone (Den Hartog 1977; Mazzella and Buia 1986). These habitats are economically important (Blasi 2009), but have suffered from widespread decline, particularly in the North West Mediterranean (Marba et al. 1996). In response, these communities have been listed as a priority habitat in Annex I of the Habitats Directive (EEC 1992).

Seagrass cover is declining at an alarming rate (7% per annum; Waycott et al. 2009) and this can be largely attributed to anthropogenic stressors such as coastal construction, eutrophication, invasive species, fishing, aquaculture and shipping (Duarte 2002). Climatic changes such as warming, sea level rise, increased wave action and storm frequency will cause added stress (Duarte 2002). However, a reduction in pH arising from OA may actually benefit seagrasses (Hall-Spencer et al. 2008).

Whilst the majority of species of seagrass appear to be able to use bicarbonate (HCO_3^-) as an inorganic carbon source, either through active transport or the presence of external carbonic anhydrase (CA), their affinity for dissolved carbon dioxide ($CO_{2(aq)}$) is greater (Invers et al. 2001; Koch et al. 2013) and many species appear to rely on $CO_{2(aq)}$ for atleast 50% of the carbon used in photosynthesis (Palacios & Zimmerman 1997). Current concentrations of $CO_{2(aq)}$, lead to a slow diffusive supply of CO_2 to the leaves (Koch et al.

2013). At a pH of 8.1-8.3 aqueous dissolved carbon dioxide ($CO_{2(aq)}$) only represents about 0.5-1% of the dissolved inorganic carbon (DIC) pool, whilst bicarbonate (HCO_3^-) represents about 90% (Stumm and Morgan 1981). If the pH of the ocean drops to ~7.8 then $CO_{2(aq)}$ levels will increase by almost 200% (Fabry et al. 2008), leading to increased diffusion potential. In response to a decrease in pH many species of seagrass have been shown to increase photosynthetic rates (Thom 1996; Invers et al. 2001; Jiang et al. 2010; Alexandre et al. 2012). This could increase seagrass production in the future. Previous studies have shown that eelgrass (*Zostera marina*) growth rates increase under low pH conditions (Thom 1996), and the plant's light requirements are reduced (Zimmerman et al. 1997). Further study observed that increased $CO_{2(aq)}$ lead to greater reproductive output, below-ground biomass and vegetative proliferation of *Z. marina* (Palacios and Zimmerman 2007). Observations at CO_2 vents have found that seagrass flourishes under acidified conditions in terms of production per square metre (Hall-Spencer et al. 2008; Fabricius et al. 2011), but leaf production decreases (MC Buia, unpublished data).

A change in pH will not only lead to changes in carbonate chemistry, but may also lead to alterations in nutrient availability, although research on this topic remains inconclusive (e.g. Breitbarth et al. 2010; Shi et al. 2010). If nitrogen availability changes under OA this may lead to a change in C:N stoichiometry. Jiang et al. (2010) found that C:N content of the above ground tissue of the seagrass *Thalassia hemprichii* increased under low pH conditions, due to a decrease in nitrogen content. Phytoplankton C:N stoichiometry has also been shown to increase under OA conditions, although this was due to increased carbon assimilation rather than reduced nitrogen uptake (Riebesell et al. 2007; Bellerby et al. 2008). Alterations in stoichiometry will have knock-on effects for primary consumers, in terms of the palatability and nutritional value of seagrass. There is some early evidence for this in the form of observations around the CO_2 vents of Ischia (Italy) where increased grazing pressure by the fish *Sarpa salpa* has been observed in acidified waters (Chapter 6).

The *Posidonia oceanica* meadows are an extremely important and biologically diverse ecosystem within the Mediterranean basin and any adverse effect of OA to the plant will impact its associated community and the ecosystem services that it provides. Using the CO_2 vents off the island of Ischia, Italy, the effect of natural acidification on *P. oceanica* structural characteristics, sediment characteristics, nutrient availability, C:N stoichiometry, and photosynthetic efficiency were examined.

3.2 Methods

3.2.1 Site description

The island of Ischia is located in the Bay of Naples, Italy. The island has a long history of volcanism and gas venting in shallow submarine sites (Tedesco 1996). The Castello Aragonese, on the east side of the island, has a number of vents which release CO_2 within the rocky sublittoral and *Posidonia oceanica* habitats on the north (N) and south (S) side of the Castello (Fig. 3.1). These CO_2 vents are unusual in that they are not heated and neither do they produce toxic compounds (Hall-Spencer et al. 2008), providing a natural laboratory for the study of the ecosystem level effects of ocean acidification. Although pH is highly variable (Kroeker et al. 2011b), a pH gradient runs parallel to the shore at each location (N and S) (Hall-Spencer et al. 2008).

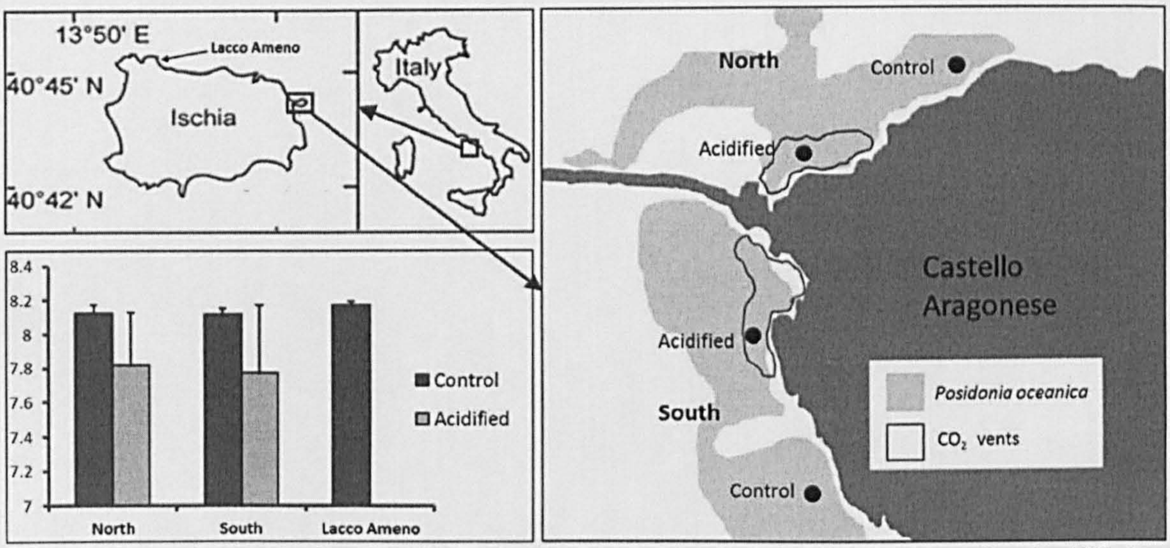


Figure 3.1 Area of the study site of Ischia (Tyrrhenian Sea, Italy), with locations of the control and acidified sampling stations at the north and south of the Castello Aragonese. Areas where *Posidonia oceanica* and CO_2 vents can be found are marked. The position of the tertiary control site (Lacco Ameno) on the north east of Ischia can be seen. Data represents the mean pH (+SD) of each station (n = 150, apart from at Lacco Ameno where n = 40) sampled at various periods between March and November 2011.

Acidified and comparable non-acidified (control) conditions can be found within the *P. oceanica* meadows at each side (N and S) of the Castello, providing data on ocean acidification, whilst allowing comparison of zones with similar environmental conditions (e.g. exposure, temperature, substratum) other than acidification. The north side of the Castello is more exposed to prevailing winds, and therefore greater wave action, whilst the south side is set in a semi-enclosed bay and sheltered from many of the prevailing winds. The seagrass beds can be found growing short distances from the shore (Chapter 1, Fig. 1.9) and acidified and control plots on the north and south of the Castello were determined through preliminary investigation of pH variation (see Chapter 1). Each plot was approximately 60 m^2, 10 m parallel to the shore and 6 m perpendicular to the shore. These dimensions were chosen as preliminary investigation showed that acidified stations were sufficiently acidified (pH < 8.0) over this area. Sites were selected between depths of 2.5-3.5 m to ensure that depth variation was not a confounding factor. A tertiary control site was situated in Lacco Ameno, in a seagrass bed situated far from any vents (Fig 3.1.). Each station was marked by buoys. The tertiary control station at Lacco Ameno was the same size and located at a depth of 3 m.

3.2.2. Carbonate chemistry

pH measurements were taken by collection of 10 samples bi-monthly between May and November (N = 150), except for Lacco Ameno (N = 40). The lower number of samples was deemed sufficient for this site, due to the lack of vent induced variation. Samples were collected from 10 cm above the canopy in falcon tubes. These were taken back to the laboratory and analysed within four hours of collection at ambient seawater temperature (Millero 1995). Measurements were taken using a Mettler Toledo SG2 pH meter which measures to 0.01 units equipped with an InLab 413 electrode and calibrated regularly using NIST-traceable buffers. This method does not determine the total hydrogen ion concentration, but the relative change in pH between sites (precision within 0.05 pH units, Zeebe and Sanyal 2002) and was considered sufficient for this study. 10 samples were collected from each site on the 04/11/11 for analysis of total alkalinity (TA). This was deemed sufficient as other studies have shown that TA shows very little variation at these sites (e.g. Cigliano et al. 2010; Rodolfo-Metalpa et al. 2010). Samples were filtered through GF/F Whatman filter paper and 0.02% mercuric chloride added. They were then stored in the dark and later analysed using the Apollo SciTech Alkalinity Titrator Model AS-ALK2 and Batch 100 certified reference materials (Dickson et al. 2007). Other carbonate variables were calculated using CO_2 SYS software (Lewis and Wallace 1998).

3.2.3 Meadow characteristics

Sampling was random but sampled areas were marked to ensure that data was independent between visits. To describe structural characteristics of the seagrass at each station, *in situ* measurements of meadow density (shoots m^{-2}) and height of the canopy (cm) were performed (4 replicates per station) using a 40 x 40cm quadrat (Buia et al. 2004). Shoot density was measured in March, July and November. Height of the canopy was recorded by measuring the longest leaf within the quadrat. Measurements were taken in March, May, July, September and November to give a picture of temporal changes.

Sediment samples were collected by inserting a manual metal corer (5.5cm diameter) 10cm into the sediment. Three sediment samples were collected from each station in May and September for chemical analysis, as this number is adequate for analysis of sedimentary features (De Falco et al. 2000). Sediments were then placed in chilled containers for transport back to the laboratory. Course debris was removed from the samples manually after visual inspection and the samples were oven dried at 60°C. Organic content of the sediments was determined from a sub-sample (1-2g) by loss-on-ignition in a muffle furnace (500°C for 2h) and then placed in a desiccator to cool at room temperature overnight. Sediments were then re-weighed and % organic content calculated. Carbonate content was estimated by dissolution of a subsample (1-2g) in 10% hydrochloric acid overnight. Samples were then washed with distilled water, oven dried at 60^0C, and placed in a dessicator to cool at room temperature. Sediments were then re-weighed and % carbonate content calculated based on weight loss. For analysis of sediment grain size coarse sediments were sieved, whilst fine sediments (< 1mm) were analysed through laser diffraction (Malvern Mastersizer 2000). Results were reported using phi scale (φ, the log base 2 scale descriptor of sediments).

3.2.4 Nutrient availability

Pore and canopy water nutrient samples, leaves for C:N analysis and PAM data were collected in May and September. These months were chosen as they correlate to periods of high and low leaf growth and nitrogen concentrations respectively (Alcoverro et al. 1995b). Pore and canopy water samples (six replicates) were collected for nutrient analysis in non-shaded patches of seagrass at each station. All samples were collected on sunny, calm days between 10.00 and 13.00 (GMT), as nutrient consumption varies dependant on time of day and is related to plant metabolism (Blackburn et al. 1994; Ziegler and Benner 1999). Canopy water samples were collected approximately 20 cm from the seafloor using a 60 ml syringe; the first syringe of water was ejected and then refilled. Pore water samples were collected using a pipette tip attached to a syringe inserted 10cm into the sediment. The end was screened with gauze to prevent coarse sediment particles from entering. The first syringe was discarded and the second syringe collected. All samples were kept on ice until returned to the laboratory and then filtered through GF/F Whatman filter paper and frozen within 4 hours of sampling. Analysis of major nutrients (ammonium, nitrates, nitrites and orthophosphates) was performed within four weeks using a Technicon autoanalyzer.

Ratios of inorganic nitrogen to phosphorous were calculated from concentrations of NO_3, NO_2, NH_4 and PO_4. The molecular weights of each of the elements ($N = 14$, $O = 16$, $P = 31$, $H = 1$) were used to calculate the mass of each compound, and the ratio of N:P calculated from the equation:

$$N = ((_{conc.}NO_3/ 62)*14) + ((_{conc.}NO_2/ 46)*14) + ((_{conc.}NH_4/ 18)*14)$$

$$P = ((_{conc.}PO_4/ 95) *31)$$

$$N: P = 100/ ((P/ N)*100))$$

3.2.5 C:N stoichiometry

For analysis of C:N content of the leaves, 6 intermediate leaves (rank 2 or 3) were collected from 4 random plots of $1m^2$ within each station (N=24). Each plot sampled was marked with buoys to ensure samples were independent. Samples were taken back to the laboratory for analysis. The leaves were scraped of epiphytes, rinsed in fresh water to remove any salt residue and freeze dried. Once freeze dried, samples were ground to a fine powder and placed in a dessicator to remove any residual moisture. Pellets of approx. 2mg were then weighed and analyzed with an elemental analyzer for the total content of both C and N (as a percentage of dry weight), using acetanilide as a standard. C:N stoichiometry was calculated from the equation: C:N = (% carbon/12) / (% nitrogen/14).

3.2.6 Photosynthetic performance

Chlorophyll fluorescence was used to determine parameters of photosynthetic performance. The measurement of chlorophyll fluorescence provides a rapid assessment of PSII photochemistry in seagrasses through the calculation of the quantum yields of both photochemical and non-photochemical energy conversion in photosystem II (PSII). When photons (quanta) within the photosynthetically active radiation (PAR) region hit photosynthetic pigments they become excited. This excitation energy can be used for photochemical processes, whilst a portion is release as heat or fluorescence. The inverse relationship between chlorophyll fluorescence and photochemistry can help elucidate aspects of the photosynthetic process (Silva et al. 2009). Estimates of photosynthetic performance of *P. oceanica* plants were obtained using in situ PAM (pulse amplitude modulated) flourometry by means of a diving-PAM instrument (Waltz, Effeltrich, Germany). Three intermediate leaves (rank 2 or 3) were chosen for each plot (three plots per station). All seagrass leaves measured were found at a depth of 2.5 – 3.5 m, therefore should be adapted to highlight conditions. PAM measurements were collected between 10.00 and 12.00 GMT, therefore light should be high at this time of day. Prior to use of the PAM, ambient PAR was calculated using a portable quantameter (QSI-140, Biospherical Instr., USA).

The following measurements were chosen to describe photosynthetic capacity of the leaves: quantum efficiency (F_v/F_m), electron transport rate (ETR_{MAX}) and alpha (α). Determinations of maximum quantum yields (Fv/Fm) were performed after submitting measured leaf spots to a dark acclimation period of 7 to 10 min prior to exposure to a saturating light pulse. Fv/Fm is a measure of maximum photosynthetic efficiency and an indicator of stress (Silva et al. 2009). It is derived from the number of electrons produced through absorption of a photon in PSII, taking into consideration minimal (F_0) and maximal (F_m) fluorescence (Kromkamp and Forster 2003). The measurement of chlorophyll fluorescence also allows the construction of irradiance vs ETR (electron transport rate past PSII) curves based on short exposures of measured leaf spots to a range of irradiances produced by the fluorometer lamp (rapid light curves, RLCs) (Ralph and Gademann, 2005; Silva et al., 2009). In that, they are used to describe the photosynthetic response to irradiance, as an analogue to the P vs E curves obtained by measurements of oxygen evolution or carbon assimilation (Enriquez & Borowitzka, 2010). Quantum yield of PSII (Y) was recorded by the PAM at each light intensity and ETR was calculated from the equation: ETR= Y* PAR*0.5*AF (Schwarz et al. 2000). An expeditive calculation of AF (the absorption factor of the leaves) was accomplished by measuring the transmittance of PAR through the leaves. Curve parameters (maximum electron transport rate, ETRmax; initial slope of the curve, α; and saturating irradiance, Ek) were calculated by fitting empirical data to an exponential function (Ralph & Gademann, 2005). The steepness of the curve in the light limiting region (α) enables us to understand the efficiency of light capture, whilst ETRmax provides information on the maximum photosynthetic capacity of the plant (Ralph and Gademann 2005).

3.2.4 Data analysis

Shoot density, canopy height, % organic and carbon content of the sediments, sediment grain size, nutrient concentrations in canopy and pore water, C:N content of the leaves and PAM fluorometry were analysed using a three-way GLM ANOVA to test the effects of pH (fixed), site (fixed) and month (fixed). Due to the incomplete design of this experiment, the tertiary control, Lacco Ameno, was excluded from these analyses. A secondary one-way ANOVA was then performed for each variable to compare control stations in the north and south with the tertiary control site at Lacco Ameno. Data was checked for homogeneity of variance using a Cochran C test. Where variances were heterogeneous, data was transformed; all percentage data was arcsine transformed, height of the canopy and nutrient concentration data were log (x+1) transformed. For some canopy water nutrient concentration data (NO_2, PO_4) although transformations reduced heterogeneity of variance, data did not become homoscedastic. Residuals were checked and found to be normally distributed and therefore, as samples were balanced and large (N=6 for 8 treatments) and therefore robust to departures from the assumptions (Underwood 1997; McGuinness 2002), this data was analysed with a GLM ANOVA. To investigate the relationship between nutrient availability and nitrogen content of the leaves, a Pearson's correlation coefficient was calculated. All analyses were carried out using Statistica 8.0 software.

3.3 Results

3.3.1 Seawater properties

Mean pH values at control stations at the north and south of the Castello Aragonese and Lacco Ameno were 8.13 and 8.12 respectively, whilst acidified sites were 7.82 and 7.78 during the study (Fig. 3.1). These reduced pH levels found at the acidified stations are ecologically relevant since these are the pH values predicted for the end of this century (Caldeira and Wickett 2003). It must be noted that pH values were extremely variable within the *P. oceanica* acidified stations (see Table 3.1). Acidified stations fell below a pH of 8.0 (the value considered as being acidified) during 55% and 67% of sampling periods in the north and south acidified site respectively. Due to the logarithmic nature of the pH scale, carbonate chemistry parameters such as pCO_2 showed high variability (Table 3.1).

Table 3.1 Mean ± SD seawater carbonate chemistry. Temperature (13-29°C throughout study period, mean = 21.1°C) was continuously recorded between March and November 2011 using a data logger. Salinity (38) was a point measurement taken in May 2011. 10 pH samples were collected from each station periodically between May and November 2011. Total alkalinity (TA) is a point measurement taken on the 04/10/11. The remaining parameters were calculated using CO_2 SYS programme using the constants of Roy et al. (1993) and Dickson (1990) for KSO4. Total alkalinity (TA) is mmol.kg^{-1}; pH_{NBS} was measured using an NBS scale; and pCO_2 is µatm.

		pH_{NBS}	TA	pCO_2	Ω_{CA}	Ω_{AR}
North	Control	8.13 ± 0.05	2.54 ± 0.04	509 ± 54	5.04 ± 0.32	3.30 ± 0.21
	Acidified	7.82 ± 0.31	2.57 ± 0.02	2158 ± 2508	3.37 ± 1.81	2.14 ± 1.18
South	Control	8.12 ± 0.04	2.54 ± 0.01	504 ± 68	5.15 ± 0.44	3.37 ± 0.29
	Acidified	7.78 ± 0.39	2.55 ± 0.01	1614 ± 1861	3.23 ± 1.51	2.11 ± 0.99
Lacco Ameno	Control	8.18 ± 0.03	2.55 ± 0.01	437 ± 25	5.61 ± 0.20	3.67 ± 0.13

3.3.2 Meadow parameters

Shoot density did not vary between months, although there was a strong effect of acidification ($p < 0.001$) and site ($p < 0.001$). Shoot density was greater in acidified stations than control stations and greater in the south than the north (Fig. 3.2a). A significant 'site x pH' interaction was found, as the effect of acidification on shoot density was much greater at the south side of the Castello Aragonese (Table 3.2). Density increased with acidification by 58% in the North side and 82% in the south side. Shoot density at Lacco Ameno (LA) was lower than the south control (Tukey HSD: $p = 0.013$) but equal to the north control station (N = LA < S).

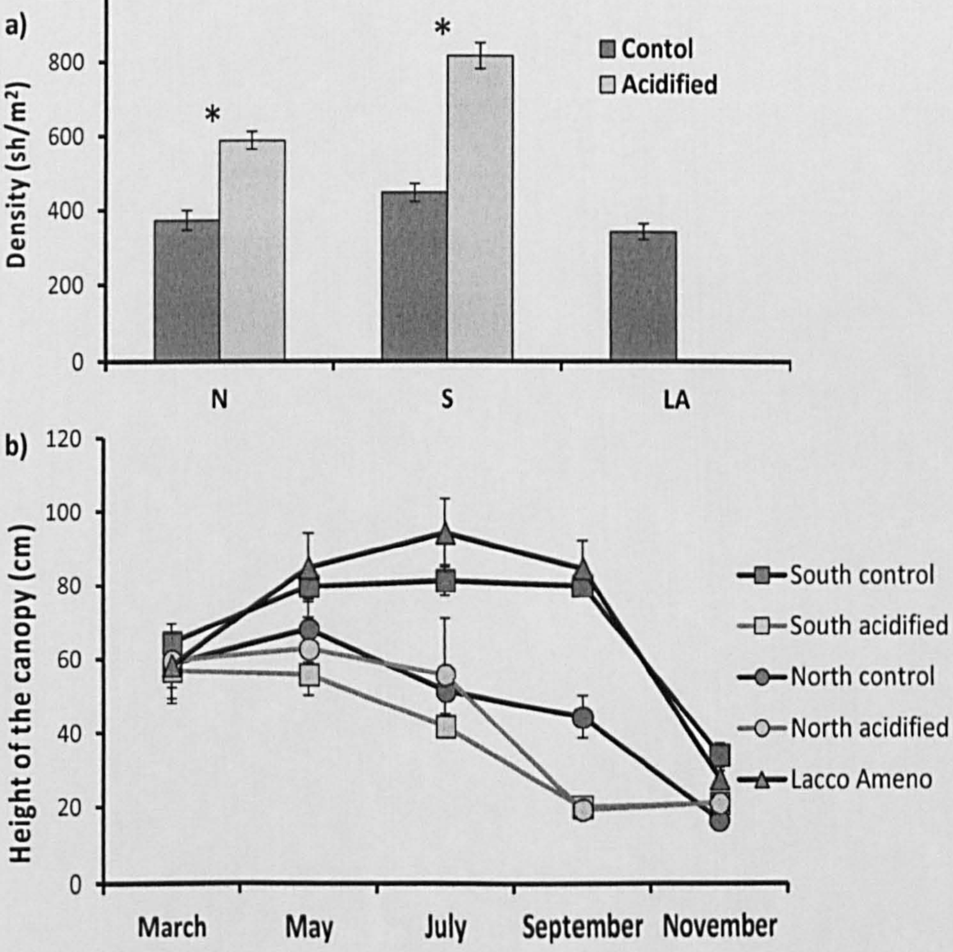


Figure 3.2 Comparison of mean (±SE): a) shoot density between control and acidified stations, and b) height of the canopy between March and Novewmber. * signifies significant results.

Height of the canopy was strongly affected by acidification ($p < 0.001$), site ($p = 0.002$) and month ($p < 0.001$), although there was a strong 'pH x month' interaction ($p < 0.001$) (Table 3.2), as no differences were found in canopy height in March, May and November (Fig. 3.2b). The canopy in the control station was taller than in the acidified station throughout July (49% taller) and September (75% taller) in the south side (Tukey HSD: $p = 0.03$, $p < 0.001$), whilst canopy height was greater in the north side only for September (56% higher) (Tukey HSD: $p = 0.004$). Canopy height was generally shorter in the north than the south, which may be because the north control station is smaller, so may exhibit edge effects. In control stations canopy height generally increased from March to July and then decreased from September to November, whilst in acidified stations canopy height generally decreased from May. Canopy height at Lacco Ameno was equal to the south control station but higher than the north control (Tukey HSD: $p = 0.024$, N < LA = S).

Table 3.2 Three-way ANOVA results of shoot density and height of the canopy between site (fixed factor), pH (fixed factor) and month (fixed factor). A secondary one-way ANOVA to compare control stations including Lacco Ameno. Height of the canopy data was LOG (X+1) transformed to meet the assumptions of ANOVA. S = site, M = month. Significant results are highlighted in red.

	Shoot density				**Height of the canopy**			
	df	**MS**	**F**	**P**	**df**	**MS**	**F**	**P**
S	1	273763	32.089	**< 0.001**	1	0.1166	10.57	**0.002**
pH	1	1024482	120.084	**< 0.001**	1	0.4715	42.74	**< 0.001**
M	2	12564	1.473	0.243	4	0.5625	50.99	**< 0.001**
S x pH	1	71302	8.358	**0.006**	1	0.2185	19.81	**< 0.001**
S x M	2	15904	1.864	0.170	4	0.0158	1.43	0.236
pH x M	2	12102	1.419	0.255	4	0.1295	11.74	**< 0.001**
S x pH x M	2	5621	0.659	0.524	4	0.0091	0.82	0.516
Err	36	8531			60	0.0110		
Controls	2	34747	4.9132	**0.014**	2	0.2104	5.093	**0.009**

Organic and carbonate content of the sediments was not affected by site, acidification or month, but was similar between all stations (Fig. 3.3), although there was a higher organic and carbonate content found at Lacco Ameno than any of the other control stations (Tukey HSD: $p < 0.01$ and $p < 0.001$, respectively) (Table 3.3). Sediment grain size was not affected by acidification (Fig. 3.3a), but was smaller in May than September ($p = 0.032$) and smaller in the south than the north ($p = 0.037$). Lacco Ameno had a larger grain size than the south control station (Tukey HSD: 0.006) but was no different from the north control station (N = LA > S).

Table 3.3 Three-way ANOVA results of % organic and carbonate content, and grain size of the sediments between site (fixed factor), pH (fixed factor) and month (fixed factor). A secondary one-way ANOVA to performed to compare control stations including Lacco Ameno. Organic and carbonate content data was arcsine transformed. S = site, M = month. Significant results are highlighted in red.

		% organic content			**% carbonate content**			**Grain size (φ)**		
	df	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
S	1	0.000075	0.265	0.614	0.002263	3.800	0.069	0.76922	5.14959	**0.037**
pH	1	0.000893	3.163	0.094	0.002012	3.378	0.085	0.12926	0.86531	0.366
M	1	0.000892	3.159	0.095	0.000860	1.444	0.247	0.82345	5.51262	**0.032**
S x pH	1	0.000377	1.334	0.265	0.001685	2.829	0.112	1.19822	8.02150	**0.012**
S x M	1	0.000000	0.001	0.979	0.002220	3.728	0.071	0.00038	0.00254	0.960
pH x M	1	0.000907	3.214	0.092	0.003659	6.144	**0.025**	0.00563	0.03766	0.849
S x pH x M	1	0.000295	1.045	0.322	0.000473	0.794	0.386	0.03327	0.22276	0.643
Err	16	0.000282			0.000595			0.14938		
Controls	2	0.006463	10.629	**0.001**	0.035102	20.147	**0.001**	1.237837	9.48300	**0.002**

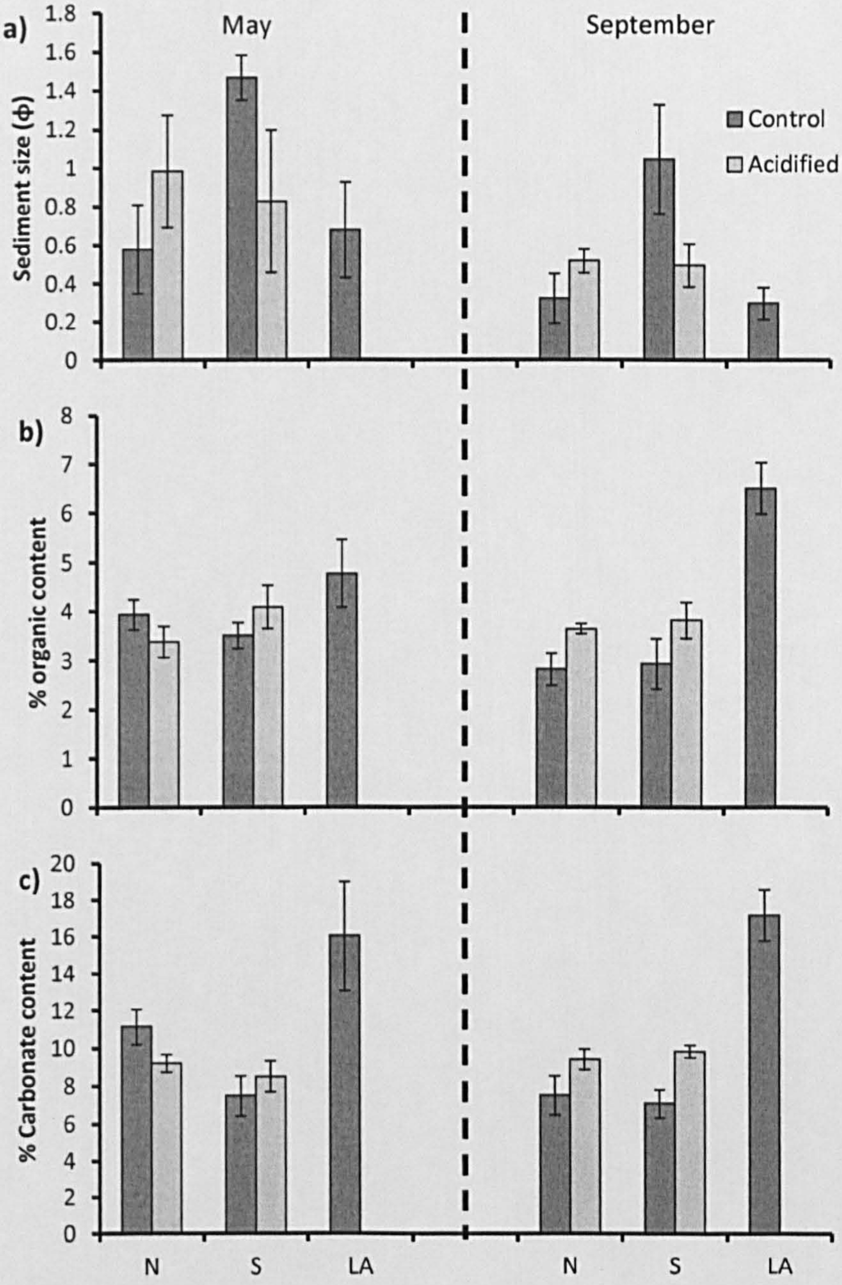


Figure 3.3 Comparison of mean (±SE) of sediment characteristics; a) sediment size (φ), b) % organic content, and c) % carbonate content between acidified and control stations in May and September.

3.3.3 Nutrient availability

Canopy water NO_3 concentrations were higher in acidified than control stations ($p < 0.01$), in the north than the south ($p < 0.01$) and in May than in September ($p < 0.01$) (Table 3.4).

Pore water NO_3 concentrations were higher in acidified than control stations ($p < 0.05$), but no differences were found for month or site (Table 3.4). In May, canopy and pore water NO_3 concentrations were greater in the acidified than the control station in the north (Tukey HSD: $p < 0.01$ and $p < 0.005$ respectively) (Fig. 3.4a). When control stations were compared to Lacco Ameno the north control station had higher concentrations of canopy water NO_3 (N > LA = S), but no differences were found for pore water concentrations (Table 3.4).

Canopy water NO_2 concentrations followed the same pattern as NO_3 concentrations and were higher in acidified than control stations ($p < 0.001$), in the north than the south ($p < 0.01$), and in September than May ($p < 0.001$) (Table 3.4). In May the canopy water NO_2 concentration in the north was greater in the acidified than the control station (Tukey HSD: $p < 0.01$) (Fig. 3.4b). A similar pattern was found between the north acidified and control stations in September although this difference was not significant (Tukey HSD: $p = 0.068$). Unlike NO_3, no differences were found between acidification, month or site for pore water concentrations. No variation in NO_2 concentrations was found between control stations and Lacco Ameno.

Canopy water NH_4 was greater in May than September ($p < 0.001$) and greater in the north than the south ($p < 0.05$). No difference was found in concentrations between acidified and control stations (Table 3.4). Pore water concentrations followed the same pattern as the canopy water; concentrations greater in May than September ($p < 0.01$) and in the north than the south ($p < 0.01$). When control sites were compared to Lacco Ameno, the north control site had higher concentrations of NH_4 (N > LA = S).

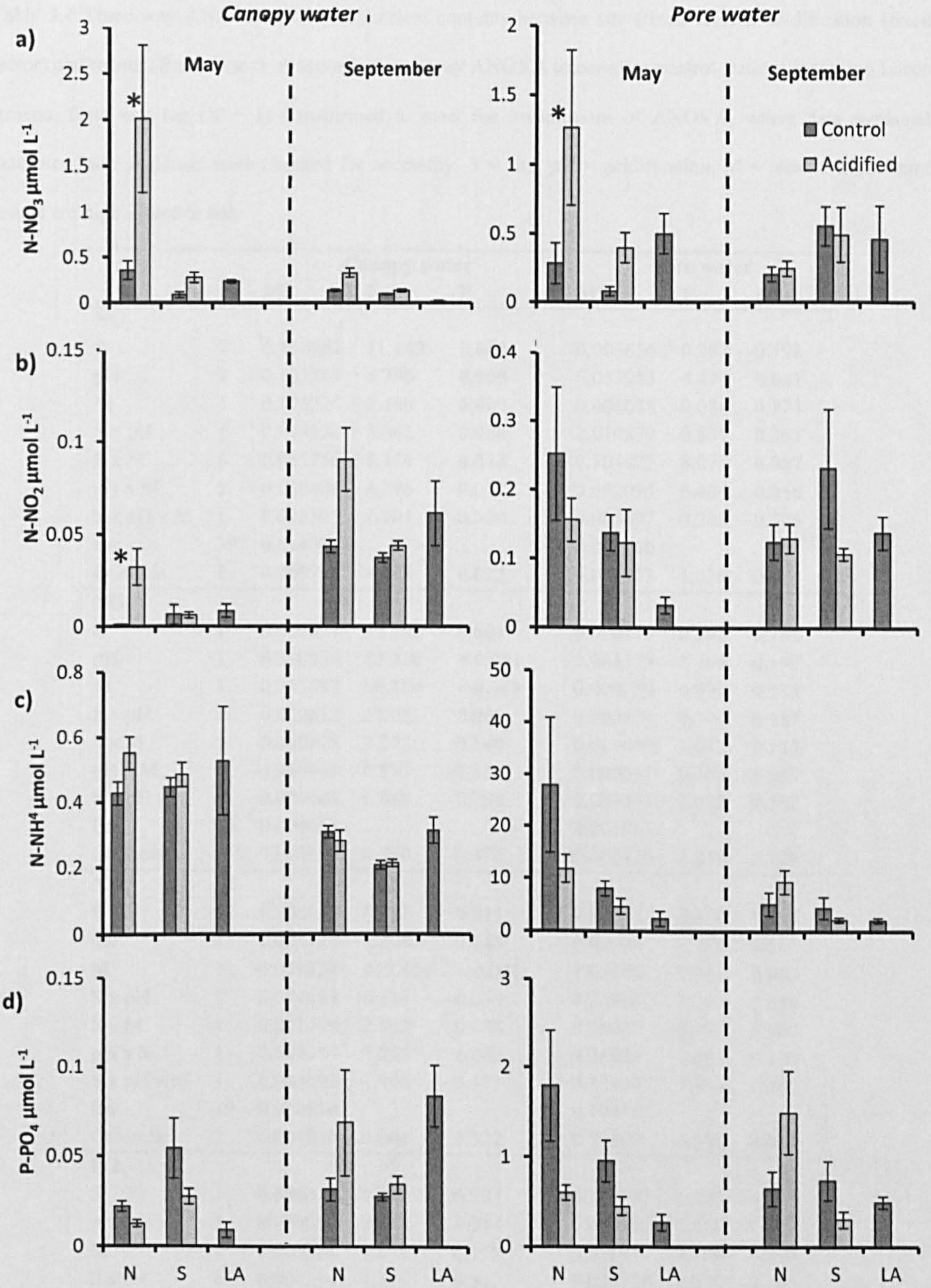


Figure 3.4 Mean (±SE) concentrations of nutrients in the canopy and pore water of *Posidonia oceanica* in May and September: a) NO_3, b) NO_2, c) NH_4, and d) PO_4. * denotes differences between control and acidified stations.

Table 3.4 Three-way ANOVA results of nutrient contents between site (fixed factor), acidification (fixed factor) and month (fixed factor). A secondary one-way ANOVA to compare control stations including Lacco Ameno. Data was log (X + 1) transformed to meet the assumptions of ANOVA, where data remained heteroscedastic residuals were checked for normality. S = site, pH = acidification, M = month. Significant results are highlighted in red.

		Canopy water			Pore water		
	df	**MS**	**F**	**P**	**MS**	**F**	**P**
NO_3							
S	1	0.156981	11.149	**0.002**	0.003656	0.282	0.598
pH	1	0.123859	8.796	**0.005**	0.057985	4.474	**0.041**
M	1	0.105326	7.480	**0.009**	0.001088	0.084	0.774
S x pH	1	0.043124	3.062	0.088	0.010879	0.839	0.365
S x M	1	0.061756	4.386	**0.043**	0.104622	8.073	**0.007**
pH x M	1	0.046408	3.296	0.077	0.082995	6.404	**0.016**
S x pH x M	1	0.015507	1.101	0.300	0.003697	0.285	0.596
Err	39	0.014081			0.012960		
Controls	2	0.009715	4.318	**0.022**	0.014323	1.430	0.254
NO_2							
S	1	0.000677	12.242	**0.001**	0.000177	0.093	0.762
pH	1	0.000856	15.480	**< 0.001**	0.003375	1.766	0.192
M	1	0.003697	66.880	**< 0.001**	0.000759	0.397	0.532
S x pH	1	0.000612	11.081	**0.002**	0.000574	0.301	0.587
S x M	1	0.000126	2.272	0.140	0.005049	2.642	0.112
pH x M	1	0.000048	0.873	0.356	0.000011	0.006	0.939
S x pH x M	1	0.000004	0.069	0.794	0.003884	2.032	0.162
Err	39	0.000055			0.001911		
Controls	2	0.000110	0.760	0.476	0.005126	2.210	0.126
NH_4							
S	1	0.003662	7.187	**0.011**	0.88348	8.470	**0.006**
pH	1	0.001124	2.206	0.146	0.02900	0.278	0.601
M	1	0.058828	115.451	**< 0.001**	1.03503	9.923	**0.003**
S x pH	1	0.000114	0.223	0.639	0.24981	2.395	0.130
S x M	1	0.001469	2.882	0.098	0.02025	0.194	0.662
pH x M	1	0.002184	4.285	**0.045**	0.24622	2.361	0.133
S x pH x M	1	0.000991	1.946	0.171	0.12439	1.193	0.282
Err	39	0.000510			0.10431		
Controls	2	0.001856	0.664	0.522	0.74507	5.295	**0.010**
PO_4							
S	1	0.000011	0.074	0.787	0.102207	6.205	**0.017**
pH	1	0.000002	0.012	0.914	0.046382	2.816	0.101
M	1	0.000278	1.873	0.179	0.018447	1.120	0.296
S x pH	1	0.000234	1.574	0.217	0.036738	2.230	0.143
S x M	1	0.000917	6.180	**0.017**	0.000905	0.055	0.816
pH x M	1	0.000737	4.967	**0.032**	0.115675	7.022	**0.012**
S x pH x M	1	0.000013	0.091	0.765	0.108360	6.578	0.014
Err	39	0.000148			0.016473		
Control	2	0.000196	1.010	0.375	0.087811	4.765	**0.015**

No significant difference was found for acidification for canopy or pore water PO_4 concentrations, although there was a significant 'pH x month' interaction ($p = 0.032$ and $p = 0.012$, respectively) as PO_4 concentrations were greater in control than acidified sites for both canopy and pore water samples in May. No differences were found between month or site for canopy water concentrations of PO_4 ($p < 0.05$), whilst pore water values were greater in the north that the south of the Castello but there were no differences for month. When control stations were compared to Lacco Ameno the north control site had higher concentrations of PO_4 in the pore water (N > LA = S), but no differences were found in the canopy (Table 3.4).

Dissolved inorganic nitrogen to phosphorous ratios were greater in acidified than control stations in May and greater in the north than the south (Table 3.5). In September the N: P ratio was lower in the acidified than control station in the north and equal in the south.

Table 3.5 Ratio of dissolved inorganic N:P concentrations in the canopy waters of control and acidified stations in May and September.

	May		September	
	North	**South**	**North**	**South**
Control	50	18	24	17
Acidified	205	42	13	16

3.3.5 C:N stoichiometry

C:N ratio of the leaves (molar) was lower in acidified than control stations ($p < 0.001$), in May than in September ($p < 0.001$) and in the south than the north ($p = 0.002$), although significant interactions suggested that this did not hold true under all combinations of factors (Table 3.6). In May C:N ratio of the leaves was lower in the acidified than the control station on the south side (Tukey HSD: $p < 0.001$) (Fig. 3.5a). Nitrogen content of the leaves showed the exact opposite pattern: it was higher in acidified than control stations ($p < 0.001$), in May than in September ($p < 0.001$) and in the south than the north ($p = 0.006$). In May nitrogen concentration of the leaves was higher in the acidified than the control station on the south side (Tukey HSD: $p < 0.001$) (Fig. 3.6c). Carbon content did not vary with acidification, site or month (Table 3.6), therefore changes in C:N stoichiometry were solely due to an increase in nitrogen content in acidified leaves. Comparisons of the control stations with Lacco Ameno revealed no differences for C:N ratio, carbon or nitrogen content of the leaves.

Table 3.6 Three-way ANOVA results of % nitrogen and % carbon content and C:N ratio (molar) of the intermediate leaves of *P. oceanica* between site (fixed factor), pH (fixed factor) and month (fixed factor). A secondary one-way ANOVA to compare control stations including Lacco Ameno. S = site, M = month. Significant results are highlighted in red.

		% nitrogen content			**% carbon content**			**C:N ratio (molar)**		
	df	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
S	1	0.7388	7.870	**0.006**	11.2692	1.441	0.231	0.0269	9.6	**0.002**
pH	1	1.5191	16.183	**< 0.001**	6.5177	0.834	0.362	0.0584	20.7	**< 0.001**
M	1	23.0951	246.020	**< 0.001**	18.9778	2.427	0.121	0.2308	81.9	**< 0.001**
S x pH	1	1.1880	12.655	**< 0.001**	0.0241	0.003	0.956	0.0275	9.8	**0.002**
S x M	1	2.9936	31.889	**< 0.001**	32.8551	4.202	0.052	0.1091	38.7	**< 0.001**
pH x M	1	2.0763	22.118	**< 0.001**	4.9589	0.634	0.427	0.0509	18.1	**< 0.001**
S x pH x M	1	2.4547	26.148	**< 0.001**	3.0780	0.394	0.531	0.0915	32.5	**< 0.001**
Err	184	0.0939			7.8195			0.0028		
Controls	2	0.5328	3.421	0.065	14.5261	2.279	0.106	19.469	1.747	0.178

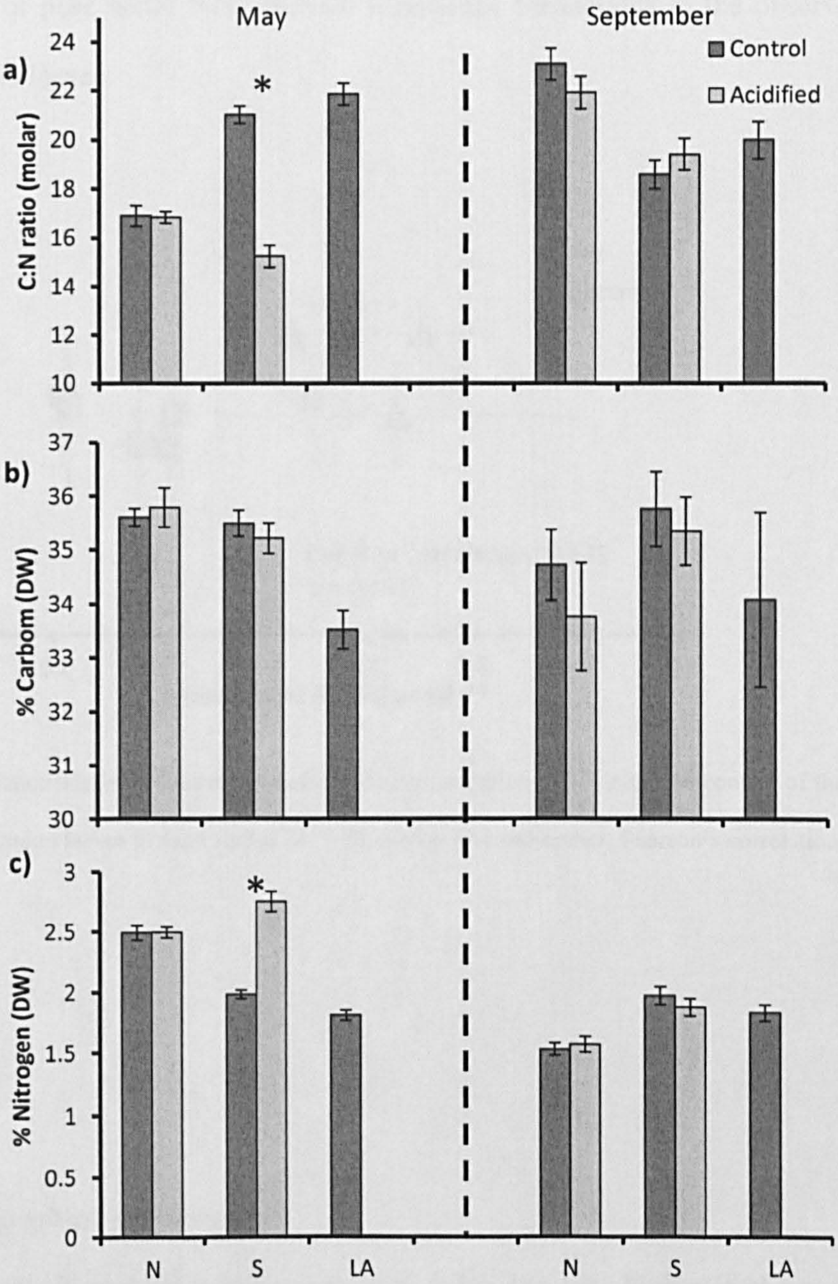


Figure 3.5 Comparison of mean (±SE): a) C:N ratio (molar), b) carbon content, and c) nitrogen content of intermediate *Posidonia oceanica* leaves between acidified and control stations in May and September.

A significant positive relationship was observed for the concentration of NH_4 in the canopy water and nitrogen concentration in the intermediate leaves (Pearson's correlation = 0.575, p = 0.041) (Fig 3.6). None of the other nutrient concentrations of the canopy or pore water

(NO_3, NO_2 or pore water NH_4) showed significant correlations to the observed nitrogen content of the leaves.

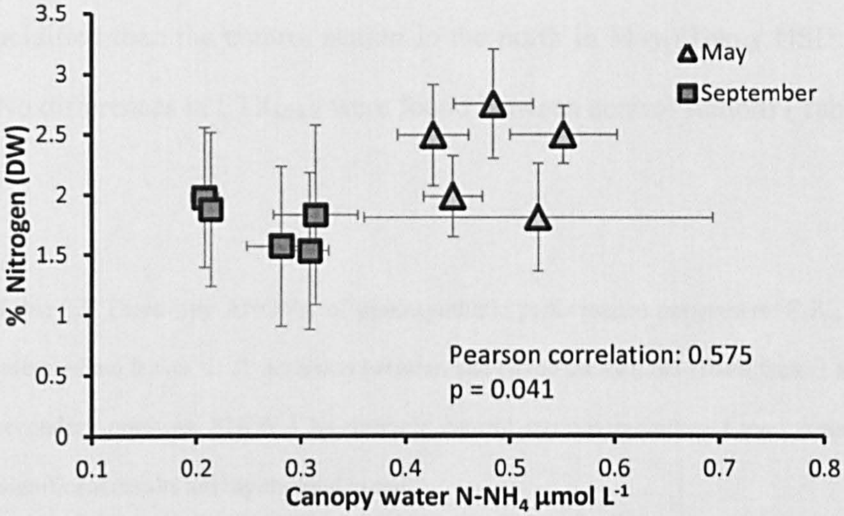


Figure 3.6 Relationship between canopy water NH_4 concentration and % nitrogen content of the intermediate *Posidonia oceanica* leaves at each station (N = 5) in May and September. Pearson's correlation coefficient is shown.

3.3.6 Photosynthetic performance

F_vF_m was not affected by acidification (Fig. 3.7a), but was higher in the north than the south side ($p < 0.001$) and higher in May than September ($p < 0.001$). There was a significant 'site x month' interaction, as the effect of month was only significant on the south side ($p = 0.002$) (Table 3.7). Comparisons of the control stations and Lacco Ameno revealed a significant effect, although this was due to the differences between the north and the south control (Tukey HSD: 0.015), as Lacco Ameno did not differ from either ($N \geq LA \geq S$).

Maximum electron transport rate (ETR_{MAX}) was not affected by acidification or site (Fig. 3.7b) but showed a similar pattern to F_vF_m and was higher in May than September ($p < 0.001$). There was a significant 'pH x month' interaction as ETR_{MAX} was higher in the acidified than the control station in the north in May (Tukey HSD: $p = 0.02$) (Fig. 3.7b). No differences in ETR_{MAX} were found between control stations (Table 3.7).

Table 3.7 Three-way ANOVA of photosynthetic performance parameters: F_vF_m, ETR_{MAX} and alpha of the intermediate leaves of *P. oceanica* between site (fixed factor), pH (fixed factor) and month (fixed factor). A secondary one-way ANOVA to compare control stations including Lacco Ameno. S = site, M = month. Significant results are highlighted in red.

			F_vF_m			**ETR_{MAX}**			**alpha**	
	df	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
S	1	8170.68	32.879	**< 0.001**	1.897	0.697	0.407	0.00009	0.073	0.788
pH	1	190.12	0.765	0.385	1.607	0.590	0.445	0.00135	1.050	0.309
M	1	3960.50	15.937	**< 0.001**	106.842	39.243	< 0.001	0.01809	14.069	**< 0.001**
S x pH	1	2.00	0.008	0.929	10.211	3.751	0.057	0.00002	0.017	0.896
S x M	1	2580.01	10.382	**0.002**	114.756	42.150	< 0.001	0.00016	0.123	0.727
pH x M	1	183.68	0.739	0.393	30.785	11.307	0.001	0.00610	4.746	**0.033**
S x pH x M	1	50.00	0.201	0.655	2.117	0.778	0.381	0.00006	0.046	0.832
Err	64	248.51			2.723			0.00129		
Controls	2	0.00199	4.26	**0.020**	1.766	0.2773	0.759	0.085677	20.219	**< 0.001**

Light harvesting efficiency (α) was not affected by acidification or site (Fig. 3.8c) but was higher in May than September ($p < 0.001$). There was an 'acidification x month' interaction as the effect of month was only noticeable in acidified stations (Tukey HSD: 0.037). When control stations were compared, Lacco Ameno had a significantly lower light harvesting efficiency than the other control stations ($p < 0.001$), due to the extremely low result obtained in May (Fig. 3.7c).

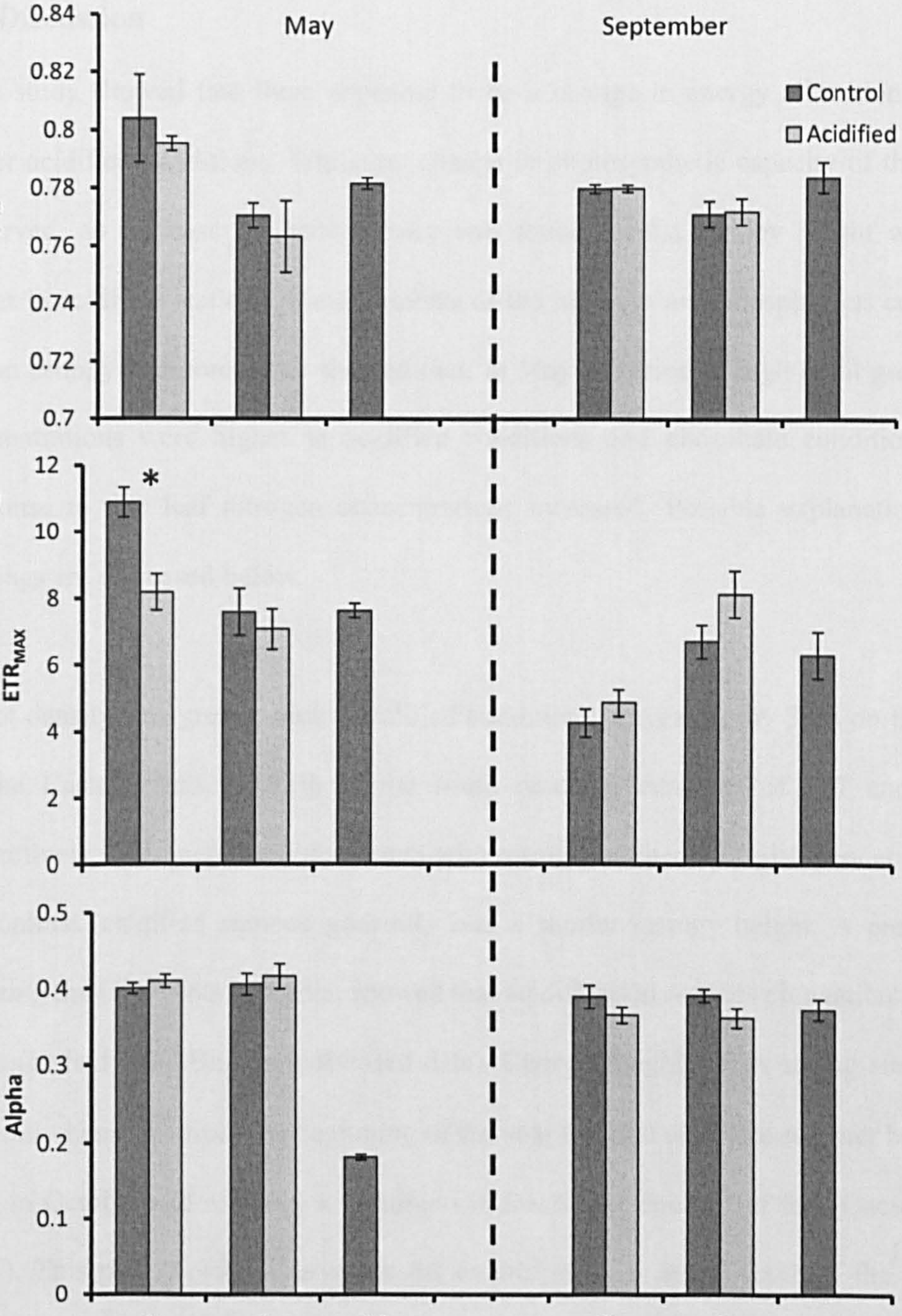


Figure 3.7 Comparison of mean (±SE) parameters of photosynthetic performance: a) F_vF_m, b)ETR_{MAX} (μmol electrons m^{-1} s^{-1}), and c) alpha of *Posidonia oceanica* leaves between acidified and control stations in May and September.

3.4 Discussion

This study showed that there appeared to be a change in energy allocation of the plant under acidified conditions. Whilst no change in photosynthetic capacity of the leaves was observed, an increase in shoot density was found, whilst canopy height was generally lower in acidified stations. Measurements of the nitrogen and phosphorous concentrations of the canopy and pore water showed that, in May (a period of high plant growth), nitrate concentrations were higher in acidified conditions and phosphate conditions lower. In response to this leaf nitrogen concentrations increased. Possible explanations for these findings are discussed below.

Shoot density was greater under acidified conditions, increasing by 58% on the north side of the Castello and by 82% in the south reaching densities of 587 and 815 sh/m^2 respectively. This increase is consistent with results obtained by Hall-Spencer et al. (2008). In contrast, acidified stations generally had a shorter canopy height. A previous study, utilizing the CO_2 vents of Ischia, showed that acidification reduces elongation rate of the *P. oceanica* leaf (MC Buia, unpublished data). Canopy height shows strong seasonality and generally increases from the beginning of the year through until late summer before a sharp drop in October and reaching a minimum in December due to leaf fall (Gacia and Duarte 2001). This pattern was observed at the control stations at the south of the Castello and Lacco Ameno, although it was not observed at the north control station, where height peaked in May. It is thought that the lower canopy height in the north control station may be due to edge effects leading to a reduced canopy height (Bologna 2006), as the meadow on the north side of the Castello is smaller. This could also explain the lower density of shoots observed at the north side, as seagrass density is generally lower nearer the edge of a meadow (Bologna 2006). Canopy height in acidified stations was peaked in March-May, and decreased to minimal height in September-November. The reason for a decrease in canopy height is most likely due to grazing pressure by the fish (*Sarpa salpa*). Extreme grazing by this fish has been recorded in the canopy of acidified stations in June and August (Chapter 6), which may account for the decrease in canopy height during these months. These results suggest that *P. oceanica* alters their energy allocation under acidified conditions, increasing vertical growth (shoots) whilst decreasing above ground production per shoot. Whether this is a stress response, an indirect effect acidification, or a positive response to a reduction in pH is unknown.

Nutrient concentration data from our study must be interpreted with caution, as the CO_2 vents are an open system, with water moving in and out of the vent areas, and pH is also more variable than values expected for the future. This said, our sampling revealed significant results. Canopy waters had a higher nutrient concentration (NO_3 and NH_4) in May than September, which would be expected for coastal stations due to nutrient fluxes (low productivity and high remineralisation in winter, high productivity in summer) (e.g. DeCasabianca et al. 1997). During the period of high nutrient concentration (May), a general increase in inorganic nitrogen (NO_3^{2-}, NO_2^-, NH_4^+) concentration was observed in canopy and pore water in acidified stations, whilst the concentration of PO_4^{3-} decreased.

Although it is generally thought that nitrogen is the limiting nutrient in the oceans (Tyrrell 1999), phosphorous is the limiting nutrient in the Mediterranean (Sala et al. 2002). The dissolved inorganic nitrogen to phosphorous ratio is approximately 21: 1 (Bethoux et al. 1992). We found that the ratio of canopy water dissolved inorganic N:P in May was extremely high in acidified stations (205: 1 in the north acidified station) in comparison to control stations. This is much greater than values found by Bethoux et al. (1992), due to the high concentrations of inorganic nitrogen and low concentrations of phosphate. There has been some concern that nitrification rates may be reduced under low pH conditions

(Behman et al. 2011), although this research suggests that, at least within *P. oceanica* meadows, concerns of nitrogen limitation will be unfounded, but under future OA conditions phosphorous limitation may become more severe. This should be investigated more thoroughly, as increased phosphate limitation will have direct impacts for all primary producers and knock on effects at all levels, from biogeochemistry to species and ecosystems.

In May the C:N ratio (molar) of the leaves was significantly lower in the acidified than control station at the south (28%). At the north side, the C:N stoichiometry was surprisingly low at both the acidified and control station. These differences were solely due to an increase in nitrogen content of the leaves, as the carbon content of leaves did not vary. This decrease is the opposite of observations by Jiang et al. (2010) for the seagrass *Thalassia hemprichii*. They found that C:N content of the leaves increased, due to a decrease in nitrogen content of the leaves. An increase in N_i was observed at acidified stations, and the north control station also had high N_i levels, therefore C:N stoichiometry of the seagrass appears to be related to the concentration of N_i found in the surrounding seawater. Jiang et al. (2010) maintained *T. hemprichii* in aquaria for 21 days, and therefore if an increase in N_i is due to other elements of the ecosystem (e.g. increase activity of nitrogen fixing bacteria, remineralisation of organic matter) the effect of increased N_i in the water column would not have been observed.

Nitrate and phosphate uptake in higher plants is thought to be through passive uptake using H^+ co-transporters, whilst ammonia uptake may be through active transport using H^+-ATPase (Fig. 3.8) (Zhu et al. 2009). A decrease in pH would be expected to increase efficiency of nitrate and phosphate uptake into the plant, whilst ammonium uptake would be expected to decrease, as the activity of H^+-ATPase is reduced under higher concentrations of H+ (Alexandre et al. 2012). An increase in canopy water concentrations of N_i and subsequent nitrogen content of the plant suggests that uptake is greater, although it is difficult to speculate on the mechanisms of uptake. Alexandre et al. (2012) found that nitrate uptake in *Zostera marina* was reduced in acidified conditions, whilst ammonium uptake remained the same. Similarly, these results appear to show that ammonium uptake is the primary path for nitrogen assimilation, as a correlation was found between NH_4^+ concentration in the canopy and nitrogen content of the leaves (% DW). It is thought that *Z. marina* uses Na^+ coupled systems to mediate the uptake of nitrate and phosphate (Garcia-Sanchez et al. 2000, Rubio et al. 2005). We suggest that this is most likely the case for *P. oceanica*, as if H^+ coupled transporters were used, a decrease in pH should lead to greater uptake of nitrate, possibly leading to a correlation between nitrate concentration and nitrogen content of the leaves, rather than the observed correlation with ammonium concentration. A further reason for an increase in nitrogen in the tissues of *P. oceanica* in acidified stations may be the loss of crustose coralline algae (CCA), which may compete with the plant for nutrients and covers a large surface area of the leaf in control stations (see Chapter 6), reducing surface area for nutrient uptake (Fig. 3.8).

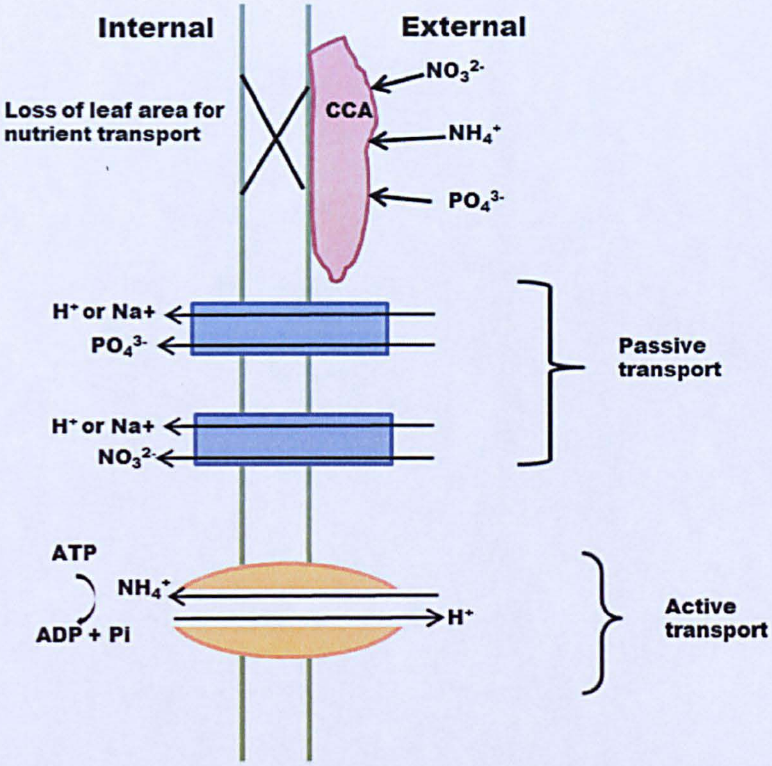


Figure 3.8 Potential mechanisms for transport of nutrients across the plasma membrane. Including coupled H^+ or Na^+ cotransporters, and H^+ ATPase. Competition with coralline crustose algae (CCA) for nutrients is shown.

The reason for the increased concentrations of nitrogen within the canopy and pore water are uncertain. As a decrease in phosphate concentration occurred, an increase in remineralization is not expected. Ocean acidification has been shown to increase nitrogen fixation in some species of cyanobacteria (Levitan et al. 2007; Levitan et al. 2010). Furthermore, ammonium (NH_4^+) and ammonia (NH_3) concentrations are pH dependent ($NH_3 + H+ \rightleftarrows NH_4^+$); under current seawater conditions approximately 5% can be found as ammonia whilst 95% can be found as ammonium, although if the pH of the oceans drops to 7.8 then the concentration of ammonia will drop by approximately 50% (Beman et al. 2011). Both of these would increase availability of NH_4^+ to P. oceanica and may lead to increases in other forms of N_i (NO_3^{2-}, NO_2^-). In order to strengthen these results more rigorous analysis is required, as nutrient concentrations were only collected at two points in the year (N = 6 for the canopy and pore water at each station). Further investigation into possibilities of increased nitrification, or seepage of N_i from the vents are suggested.

Although the decrease in calcareous epiphytes and increase in filamentous algal epiphytes on *P. oceanica* along a pH gradient has been documented (Martin et al. 2008; Gambi et al. 2011), this is the first study to be directed at solely studying the impact of ocean acidification on *P. oceanica.* The pH values observed during this study were highly variable, corresponding to values found during other studies at acidified stations at the Castello Aragonese, Ischia (e.g. Hall-Spencer et al. 2008; Kroeker et al. 2011b). This is primarily because, whilst a pH gradient can be found in this habitat (Martin et al. 2008), water movements can disperse the developing gradients (Meyer et al. 2012). Although the north acidified station showed a greater pH range, the acidified station at the south was acidified for longer and therefore we would expect that any effects of acidification would be more pronounced at this site. It was observed that during high periods of wave movement the pH of the acidified sites generally increased to values similar to the control stations. It is thought that this effect was due to mixing of external waters, leading to a dilution effect.

Parameters related to photosynthetic performance of the leaves (F_vF_m, ETR_{MAX}, alpha) were not affected by low pH. This agrees with the study by Hall-Spencer et al. (2008) at these vent sites, although contrasts with expected findings if seagrass is CO_2 limited (Invers et al. 2001). Studies of both *Zostera marina* (Thom 1996; Zimmerman et al. 1997), *Zostera noltii* (Alexandre et al. 2012) and *Thalassia hemprichii* (Jiang et al. 2010) showed an increase in photosynthetic rates under low pH, although these results were collected from short-term studies(days to months). This species-specific response to ocean acidification is common, with closely related species showing very different responses

(Garrard et al. 2013), although our research shows that these differences do not just refer to marine calcifiers but that seagrasses, which would be expected to thrive under low pH conditions, show differential responses also. A similarly mixed response has also been found for macroalgae. For example under decreased pH growth rates were enhanced for the red macroalga *Porphyra yezoensis* (Gao et al. 1991) but decreased for *Porphyra leucostica* (Mercado et al. 1999) and *Porphyra linearis* (Israel et al. 1999). A second consideration is that whilst seagrasses subjected to short-term acidification (days) (e.g. Thom 1996, Jiang et al. 2010) may show elevated rates of photosynthesis, those exposed to chronic acidification may adapt.

As well as designating control stations at the north and south of the Castello Aragonese, we recorded data from a tertiary control (Lacco Ameno), a seagrass bed far from any vents. This tertiary control was designated to ensure that the control stations at the Castello were similar to other seagrass beds and did not exhibit any effects of acidification. For the most part, results from Lacco Ameno were the same as the north, south or both control stations (density, canopy height, nutrient concentrations, C:N ratio of the leaves, sediment grain size, F_vF_m, ETR_{MAX}). Lacco Ameno had a significantly higher organic and carbonate content of the sediment. *P. oceanica* beds surrounding Ischia show high spatial variation in terms of canopy structure and the associated communities (Vasapollo 2009; Vasapollo and Gambi 2012). Therefore we suggest that these differences in sedimentary properties were due to spatial variation in environmental conditions, shoot density and the associated communities of these seagrass beds. A surprising observation was that α (light harvesting efficiency) was much lower in May at Lacco Ameno, although this is thought to be due to an operational error. Overall, Lacco Ameno exhibited very similar characteristics to the other controls, suggesting that acidification did not have an effect on our control stations.

P. oceanica appears to alter energy allocation under acidified conditions, leading to an increase in density and decrease in canopy height. Whether this is a stress response, an indirect effect acidification, or a positive response to a reduction in pH is unknown. Seagrasses evolved from terrestrial plants to live in the sea approximately 90 million years ago (Beer and Koch 1996), when the mean surface water of the oceans was approximately pH 7.7 (Ridgwell and Schmidt 2010). This suggests that perhaps they should physiologically be able to withstand a decrease in pH, although evidence of physiological evolution in this time frame can be seen. For example, whilst land plants use H^+ transporters for nutrient uptake, there is evidence that some seagrasses may have adapted to using Na^+ transporters (discussed above, Garcia-Sanchez et al. 2000, Rubio et al. 2005).

Whether a stress or a positive response to acidification; an increase in shoot density may lead to positive outcomes for the future of *P. oceanica.* This species is at risk from many different anthropogenic and climatic stressors. Anthropogenic stressors such as fish farming (Cancemi et al. 2003), anchoring (Francour et al. 1999) and water degradation (Fernandez-Torquemada and Sanchez-Lizaso 2005) have been shown to reduce shoot density. Warming has also been shown to increase shoot mortality and hence lead to a decrease in shoot density (Marba and Duarte 2010). If density increases under OA then this may help to ameliorate other human and climatic impacts in the future. A second observation is that although both the invasive *Caulerpa racemosa* and *Asparagopsis sp.* and the indigenous *Caulerpa prolifera* have been observed in low pH waters around the vents at the Castello (Hall-Spencer et al. 2008), they have not invaded the *P. oceanica* meadows, as has been observed at many sites in the NW Mediterranean (Montefalcone et al. 2010). Invasion of these macroalgal species has been linked to seagrass decline, through an increase in sediment sulphate reduction rates (Holmer et al. 2004) leading to increased sulphide pore water concentrations (Calleja et al. 2007). Research has shown that *Caulerpa* *spp.* generally only invades *P. oceanica* meadows with low shoot density (Ceccherelli et al. 2000), therefore OA may prevent further invasion of meadows in the future.

4. Ocean acidification leads to altered invertebrate assemblage and food web dynamics in a temperate seagrass system

4.1 Introduction

Rising CO_2 levels have pushed the pH of ocean surface waters from approximately 8.2 in pre-industrial times down to current values of 8.1 (Raven et al. 2005). A further drop of 0.2-0.4 units is expected by the end of this century (Caldeira and Wickett 2005). A reduction in pH leads to a subsequent reduction in carbonate ions (CO_3^{2-}) and the calcite (Ω_{cal}) and aragonite (Ω_{arg}) saturation state of the water (Fabry et al. 2008). Research suggests that calcification in some invertebrates is dependent on the carbonate saturation state of the seawater (e.g. Marubini et al. 2001; Ohde and Hossain 2004; Langdon and Atkinson 2005). A reduction in calcification and increase in carbonate dissolution rates under enhanced CO_2 conditions has been observed in different calcifying invertebrate taxa such as corals (Hoegh-Guldberg et al. 2007), calcareous algae (Price et al. 2011), echinoderms (Dupont et al. 2008), and molluscs (Gazeau et al. 2007). This decline in calcification is not a uniform response and some species have shown no response to OA (Rodolfo-Metalpa et al. 2011; Comeau et al. 2013b), whilst others have been shown to increase their calcification rates, such as the cephalopod *Sepia officinalis* (Gutowska et al. 2010), the barnacle *Amphibalanus amphitrite* (McDonald et al. 2009) and the coral *Balanophyllia europaea* (Rodolfo-Metalpa et al. 2011). Ries et al. (2009) tested the calcification response of 18 different invertebrates to different pCO_2 conditions and associated saturation states and found that calcification was not negatively affected in 12 species when Ω_{arg} was above 1. When Ω_{arg} fell below 1.0 eight of those species still exhibited either no response or increased calcification, whilst the rest showed negative calcification responses. Similarly mixed responses have been found for marine phytoplankton (Iglesias-Rodriguez et al. 2008). Meta-analysis revealed that calcification in crustaceans generally exhibited a positive response to acidification (Kroeker et al. 2010).

So why are responses so varied? Evidence suggests that some species may be able to actively control extracellular pH through ion transport, actively converting HCO_3^- to CO_3^{2-} at the site of calcification (Melzner et al. 2009; Ries et al. 2009; Calosi et al. 2013). Further evidence suggests that some marine calcifiers may be able to tolerate low pH by utilising HCO_3^- for calcification, and therefore compensate for the decrease in CO_3^{2-} caused by OA (Iglesias-Rodriguez et al. 2008; Comeau et al. 2013a). Organisms that have a protective external organic layer may be more tolerant to low pH (Ries et al. 2009; Lombardi et al. 2011a; Rodolfo-Metalpa et al. 2011). However, tolerance to OA may come at a cost. For example the brittlestar, *Amphiura filiformis*, was demonstrated to up-regulate metabolism and calcification in response to OA but this response leads to muscle loss, making long-term tolerance unsustainable (Wood et al. 2008).

OA will not only affect calcification but can have negative effects on the survival, growth, reproduction, metabolic function and respiration in many marine organisms (reviewed in Kroeker et al. 2010; Widdicombe et al. 2010). Again, responses can be extremely varied. For example growth and reproduction were depressed in the shrimp, *Palaemon pacificus*, in response to long-term acidification (Kurihara et al. 2008), whilst no response in terms of growth and reproduction were observed in the barnacle, *Amphibalanus amphitrite* (McDonald et al. 2009). Metabolic depression was observed in the mussel, *Mytilus chilensis* (Navarro et al. 2013), whilst increased metabolism was observed in Antarctic krill, *Euphausia superba* (Saba et al. 2012). Both of these metabolic responses may be caused by stress: the mussel's decreased metabolic rate may be due to extracellular acidosis (Michaelidis et al. 2005), whilst the krill may increase metabolic function to cope with increased physiological costs of maintaining homeostasis (Saba et al. 2012).

Whilst direct effects of changes in carbonate chemistry will be important in structuring marine communities, indirect effects will also play an important role (Russell et al. 2012).

These effects may include changes to the attributes of habitat-forming species (such as seagrasses) and other associated communities (epiphytes), thus affecting food and shelter availability for mesograzers (Gartner et al. 2013). Other indirect effects may occur through changes in biotic relationships (competition, predation etc), due to changes in species distribution and abundance (Hofmann et al. 2010). This variation in species 'tolerance' or 'sensitivity' to low pH, coupled with uncertainties in how species interactions will change, makes it difficult to predict the outcome for invertebrates at the community level (Garrard et al. 2013). The majority of studies on the impacts of OA have focussed on single species experiments, giving a good understanding of the physiological and developmental impacts of OA (e.g. Bibby et al. 2008; Ellis et al. 2009; Kurihara et al. 2009; Walther et al. 2009), although there are now many laboratory or mesocosm studies that incorporate two or more species (e.g. Andersson et al. 2009; Ferrari et al. 2011b). In order to anticipate the effects of OA we must understand the structure and function of biotic interactions at the community level (Wootton et al. 2008).

Although not direct analogues of OA, due to high pH variation and close proximity of acidified zones to areas of ambient pH, natural CO_2 vents can be useful in examining the long-term community level response to exposure to high CO_2 (Kroeker et al. 2011b). Subtidal CO_2 vents on the island of Ischia (Italy) have been used for this purpose. Hall-Spencer et al. (2008) showed that in areas of CO_2 venting calcifying macrobenthic taxa such as sea urchins, limpets, barnacles, and corals disappear, whilst non-calcifying taxa such as anemones, seagrass, and fleshy algae may actually benefit from acidified conditions, either through direct effects (changes in carbonate chemistry) or indirect effects (reduced competition, predation). Kroeker et al. (2011b) showed that in areas of rocky reef, increasing CO_2 venting (and reduced pH) lead to a reduced taxonomic richness of benthic invertebrates, although abundance was not affected. Trophic analysis showed a decrease in the abundance of herbivores, whilst detritivores and omnivores increased. Comparisons of the abundance of different taxonomic groups showed that the abundance of some groups such as gastropods and decapods were negatively affected by acidification, whilst others such as polychaetes and amphipods were not.

CO_2 vents at the Castello Aragonese can be found venting into the seagrass, *Posidonia oceanica*, as well as the rocky sublittoral. *P. oceanica* meadows are a pivotal shallow water system in the Mediterranean Sea, which harbours a high biodiversity of species (reviewed in Chapter 2). Many calcifying species of gastropods, bivalves, decapods and echinoderms can be found within the shoots. These CO_2 vents provide an important opportunity to investigate how *P. oceanica*-associated benthic invertebrate communities respond to OA. Previously described studies at the Castello Aragonese were conducted at a single time point (June: Hall-Spencer et al. 2008; November: Kroeker et al. 2011b). However, *P. oceanica*-associated benthic communities show high seasonal variation (reviewed in Chapter 2) and results can vary at different times of the year. The importance of conducting temporal studies can be exemplified by algal studies: an increase in turf algae was observed in summer (Kroeker et al. 2013b), whilst a decrease in turf algae was observed in autumn (Porzio et al. 2011) in response to acidification at these CO_2 vents. This chapter will provide a temporal overview of the response of benthic invertebrates to acidification by sampling at three time points during the year to incorporate cold (March), warm (July) and intermediate (November) seawater temperatures, alongside accounting for temporal changes in the morphological features of the *Posidonia* meadow. Due to the increased sampling needed to provide a temporal overview of invertebrate communities, rather than simple comparison between control, low and extremely low pH sites (Kroeker et al. 2011b; Porzio et al. 2011; Kroeker et al. 2013b), the work was focussed on only two pH zones: control (mean pH 8.1) and acidified (mean pH 7.8). These values correspond to control and low pH zones of previous studies and provide ecologically relevant data on current pH values and those predicted for the end of this century (Caldeira and Wickett 2003). It is expected that 1) ocean acidification will change the structure and function of invertebrate communities, and 2) heavily calcified species such as gastropods, bivalves, and decapods will be will be most negatively affected by acidification, leading to decreases in both abundance and richness of these groups of taxa.

4.2 Methods

4.2.1 Site description

The island of Ischia, is located in the eastern Tyrrhenian Sea, off the coast of Italy. CO_2 vents can be found at the north and south side of the Castello Aragonese, Ischia, between a depth of 0.5 and 3 m, leading to acidification of the water column in the surrounding area. Sampling stations were the same as those described in Chapter 3; control and acidified stations at both the north and south of the Castello and a tertiary control site at Lacco Ameno (Chapter 3, Fig. 3.1). Control and acidified stations at each location (north and south) were located in the same seagrass meadow at depths of 2.5 - 3.5 m. A more comprehensive description of sites can be found in section 3.2.1 (Chapter 3). Sampling took place in March, July and November of 2011. pH and carbonate chemistry are described in Chapter 3 (Fig. 3.1, Table 3.1). Mean shoot density in south and north control stations was 446 (±24) and 372 (±27), respectively, whilst mean density in the south and north acidified stations was 815 (±34) and 587 (±24), respectively (see Chapter 3).

4.2.2 Sampling methods

To investigate benthic invertebrate composition, four randomly selected plots (40 x 40 cm) were sampled at each station in each month. Two of the plots were located approximately 2 m apart and 10 m from the other two to take into consideration spatial variability within the designated plots (Vasapollo 2009). These plots were marked with buoys to ensure that different plots were sampled during each sampling period. Samples were collected with an airlift sampler, as this is a non-destructive, quantitative and reproducible method which uses the discharged air from a scuba tank to dislodge and lift the macrofauna from around the seagrass blades, rhizomes and sediment surface into the net for collection (Brook 1978; Buia et al. 2004). Some studies suggest this method may show a greater efficiency for collection of species found within the rhizomes than those in the leaf canopy (Terlizzi and Russo 1996), and lacks the power to collect larger invertebrates such as some holothurians and sea-urchins (pers. obs.). As this is a comparative study, enhanced collection of species within the rhizomes was not seen as a drawback, but was recognised. Samples were collected from each plot using an airlift sampler attached to a 400μm collection net (Terlizzi et al. 2010), whilst the quadrat frame (40 x 40 cm) was attached to the airlift sampler by a 1mm net to contain invertebrates within the quadrat (Fig. 5.1). Each quadrat was sampled continually with the airlift sampler for a period of two minutes to obtain a standardized procedure for faunal comparison. Once the fauna were collected, the shoot density and height of the canopy were measured within the sample quadrat. Samples were fixed in 4% formalin for preservation and subsequently stored in 70% ethanol prior to sorting and identification. Samples were examined under a dissecting microscope and organisms separated from the sediment, algae and seagrass detritus. Gastropods, bivalves, tanaids, isopods, amphipods, decapods and polychaetes were identified by specialized taxonomists (researchers at the SZN, see acknowledgements) to the lowest taxonomical resolution possible, whilst rarer organisms (echinoderms, pycnogonids, cumaceans, mysids, opistobranchs, nudibranchs and polyplacophora) were identified only to their taxonomic group. These are hereafter referred to as operational taxonomic unit (OTU) (Kroeker et al. 2011b).

Figure 5.1 Demonstration of the airlift sampler collecting benthic invertebrates in *P. oceanica*.

4.2.3 Statistical analysis

The abundance (no. individuals/0.16 m^2), richness (no. of species/0.16 m^2), diversity (N1/0.16 m^2 and N2/0.16 m^2), and evenness (N21'/0.16 m^2) were calculated for each samples. Hill's numbers (N1 and N2) were used as each metric describes a different aspect of community structure (Heip et al. 1998). In response to OA, species loss (a decrease in the number of species) has been predicted (Hall-Spencer et al. 2008; Fabricius et al. 2011). This can be calculated from this study using richness, but this does not take into consideration the abundance of each species. For example the ecological interactions present in an assemblage with 1 dominant species and 19 rare species are fewer than those in an assemblage of 20 equally abundant species (Heip et al. 1998). The Shannon-Weiner diversity index (H') and the Simpsons dominance index (λ) can be converted to true diversity measures: Hill's N1 (Exp H') and Hill's N2 ($1/\lambda$) (Hill 1973), which transforms the indices into the effective number of species, producing stable, sensitive and easily interpreted measures (Jost 2006). Evenness (N21') was calculated by the equation: N21' = (N2-1) / (N1-1), as evenness should be independent of species richness (Heip et al. 1998), and unlike many other evenness measures (e.g Pielou's J') this equation is not reliant on species richness and gives a simply interpreted result of the equitability of abundances within a community (Alatalo 1981). Richness (number of species) weighs all species equally, N2 gives most weight to the most abundant species and N1 is in between the two, giving weight to common species (Heip et al. 1998).

Comparisons for each of the taxonomic groups and the community as a whole were examined by means of a three-way GLM ANOVA to test the effects of pH (fixed), site (fixed) and month (fixed). Differences in the abundances of individual OTUs between control and acidified zones were tested using a one-way GLM ANOVA. Data was checked for homogeneity of variance using a Cochran C test ($p > 0.05$). Where variance was found to be heterogeneous, data was $\sqrt{(X + 1)}$ transformed (Underwood 1997). Although a few samples still tested positive in the heterogeneity test (Cochran C: $p < 0.05$), GLM ANOVA was preferred to a less powerful non-parametric approach since the Cochran C test (and other heterogeneity tests) are considerably more sensitive to heterogeneity of variance than ANOVA (McGuinness 2002), plus samples were balanced and relatively large (12 replicates for each 4 treatments), and therefore robust to deviations from the assumptions of ANOVA (Underwood 1997). All univariate analyses were carried out using Statistica 8.0 software.

Community composition and structure were analysed for all data, and separately for each month of sampling, Community composition was analysed by applying a Bray-Curtis similarity matrix on presence/ absence data. PERMANOVA was used to test for significant differences, with site, pH and month as fixed factors. Community structure was analysed by applying a Bray-Curtis similarity matrix to square root transformed abundance data (to reduce the influence of abundant OTUs). PERMANOVA was used to test for significant differences, with site, pH and month as fixed factors. All PERMANOVA analyses used Type III SS and 9,999 unrestricted permutations. Where significant differences occurred between control and acidified stations, pairwise 'site x pH' tests were used to compare control and acidified stations in the north and south of the Castello. Non-metric multidimensional scaling (nMDS) ordinations of Bray-Curtis similarity matrices were plotted for each month. To determine the most representative species in each pH zone a SIMPER analysis was used. All multivariate analyses were performed using Primer v6 with PERMANOVA+ (Plymouth Marine Laboratory).

Invertebrates were assigned to trophic groups based on food preferences documented in the literature and expert judgement (by SZN researchers, see acknowledgements). The abundance and proportion of each trophic guild (suspension feeders, suspension feeders/detritivores, detritivores, herbivore/detrivores, herbivores, carnivore/detritivores, carnivores, omnivores, scavengers, parasites and commensals) were tested by means of a three-way GLM ANOVA to test the effects of pH (fixed), site (fixed) and month (fixed). Multivariate analysis of trophic structure was determined by applying a Bray-Curtis similarity matrix to square-root transformed abundance data (to reduce the influence of abundant trophic guilds). PERMANOVA was used to test for significant differences, with site, pH and month as fixed factors.

Seagrass density was measured within the quadrat after invertebrate sampling; therefore it is possible to determine whether increases (or decreases) in the different taxonomic groups may be due to the indirect effect of a change in seagrass shoot density. Associations between shoot density and the different taxonomic groups, abundance, richness, diversity and evenness were tested by means of non-linear Spearman Rank correlations, as graphical representation did not rule out non-linear relationships, and, in addition, this enables comparison with previous data (Scipione et al. 1996).

4.3 Results

Values of pH and carbonate chemistry are described in Chapter 3. Control stations in the north and south had a mean pH of 8.13 and 8.12, respectively, whilst acidified stations had a mean pH of 7.82 and 7.78 reflecting current ocean conditions and those predicted for the end of this century (Caldeira and Wickett 2003). From calculations of saturation states using the CO_2 SYS software it was estimated that $\Omega_{ARAGONITE} \leq 1.0$ in 17% and 30% of pH samples in the north and south respectively, whilst it was estimated that $\Omega_{CALCITE} \leq 1.0$ in 10% and 21% of pH samples collected in the north and south. A pH of below 7.8 (expected average value for the end of this century, Caldeira and Wickett 2003) was observed in 38% of pH measurements for the north acidified station and 40% for the south acidified station.

During sampling at the Castello Aragonese over 38,000 individual invertebrates were collected and identified to a total of 270 OTUs, from 162 families, over 80% of which were identified to either genus or species level. Results of analyses of the main taxonomic groups and the community as a whole are presented.

4.3.1 Gastropods

A total of 6,036 gastropods were collected from samples at the Castello Aragonese and identified to 51 OTUs (Table 4.1). 3,994 individuals were collected from acidified stations, whilst the other 2,042 were collected from control stations. 10 species increased in response to acidification: *Rissoa italiensis* ($F_{1,46} = 7.28$, $p = 0.010$), *Rissoa guerinii* ($F_{1,46} = 4.92$, $p = 0.031$), *Rissoa variabilis* ($F_{1,46} = 4.65$ $p = 0.036$), *Columbella rustica* ($F_{1,46} = 12.82$, $p < 0.001$), *Gibberula miliaria* ($F_{1,46} = 6.21$ $p = 0.016$), *Jujubinus striatus* ($F_{1,46} = 5.75$, $p = 0.021$), *Mitrella scripta* ($F_{1,46} = 13.39$ $p < 0.001$), *Alvania lineata* ($F_{1,46} = 12.72$, $p < 0.001$), *Gibberula philippii* ($F_{1,46} = 6.98$ $p < 0.011$), and *Nassarius corniculum* ($F_{1,46} = 7.46$, $p = 0.008$). Of these, three were the most abundant gastropod species collected (*A.*

lineata, R. variabilis and *R. italiensis*). *N. corniculum* was present in 42% of samples from acidified stations (N = 24) but was completely absent from control samples. Three of the four species from the genus *Rissoa* and both species from the genus *Gibberula* increased in abundance in response to acidification. Although *Columbella rustica* was more abundant in the acidified zones, dissolution of the shell and loss of the periostracum was noticeable (Fig. 4.2).

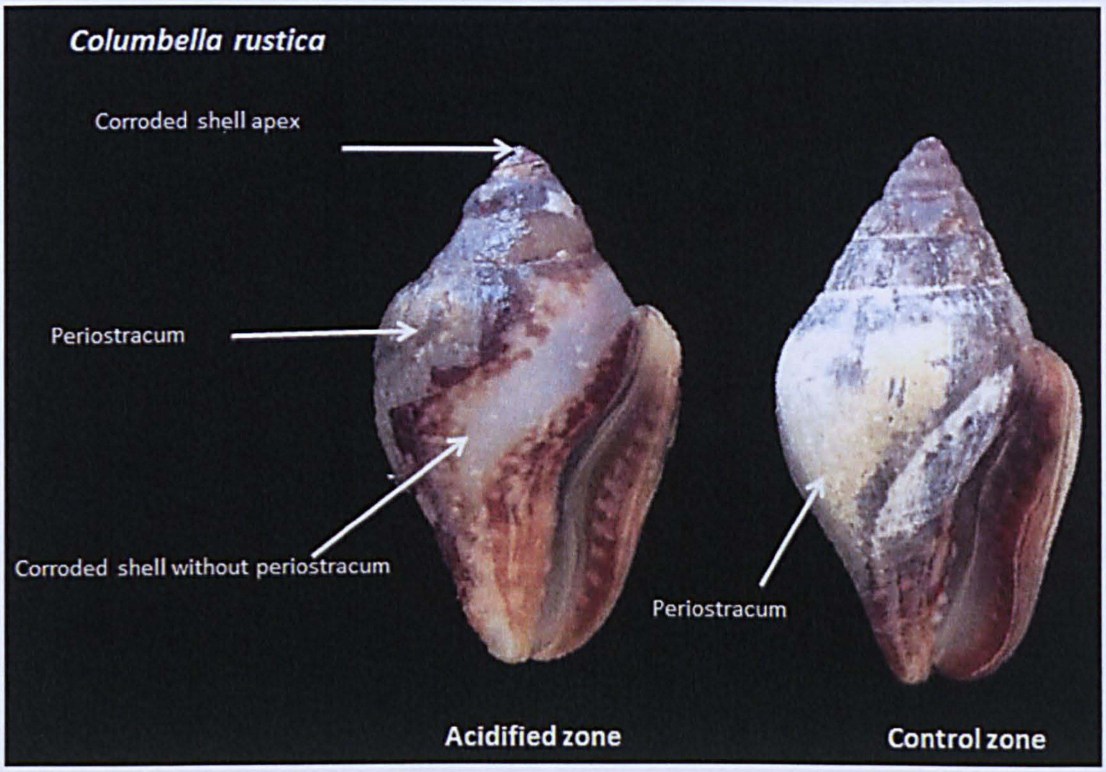


Fig. 4.2 *Columbella rustica* individuals collected in control and acidified zones, showing the loss of periostracum and corroded shell apex that occurred in acidified zones.

Only three species significantly decreased in abundance in response to acidification: *Bittium latreillii* ($F_{1,46} = 7.19$, p = 0.010), *Vexillium tricolor* ($F_{1,46} = 5.66$, p = 0.022), and *Mangelia costulata* ($F_{1,46} = 4.36$, p = 0.042). *B. latreillii* was present in 100% of control samples but only occurred in 42% of acidified samples. No common species (present in four or more samples) disappeared completely from acidified stations. Although not classified as rare, less than 10 individuals were collected for both *V. tricolor* and *M. costulata* during sampling (Table 4.1).

Table 4.1 Mean abundance (± SE) of each species of gastropod per sample (N = 12) at each station, and total abundance collected. Arrows in the final column signify significant increase (↑) or decrease (↓) in the abundance in response to acidification, obtained by a one-way GLM ANOVA.

Species	**South control**	**South acidified**	**North control**	**North acidified**	**Total no. of individuals**	**Change**
Alvania lineata (Risso, 1826)	30.50 ± 5.30	94.67 ± 24.70	14.00 ± 2.83	59.92 ± 11.20	**2,389**	↑
Rissoa variabilis (Von Mühlfeldt, 1824)	0.50 ± 0.19	31.92 ± 13.52	0.17 ± 0.17	0.58 ± 0.40	**398**	↑
Rissoa italiensis (Verduin, 1985)	2.92 ± 0.79	6.82 ± 2.15	3.83 ± 1.73	17.83 ± 5.69	**377**	↑
Bittium latreillii (Payraudeau, 1826)	5.42 ± 1.74	0.75 ± 0.30	22.17 ± 8.72	1.58 ± 0.85	**359**	↓
Jujubinus striatus (Linnaeus, 1758)	4.58 ± 0.87	8.08 ± 3.38	4.08 ± 0.79	11.58 ± 2.94	**340**	↑
Alvania cimex (Linnaeus, 1758)	7.42 ± 1.29	7.75 ± 1.46	7.42 ± 1.83	4.33 ± 1.46	**323**	
Gibbula umbilicaris (Linnaeus, 1758)	4.33 ± 1.57	4.25 ± 1.16	10.58 ± 2.38	5.17 ± 1.04	**292**	
Rissoa auriscalpium (Linnaeus, 1758)	9.75 ± 3.97	4.25 1.60	4.08 ± 1.31	2.33 ± 0.44	**245**	
Columbella rustica (Linnaeus, 1758)	1.25 ± 0.57	12.17 ± 2.84	1.42 ± 0.50	3.17 ± 0.86	**216**	↑
Gibberula philippii (Monterosato, 1878)	0.42 ± 0.33	12.25 ± 3.67	0.17 ± 0.11	0.00	**154**	↑
Tricolia pullus (Linnaeus, 1758)	2.67 ± 0.77	3.17 ± 1.77	2.25 ± 0.57	2.25 ± 0.64	**124**	
Mitrella scripta (Linnaeus, 1758)	1.00 ± 0.52	6.08 ± 1.11	0.50 ± 0.26	1.42 ± 0.48	**108**	↑
Gibberula miliaria (Linnaeus, 1758)	0.33 ± 0.14	2.67 ± 0.81	1.92 ± 1.12	3.33 ± 0.54	**99**	↑
Nassarius incrassatus (Strøm, 1768)	0.58 ± 0.23	1.08 ± 0.26	3.42 ± 0.85	2.75 ± 0.65	**94**	
Clanculus cruciatus (Linnaeus, 1758)	0.92 ± 0.50	1.67 ± 0.75	3.25 ± 0.92	1.75 ± 0.71	**91**	
Rissoa guerinii (Récluz, 1843)	0.42 ± 0.15	3.92 ± 1.42	0.42 ± 0.29	0.50 ± 0.26	**63**	↑
Calliostoma laugieri (Payraudeau, 1826)	0.83 ± 0.24	1.00 ± 0.51	1.75 ± 0.49	1.08 ± 0.36	**56**	
Chauvetia brunnea (Donovan, 1804)	1.75 ± 0.78	0.17 ± 0.11	0.75 ± 0.35	0.75 ± 0.28	**41**	

Species	South control	South acidified	North control	North acidified	No. individuals	Change
Smaragdia viridis (Linnaeus, 1758)	1.50 ± 0.67	0.67 ± 0.28	0.33 ± 0.19	0.58 ± 0.29	**37**	
Marshallora adversa (Montagu, 1803)	0.50 ± 0.15	0.25 ± 0.13	0.92 ± 0.38	0.42 ± 0.26	**25**	
Nassarius corniculum (Olivi, 1792)	0.00	1.17 ± 0.55	0.00	0.58 ± 0.34	**21**	↑
Fusinus pulchellus (Philippi, 1844)	0.17 ± 0.11	0.17 ± 0.11	0.92 ± 0.34	0.42 ± 0.15	**20**	
Tricolia speciosa (Mühlfeld, 1824)	0.33 ± 0.26	0.92 ± 0.66	0.00	0.42 ± 0.23	**20**	
Rissoa violacea (Desmarest, 1814)	0.50 ± 0.26	0.17 ± 0.17	0.50 ± 0.23	0.33 ± 0.19	**18**	
Muricopsis cristata (Brocchi, 1814)	0.92 ± 0.66	0.25 ± 0.13	0.00	0.17 ± 0.11	**16**	
Turbonilla lactea (Linnaeus, 1758)	0.42 ± 0.19	0.42 ± 0.23	0.17 ± 0.17	0.25 ± 0.13	**15**	
Hexaplex trunculus (Linnaeus, 1758)	0.50 ± 0.42	0.00	0.42 ± 0.15	0.25 ± 0.13	**14**	
Eulimella cerullii (Cossmann, 1916)	0.42 ± 0.34	0.17 ± 0.11	0.25 ± 0.25	0.17 ± 0.11	**12**	
Vexillum tricolor (Gmelin, 1791)	0.33 ± 0.19	0.00	0.33 ± 0.14	0.08 ± 0.08	**9**	↓
Cerithiopsis micalii (Cecalupo & Villari, 1997)	0.25 ± 0.13	0.17 ± 0.11	0.25 ± 0.18	0.25 ± 1.78	**9**	
Vexillum (Pusia) ebenus (Lamarck, 1811)	0.17 ± 0.11	0.00	0.25 ± 0.18	0.08 ± 0.08	**6**	
Conus ventricosus mediterraneus (Hwass in Bruguière, 1792)	0.00	0.00	0.42 ± 0.19	0.00	**5**	
Haliotis tuberculata tuberculata (Linnaeus, 1758)	0.17 ± 0.17	0.17 ± 0.11	0.00	0.00	**4**	
Diodora gibberula (Lamarck, 1822)	0.00	0.00	1.67 ± 1.67	1.67 ± 1.67	**4**	
Ocenebra erinaceus (Linnaeus, 1758)	0.08 ± 0.08	0.25 ± 0.18	0.00	0.00	**4**	
Enginella leucozona (Philippi, 1843)	0.00	0.25 ± 0.13	0.00	0.00	**3**	
Cerithium scabridum (Philippi, 1848)	0.08 ± 0.08	0.00	0.08 ± 0.08	0.00	**2**	
Naticarius hebraeus (Martyn, 1786)	0.08 ± 0.08	0.00	0.00	0.08 ± 0.08	**2**	
Haminoea hydatis (Linnaeus, 1758)	0.00	0.17 ± 0.17	0.00	0.00	**2**	
Chrysallida indistincta (Henn & Brazier, 1894)	0.00	0.00	0.08 ± 0.08	0.00	**1**	
Cerithiopsis diadema (Monterosato, 1874)	0.00	0.00	0.08 ± 0.08	0.00	**1**	
Luria lurida (Linnaeus, 1758)	0.00	0.00	0.08 ± 0.08	0.00	**1**	

Species	**South control**	**South acidified**	**North control**	**North acidified**	**No. individuals**	**Change**
Jujubinus exasperatus (Pennant, 1777)	0.00	0.08 ± 0.08	0.00	0.00	1	
Retusa truncatula (Bruguière, 1792)	0.00	0.00	0.00	0.08 ± 0.08	1	
Mitra cornicula (Linnaeus, 1758)	0.00	0.08 ± 0.08	0.00	0.00	1	
Erosaria spurca (Linnaeus, 1758)	0.08 ± 0.08	0.00	0.00	0.00	1	
Bulla sp.	0.00	0.00	0.00	0.08 ± 0.08	1	
Raphitoma sp.	0.08 ± 0.08	0.00	0.00	0.00	1	
Cylichna cylindracea (Pennant, 1777)	0.00	0.00	0.00	0.08 ± 0.08	1	
Gibbula turbinoides (Deshayes, 1835)	0.00	0.08 ± 0.08	0.00	0.00	1	

Abundance was greater in acidified stations ($F_{1,36} = 26.46$, $p < 0.001$), although there was a significant 'site x pH' interaction ($F_{1,36} = 7.89$, $p = 0.008$), as this increase was only significant at the south side (Tukey HSD: $p < 0.001$). This difference was primarily driven by the large increase in the abundance of gastropods that occurred in November at the south acidified station (Fig. 4.3a). In contrast, diversity (N1 and N2) and evenness (N21') were significantly lower in acidified than control stations (N1: $F_{1,36} = 13.74$, $p < 0.001$, N2: $F_{1,36} = 16.03$, $p < 0.001$, and N21': $F_{1,36} = 9.92$, $p = 0.003$), although there was a significant 'site x pH' interaction for each (N1: $F_{1,36} = 7.77$, $p = 0.008$, N2: $F_{1,36} = 10.45$, $p = 0.003$, and N21': $F_{1,36} = 5.58$, $p = 0.023$) as these increases were only significant on the north side (Tukey HSD: $p < 0.001$, $p < 0.001$, and $p = 0.002$, respectively) (Fig. 4.3c-e). Species richness was not affected by pH ($F_{1,36} = 0.35$, $p = 0.555$). Abundance and richness were higher in November than March and July (abundance: Tukey HSD: $p < 0.001$ and $p < 0.001$, M = J < N; richness: Tukey HSD: $p = 0.004$ and $p < 0.001$, M = J < N), whilst evenness was higher in July than March and November (Tukey HSD: $p = 0.017$ and $p = 0.003$, N = M > J). Diversity (N1 and N2) did not vary among months (Table 4.2).

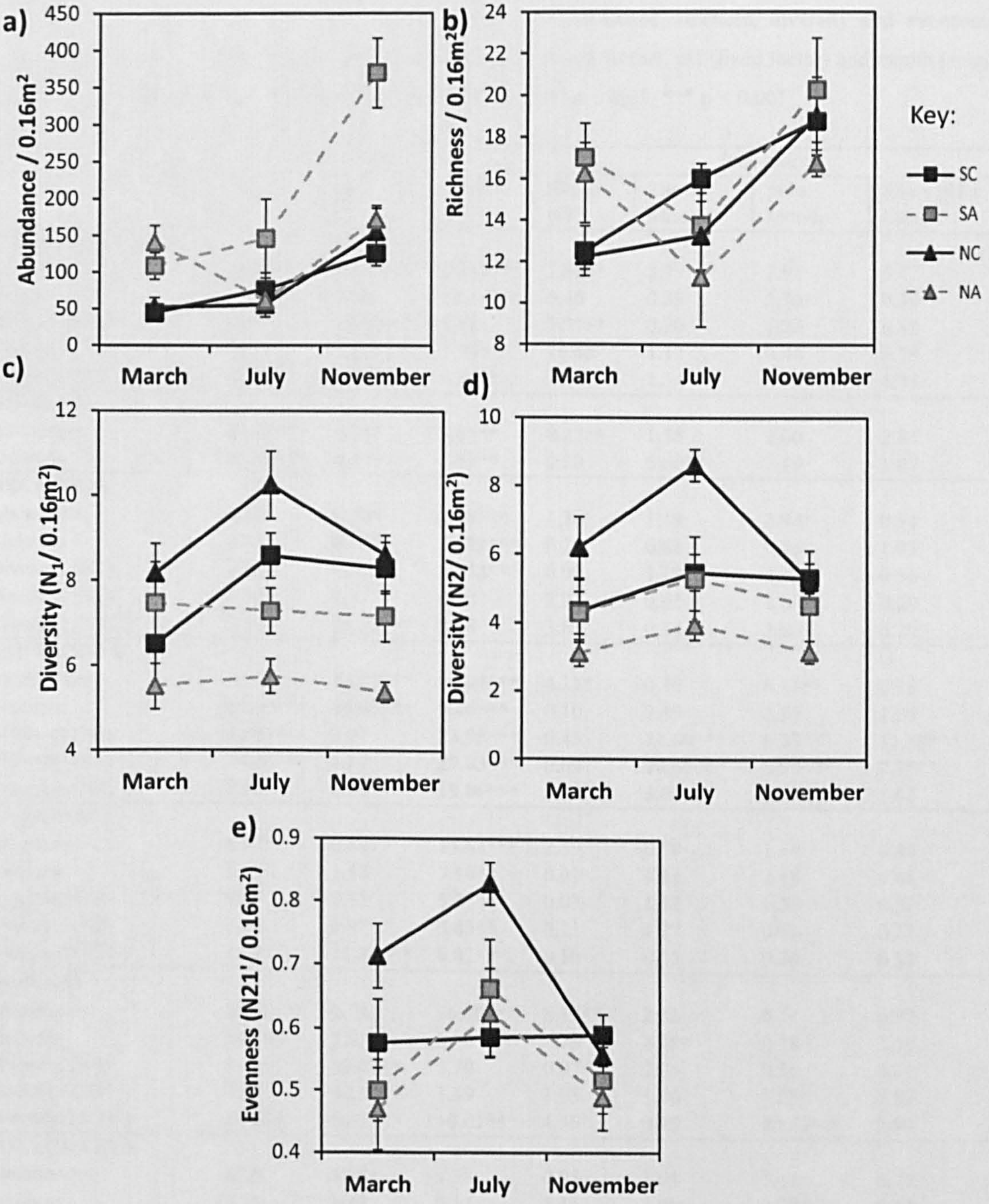


Figure 4.3 Temporal changes in a) abundance, b) richness, c) diversity (N1), d) diversity (N2), and e) evenness (N21') of gastropods at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

Table 4.2 Results for each taxonomic group in terms of abundance, richness, diversity and evenness, analysed by means of a three-way GLM ANOVA with site (fixed factor), pH (fixed factor) and month (fixed factor). Significant results are highlighted in red. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	Factors			Interactions			
	Site	pH	Month	Site x pH	Site x Month	pH x Month	Site x pH x Month
GASTROPODA							
Abundance	**6.11***	**24.46*****	**25.52*****	**7.89****	**3.79***	2.91	5.47
Richness	2.61	0.35	**10.34*****	0.46	0.39	**3.76***	0.39
Diversity (N1)	0.27	**13.74*****	1.32	**7.77****	0.20	1.26	0.18
Diversity (N2)	0.14	**16.03*****	**3.75***	**10.45****	1.12	0.48	0.38
Evenness (N21')	1.76	**9.92****	**7.25****	**5.58***	1.54	0.98	1.77
BIVALVA							
Abundance	**14.16*****	**6.30***	**5.41****	**8.27****	1.55	2.00	2.81
Richness	**33.80*****	**6.42***	**7.02****	0.20	5.60**	2.49	1.07
DECAPODA							
Abundance	0.39	**6.30***	**9.18*****	1.20	1.19	**3.93***	0.84
Richness	0.16	**8.75****	**13.92*****	0.29	0.83	0.76	1.93
Diversity (N1)	0.83	0.02	**10.13*****	0.99	1.12	2.47	0.36
Diversity (N2)	0.18	2.31	6.72	2.22	0.92	2.83	0.29
Evenness (N21')	0.50	**21.35*****	0.18	3.63	0.34	2.60	0.20
AMPHIPODA							
Abundance	0.07	**44.87*****	**15.04*****	**4.21***	0.46	**8.10****	0.18
Richness	**15.49*****	**15.49*****	**9.46*****	0.10	2.89	2.55	1.54
Diversity (N1)	**44.51*****	2.57	**23.95*****	0.45	**11.60*****	**6.37****	**11.98*****
Diversity (N2)	**24.10*****	1.37	**27.83*****	0.69	**10.67*****	**8.95*****	**7.75****
Evenness (N21')	0.02	0.60	**19.06*****	0.45	3.01	**12.17*****	1.62
TANAIDACEA							
Abundance	**6.94***	0.84	**15.62*****	2.39	0.59	1.14	0.88
Richness	1.48	1.48	**7.10****	0.01	1.24	2.18	0.81
Diversity (N1)	0.97	0.15	**5.37****	0.03	1.12	1.34	0.31
Diversity (N2)	1.49	0.97	**5.43****	0.11	1.27	0.58	0.21
Evenness (N21')	1.62	**11.84*****	**8.02****	0.10	0.35	0.10	0.33
ISOPODA							
Abundance	**26.69*****	1.73	**16.70*****	**8.15****	2.82	0.71	0.77
Richness	**15.79*****	**7.57****	**8.76*****	1.75	**4.48***	0.38	3.16
Diversity (N1)	3.35	**10.47****	2.70	0.07	2.94	0.50	**3.47***
Diversity (N2)	0.25	**9.22****	1.49	1.05	1.66	1.25	2.83
Evenness (N21')	**10.96****	0.03	**10.01*****	**4.36***	0.97	**10.53*****	2.99
POLYCHAETA							
Abundance	0.20	**4.98***	1.38	0.95	3.01	1.62	0.77
Richness	2.31	1.42	3.17	3.41	**3.89***	0.29	0.42
Diversity (N1)	2.91	0.95	2.13	1.34	**4.62***	0.21	0.33
Diversity (N2)	2.58	0.94	0.84	0.62	**3.86***	0.42	0.61
Evenness (N21')	0.01	0.14	0.41	1.68	0.35	0.53	0.07
OPHIURIODEA							
Abundance	0.21	**59.15*****	**7.59****	**7.34***	**3.41***	**3.41***	1.96
PYCNOGONIDAE							
Abundance	**7.20***	**36.54*****	0.76	**7.55****	0.08	1.54	0.41
CUMACEA							
Abundance	0.76	0.89	**5.40****	**6.50***	0.78	0.60	2.67
MYSIDACEA							
Abundance	1.15	2.26	**48.25*****	**5.51***	1.15	2.26	**5.51***
POLYPLACOPHORA							
Abundance	4.05	**5.84***	0.16	1.46	0.65	1.46	1.95

4.3.2 Bivalves

A total of 326 bivalves were collected from stations around the Castello Aragonese and identified to 27 species. 16 of these species were rare (present in less than four samples) and more than 10 individuals were only collected for 6 species (Table 4.3). A total of 115 individuals were collected from control stations and 211 individuals from acidified stations. Only two species showed a significant response to pH: the bivalves *Abra alba* ($F_{1,46} = 7.51$, $p = 0.009$) and *Musculus subpictus* ($F_{1,46} = 15.25$, $p < 0.001$) significantly increased in response to acidification. *A. alba* and *M. subpictus* were the two most abundant species, accounting for over half of all individuals collected from the Castello Aragonese. *Mytilus galloprovincialis* was present in control stations but completely absent from acidified stations, although the patchy distribution of this species, primarily in the northern control station, meant that this difference in abundance was not significant ($F_{1,46} = 2.94$, $p = 0.093$).

Table 4.3 Mean abundance (± SE) of each species of bivalve per sample (N = 12) at each station, and total abundance collected. Arrows in the final column signify significant increase (↑) or decrease (↓) in the abundance in response to acidification, obtained by a one-way GLM ANOVA.

Species	**South control**	**South acidified**	**North control**	**North acidified**	**No. individuals**	**Change**
Abra alba (W. Wood, 1802)	1.42 ± 0.61	8.12 ± 2.42	0.00	0.25 ± 0.25	**118**	↑
Musculus subpictus (Cantraine, 1835)	0.17 ± 0.11	3.92 ± 1.45	0.17 ± 0.11	1.75 ± 0.68	**72**	↑
Mytilus galloprovincialis (Lamarck, 1819)	0.25 ± 0.18	0.00	2.33 ± 1.46	0.00	**31**	
Striarca lactea (Linnaeus, 1758)	0.50 ± 0.15	0.25 ± 0.13	0.25 ± 0.13	0.50 ± 0.23	**18**	
Acanthocardia tuberculata (Linnaeus, 1758)	0.25 ± 0.13	0.17 ± 0.17	0.17 ± 0.11	0.83 ± 0.51	**17**	
Venus casina (Linnaeus, 1758)	0.58 ± 0.29	0.17 ± 0.11	0.25 ± 0.18	0.00	**12**	
Glans trapezia (Linnaeus, 1767)	0.67 ± 0.26	0.08 ± 0.08	0.00	0.00	**9**	
Arca noae (Linnaeus, 1758)	0.33 ± 0.14	0.08 ± 0.08	0.17 ± 0.17	0.00	**7**	

Species	South control	South acidified	North control	North acidified	No. individuals	Change
Barbatia barbata (Linnaeus, 1758)	0.00	0.58 ± 0.29	0.00	0.00	7	
Parvicardium exiguum (Gmelin, 1791)	0.42 ± 0.19	0.00	0.08 ± 0.08	0.00	6	
Limaria tuberculata (Olivi, 1792)	0.08 ± 0.08	0.08 ± 0.08	0.25 ± 0.18	0.00	5	
Psammotreta cumana (Costa O.G., 1829)	0.33 ± 0.19	0.00	0.00	0.00	4	
Parvicardium pinnulatum (Conrad, 1831)	0.08 ± 0.08	0.16 ± 0.11	0.00	0.00	3	
Angulus tenuis (da Costa, 1778)	0.00	0.16 ± 0.16	0.00	0.00	2	
Hiatella rugosa (Linnaeus, 1767)	0.00	0.00	0.17 ± 0.17	0.00	2	
Hiatella arctica (Linnaeus, 1767)	0.17 ± 0.11	0.00	0.00	0.00	2	
Mimachlamys varia (Linnaeus, 1758)	0.00	0.00	0.08 ± 0.08	0.00	1	
Irus irus (Linnaeus, 1758)	0.08 ± 0.08	0.00	0.00	0.00	1	
Venerupis corrugata (Gmelin, 1791)	0.00	0.00	0.08 ± 0.08	0.00	1	
Donax semistriatus (Poli, 1795)	0.00	0.00	0.08 ± 0.08	0.00	1	
Lutraria oblonga (Gmelin, 1791)	0.00	0.00	0.00	0.008 ± 0.008	1	
Chama gryphoides (Linnaeus, 1758)	0.00	0.00	0.00	0.008 ± 0.008	1	
Lima lima (Linnaeus, 1758)	0.00	0.08 ± 0.08	0.00	0.00	1	
Modiolus barbatus (Linnaeus, 1758)	0.00	0.08 ± 0.08	0.00	0.00	1	
Arcuatula perfragilis (Dunker, 1857)	0.08 ± 0.08	0.00	0.00	0.00	1	
Moerella donacina (Linnaeus, 1758)	0.08 ± 0.08	0.00	0.00	0.00	1	
Timoclea ovata (Pennant, 1777)	0.00	0.08 ± 0.08	0.00	0.00	1	

Species richness was significantly lower in acidified stations ($F_{1,36} = 6.42$, $p = 0.016$). Abundance was greater in acidified stations ($F_{1,36} = 6.30$, $p = 0.017$), although there was a significant 'site x pH' interaction ($F_{1,36} = 8.27$, $p = 0.007$), as this increase was only significant at the south side (Tukey HSD: $p = 0.003$). This is a similar pattern to that observed for gastropods, although this difference was driven by the large increase in the abundance of bivalves that occurred in both July and November at the south acidified station (Fig. 4.4a). The species primarily responsible for this increase was *A. alba* (Table 4.3). Both abundance and richness were significantly different between months (Table 4.2). Abundance was lower in March than in July and November (Tukey HSD: $p = 0.007$ and $p < 0.001$, $M < J = N$). Richness was lower in March than July and November (Tukey HSD: $p = 0.039$ and $p = 0.002$, $M < J = N$). Abundance and richness were higher in the south than the north side (Fig. 4.4). Diversity and evenness could not be analysed as bivalves were not present in all samples. All collected bivalves were suspension feeders, so there was no difference in trophic structure between stations.

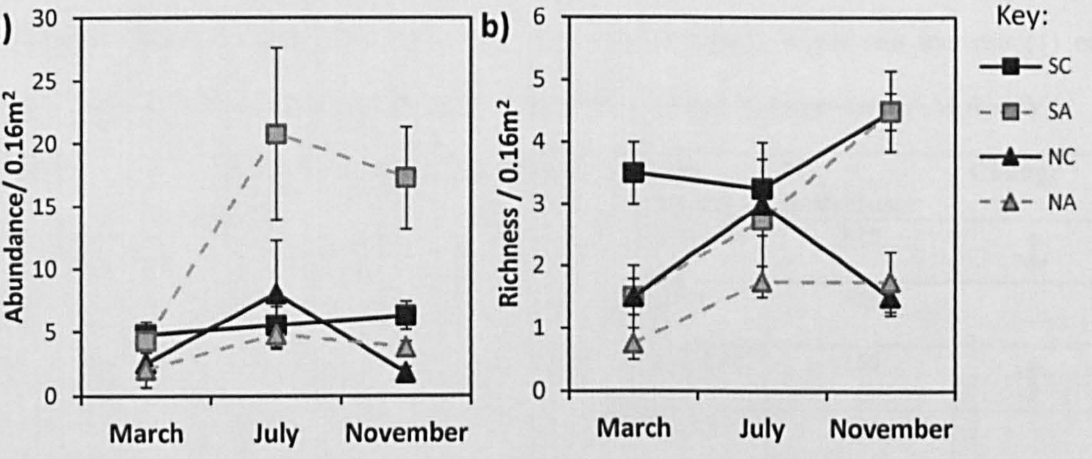


Figure 4.4 Temporal changes in a) abundance, and b) richness of bivalves at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

4.3.3 Decapods

A total of 2,235 decapods were collected during sampling at the Castello, 1,396 in control stations and 839 in acidified stations. These were identified to 36 species (Table 4.4). Five species decreased in abundance in response to ocean acidification. Most notable was the decreased abundance of the hermit crab *Cestopagurus timidus* ($F_{1,46} = 19.16$, $p < 0.001$), which decreased from 697 collected in control stations to 128 collected in acidified stations. This species accounted for over one third of all decapods collected. Other species that showed a negative response to acidification were: the shrimp *Eualus cranchii* ($F_{1,46}$ = 13.09, $p < 0.001$), the crab *Galathea bolivari (*$F_{1,46}$ = 12.91, $p < 0.001$), the crab *Calcinus tubularis* ($F_{1,46}$ = 4.73, $p = 0.035$), and the shrimp *Processa canaliculata* ($F_{1,46}$ = 5.31, $p = 0.026$). *G. bolivari* was present in 58% of samples in control stations and only 12.5% of samples in acidified stations, whilst *C. tubularis* decreased from 38% of control samples to 4% of acidified samples. Only one species of decapod increased in abundance in response to OA: the shrimp *Hippolyte leptocerus* ($F_{1,46}$ = 4.18, $p = 0.047$).

Table 4.4 Mean abundance (± SE) of each species of decapod per sample (N = 12) at each station, and total abundance collected. Arrows in the final column signify significant increase (↑) or decrease (↓) in the abundance in response to acidification, obtained by a one-way GLM ANOVA.

Species	**South control**	**South acidified**	**North control**	**North acidified**	**No. individuals**	**Change**
Cestopagurus timidus (Roux, 1830)	30.5 ± 8.99	4.08 ± 0.81	27.58 ± 6.10	6.58 ± 1.84	**825**	↓
Athanas nitescens (Leach, 1813)	14.5 ± 4.81	9.33 ± 2.88	10.08 ± 2.13	18.25 ± 7.43	**626**	
Hippolyte leptocerus (Heller, 1863)	2.0 ± 0.89	5.58 ± 2.33	1.5 ± 0.57	3.83 ± 1.43	**155**	↑
Hippolyte inermis (Leach, 1816)	1.92 ± 0.75	2.83 ± 1.33	2.42 ± 1.32	4.0 ± 1.41	**134**	
Alpheus dentipes (Guérin, 1832)	1.42 ± 0.77	1.08 ± 0.66	2.92 ± 0.73	5.5 ± 1.83	**131**	
Eualus cranchii (Leach, 1817)	1.92 ± 0.45	0.92 ± 0.23	3.75 ± 0.89	0.75 ± 0.28	**88**	↓
Galathea bolivari (Zariquiey Álvarez, 1950)	0.5 ± 0.29	0.00	3.75 ± 0.80	0.33 ± 0.19	**55**	↓
Pilumnus hirtellus (Linnaeus, 1761)	3.0 ± 0.74	0.75 ± 0.28	0.33 ± 0.14	0.5 ± 0.42	**55**	
Pisidia bluteli (Risso, 1816)	0.83 ± 0.58	0.67 ± 0.19	1.17 ± 0.42	0.00	**32**	
Xantho poressa (Olivi, 1792)	0.25 ± 0.13	0.33 ± 0.14	0.67 ± 0.31	1.0 ± 0.37	**27**	
Brachynotus sexdentatus (Risso, 1827)	0.5 ± 0.26	0.58 ± 0.31	0.25 ± 0.13	0.75 ± 0.45	**25**	
Calcinus tubularis (Linnaeus, 1767)	0.83 ± 0.58	0.00	0.58 ± 0.23	0.08 ± 0.08	**18**	↓
Xantho pilipes (Milne-Edwards, 1867)	0.42 ± 0.22	0.00	0.17 ± 0.11	0.5 ± 0.42	**13**	

Species	South control	South acidified	North control	North acidified	No. individuals	Change
Achaeus gracilis (Costa, 1839)	0.5 ± 0.19	0.00	0.17 ± 0.11	0.17 ± 0.11	**10**	
Processa canaliculata (Leach, 1815)	0.00	0.00	0.5 ± 0.19	0.00	**6**	↓
Lysmata seticaudata (Risso, 1816)	0.17 ± 0.11	0.25 ± 0.18	0.00	0.00	**5**	
Acanthonyx lunulatus (Risso, 1816)	0.00	0.25 ± 0.18	0.00	0.08 ± 0.08	**4**	
Philocheras fasciatus (Risso, 1816)	0.17 ± 0.11	0.00	0.00	0.08 ± 0.08	**3**	
Dromia personata (Linnaeus, 1758)	0.00	0.08 ± 0.08	0.00	0.08 ± 0.08	**2**	
Ilia nucleus (Linnaeus, 1758)	0.17 ± 0.11	0.00	0.00	0.00	**2**	
Munida sp.	0.00	0.00	0.00	0.17 ± 0.17	**2**	
Pisa nodipes (Leach, 1815)	0.08 ± 0.08	0.00	0.00	0.08 ± 0.08	**2**	
Scyllarus sp.	0.08 ± 0.08	0.00	0.00	0.08 ± 0.08	**2**	
Eriphia verrucosa (Forskål, 1775)	0.00	0.00	0.00	0.08 ± 0.08	**1**	
Eualus pusiolus (Krøyer, 1841)	0.00	0.00	0.00	0.08 ± 0.08	**1**	
Liocarcinus navigator (Herbst, 1794)	0.08 ± 0.08	0.00	0.00	0.00	**1**	
Microcassiope minor (Dana, 1852)	0.08 ± 0.08	0.00	0.00	0.00	**1**	
Munida curvimana (Milne Edwards & Bouvier, 1894)	0.08 ± 0.08	0.00	0.00	0.00	**1**	
Pagurus anachoretus (Risso, 1827)	0.08 ± 0.08	0.00	0.00	0.00	**1**	
Palaemon serratus (Pennant, 1777)	0.00	0.08 ± 0.08	0.00	0.00	**1**	
Parthenopoides massena (Roux, 1830)	0.00	0.00	0.08 ± 0.08	0.00	**1**	
Pasiphaea multidentata (Esmark, 1866)	0.08 ± 0.08	0.00	0.00	0.00	**1**	
Pisa carinimana (Miers, 1879)	0.00	0.08 ± 0.08	0.00	0.00	**1**	
Pisa tetraodon (Pennant, 1777)	0.08 ± 0.08	0.00	0.00	0.00	**1**	
Sicyonia carinata (Brünnich, 1768)	0.00	0.08 ± 0.08	0.00	0.00	**1**	
Upogedia deltaura (Leach, 1815	0.08 ± 0.08	0.00	0.00	0.00	**1**	

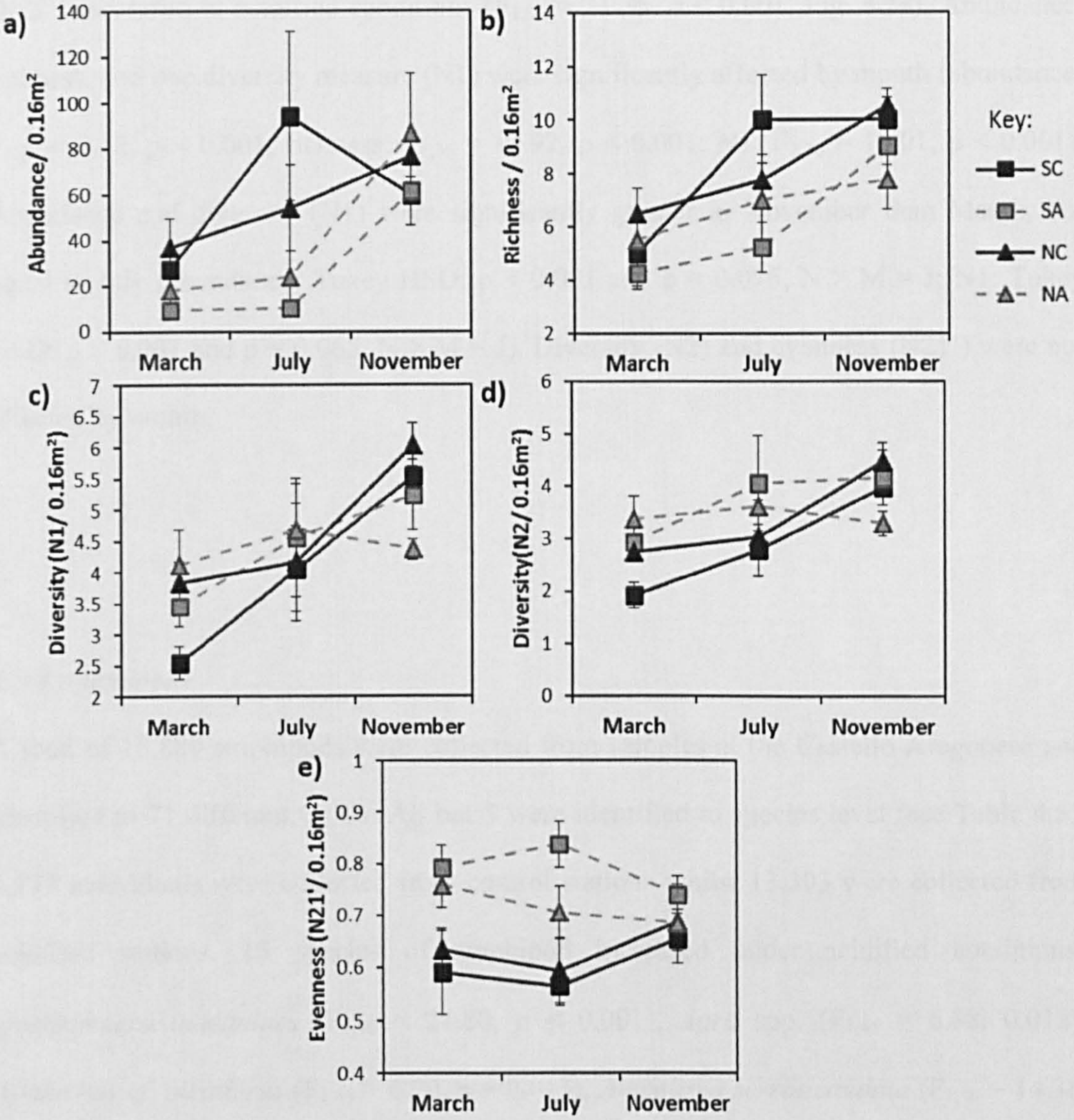


Figure 4.5 Temporal changes in a) abundance, b) richness, c) diversity (N1), d) diversity (N2), and e) evenness (N21') of decapods at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

The abundance of decapods was lower in acidified stations ($F_{1,36}$ = 6.30, p = 0.017), although there was a significant 'pH x month' interaction ($F_{1,36}$ = 3.93, p = 0.029), as this decrease was only significant in July (Tukey HSD: p = 0.012) (Fig. 4.5a). In response to acidification, a significant decrease in species richness was observed ($F_{1,36}$ = 8.75, p =

0.005). Diversity (N1 and N2) were not affected by pH (Table 4.2). In contrast evenness (N21') increased in acidified conditions ($F_{1,36} = 21.35$, $p < 0.001$, Fig. 4.5e). Abundance, richness, and one diversity measure (N1) were significantly affected by month (abundance: $F_{1,36} = 9.18$, $p < 0.001$; richness: $F_{1,36} = 13.92$, $p < 0.001$; N1: $F_{1,36} = 10.01$, $p < 0.001$). Abundance and diversity (N1) were significantly greater in November than March, but equal to July (abundance: Tukey HSD: $p < 0.001$ and $p = 0.075$, N > M = J; N1: Tukey HSD: $p < 0.001$ and $p = 0.065$, N > M = J). Diversity (N2) and evenness (N21') were not affected by month.

4.3.4 Amphipods

A total of 18,880 amphipods were collected from samples at the Castello Aragonese and identified to 71 different OTUs. All but 5 were identified to species level (see Table 4.5). 5,577 individuals were collected from control stations whilst 13,303 were collected from acidified stations. 15 species of amphipod increased under acidified conditions: *Quadrimaera inaequipes* ($F_{1,46} = 21.80$, $p < 0.001$), *Aora* spp. ($F_{1,46} = 6.88$, 0.012), *Apolochus cf. picadurus* ($F_{1,46} = 6.70$, $p = 0.013$), *Ampelisca serraticaudata* ($F_{1,46} = 14.38$, $p < 0.001$), *Metaphoxus simplex* ($F_{1,46} = 24.72$, $p < 0.001$), *Lembos websteri* ($F_{1,46} = 20.26$, $p < 0.001$), *Liljeborgia dellavallei* ($F_{1,46} = 5.75$, $p = 0.021$), *Ericthonius punctatus* ($F_{1,46} = 7.59$, $p = 0.008$), Maeridae sp. ($F_{1,46} = 17.12$, $p < 0.001$), *Tethylembos viguieri* ($F_{1,46} = 17.33$, $p < 0.001$), *Lysianassa pilicornis* ($F_{1,46} = 8.35$, $p = 0.006$), *Ampithoe ramondi* ($F_{1,46} = 4.25$, $p = 0.025$), *Caprella acanthifera* ($F_{1,46} = 4.48$, $p = 0.040$), *Protohyale schmidtii* ($F_{1,46} = 7.71$, $p = 0.008$), *Ericthonius difformis* ($F_{1,46} = 5.28$, $p = 0.026$). The increase in abundance of many of these species in response to acidification was substantial (Table 4.5). For example the number of individuals collected in control and acidified stations of

Q. inaequipes increased from 438 to 1,896, *Aora* spp. from 323 to 1,523, *A. serraticaudata* from 37 to 1,229 and *M. simplex* from 43 to 932. Only two species of amphipods decreased in response to acidification: *Dexamine spinosa* ($F_{1,46}$ = 13.37, $p < 0.001$), and *Ampithoe helleri* ($F_{1,46}$ = 6.90, $p = 0.011$). No common species of amphipods (present in > 4 samples) disappeared from acidified stations.

Table 4.5 Mean abundance (± SE) of each species of amphipod per sample (N = 12) at each station, and total abundance collected. Arrows in the final column signify significant increase (↑) or decrease (↓) in the abundance in response to acidification, obtained by a one-way GLM ANOVA.

Species	**South control**	**South acidified**	**North control**	**North acidified**	**No. individuals**	**Change**
Quadrimaera inaequipes (A. Costa, 1857)	11.0 ± 3.78	95.33 ± 16.79	25.50 ± 6.57	62.67± 17.62	**2,334**	↑
Apherusa cf. *chiereghinii* (Giordani-Soika, 1950)	48.33± 9.79	21.42± 7.92	40.67 ± 12.67	57.83 ± 24.33	**2,009**	
Aora spp.	17.83 ± 4.85	108.58 ± 33.15	9.08 ± 1.85	18.33 ± 4.47	**1,846**	↑
Apolochus cf. *picadurus* (J.L. Barnard, 1962)	11.83 ± 3.58	41.75 ± 8.72	28.0 ± 4.92	32.25 ± 7.29	**1,366**	↑
Ampelisca serraticaudata (Chevreux, 1888)	0.42 ± 0.15	73.50 ± 21.76	2.67 ± 2.06	28.92 ± 12.21	**1,266**	↑
Metaphoxus simplex (Bate, 1857)	1.50 ± 0.62	54.75 ± 12.47	2.08 ± 0.62	22.92 ± 5.46	**975**	↑
Lembos websteri (Bate, 1857)	12.00 ± 1.86	39.00 ± 8.12	5.92 ± 1.20	23.33 ± 4.27	**963**	↑
Melita hergensis (Reid, 1939)	3.08 ± 1.94	2.42 ± 1.03	19.33 ± 5.22	35.00 ± 11.01	**718**	
Elasmopus pocillimanus (Bate, 1862)	6.67 ± 2.67	8.08 ± 2.43	12.42 ± 1.91	28.25 ± 7.76	**665**	
Liljeborgia dellavallei (Stebbing, 1906)	6.42 ± 1.97	19.25 ± 4.59	11.17 ± 3.21	14.75 ± 3.43	**619**	↑
Gammarella fucicola (Leach, 1814)	9.00 ± 3.02	18.33 ± 6.71	5.50 ± 0.81	8.75 ± 1.57	**499**	
Ericthonius punctatus (Bate, 1857)	3.75 ± 1.08	10.42 ± 2.98	7.42 ± 1.94	18.33 ± 5.03	**479**	↑
Megamphopus cornutus (Norman, 1869)	8.92 ± 2.44	11.92 ± 2.96	6.00 ± 1.78	12.58 ± 2.77	**473**	

Species	South control	South acidified	North control	North acidified	No. individuals	Change
Maeridae sp.	0.75 ± 0.49	17.00 ± 4.28	4.42 ± 1.38	12.67 ± 3.84	**418**	↑
Gammaropsis palmata (Stebbing & Robertson, 1891)	5.83 ± 1.81	9.50 ± 1.72	8.33 ± 2.23	9.67 ± 2.12	**400**	
Maera grossimana (Montagu, 1808)	3.17 ± 0.97	6.25 ± 1.83	7.67 ± 3.62	12.00 ± 3.95	**349**	
Monocorophium sextonae (Crawford, 1937)	0.00	0.92 ± 0.92	23.25 ± 6.71	3.50 ± 0.81	**332**	
Perioculodes aequimanus (Kossmann, 1880)	4.00 ± 1.04	3.92 ± 1.30	8.08 ± 2.04	11.17 ± 1.57	**326**	
Hyale camptonyx (Heller, 1866)	4.67 ± 1.60	2.00 ± 0.75	3.75 ± 1.09	15.67 ± 5.40	**313**	
Orchomene humilis (A. Costa, 1853)	1.92 ± 0.87	3.17 ± 0.66	7.67 ± 1.27	7.92 ± 4.09	**248**	
Tethylembos viguieri (Chevreux, 1911)	0.75 ± 0.37	14.83 ± 3.33	0.25 ± 0.18	4.00 ± 1.60	**238**	↑
Iphimedia minuta (G.O. Sars, 1882)	5.92 ± 3.75	1.25 ± 0.73	2.67 ± 0.91	6.50 ± 2.78	**196**	
Ischyrocerus inexpectatus (Ruffo, 1959)	0.75 ± 0.51	5.33 ± 1.85	3.67 ± 1.91	6.58 ± 2.97	**196**	
Lysianassa pilicornis (Heller, 1866)	0.92 ± 0.29	10.42 ± 3.34	0.58 ± 0.23	2.08 ± 0.81	**168**	↑
Microdeutopus chelifer (Bate, 1862)	0.92 ± 0.67	1.42 ± 0.68	2.75 ± 1.88	7.42 ± 1.78	**150**	
Leucothoe denticulata (A. Costa, 1851)	0.58 ± 0.26	1.67 ± 0.75	3.17 ± 0.74	6.17 ± 1.96	**139**	
Leptocheirus pectinatus (Norman, 1869)	2.50 ± 0.65	1.50 ± 0.66	1.58 ± 0.58	5.75 ± 1.61	**136**	
Ampithoe ramondi (Audouin, 1826)	0.42 ± 0.29	7.58 ± 3.79	0.17 ± 0.17	1.25 ± 0.68	**113**	↑
Caprella acanthifera (Leach, 1814)	0.33 ± 0.14	2.17 ± 0.93	1.83 ± 0.71	4.17 ± 1.55	**102**	↑
Aoridae gen.sp.	2.67 ± 0.57	0.17 ± 0.11	1.92 ± 0.69	2.83 ± 0.59	**91**	
Leptocheirus guttatus (Grube, 1864)	2.83 ± 0.71	4.17 ± 1.49	0.00	0.08 ± 0.08	**85**	
Urothoe elegans (Bate, 1857)	0.17 ± 0.11	0.00	2.17 ± 0.74	3.42 ± 0.76	**69**	
Apocorophium acutum (Chevreux, 1908)	0.25 ± 0.18	1.42 ± 0.77	2.17 ± 0.77	1.67 ± 0.64	**66**	
Peltocoxa marioni (Catta, 1875)	0.42 ± 0.23	1.25 ± 0.45	2.25 ± 0.87	0.92 ± 0.34	**58**	
Tmetonyx nardonis (Heller, 1866)	0.75 ± 0.28	0.25 ± 0.13	1.92 ± 0.91	0.50 ± 0.26	**41**	
Aoridae ind..	2.92 ± 1.64	0.00	0.00	0.00	**35**	
Gammaropsis crenulata (Krapp-Schickel & Myers, 1979)	0.00	0.08 ± 0.08	2.58 ± 1.08	0.25 ± 0.18	**35**	
Dexamine spiniventris (A. Costa, 1853)	0.33 ± 0.19	0.83 ± 0.39	0.25 ± 0.13	1.00 ± 0.58	**29**	

Species	**South control**	**South acidified**	**North control**	**North acidified**	**No. individuals**	**Change**
Gitana sarsi (Boeck, 1871)	0.17 ± 0.11	0.67 ± 0.47	1.25 ± 0.68	0.17 ± 0.17	**27**	
Dexamine spinosa (Montagu, 1813)	1.50 ± 0.47	0.00	0.67 ± 0.28	0.08 ± 0.08	**27**	↓
Protohyale schmidtii (Heller, 1866)	0.00	1.83 ± 0.73	0.00	0.42 ± 0.26	**27**	↑
Amphipoda ind.	0.33 ± 0.33	0.17 ± 0.17	0.17 ± 0.11	1.58 ± 0.71	**27**	
Ampelisca rubella (A. Costa, 1864)	0.00	0.00	1.67 ± 0.54	0.42 ± 0.19	**25**	
Caprella cf. rapax (Mayer, 1890)	0.00	0.00	0.00	1.75 ± 1.66	**21**	
Phtisica marina (Slabber, 1769)	0.50 ± 0.23	0.42 ± 0.26	0.25 ± 0.18	0.42 ± 0.15	**19**	
Caprella acanthifera cf. var. *discrepans* (Leach, 1814)	0.25 ± 0.13	0.50 ± 0.42	0.67 ± 0.36	0.08 ± 0.08	**18**	
Stenothoe tergestina (Nebeski, 1880)	0.08 ± 0.08	1.00 ± 0.75	0.08 ± 0.08	0.17 ± 0.11	**16**	
Leucothoe richiardii (Lessona, 1865)	0.00	0.83 ± 0.39	0.25 ± 0.13	0.17 ± 0.11	**15**	
Stenothoe monoculoides (Montagu, 1813)	0.33 ± 0.14	0.08 ± 0.08	0.33 ± 0.14	0.42 ± 0.23	**14**	
Ampelisca sp.	0.00	0.00	0.50 ± 0.19	0.50 ± 0.26	**12**	
Ampithoe helleri (G. Karaman, 1975)	0.92 ± 0.34	0.00	0.08 ± 0.08	0.00	**12**	↓
Ericthonius difformis (Milne Edwards, 1830)	0.00	0.83 ± 0.37	0.00	0.08 ± 0.08	**11**	↑
Melitidae ind.	0.00	0.00	0.00	0.75 ± 0.75	**9**	
Pereionotus testudo (Montagu, 1808)	0.00	0.33 ± 0.19	0.08 ± 0.08	0.25 ± 0.18	**8**	
Peltocoxa gibbosa (Schiecke, 1977)	0.00	0.42 ± 0.19	0.17 ± 0.11	0.00	**7**	
Photis longicaudata (Bate & Westwood, 1862)	0.00	0.42 ± 0.26	0.00	0.00	**5**	
Synchelidium longidigitatum (Ruffo, 1947)	0.17 ± 0.11	0.08 ± 0.08	0.00	0.17 ± 0.11	**5**	
Ampelisca diadema (A. Costa, 1853)	0.17 ± 0.11	0.00	0.08 ± 0.08	0.00	**3**	
Cymadusa crassicornis (A. Costa, 1857)	0.17 ± 0.17	0.00	0.00	0.00	**2**	
Atylus vedlomensis (Bate & Westwood, 1862)	0.00	0.00	0.17 ± 0.11	0.00	**2**	
Maera pachytelson (G. Karaman & Ruffo, 1971)	0.00	0.17 ± 0.17	0.00	0.00	**2**	
Deutella schieckei (Cavedini, 1982)	0.00	0.17 ± 0.11	0.00	0.00	**2**	

Species	**South control**	**South acidified**	**North control**	**North acidified**	**No. individuals**	**Change**
Ampelisca unidentata (Schellenberg, 1936)	0.00	0.00	0.08 ± 0.08	0.00	1	
Atylus guttatus (A. Costa, 1851)	0.00	0.00	0.00	0.08 ± 0.08	1	
Jassa cf. marmorata (Holmes, 1903)	0.00	0.00	0.00	0.08 ± 0.08	1	
Lysianella dellavallei (Stebbing, 1906)	0.00	0.08 ± 0.08	0.00	0.00	1	
Ceradocus semiserratus (Bate, 1862)	0.00	0.00	0.08 ± 0.08	0.00	1	
Perioculodes longimanus (Bate & Westwood, 1868)	0.08 ± 0.08	0.00	0.00	0.00	1	
Caprella tavolarensis (Sturaro & Guerra-García, 2011)	0.08 ± 0.08	0.00	0.00	0.00	1	
Caprella sp.	0.08 ± 0.08	0.00	0.00	0.00	1	
Pariambus typicus (Krøyer, 1844)	0.00	0.00	0.00	0.08 ± 0.08	1	

Amphipod richness increased in response to acidification ($F_{1,36}$ = 15.48, $p < 0.001$). Their abundance also increased in response to acidification ($F_{1,36}$ = 44.87, $p < 0.001$), although there was a 'site x month' interaction ($F_{1,36}$ = 8.10, $p = 0.001$) as this increase was only observed in July and November (Tukey HSD March: $p = 0.988$, July: $p < 0.001$, November: $p < 0.001$) (Fig. 4.6b). Diversity (N1 and N2) and evenness (N21') were not affected by acidification (Fig. 4.6c-e). All variables were affected by month (Table 4.2), abundance and richness were lower in March than July and November (abundance: Tukey HSD: $p < 0.001$ and $p < 0.001$, M < J = N; richness: Tukey HSD: $p = 0.001$ and $p = 0.003$, M < J = N). Diversity (N1 and N2) and evenness (N21') were higher in July than March and November (N1: Tukey HSD: $p = 0.008$ and $p < 0.001$, J > M > N; N2: Tukey HSD: $p = 0.008$ and $p < 0.001$, J > M > N) Diversity was lower in November than March and July (Tukey HSD: $p < 0.001$ and $p < 0.001$, N < M = J). Richness and diversity (N1 and N2) were greater at the north than the south side (Table 4.2 and Fig. 4.6b-d).

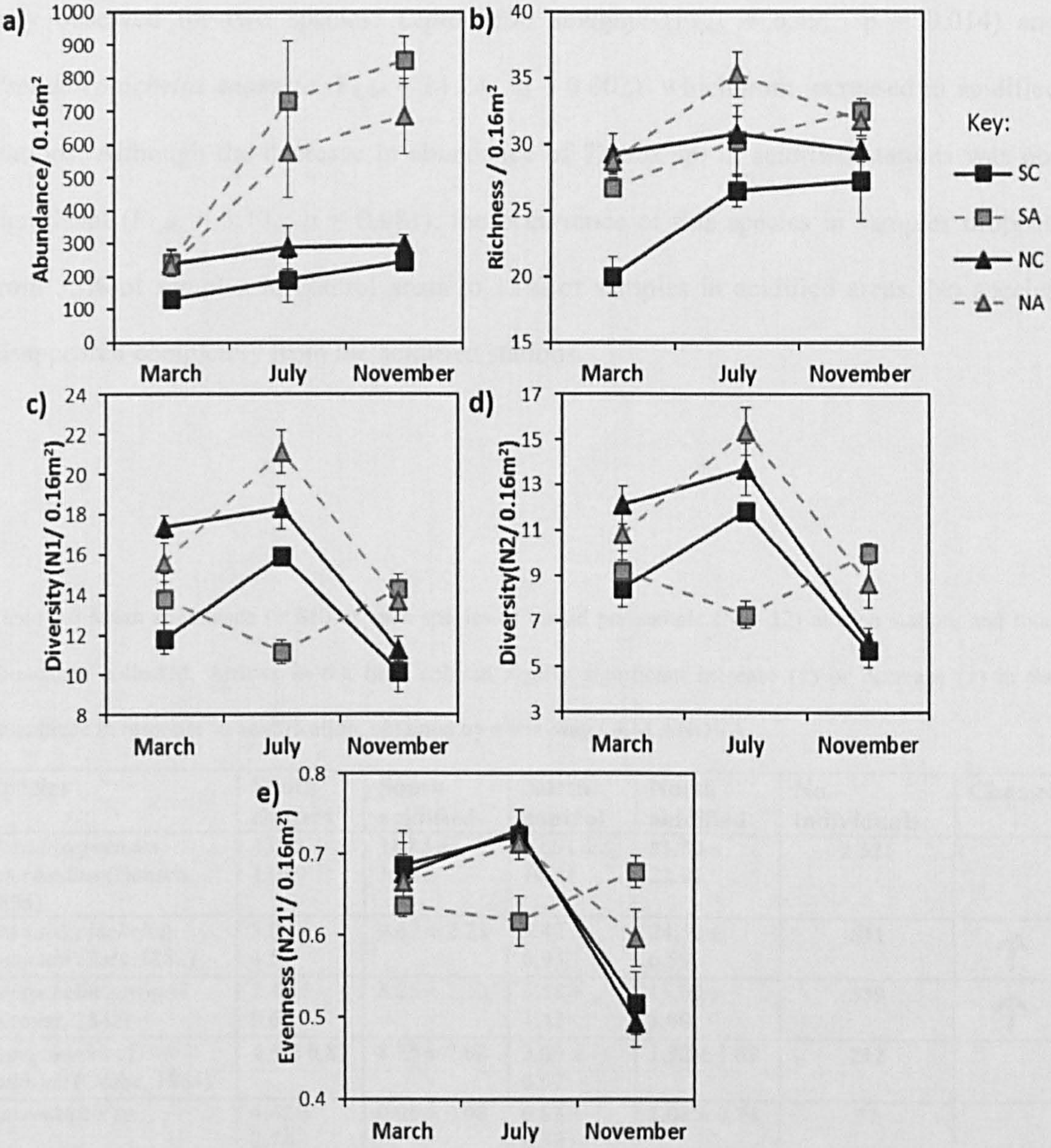


Figure 4.6 Temporal changes in a) abundance, b) richness, c) diversity (N1), d) diversity (N2), and e) evenness (N21') of amphipods at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

4.3.5 Tanaids

A total of 3,748 tanaids which were identified to 7 OTUs from samples collected at the Castello Aragonese (Table 4.6). 1,556 individuals were collected from control stations, whilst 2,194 were collected from acidified stations. Of these, a significant effect of pH was only observed for two species: *Leptochelia savignyi* ($F_{1,46} = 6.49$, $p = 0.014$) and *Pseudoleptochelia anomala* ($F_{1,46} = 11.24$, $p = 0.002$) which both increased in acidified stations. Although the decrease in abundance of *Tanais* sp. in acidified stations was not significant ($F_{1,46} = 3.17$, $p = 0.081$), the occurrence of this species in samples dropped from 35% of samples in control areas to 13% of samples in acidified areas. No species disappeared completely from the acidified stations.

Table 4.6 Mean abundance (± SE) of each species of tanaid per sample (N = 12) at each station, and total abundance collected. Arrows in the final column signify significant increase (↑) or decrease (↓) in the abundance in response to acidification, obtained by a one-way GLM ANOVA.

Species	**South control**	**South acidified**	**North control**	**North acidified**	**No. individuals**	**Change**
Paradoxapseudes intermedius (Hansen, 1895)	43.83 ± 13.19	31.83 ± 16.81	52.91 ± 10.21	81.50 ± 22.01	**2,521**	
Pseudoleptochelia anomala (Sars, 1882)	5.00 ± 1.51	9.67 ± 2.23	3.42 ± 0.93	24.50 ± 6.59	**511**	↑
Leptochelia savignyi (Kroyer, 1842)	2.42 ± 0.61	8.25 ± 2.10	6.58 ± 1.42	11.00 ± 3.00	**339**	↑
Parapseudes cf. *latifrons* (Grube, 1864)	4.5 ± 0.87	8.25 ± 2.68	3.00 ± 0.67	1.92 ± 1.09	**212**	
Apseudopsis sp.	4.42 ± 2.74	0.08 ± 0.08	0.83 ± 0.32	1.08 ± 0.74	**77**	
Tanaidomorpha indet.	0.92 ± 0.42	2.08 ± 1.18	0.82 ± 0.41	2.17 ± 1.00	**72**	
Tanais sp.	0.58 ± 0.29	0.17 ± 0.11	0.42 ± 0.15	0.17 ± 0.17	**16**	

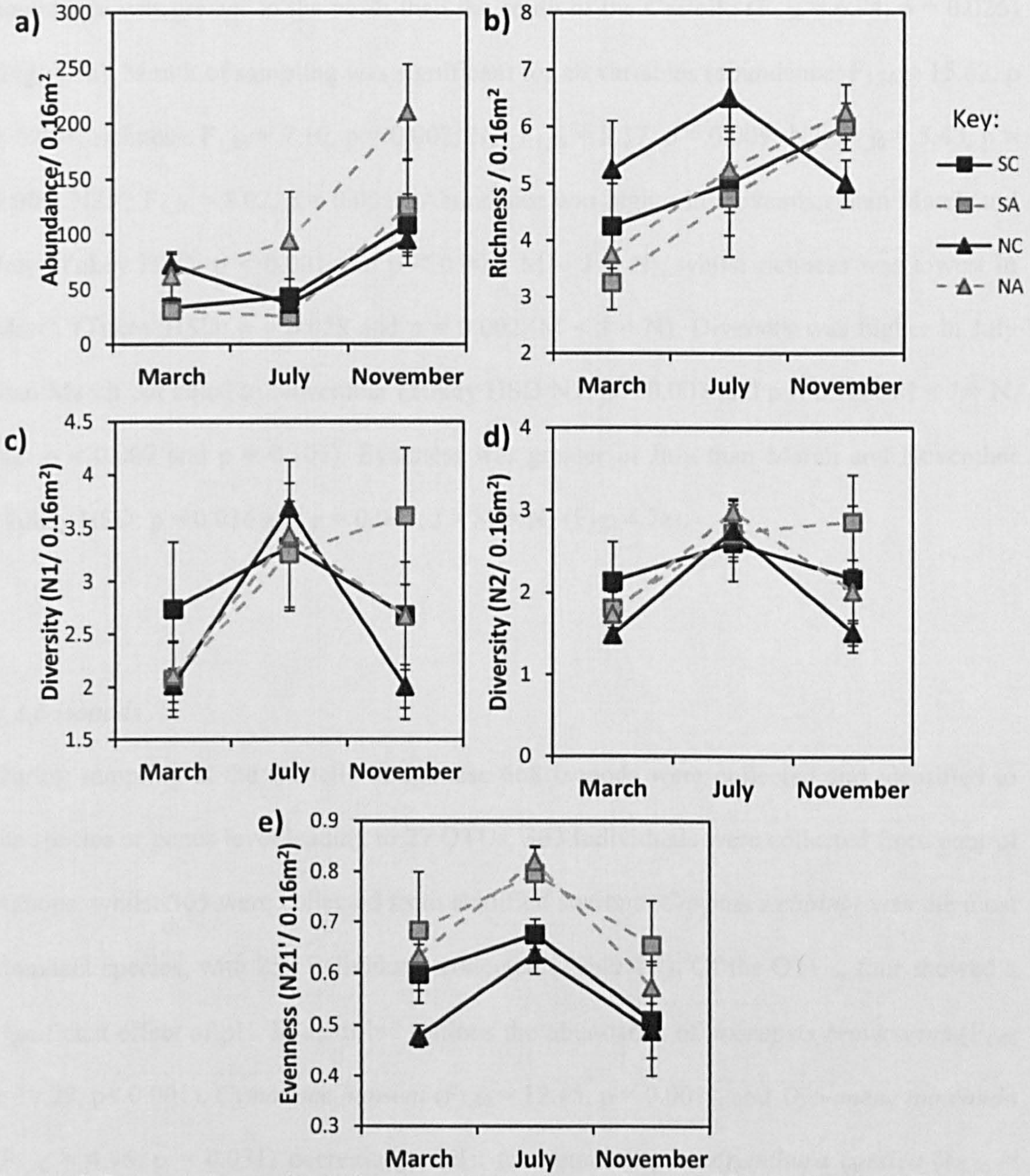


Figure 4.7 Temporal changes in a) abundance, b) richness, c) diversity (N1), d) diversity (N2), and e) evenness (N21') of tanaids at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

The abundance, richness, and diversity (N1 and N2) of tanaids did not differ between acidified and control stations (Table 4.2). The only variable that was affected by pH was evenness (N21'), which was higher in acidified stations ($F_{1,36} = 11.84$, $p = 0.001$). Tanaid abundance was greater in the north than the south of the Castello ($F_{1,36} = 6.94$, $p = 0.026$) (Fig. 4.7a). Month of sampling was significant for all variables (abundance: $F_{1,36} = 15.62$, $p < 0.001$; richness: $F_{1,36} = 7.10$, $p = 0.002$; N1: $F_{1,36} = 5.37$, $p = 0.009$; N2: $F_{1,36} = 5.43$, $p = 0.009$; N21': $F_{1,36} = 8.02$, $p = 0.001$). Abundance was higher in November than March and July (Tukey HSD: $p < 0.001$ and $p < 0.001$, $M = J < N$), whilst richness was lowest in March (Tukey HSD: $p = 0.028$ and $p = 0.002$, $M < J = N$). Diversity was higher in July than March but equal to November (Tukey HSD N1: $p = 0.007$ and $p = 0.160$, $M < J = N$, N2: $p = 0.007$ and $p = 0.101$). Evenness was greater in July than March and November (Tukey HSD: $p = 0.016$ and $p = 0.001$, $J > M = N$) (Fig. 4.7e).

4.3.6 Isopods

During sampling at the Castello Aragonese 668 isopods were collected and identified to the species or genus level leading to 27 OTUs. 363 individuals were collected from control stations, whilst 305 were collected from acidified stations. *Carpias stebbingi* was the most abundant species, with 258 individuals collected (Table 4.7). Of the OTUs, four showed a significant effect of pH. In acidified stations the abundance of *Joeropsis brevicornis* ($F_{1,46} = 19.27$, $p < 0.001$), *Cymodoce hanseni* ($F_{1,46} = 12.15$, $p = 0.001$), and *Dynamene tubicauda* ($F_{1,46} = 4.96$, $p = 0.031$) decreased, whilst the abundance of *Apanthura corsica* ($F_{1,46} = 5.78$, $p = 0.020$) increased. *Carpias stebbingi* increased in acidified areas, although this increase was not significant ($F_{1,46} = 3.69$, $p = 0.06$). Not only did *Joeropsis brevicornis* show a significant decrease in overall abundance but it dropped from being present in 75% of samples in control stations to only 4% of samples in acidified stations. Similarly the presence of *Cymodoce hanseni* in samples dropped from 71% in control stations to 17% in acidified stations.

Table 4.7 Mean abundance (± SE) of each species of isopod per sample (N = 12) at each station, and total abundance collected. Arrows in the final column signify significant increase (↑) or decrease (↓) in the abundance in response to acidification, obtained by a one-way GLM ANOVA.

Species	**South control**	**South acidified**	**North control**	**North acidified**	**No. individuals**	**Change**
Carpias stebbingi (Monod, 1933)	2.08 ± 0.91	6.50 ± 2.91	5.00 ± 1.82	7.92 ± 2.91	**258**	
Joeropsis brevicornis (Koehler, 1885)	0.50 ± 0.15	0.08 ± 0.08	10.17 ± 1.34	0.00	**129**	↓
Gnathia spp.	1.33 ± 0.33	1.08 ± 0.36	1.33 ± 0.31	2.08 ± 0.61	**70**	
Cymodoce hanseni (Dumay, 1972)	2.50 ± 0.91	0.00	2.08 ± 0.58	0.67 ± 0.35	**63**	↓
Jaera nordmanni massiliensis (Lemercier, 1960)	0.00	0.00	0.25 ± 0.18	1.67 ± 1.00	**23**	
Apanthura corsica (Amar, 1953)	0.00	0.75 ± 0.27	0.17 ± 0.11	0.27 ± 0.18	**14**	↑
Paranthura nigropunctata (Lucas, 1846)	0.08 ± 0.08	0.00	0.75 ±0.35	0.33 ± 0.18	**14**	
Dynamene bifida (Torelli, 1930)	0.00	0.67 ± 0.43	0.42 ± 0.22	0.00	**13**	
Uromunna sp.	0.00	0.67 ± 0.35	0.33 ± 0.26	0.00	**12**	
Dynamene tubicauda (Holdich, 1968)	0.25 ± 0.13	0.08 ± 0.08	0.50 ± 0.19	0.00	**11**	↓
Paranthuridae. sp.	0.17 ± 0.11	0.00	0.08 ± 0.08	0.67 ± 0.28	**11**	
Limnoria mazzellae (Cookson & Lorenti, 2001)	0.08 ± 0.08	0.08 ± 0.08	0.58 ± 0.50	0.00	**9**	
Stenosoma appendiculatum (Risso, 1826)	0.08 ± 0.08	0.08 ± 0.08	0.08 ± 0.08	0.33 ± 0.14	**7**	
Cymodoce truncata (Leach, 1814)	0.00	0.00	0.33 ± 0.14	0.08 ± 0.08	**5**	
Mesanthura sp.	0.08 ± 0.08	0.00	0.16 ± 0.11	0.08 ± 0.08	**4**	
Cleantis prismatica (Risso, 1826)	0.00	0.08 ± 0.08	0.08 ± 0.08	0.08 ± 0.08	**3**	
Kupellonura serritelson (Wagele, 1981)	0.08 ± 0.08	0.00	0.08 ± 0.08	0.08 ± 0.08	**3**	
Stenosoma capito (Rathke, 1837)	0.00	1.17 ± 1.17	0.00	0.08 ± 0.08	**3**	
Dynamene torelliae (Holdich, 1968)	0.00	0.17 ± 0.11	0.00	0.08 ± 0.08	**3**	
Eurydice inermis (Hansen, 1890)	0.00	0.00	0.08 ± 0.08	0.08 ± 0.08	**2**	
Gnathia inopinata (Monod, 1925)	0.00	0.08 ± 0.08	0.00	0.08 ± 0.08	**2**	
Limnoria sp.	0.17 ± 0.17	0.00	0.00	0.00	**2**	

Species	South control	South acidified	North control	North acidified	No. individuals	Change
Astacilla sp.	0.00	0.08 ± 0.08	0.00	0.08 ± 0.08	2	
Synischia hectica (Pallas, 1772)	0.17 ± 0.11	0.00	0.00	0.00	2	
Gnathia vorax (Lucas, 1849)	0.00	0.00	0.08 ± 0.08	0.00	1	
Astacilla mediterranea (Koehler, 1911)	0.00	0.00	0.00	0.08 ± 0.08	1	

The abundance and evenness (N21') of isopods did not differ between pH zones (Table 4.2; Fig 4.8). Richness and diversity (N1 and N2) of isopods was significantly lower in acidified stations (richness: $F_{1,36} = 7.57$, $p = 0.009$; N1: $F_{1,36} = 10.47$, $p = 0.003$; N2: $F_{1,36} = 9.22$, $p = 0.004$). Month of sampling was significant for abundance, richness, and evenness (N21') (abundance: $F_{1,36}= 16.70$, $p < 0.001$; richness: $F_{1,36}= 8.76$, $p < 0.001$; N21': $F_{1,36}= 10.01$, $p < 0.001$). Abundance and richness were lower in March than July and November (abundance: Tukey HSD: $p < 0.001$ and $p < 0.001$, M < J = N; richness: Tukey HSD: $p = 0.004$ and $p = 0.001$, M < J = N), whilst evenness was lowest in November (Tukey HSD: $p = 0.017$ and $p < 0.001$, N < J = M). Diversity (N1and N2) did not vary between months or stations. Abundance was greater in the north than the south side ($F_{1,36} = 26.67$, $p < 0.001$).

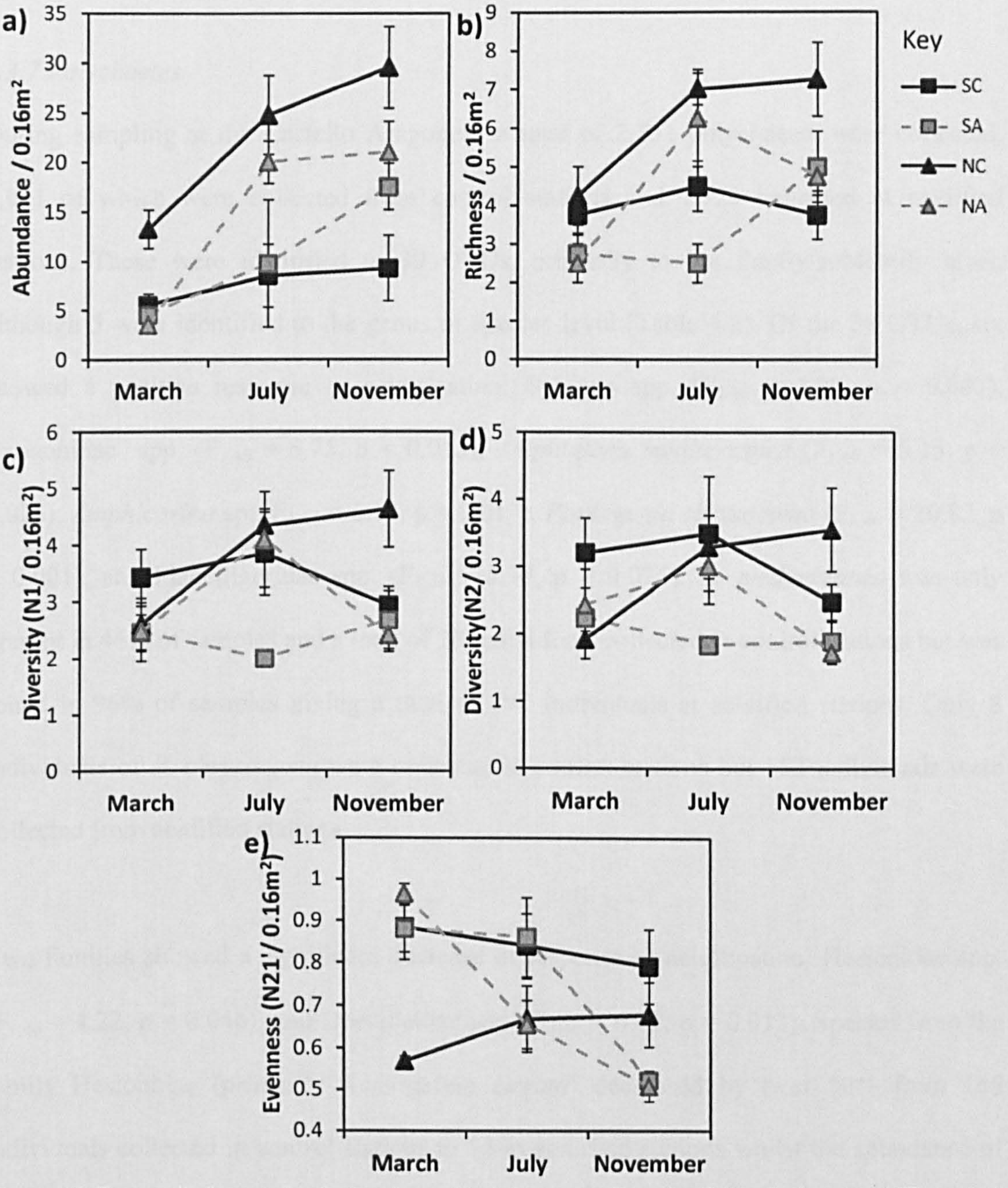


Figure 4.8 Temporal changes in a) abundance, b) richness, c) diversity (N1), d) diversity (N2), and e) evenness (N21') of isopods at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

4.3.7 Polychaetes

During sampling at the Castello Aragonese a total of 2,731 polychaetes were collected, 1,011 of which were collected from control stations and 1,720 collected at acidified stations. These were identified to 39 OTUs, primarily to the family/subfamily level, although 5 were identified to the genus or species level (Table 4.8). Of the 39 OTUs, six showed a positive response to acidification: Syllinae spp. ($F_{1,46} = 7.90$, $p = 0.007$), Exogoninae spp. ($F_{1,46} = 6.75$, $p = 0.013$), *Amphiglena mediterranea* ($F_{1,46} = 5.15$, $p = 0.028$), *Amphicorina* sp. ($F_{1,46} = 6.12$, $p = 0.017$), *Pontogenia chrysocoma* ($F_{1,46} = 20.82$, $p < 0.001$), and Flabelligeridae spp. ($F_{1,46} = 5.38$, $p = 0.025$). A. *mediterranea* was only present in 46% of samples and a total of 28 individuals collected in control stations but was found in 96% of samples giving a total of 291 individuals at acidified stations. Only 8 individuals of *P. chrysocoma* were collected in control stations but 152 individuals were collected from acidified stations.

Two families showed a significant decrease in response to acidification: Hesionidae spp. ($F_{1,46} = 4.22$, $p = 0.046$), and Dorvilleidae spp. ($F_{1,46} = 6.62$, $p = 0.013$). Species from the family Hesionidae (primarily *Kefersteinia cirrata*) decreased by over 50% from 165 individuals collected in control stations to 74 in acidified stations whilst the abundance of species from the family Dorvilleidae decreased from 44 in control stations to 7 in acidified stations. There was a decrease in the abundance of species from the calcifying tubiculous Serpullidae (from 74 to 7) and Spirorbidae (from 66 to 11) in acidified stations, although their abundance was patchy and therefore these decreases were not significant.

Table 4.8 Mean abundance (± SE) of each polychaete OTU per sample (N = 12) at each station, and total abundance collected. Arrows in the final column signify significant increase (↑) or decrease (↓) in the abundance in response to acidification, obtained by a one-way GLM ANOVA.

Species	**South control**	**South acidified**	**North control**	**North acidified**	**No. individuals**	**Change**
Syllinae spp.	7.67 ± 1.55	14.5 ± 2.15	8.17 ± 1.07	13.17 ± 3.20	**522**	↑
Exogoninae spp.	5.58. ±.1.28	9.25. ±.2.58	7.0 ± 1.86	15.5 ± 3.05	**448**	↑
Amphiglena mediterranea (Leydig, 1851)	0.5 ± 0.23	9.41 ± 2.38	1.83 ± 0.74	14.83 ± 9.48	**319**	↑
Hesionidae spp.	7.92 ± 3.17	4.0 ± 1.17	5.83 ± 1.44	2.17 ± 0.63	**239**	↓
Amphicorina sp.	2.0 ± 0.73	6.5 ± 1.83	0.58 ± 0.29	6.92 ± 4.0	**192**	↑
Pontogenia chrysocoma (Baird, 1865)	0.42 ± 0.19	9.58 ± 2.01	0.25 ± 0.13	3.08 ± 1.10	**160**	↑
Fabriciidae spp.	1.17 ± 0.39	2.5 ± 0.73	1.0 ± 0.32	7.0 ± 4.04	**140**	
Paraonidae spp.	3.42 ± 1.14	2.83 ± 1.50	0.33 ± 0.22	2.08 ± 0.65	**104**	
Serpulidae spp.	4.67 ± 2.99	0.08 ± 0.08	1.5 ± 0.66	0.5 ± 0.23	**81**	
Spirorbidae spp.	4.92 ± 3.66	0.83 ± 0.30	0.58 ± 0.26	0.08 ± 0.08	**77**	
Spionidae spp.	1.67 ± 0.56	1.92 ± 0.80	0.33 ± 0.26	1.75 ± 0.49	**68**	
Dorvilleidae spp.	2.83 ± 1.01	0.25 ± 0.13	0.83 ± 0.49	0.33 ± 0.22	**51**	↓
Ctenodriliidae spp.	0.42 ± 0.19	0.42 ± 0.19	0.92 ± 0.83	1.92 ± 0.91	**44**	
Cirratulidae spp.	0.42 ± 0.58	0.5 ± 0.19	0.42 ± 0.15	1.17 ± 0.60	**42**	
Nereididae spp.	1.33 ± 0.81	0.83 ± 0.39	0.58 ± 0.31	0.75 ± 0.28	**42**	
Flabelligeridae spp.	0.25 ± 0.25	1.92 ± 0.61	0.00	0.08 ± 0.08	**27**	↑
Polyophthalmus pictus (Dujardin, 1839)	1.17 ± 0.52	0.17 ± 0.11	0.25 ± 0.18	0.33 ± 0.22	**23**	
Chrysopetalidae spp.	0.42 ± 0.26	0.00	0.75 ± 0.22	0.5 ± 0.23	**20**	
Polynoidae spp.	0.83 ± 0.34	0.17 ± 0.11	0.17 ± 0.11	0.5 ± 0.19	**20**	
Pholoididae spp.	0.33 ± 0.14	0.58 ± 0.15	0.25 ± 0.13	0.17 ± 0.17	**16**	
Capitellidae spp.	0.42 ± 0.19	0.17 ± 0.11	0.00	0.5 ± 0.11	**13**	
Sabellariidae spp	0.08 ± 0.08	0.5 ± 0.42	0.00	0.42 ± 0.26	**12**	
Phyllodocidae spp.	0.33 ± 0.19	0.33 ± 0.14	0.08 ± 0.08	0.08 ± 0.08	**10**	

Species	**South control**	**South acidified**	**North control**	**North acidified**	**No. individuals**	**Change**
Sabellidae spp.	0.5 ± 0.29	0.00	0.08 ± 0.08	0.08 ± 0.08	8	
Branchiomma spp.	0.17 ± 0.11	0.17 ± 0.11	0.17 ± 0.11	0.08 ± 0.08	7	
Lumbrineridae spp	0.08 ± 0.08	0.00	0.00	0.42 ± 0.19	6	
Pilargidae spp.	0.08 ± 0.08	0.33 ± 0.33	0.00	0.00	5	
Sigalionidae spp.	0.33 ± 0.19	0.00	0.00	0.00	4	
Trichobranchiidae spp.	0.25 ± 0.13	0.08 ± 0.08	0.00	0.00	4	
Sphaerodoridae spp.	0.00	0.00	0.08 ± 0.08	0.25 ± 0.25	4	
Maldanidae spp.	0.17 ± 0.17	0.00	0.00	0.17 ± 0.17	4	
Euphrosinidae spp.	0.00	0.17 ± 0.11	0.08 ± 0.08	0.00	3	
Orbiniidae spp.	0.00	0.17 ± 0.17	0.08 ± 0.08	0.00	3	
Autolytinae spp.	0.08 ± 0.08	0.00	0.17 ± 0.11	0.00	3	
Scalibregmatidae spp.	0.00	0.00	0.25 ± 0.25	0.00	3	
Eunicidae spp.	0.00	0.00	0.08 ± 0.08	0.08 ± 0.08	2	
Opheliidae spp.	0.00	0.00	0.08 ± 0.08	0.08 ± 0.08	2	
Nephtyidae spp.	0.08 ± 0.08	0.00	0.00	0.08 ± 0.08	2	
Terebellidae spp.	0.00	0.00	0.00	0.08 ± 0.08	1	

The abundance of polychaetes was significantly affected by pH ($F_{1,36} = 4.98$, $p = 0.032$), as an increase in abundance was observed in acidified stations (Fig. 4.9a). Richness, diversity and evenness were not affected by pH (Table 4.2). Unlike other groups of taxa, time of year did not affect the abundance, richness or diversity of polychaetes (Table 4.2), although a significant 'site x month' interaction was observed for richness and diversity (N1 and N2) (richness: $F_{1,36} = 3.89$, $p = 0.030$; N1: $F_{1,36} = 4.62$, $p = 0.016$; N2: $F_{1,36} = 3.86$, $p = 0.030$). This interaction was observed as these variables were greater in the south than the north side in November (Tukey HSD: richness $p = 0.044$; N1 $p = 0.034$; N2 $p = 0.046$), whilst this pattern was not observed in other months (Fig. 4.9).

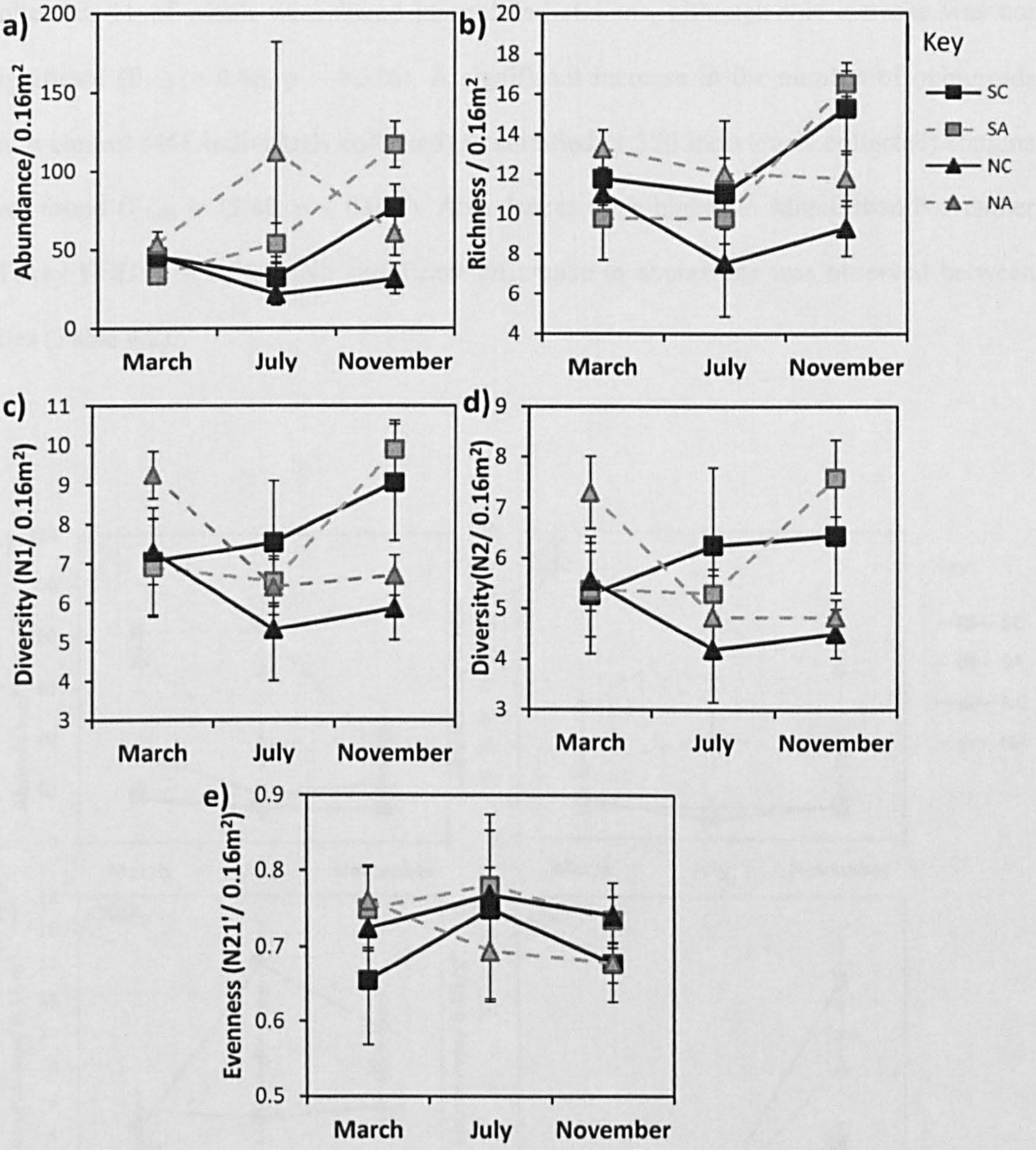


Figure 4.9 Temporal changes in a) abundance, b) richness, c) diversity (N1), d) diversity (N2), and e) evenness (N21') of polychaetes at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

6.3.8 Other groups

During sampling at the Castello a total of 1,869 echinoderms were collected, 1,841 of which were ophiuroids (primarily *Amphipholis squamata*). Only 10 echinoids and 2 holothurians were collected. Although these species are present, they are generally quite large (> 5 cm) so less likely to get collected by the suction sampler. Sixteen starfish were collected, 11 of which were found in acidified stations, although this increase was not significant ($F_{1,46}$ = 0.86, p = 0.376). A significant increase in the number of ophiuroids from control (465 individuals collected) to acidified (1,376 individuals collected) stations was found ($F_{1,46}$ = 35.48, p < 0.001). Abundances were higher in March than November (Tukey HSD: p = 0.001). No significant difference in abundance was observed between sites (Table 4.2).

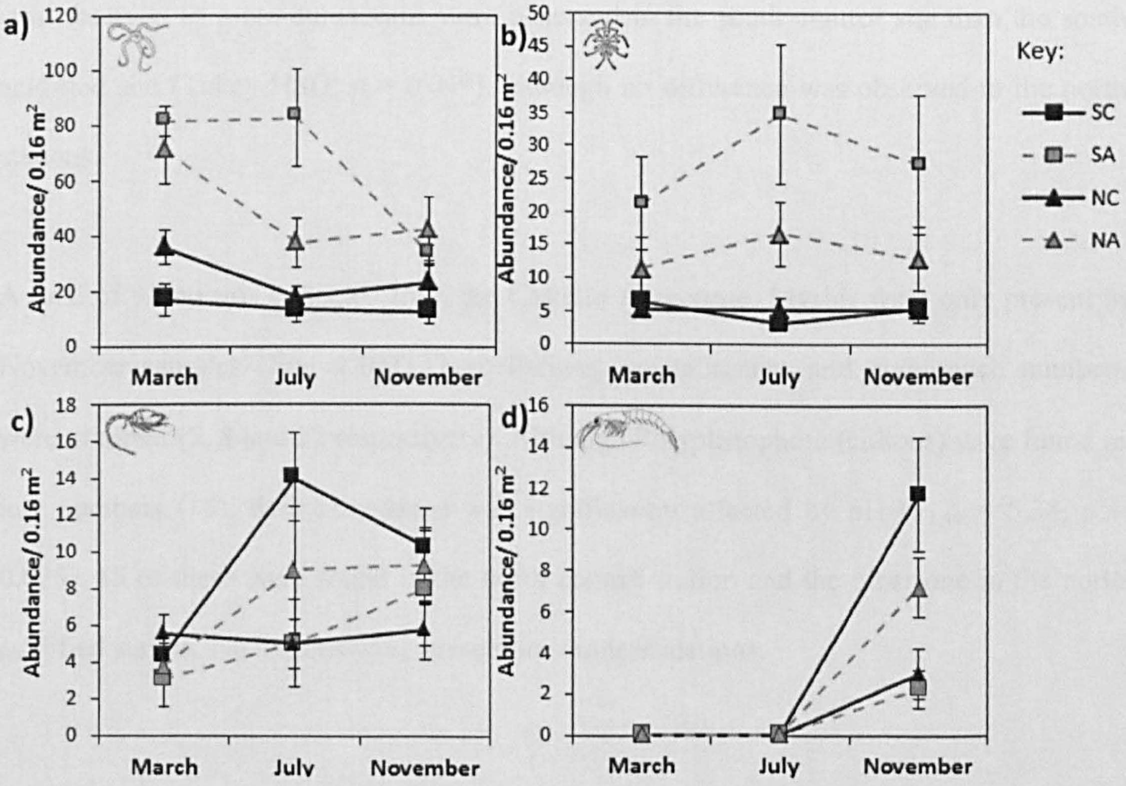


Figure 4.10 Temporal changes in the abundance of: a) echinoderms, b) pycnogonids, c) cumaceans, and d) mysids at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

A total of 622 pycnogonids (sea spiders) were collected from the Castello Aragonese. Their abundance significantly increase from control to acidified stations ($F_{1,46} = 29.83$, $p < 0.001$) as 80% were collected from acidified stations. There was a 'site x pH' interaction ($F_{1,36} = 7.55$, $p = 0.009$), as the increase in abundance observed at acidified stations was more pronounced on the south side (Fig. 4.10b).

A total of 331 cumaceans were collected from the Castello Aragonese. Cumaceans were not significantly affected by pH (Table 4.2), but differed between months ($F_{1,36} = 5.40$, $p = 0.009$). The lowest numbers of cumaceans were found in March in comparison to July and November (Tukey HSD: $p = 0.021$ and $p = 0.018$, $M < J = N$). A 'site x pH' interaction was observed as more cumaceans were observed in the south control site than the south acidified site (Tukey HSD: $p = 0.049$), although no difference was observed in the north stations.

A total of 91 mysids collected from the Castello Aragonese. Mysids were only present in November samples (Fig. 4.10d). Turbellarians, opistobranchs and nudibranch numbers were minimal (5, 8 and 22 respectively). Although Polyplacophora (chitons) were found in low numbers (16), their abundance was significantly affected by pH ($F_{1,46} = 5.38$, $p = 0.025$). 15 of these were found in the north control station and the other one in the north acidified station. No chitons were present in southern stations.

4.3.9 Community analysis

A total of 13,116 invertebrates were collected at control stations and 24,997 at acidified stations whilst sampling at the Castello Aragonese. The abundance of invertebrates was positively affected by pH ($F_{1,36} = 36.67$, $p < 0.001$), although there was a 'pH x month' interaction ($F_{1,36} = 4.93$, $p = 0.013$), as this increase was only observed in July and November (Fig. 4.11a). Diversity (N1 and N2) decreased in acidified stations (N1: $F_{1,36} = 12.04$, $p = 0.001$; N2: $F_{1,36} = 4.16$, $p = 0.05$) (Fig. 4.11c-d). Acidification did not affect richness or evenness (Table 4.9). Abundance and richness increased from March through to November (abundance: Tukey HSD $p = 0.024$ and $p < 0.001$, M < J < N; richness: Tukey HSD $p = 0.024$ and $p < 0.001$, M < J < N), whilst diversity (N2) and evenness (N21') were lower in November than July (N1: Tukey HSD $p = 0.044$, N < J = M; N21': Tukey HSD $p = 0.011$, N < J = M). Diversity (N1) was not affected by month (Table 4.9). Diversity was higher on the north side (N1: $F_{1,36} = 9.60$, $p = 0.004$; N2: $F_{1,36} = 7.08$, $p = 0.012$), although there was a 'site x month' interaction (N1: $F_{1,36} = 8.62$, $p < 0.001$; N2: $F_{1,36} = 5.40$, $p = 0.009$), as this effect was only observed in July (Fig. 4.11c-d). No difference in richness or evenness was observed between pH zones (Fig. 4.11).

Table 4.9 Comparison of abundance, richness, diversity and evenness of the invertebrate community, analysed by means of a three-way GLM ANOVA with site (fixed factor), pH (fixed factor) and month (fixed factor). Significant results are highlighted in red. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	Factors			Interactions			
	Site	pH	Month	Site x pH	Site x Month	pH x Month	Site x pH x Month
Abundance	0.05	36.67***	17.53***	1.99	1.01	4.93*	0.20
Richness	0.50	0.17	20.58***	0.01	3.54*	0.30	1.66
Diversity (N1)	9.60**	12.04**	2.41	0.69	8.62***	1.16	3.01
Diversity (N2)	7.08*	4.16*	3.47*	0.96	5.40**	2.35	1.93
Evenness (N21')	2.44	0.01	5.06*	0.88	1.90	4.24*	0.39

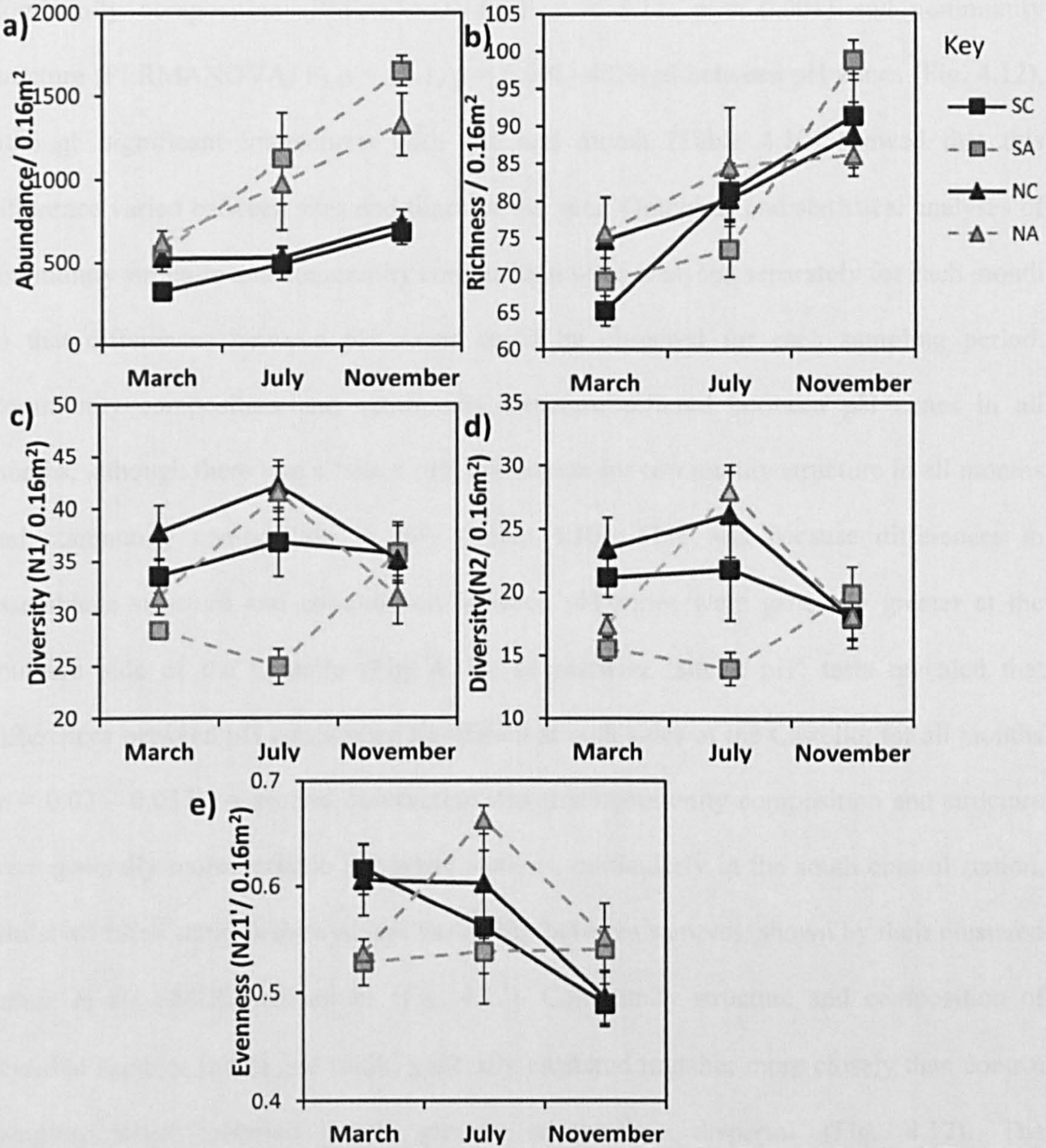


Figure 4.11 Temporal changes in a) abundance, b) richness, c) diversity (N1), d) diversity (N2), and e) evenness (N21') of the whole invertebrate community at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

Community composition (PERMANOVA: $F_{1,47} = 8.11$, $p = 0.001$) and community structure (PERMANOVA: $F_{1,47} = 14.1$, $p = 0.001$) differed between pH zones (Fig. 4.12), although significant interactions with site and month (Table 4.10) showed that this difference varied between sites and times of the year. Graphical and statistical analyses of community structure and community composition were analysed separately for each month so that differences between pH zones could be observed for each sampling period. Community composition and community structure differed between pH zones in all months, although there was a 'site x pH' interaction for community structure in all months and community composition in July (Table 4.10). This was because differences in assemblage structure and composition between pH zones were generally greater at the southern side of the Castello (Fig. 4.12), as pairwise 'site x pH' tests revealed that differences between pH zones were significant at both sides of the Castello, for all months ($p = 0.02 - 0.037$). A second observation was that community composition and structure were generally more variable in control stations, particularly in the south control station, whilst acidified stations showed less variability between samples, shown by their clustered nature in the nMDS ordinations (Fig. 4.12). Community structure and composition of acidified samples (north and south) generally clustered together more closely than control samples, which showed much greater multivariate dispersal (Fig. 4.12). The PERMANOVA high pseudo-F value and nMDS ordination show that although community structure and composition differed in all months, the greatest difference between control and acidified stations occurred in November.

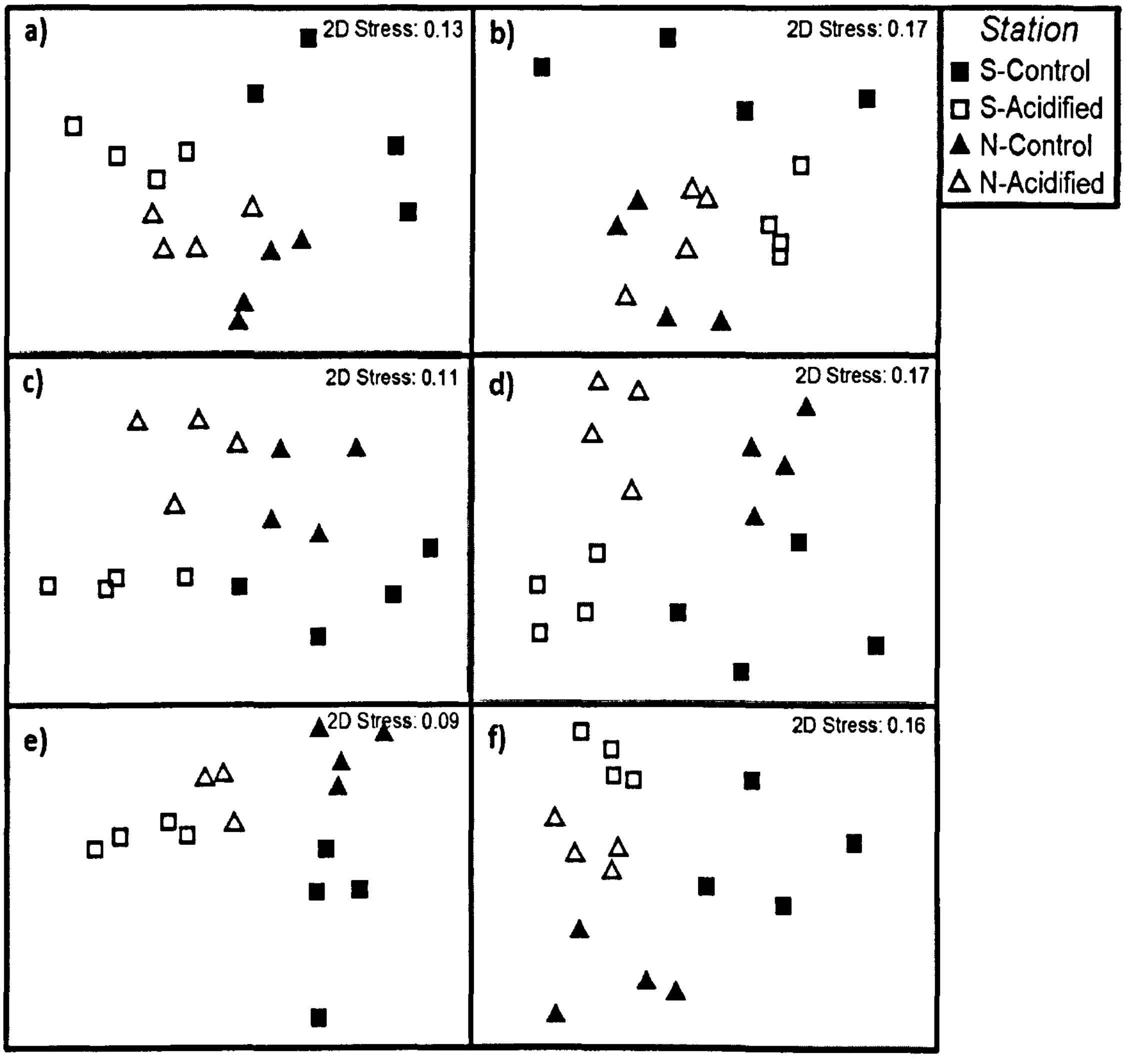


Figure 4.12 nMDS ordinations of community structure in control and acidified *P. oceanica* stations at the north and south of the Castello Aragonese in a) March, c) July and e) November and community composition at the control and acidified stations at the north and south of the Castello Aragonese in b) March, d) July, and f) November.

Table 4.10 Results of community analysis for the invertebrate community, collected by airlift sampler, pooled for all months and for each separate month. Community structure and community composition were analysed using a three-factor PERMANOVA with site (fixed factor), pH (fixed factor) and month (fixed factor) for pooled data, and each month was analysed using a two-factor PERMANOVA with site (fixed factor), and pH (fixed factor). Significant results are highlighted in red.

		Community composition		**Community structure**	
	df	**Pseudo-F**	**p_{perm}**	**Pseudo-F**	**p_{perm}**
ALL MONTHS					
Site	1	6.77	**0.001**	7.54	**0.001**
pH	1	8.11	**0.001**	14.1	**0.001**
Month	2	5.59	**0.001**	8.56	**0.001**
Site x pH	1	3.32	**0.001**	4.11	**0.001**
Site x Month	2	1.50	**0.015**	1.67	**0.005**
pH x Month	2	1.37	**0.046**	1.88	**0.001**
Site x pH x Month	2	1.37	**0.048**	1.23	0.122
Total	47				
MARCH					
Site	1	3.26	**0.001**	3.36	**0.001**
pH	1	6.78	**0.001**	4.37	**0.001**
Site x pH	1	1.62	0.072	1.84	**0.025**
Total	15				
JULY					
Site	1	3.06	**0.001**	3.49	**0.002**
pH	1	4.12	**0.002**	5.86	**0.002**
Site x pH	1	2.37	**0.003**	2.38	**0.001**
Total	15				
NOVEMBER					
Site	**1**	3.51	**0.001**	4.16	**0.001**
pH	**1**	4.25	**0.001**	8.04	**0.001**
Site x pH	1	1.44	0.113	2.34	**0.001**
Total	15				

SIMPER analysis showed that the most representative OTUs characterizing the control stations were the tanaid *Paradoxapseudes intermedius*, the amphipod *Apherusa chiereghinii* and the hermit crab *Cestopagurus timidus*, whilst the most representative OTUs characterizing the acidified stations were Ophiuroidea (primarily *Amphipholis squamata*), the amphipod *Quadrimaera inaequipes*, and the gastropod *Alvania lineata* (Table 4.11).

Table 4.11 SIMPER analysis of the abundance of each OTU in each sample (N=48) to determine which OTUs, contribute > 3% similarity for each pH zone (control and acidified). Data has been pooled across sites and months. % SC = % similarity contribution

Control		**Acidified**	
OTU	**%SC**	**OTU**	**%SC**
Paradoxapseudes intermedius	5.12	Ophiuroidea spp.	5.25
Apherusa chiereghinii	4.66	*Quadrimaera inaequipes*	4.78
Cestopagurus timidus	4.37	*Alvania lineata*	4.42
Ophiuroidea spp.	3.82	*Apolochus picadurus*	3.59
Alvania lineata	3.69	*Metaphoxus simplex*	3.30
Apolochus picadurus	3.33	*Lembos websteri*	3.22

To summarise, although there was an increase in the abundance of invertebrates, with almost double the number of individuals being collected from acidified stations, not all groups of taxa increased in response to acidification (Fig. 4.13). The abundance of gastropods, bivalves, polychaetes, and amphipods increased, whilst no difference was found for isopods and tanaids and the abundance of decapods decreased. There was no overall difference in species richness between control and acidified stations, although a significant a decrease in the richness of decapods, bivalves and isopods was found whilst the richness of amphipods increased (Fig 4.13). In comparison to species richness, diversity decreased in response to acidification for both diversity measures (N1 and N2), due to a decrease in the diversity of gastropods and isopods (Fig. 4.14). Overall evenness was not affected by acidification, although a significant increase in the evenness of decapods and tanaids was found, whilst the evenness of gastropods decreased (Fig. 4.14). 'Site x pH' interactions were observed for the abundance of gastropods and bivalves, as an increase in response to acidification was only observed on the southern side, whilst significant 'site x pH' interactions were found for gastropod diversity and evenness, as the decrease in response to acidification was only observed for the northern side of the Castello.

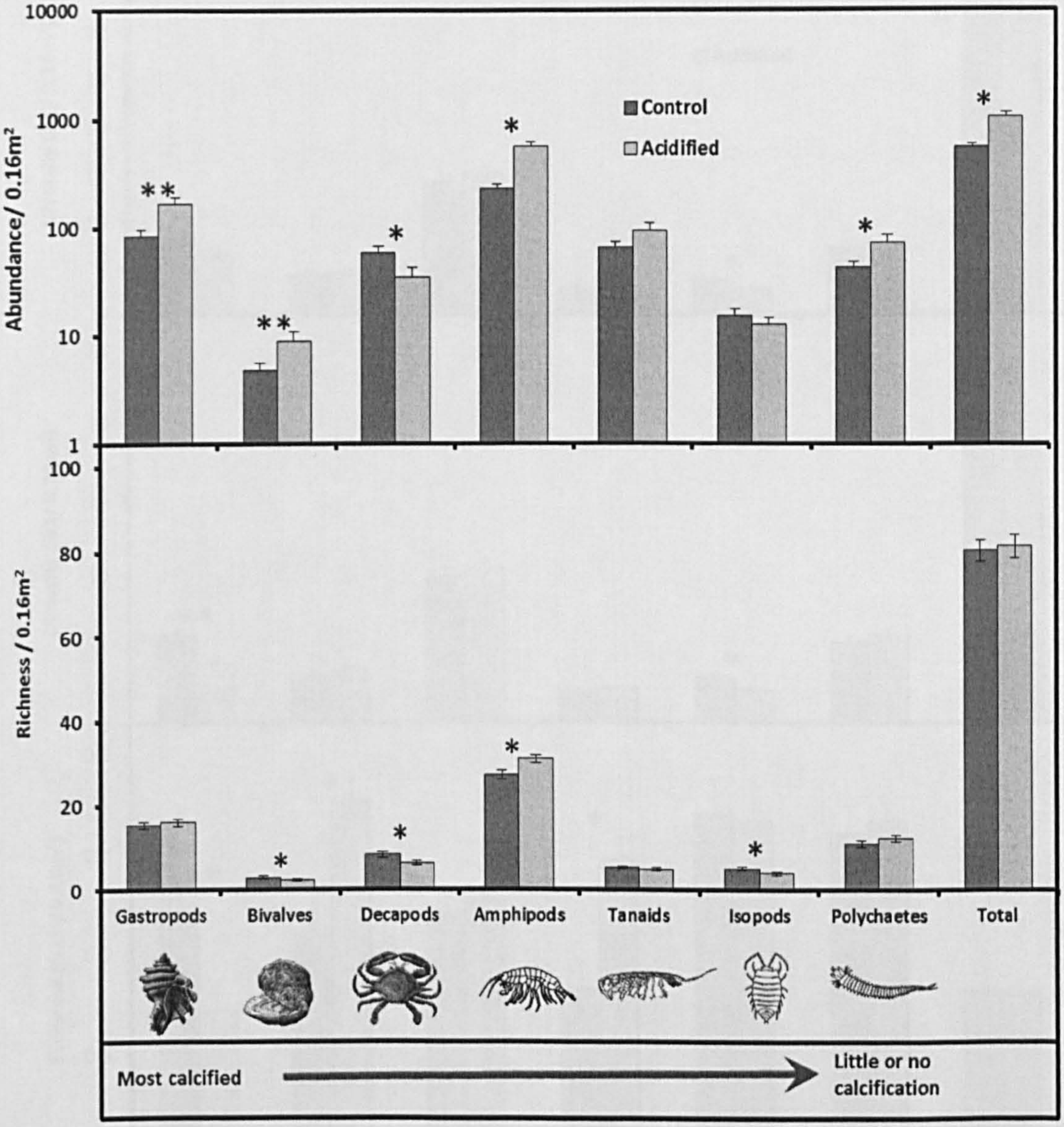


Figure 4.13 Mean abundance, and species richness (± SE), per sample ($0.16m^2$, N = 24), of each of the main taxonomic groups. Data is pooled for control and acidified stations at the north and south of the Castello Aragonese. * = significant difference between pH zones ($p < 0.05$), ** significant 'site x pH' interaction ($p < 0.05$).

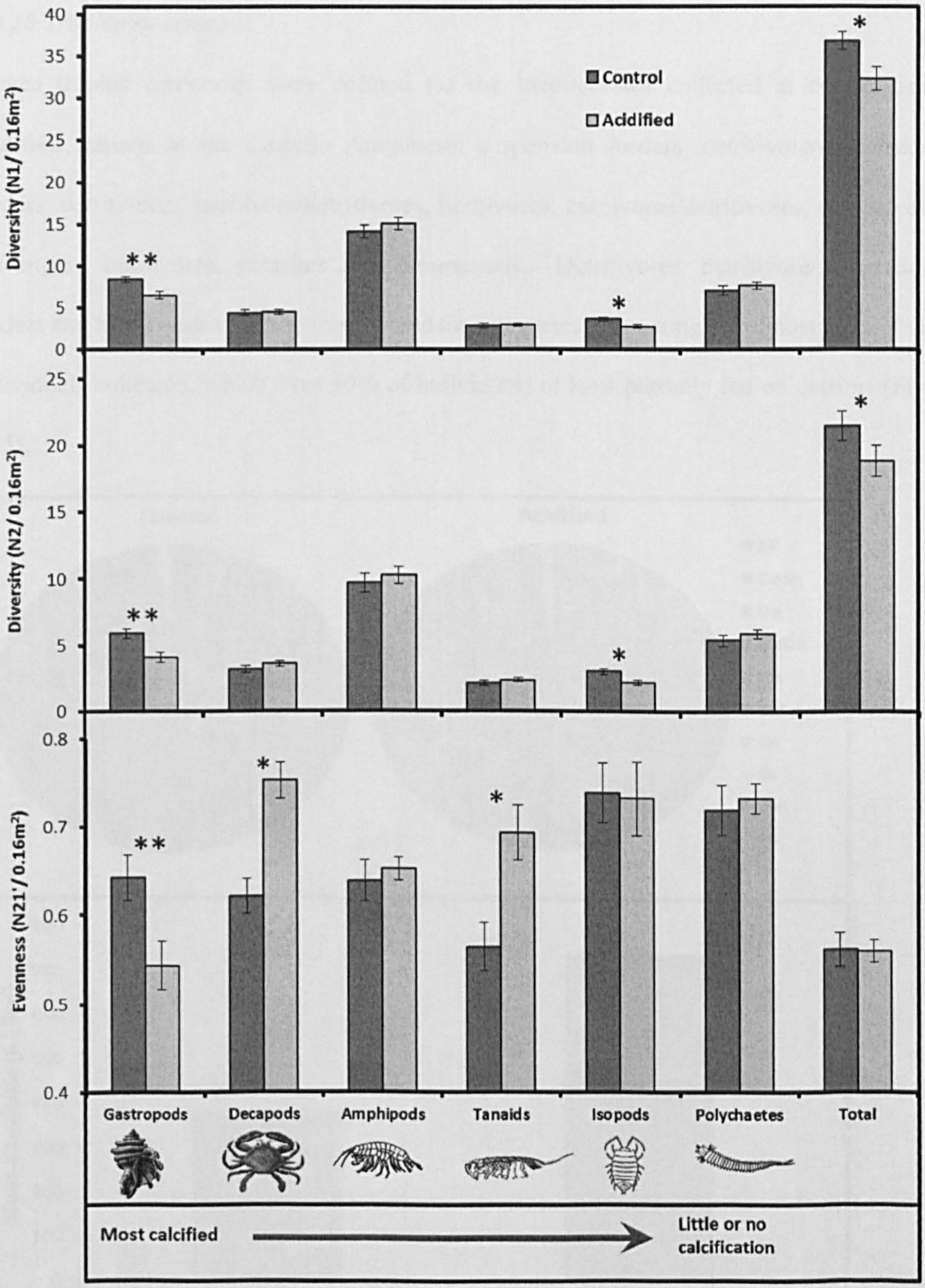


Figure 4.14 Mean diversity (N1 and N2), and evenness (N21') (± SE), per sample ($0.16m^2$, $N = 24$), of each of the main taxonomic groups. Data is pooled for control and acidified stations at the north and south of the Castello Aragonese. * = significant difference between pH zones ($p < 0.05$), ** significant 'site x pH' interaction ($p < 0.05$).

4.3.10 Trophic structure

Eleven trophic categories were defined for the invertebrates collected at control and acidified stations at the Castello Aragonese: suspension feeders, detritivore/suspension feeders, detritivores, herbivore/detritivores, herbivores, carnivores/detritivores, carnivores, scavengers, omnivores, parasites and commensals. Detritivores, detritivore/suspension feeders and herbivores were the most abundant categories accounting for almost 60% of all individuals collected, whilst over 50% of individuals at least partially fed on detritus (Fig. 4.15).

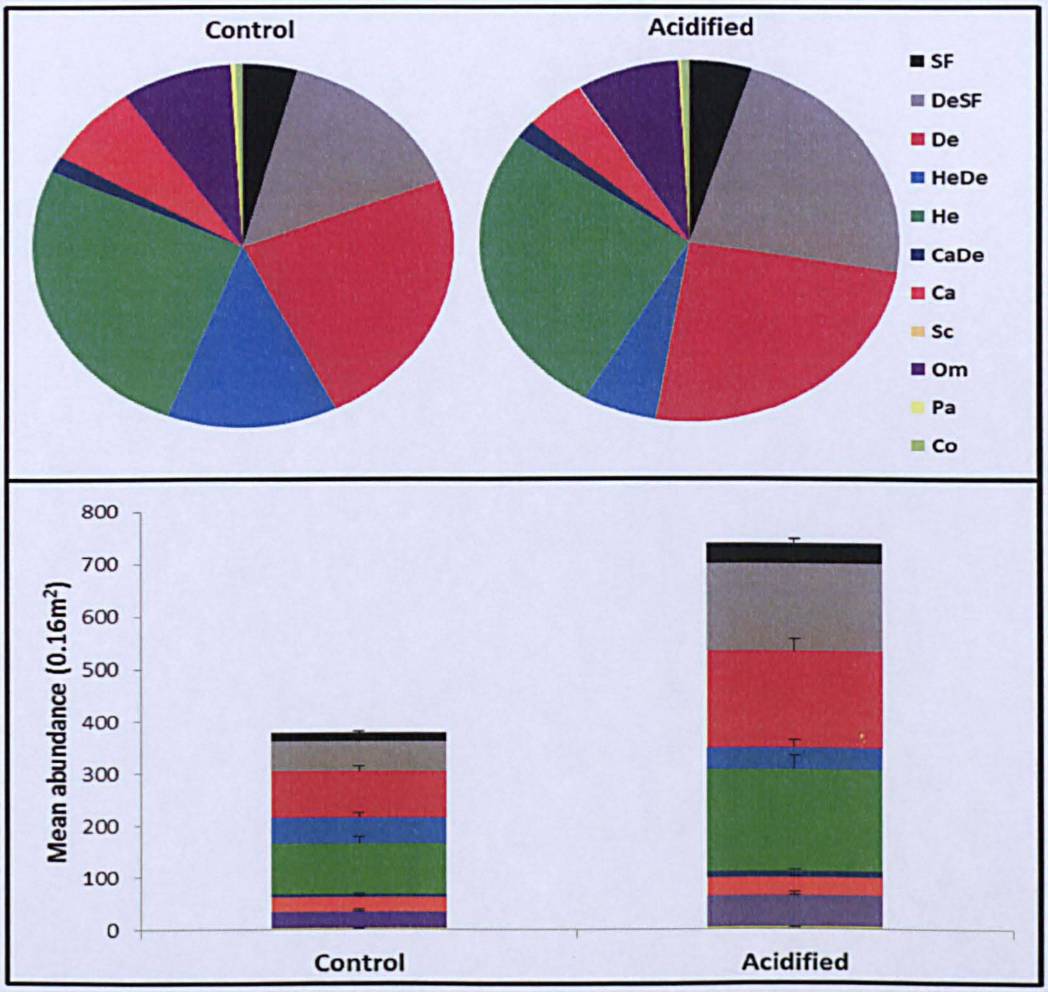


Figure 4.15 Trophic structure of the invertebrate community in *Posidonia oceanica* pooled for control and acidified zones (N = 24) at the north and south of the Castello Aragonese and shown in a) proportions, and b) mean abundance (±SE). SF = suspension feeders, DeSF = detritivore/suspension feeders, De = detritivores, HeDe = herbivore/detritivores, He = herbivores, CaDe = carnivore/detritivores, Ca = carnivores, Sc = scavengers, Om = omnivores, Pa = parasites, Co = commensals.

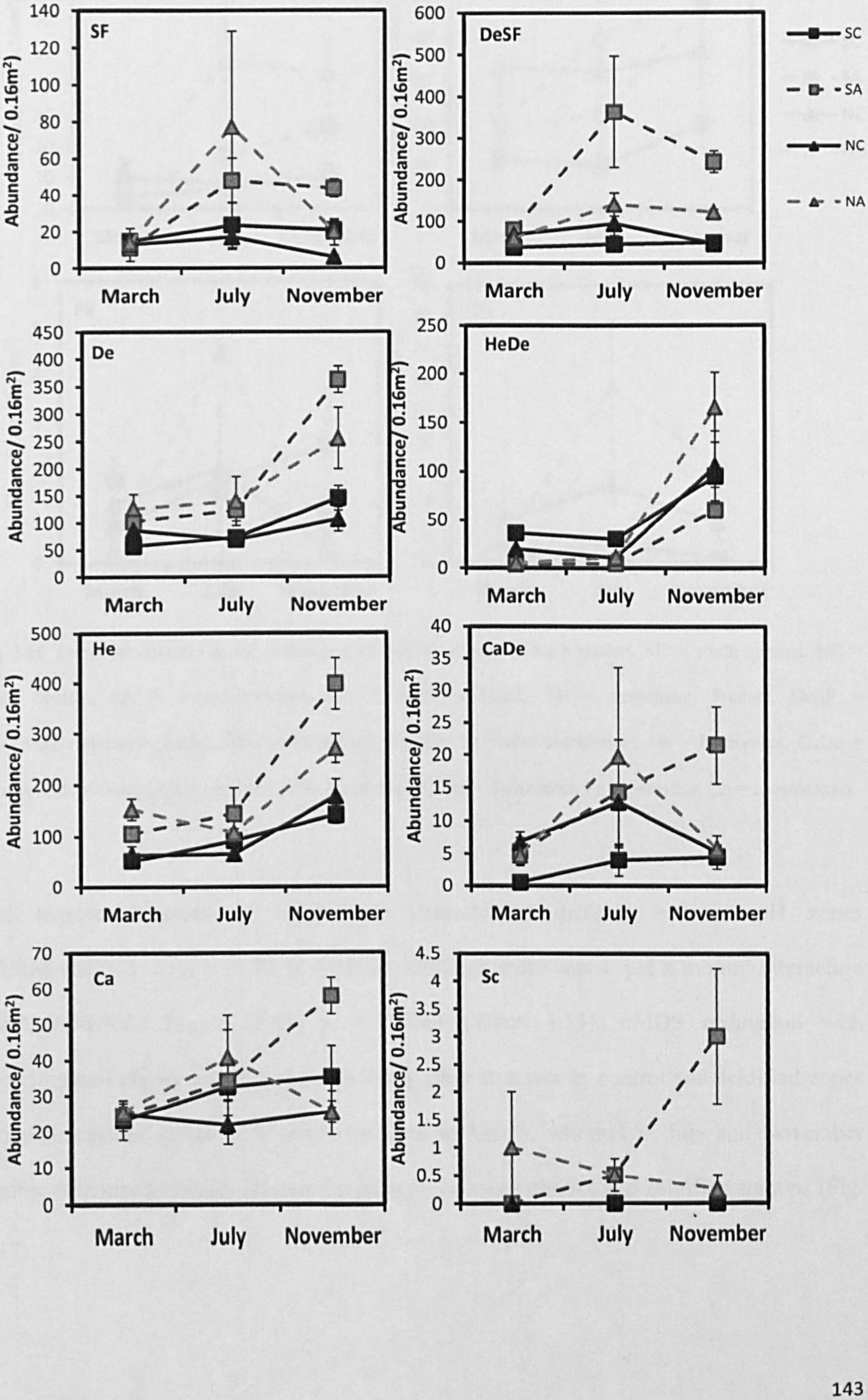
All trophic groups other than carnivores, herbivore/detritivores, and parasites increased in abundance in response to acidification (Table 4.12; Fig. 4.15), although there was a 'site x pH' interaction for the abundance of detritivore/suspension feeders (Tukey HSD: $p < 0.001$ and $p = 0.129$, respectively), carnivore/detritivores (Tukey HSD: $p < 0.001$ and $p = 0.918$, respectively), and omnivores (Tukey HSD: $p = 0.009$ and $p = 0.999$), as the increase in abundance in response to acidification was only observed on the southern side of the Castello (Fig. 4.16). A 'pH x month' interaction was observed for the abundance of commensals, as the effect of pH was only observed in November (Tukey HSD: $p = 0.012$). The abundance of carnivores or parasites was not affected by pH (Table 4.12, Fig 4.16). Herbivore/detritivores were the only trophic group which decreased in abundance in response to acidification, although there was 'site x pH' interaction, as this effect was only observed on the south side (Tukey HSD: $p < 0.001$ and $p = 0.212$, Fig. 4.16).

Even though the abundance of invertebrates almost doubled in acidified stations, the proportion of many of the trophic groups remained the same (Table 4.12, Fig. 4.15). The proportion of carnivores, herbivore/detritivores, and omnivores decreased, although there was a 'site x pH' interaction for the proportion of herbivore/detritivores, as this decrease was more prominent on the south side (Tukey HSD $p < 0.001$ and $p = 0.002$, respectively). Conversely a 'site x pH' interaction was observed for proportion of omnivores as their decrease was only significant on the north side (Tukey HSD $p = 0.015$ and $p = 0.997$, respectively). The abundance of detritivore/suspension feeders, and scavengers increased in response to acidification. Increased numbers of scavengers was solely related to the presence of the gastropod *Nassarius incrassatus* in acidified stations. Detritivore/suspension feeders were abundant in all stations although the increased proportion in acidified stations was only significant at the south side (Tukey HSD p <

0.001 and p = 0.994, respectively). The proportion of scavengers, parasites and commensals collected during this study was minimal (< 1%, Fig. 4.15).

Table 4.12 Analysis of abundance and proportion of each trophic group, analysed by means of a three-way GLM ANOVA with site (fixed factor), pH (fixed factor) and month (fixed factor). All abundance data was LOg (X + 1) transformed, whilst proportion data was arcsine transformed to conform to the assumptions of ANOVA. Significant results are highlighted in red. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	Factors			Interactions			
	Site	pH	Month	Site x pH	Site x Month	pH x Month	Site x pH x Month
ABUNDANCE							
Suspension feeder	3.20	8.18**	3.83*	0.27	2.57	1.60	0.01
Detritivore/ suspension feeder	0.95	46.56***	7.05**	13.25***	1.37	4.69*	1.09
Detritivore	0.06	24.92***	13.81***	0.29	2.08	1.10	0.05
Herbivore/ detritivore	0.26	63.83***	181.14***	26.84***	10.97**	4.81*	19.08***
Herbivore	0.18	23.30***	24.15***	0.22	1.57	1.40	0.77
Carnivore/ detritivore	2.96	6.95*	4.09*	12.55**	3.21	0.20	0.03
Carnivore	1.73	3.61	1.99	0.48	2.90	0.62	1.03
Scavenger	1.16	16.73***	2.15	1.16	4.58*	2.15	4.14*
Omnivore	17.05***	6.06*	5.81**	5.47*	162	1.86	0.36
Parasite	0.55	0.14	4.12*	0.50	3.51*	1.08	3.58*
Commensal	19.32***	5.12*	5.74**	0.27	2.19	3.48*	1.34
PROPORTION							
Suspension feeder	2.58	0.07	7.43**	2.91	1.18	1.25	0.09
Detritivore/ suspension feeder	3.08	18.87***	26.87***	22.19***	0.22	5.09*	2.28*
Detritivore	0.18	0.94	4.40*	0.31	1.65	1.12	0.60
Herbivore/ detritivore	0.01	136.57***	89.82***	37.31***	18.60***	3.39*	0.17
Herbivore	0.28	0.55	9.27***	0.84	2.31	11.23***	2.41
Carnivore/ detritivore	7.19*	0.21	3.52*	11.77**	3.22	0.36	0.46
Carnivore	2.32	10.82**	8.41**	0.58	0.91	0.26	1.12
Scavenger	0.09	13.83***	0.87	0.09	2.74	0.87	2.74
Omnivore	30.39***	5.74*	1.56	4.53*	3.35*	0.56	1.58
Parasite	0.49	0.50	4.77*	0.41	3.25	0.28	3.00
Commensal	19.55***	0.22	5.94**	0.47	1.63	1.63	1.23


SF
DeSF
De
HeDe
He
CaDe
Ca
Sc
Abundance/ 0.16m²
March
July
November
SC
SA
NC
NA
0
20
40
60
80
100
120
140
200
250
300
350
400
450
500
600
5
10
15
25
30
35
70
0.5
1
1.5
2
2.5
3
3.5
4
4.5

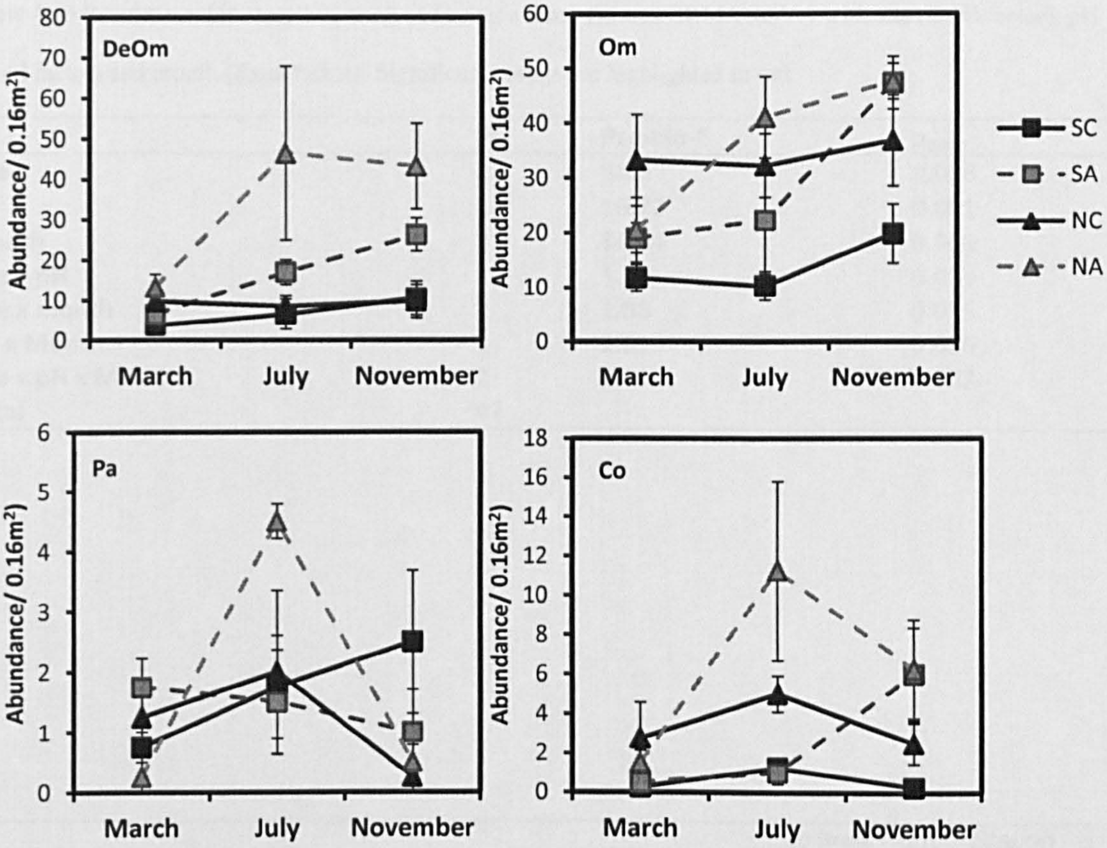


Fig 4.16 Temporal changes in the abundance of trophic groups at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified. SF = suspension feeders, DeSF = detritivore/suspension feeders, De = detritivores, HeDe = herbivore/detritivores, He = herbivores, CaDe = carnivore/detritivores, Ca = carnivores, Sc = scavengers, Om = omnivores, Pa = parasites, Co = commensals.

The trophic structure of invertebrate assemblages differed between pH zones (PERMANOVA: $F_{1,47} = 19.72$, $p = 0.001$), although there was a 'pH x month' interaction (PERMANOVA: $F_{1,47} = 2.61$, $p = 0.026$) (Table 4.13). nMDS ordination with superimposed cluster analysis showed that trophic structure in control and acidified zones grouped together at the 75% similarity level in March, whereas in July and November trophic structure generally clustered separately between control and acidified stations (Fig. 4.17).

Table 4.13 Results trophic structure, analysed using a three-factor PERMANOVA with site (fixed factor), pH (fixed factor) and month (fixed factor). Significant results are highlighted in red.

	df	Pseudo-F	p_{perm}
Site	1	3.86	0.008
pH	1	19.72	0.001
Month	2	14.64	0.001
Site x pH	1	3.31	0.02
Site x month	2	1.88	0.085
pH x Month	2	2.61	0.026
Site x pH x Month	2	0.71	0.667
Total	47		

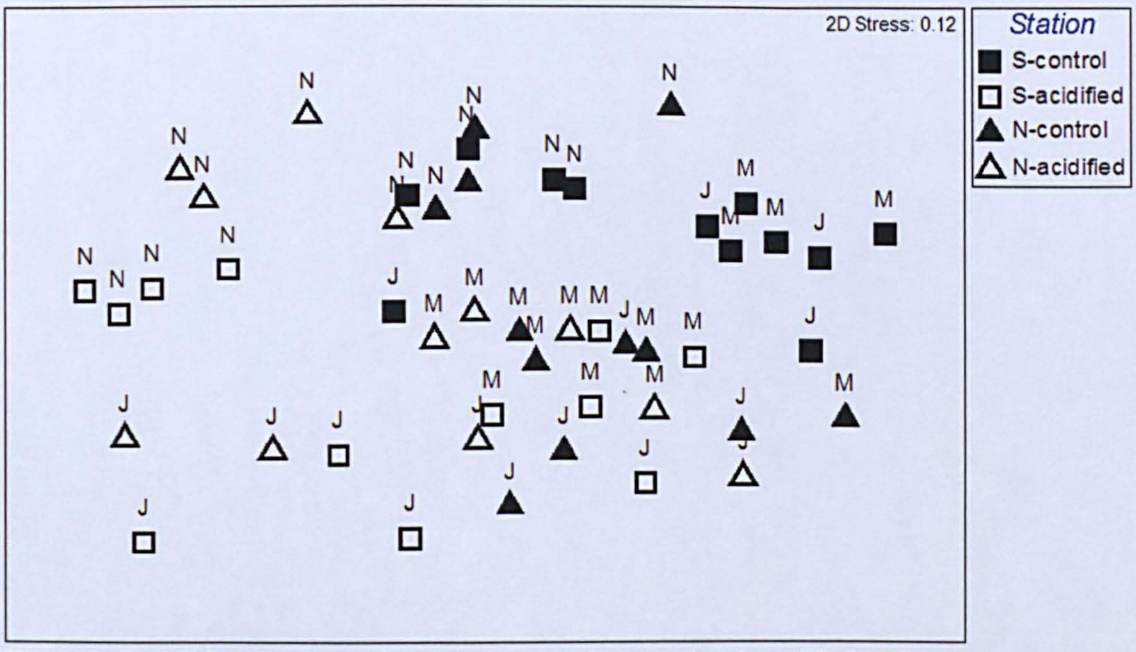


Figure 4.17 nMDS ordinations of trophic structure in control and acidified stations at the north and south of the Castello Aragonese. M = March, J = June, N = November.

4.3.11 Relationship between the abundance of different taxonomic groups and Posidonia oceanica shoot density

There is an increase in *P. oceanica* shoot density in response to acidification, and density increased by 58% in the north acidified station and 82% in the south acidified station (see Chapter 3). Positive correlations were found for the abundance of gastropods, bivalves, amphipods, pycnogonids and ophiuroids in relation to *P. oceanica* shoot density (Fig. 4.18), although R values were low for gastropods and bivalves, suggesting that factors other than shoot density played an important role in determining the abundance of these groups (Table 4.14). Each of these groups of taxa showed a significant increase in abundance in response to acidification. No relationship was found between the abundance of polychaetes and *P. oceanica* density, although this group of taxa also showed a significant increase in response to acidification. A significant negative correlation was observed for decapods, although the R value was low (Table 4.14).

Table 4.14 Results of Spearman Rank Correlations between total abundance, richness, diversity, evenness and abundance of each of the taxonomic invertebrate groups, and shoot density of *Posidonia oceanica.*

Taxa	**R**	**t**	**p**
Gastropods	0.347	2.505	**0.015**
Bivalves	0.289	2.044	**0.047**
Decapods	-0.351	-2.546	**0.014**
Amphipods	0.438	3.308	**0.002**
Tanaids	-0.099	-0.675	0.503
Isopods	-0.231	-1.612	0.114
Polychaetes	0.190	1.315	0.195
Ophiuroids	0.430	3.228	**0.002**
Pycnogonids	0.584	4.884	**<0.001**
Cumaceans	-0.269	-1.893	0.065
Mysids	0.141	0.963	0.341
Total abundance	0.357	2.595	**0.013**
Richness	-0.067	-0.453	0.652
Diversity (N1)	-0.449	-3.409	**0.001**
Diversity (N2)	-0.433	-3.260	**0.002**
Evenness (N21')	-0.183	-1.262	0.213

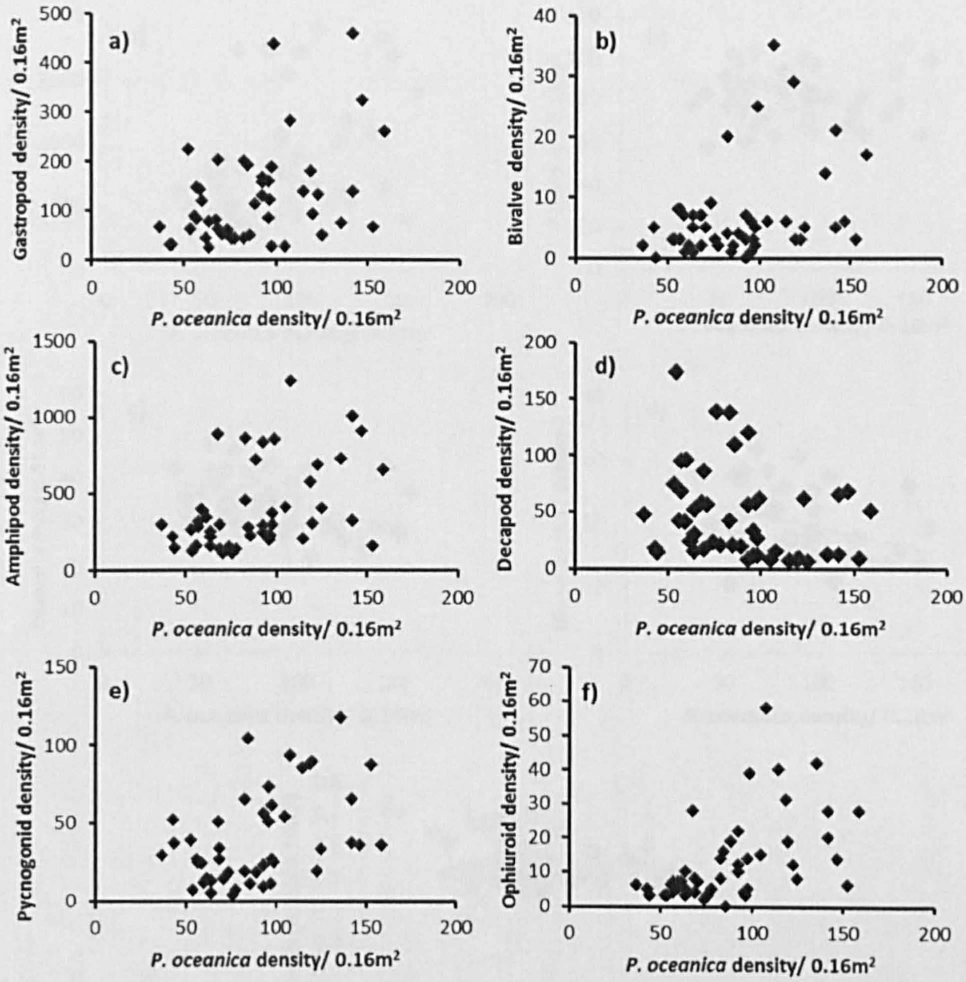


Figure 4.18 Relationship between a) gastropod, b) bivalve, c) amphipod, d) decapod, e) pycnogonid, and f) ophiuroid abundance with *P. oceanica* shoot density.

A positive correlation was found for the abundance of invertebrates and *P. oceanica* shoot density (Table 4.14), although the R value was low and the abundance of invertebrates appeared to be unaffected by shoot density in many samples (Fig. 4.19a). Diversity (N1 and N2, Fig. 4.19b-c) showed a negative correlation with shoot density. No relationship was found between shoot density and species richness or evenness (Table 4.14)

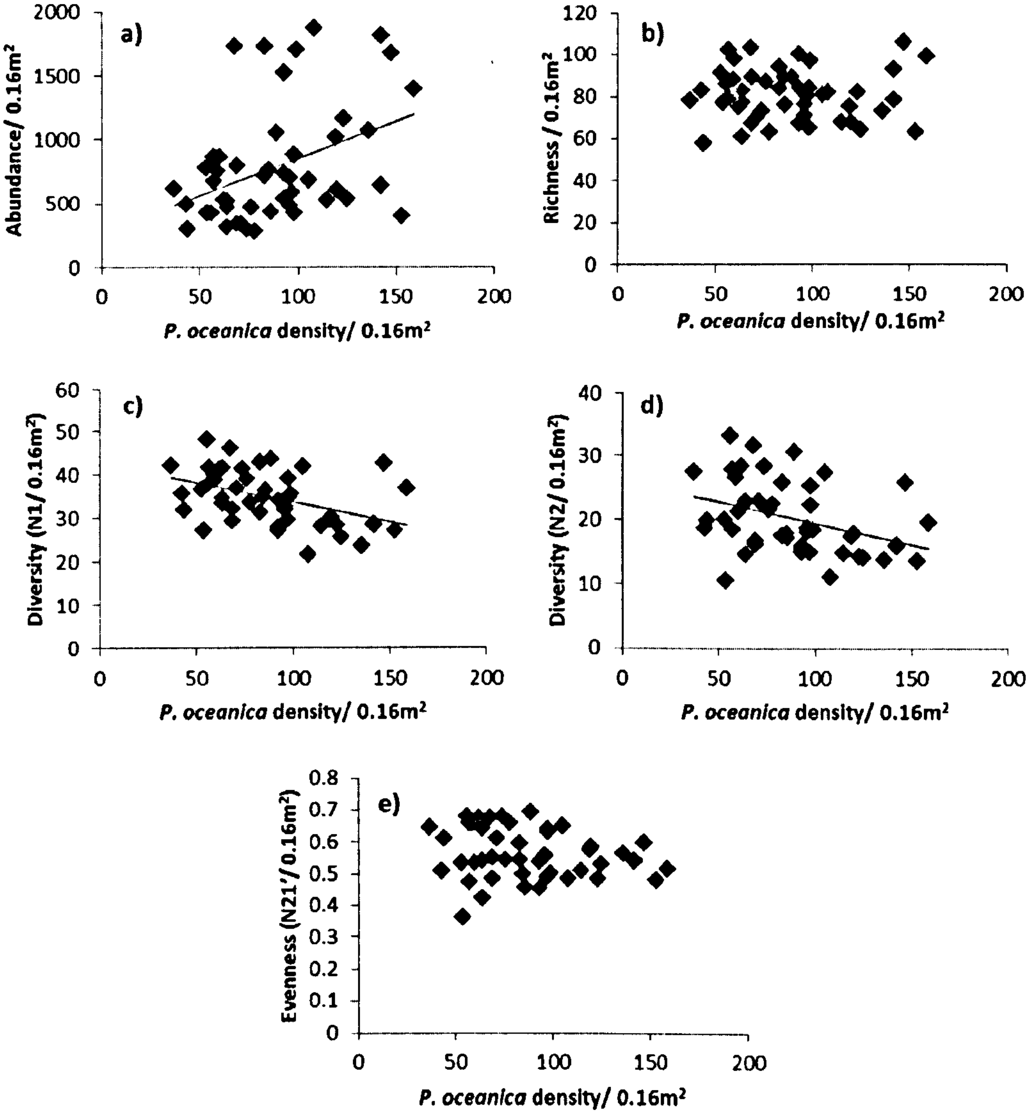


Figure 4.19 Relationship between a) abundance, b) richness, c) diversity (N1), d) diversity (N2), and e) evenness (N21') with *P. oceanica* shoot density.

4.4 Discussion

These results are surprising; in that they suggest that the majority of heavily calcified *P. oceanica*-associated invertebrates will be robust and even thrive in response to ocean acidification. Rather than a decrease in abundance and richness, as would be expected, an increase in abundance was observed, whilst species richness did not differ between pH zones. In fact, of the 270 OTUs identified during this study, 15 suffered a significant decrease in their abundance in response to acidification, whilst 36 OTUs significantly increased in abundance and the total abundance of invertebrates increased by over 90% in acidified zones. These results contrast to results from laboratory experiments, which show that survival decreases for many species of calcifying invertebrates in response to OA (Kroeker et al. 2010), and suggest that a complex interplay of direct and indirect effects may lead to positive responses of invertebrate communities to OA in the future.

Multivariate analysis indicated that *P. oceanica*-associated communities differed between control and acidified stations, with changes reflected in both variations in presence and absence and changes in relative abundances of species, suggesting that there will be an ecological shift in assemblages under future OA. Similar shifts in community structure have been observed for invertebrate settlement (Ricevuto et al. 2012) and algal populations (Kroeker et al. 2013b) in response to acidification at the vents of the Castello Aragonese. In terms of biodiversity measures, whilst some taxa experienced a decrease in species richness (bivalves, decapods and isopods), overall community richness was not affected by acidification. This may be because the decrease in richness of some groups of taxa was compensated by the increase in the number of amphipod species. Evenness was not affected by acidification, as a decrease in gastropod evenness was balanced by an increase in decapod and tanaid evenness. Similarly, no difference in species richness and evenness was observed between control and acidified (pH 7.8) stations in the rocky subtidal at the Castello Aragonese (Kroeker et al. 2011b). Diversity (N1 and N2) decreased in response to acidification. This decrease was primarily due to the decrease in gastropods and isopod diversity. Significance of this decrease in diversity was greater for N1 than for N2, suggesting that changes in diversity may be primarily due to a decrease in the number of common (rather than abundant) species (Heip et al. 1998). Whilst this decrease must be taken into consideration, many authors suggest that species richness and evenness are the most important independent characteristics of biological communities that together are most linked to biodiversity (Heip and Engels 1974; Peet 1974; Magurran 2004).

There is some concern with research at CO_2 vents that, due to the open nature of the system and close proximity of habitat at ambient pH, supply of larvae from areas of ambient pH may lead to an underestimation of the impacts of OA. The larval/ juvenile phase of calcifying invertebrates may be the most susceptible stage to OA (Dupont et al. 2010; (Ross et al. 2011)), and supply of larvae from non-acidified areas may limit the negative effects experienced by individuals. Secondly, reproductive output may decrease in acidified waters (Fitzer et al. 2013), a response that may be masked at CO_2 vents. This must be taken into consideration when assessing the effect of vent induced acidification on invertebrate communities and may be why very little. However many groups of taxa (amphipods, tanaids, some polychaetes) are direct developers and therefore the reproductive success of these taxa in acidifed conditions can be seen (Kroeker et al. 2011). Amphipods imore than doubled in numbers in acidified stations, showing that reproductive output was not limited by acidified conditions.

A previous study by Cigliano et al. (2010) placing artificial collectors (scouring pads) in the water column in three different pH zones (control, acidified and very acidified), looked at larval settlement at the CO_2 vents of Ischia. Of the species common to both studies, the juveniles of those that increased in this study either increased (the tanaid *Leptochelia savignyi,* the amphipod *Caprella acanthifera,* the polychaete *Amphiglena mediterranea* and other polychaetes from the subfamily Syllinae) or remained abundant (polychaetes from the subfamily Exogoninae, the gastropod *Rissoa variabilis,* and the amphipod *Ampithoe ramondi*) in acidified stations in the previous study, suggesting that the juvenile stage of many species of invertebrate may be robust to future acidification. Other OTUs which showed a positive response to acidification in this study were not collected by Cigliano et al. (2010); this may be because scouring pads do not have the same spatial complexity of natural substrata, and in addition, they stay in the field only for a short period (one month); therefore they select only a subset of the potential diversity of species occurring in the natural community.

Decapods were the taxonomic group most affected by acidification in this study. They were the only group whose abundance and taxonomic richness decreased in acidified zones; more species in this group exhibited a significant decrease in abundance in response to acidification than other taxonomic groups. This leads to a decreased number of species being collected in acidified stations. The carapace of decapods generally contains high magnesium-calcite (Ries et al. 2009), which is more soluble than low magnesium-calcite and aragonite, and therefore more susceptible to OA-based dissolution than the shells or skeletons of organisms (Andersson et al. 2008). Many species of decapods produce an external organic layer (Ries et al. 2009), which may protect against dissolution (Rodolfo-Metalpa et al. 2011). Ries et al. (2009) found that over a period of 60 days, three species of decapods which were exposed to high levels of acidification responded by increasing net calcification rates, suggesting some degree of tolerance to OA. Decapod susceptibility to OA is not only due to their high magnesium-calcite carapace. Acidification has been shown to disrupt chemo-responsive behaviour in the hermit crab *Pagurus bernhardus* (de la Haye et al. 2012), and to decrease metabolic rates in the velvet swimming crab *Necora puber* (Small et al. 2010). If the decrease in decapods is due to physiological intolerance to OA, then loss of decapods may lead to simplification of the *P. oceanica* food web, as they are the preferred prey for many species of fish (Zupo and Stubbing 2010). Fish may end up foraging for smaller prey items, and lead to a reduction in energy transfer efficiency between invertebrates and fish (Sherwood et al. 2002). A second consideration is that the decrease in decapods may not be due to a physiological intolerance to OA, but due to indirect effects. Extreme grazing by the fish, *Sarpa salpa*, lead to a decrease in seagrass canopy height in acidified stations in late spring/ summer (see Chapters 3 and 6), corresponding to the time when decapod populations were extremely low in acidified stations, but high in control stations. A reduction in canopy height, through extreme grazing by *S. salpa*, has shown to intensely increase predation risk of invertebrates in *P. oceanica* (Pages et al. 2012), so loss of protection may account for the decline of decapod populations.

There was a 82% decrease in the number of the hermit crab *Cestopagurus timidus* collected from control to acidified stations, although it is uncertain whether this was a direct result of acidification or whether it resulted from the indirect effect of a lack of suitable shells to occupy. The preferred shell for juvenile C. *timidus* is from the gastropod *Bittium latreillii* (Pessani and Premoli 1993). This species suffered a drastic decrease of 94% from control to acidified stations; therefore it is possible that juvenile hermit crabs were unable to find suitable shells to occupy and were unable to survive, although they are also known to inhabit shells of the gastropods *Jujubinus striatus*, *Rissoa spp. or Alvania spp.* (Zupo et al. 1985; Pessani and Premoli 1993), which increased in response to acidification. The only other hermit crab found during sampling was *Pagurus anachoretus*, and only one individual was collected so it was not possible to determine whether this species is adversely affected by acidification. A second consideration is that some species of mollusc are protected from shell dissolution, in a low pH environment, through covering of an organic layer (Rodolfo-Metalpa et al. 2011). When the animal dies, the organic layer disappears. Dissolution of empty shells (of even abundant species), may lead to a lack of suitable shells for hermit crabs.

A second group of taxa that may suffer negative effects from OA are isopods. Although this group did not decrease in abundance in response to acidification during this study, they suffered a decrease in species richness and diversity. *Cymodoce hanseni* feeds on crustose coralline algae (M. Lorenti, pers. comm.), which is highly reduced at acidified stations (Martin et al. 2008; Chapter 5), therefore a reduction in this species may be due to a loss of food availability. A second consideration is that many shallow water isopods are generally cryptic, and camouflaged within their habitat (Poore and Bruce 2012). All species with reduced abundances (*Joeropsis brevicornis, Cymodoce hanseni,* and *Dynamene tubicauda*) are associated with the leaves, rather than the rhizomes of *P. oceanica.* Through loss of crustose coralline algae the colouration and complexity of the leaves changes under acidified conditions (see Chapter 5), which may lead to loss of refuge for this group of taxa. Similarly, a reduction in canopy height, through increased grazing, may lead to a loss of habitat for this group of species. A similar reduction in richness of isopods was observed in other studies of juvenile settlement (Cigliano et al. 2010) and the rocky subtidal (Kroeker et al. 2011b) in response to vent induced acidification at the Castello Aragonese.

Gastropods and bivalves are heavily calcified groups of taxa, and therefore at risk of dissolution in low pH conditions, yet each of these groups increased in abundance in response to acidification. When the saturation state of calcite or aragonite (Ω) falls below 1.0 calcium carbonate shell or skeleton dissolution will occur (Fabry et al. 2008), although evidence suggests that external organic layers can protect shells and skeletons against this (Rodolfo-Metalpa et al. 2011). Some species may up-regulate calcification rates in response to acidification in order to offset rates of dissolution (Ries et al. 2009), although this may be energetically costly (Portner et al. 2004), leading to a decrease in fitness and survival (Wood et al. 2008). Some species of molluscs have been observed to be tolerant to low pH but at the cost of loss of induced defences (Bibby et al. 2007) or immune response (Bibby et al. 2008).

Although it was estimated that calcite and aragonite saturation states fell below 1.0 between 10-30% of the time at acidified stations at the Castello Aragonese, evidence of dissolution was only visually apparent for one species: the gastropod *Columbella rustica.* A loss of periostracum (external organic layer) was also noticeable, although this and shell dissolution did not appear to negatively affect this species and the mean number of individuals collected in acidified stations were much greater than in control stations (7.7±1.9 and 1.3±0.5, respectively). All species from the genus *Rissoa* increased in abundance in response to acidification. Tolerance to OA in this species may be because they produce a mucous layer which may protect their shell from dissolution (F. Patti, pers. comm.). Increases in the abundance of these species are likely to be due to the indirect effects of acidification. Although abundance of molluscs increased, gastropod diversity and evenness decreased, whilst a reduction in the number of species of bivalves was observed in response to low pH suggesting that this group will suffer some detrimental effects in response to acidification.

Two groups of taxa which appeared extremely robust to acidification are amphipods and polychaetes. Some of the species of polychaete that occur at these vents have been shown to be tolerant to low pH due to physiological acclimation and adaptation (Calosi et al. *in press*). Amphipod abundance and richness and polychaete abundance increased in response to acidification, whilst no negative effects on diversity or evenness were observed for either of these groups. Two OTUs from each of these groups of taxa decreased in response to acidification, although reasons for these decreases are uncertain. A decrease in the abundance of polychaetes from the family Dorvilleidae (dominated by the species *Protodorvillea kefersteini)* is surprising, as this group is known to thrive in less favourable marine environments such as methane seeps (Levin et al. 2003), hydrothermal vents (Van Dover et al. 1988) and highly polluted sediments (Bailey-Brock et al. 2001).

Whilst the effects of OA have been demonstrated on individuals at the physiological level (e.g. Bibby et al. 2008; Calosi et al. 2009; Saphorster et al. 2009; Dupont and Thorndyke 2012; Calosi et al. *in press*), impacts of OA on marine communities will be more complex than direct impacts of changes in carbonate chemistry alone. Indirect effects of changes in biotic interactions (e.g. competition and predation) or habitat characteristics (e.g. seagrass density and canopy features) will be important in structuring invertebrate communities (Russell et al. 2012). A general pattern of community homogeneity/simplification, and reduced spatial variability was experimentally demonstrated recently by Kroeker et al. (2013a; 2013b) on the benthic rocky reef community in response to vent induced acidification, considering both artificial and natural substrata. Whilst species richness remained high in acidified stations in this study, multivariate dispersion between samples decreased, suggesting that invertebrate samples were more similar to each other in acidified than control stations.

Invertebrate abundance showed a positive, although weak, association to seagrass density, similar to previous studies (Bostrom and Bonsdorff 2000; Bedini et al. 2011), and no relationship was found for species richness. Increases in shoot density increase habitat heterogeneity and niche availability (Orth et al. 1984), whilst providing an increase in primary production of the seagrass and associated epiphytes (Van Montfrans et al. 1984). Although species richness of some groups of pH tolerant taxa, such as amphipods, may respond to this increase in density, the loss of species susceptible to low pH, such as bivalves, decapods and isopods, may cancel out any increase in species richness in response to shoot density. Surprisingly, diversity decreased with an increase in shoot density. A significant correlation between pH and shoot density exists (see Chapter 5) so it is suspected that this decrease in diversity is in response to a decrease in pH rather than the indirect effect of an increase in shoot density. Similar to results of Sanchez-Jerez (2000) we found that amphipod abundance showed a significant positive relationship with *P. oceanica* shoot density whilst decapod abundance was independent of density. Similarly, Mazzella et al. (1989) found that the abundance of amphipods and molluscs increased with increasing shoot density. The taxa that showed a positive relationship to shoot density during this study were: amphipods, gastropods, bivalves, pycnogonids and ophiuroids. Although polychaetes increased in response to acidification, no relationship was found between this group of taxa and shoot density. An increase in shoot density was not the only change in seagrass structure. A decrease in leaf length in acidified stations was observed in summer. As discussed, this may have lead to the decrease in abundance of decapods observed in July. Although not measured, leaf biomass is thought to be higher acidified than control stations in March and November (due to increased density in acidified stations and equal canopy height during these months) but may be equal to or lower than control stations in July (due to increased density in acidified stations but reduced canopy height during July; see Chapter 3). In acidified stations, this may lead to a decrease in habitat availability for species associated with the leaf in the summer months, whilst species associated with the rhizomes will benefit from increased habitat complexity throughout the year.

Kroeker et al. (2011b) observed a simplification of the trophic structure in response to acidification through a reduction in trophic groups. We did not observe this in *P. oceanica*, although, similar to the rocky subtidal (Kroeker et al. 2011b), there was a notable decrease in the proportion of carnivores, in comparison to other trophic groups (discussed in Chapter 5). Primary consumers increased dramatically, which may be due to a combination of both a decrease in top-down and bottom-up control. For example a reduction in species that regulate macrobenthic taxa could lead to sharp increases in the abundances of these populations. Carnivorous decapods (Nelson 1981) and polychaetes from the family Hesionidae (Shaffer 1979; Oug 1980) both feed on polychaetes and small crustaceans. The number of carnivorous decapods decreased from 766 individuals collected in control stations to 166 in acidified stations, whilst the number of Hesionidae polychaetes decreased from 165 individuals collected in control stations to 74 in acidified stations. This may at least partially explain the increase in primary consumers, such as many species of amphipods and small polychaetes. Secondly, increases in shoot density may lead may lead to an increase in abundance of palatable, non-calcareous epiphytic algae (Porzio et al. 2011), or other benefits such as protection from predators. Mesograzers are important in controlling epiphytic algal cover in seagrass beds, allowing increased light penetration and enhancing leaf survival (Jernakoff and Nielsen 1997). Alsterberg et al. (2013) suggest that in the absence of mesograzers the effects of ocean acidification and warming could pose a threat to seagrasses. These results show that the majority of species of mesograzers are robust and even thrive in response to acidification.

Of the primary consumers, only herbivore/detritivores decreased in abundance in response to acidification. As both herbivores and detritivores increased in response to acidification it is suggested that this decrease is due to a physiological intolerance to low pH, rather than a loss of food items. An increase in the abundance of omnivores is often observed in stressed communities (Polis and Strong 1996; Kroeker et al. 2011b). An increase in the number of omnivores was observed during this study, although proportionally there was no increase in comparison to other trophic groups.

Many variables measured during this study were dependent on the month sampled. Differences in community and trophic structure and abundance between pH zones were lowest in March, whilst differences in diversity and evenness were most prominent. Conversely, differences in abundance, community and trophic structure were high in November, whilst there was no difference in diversity or evenness between pH zones in this month. The temporal differences found in community dynamics, during this study, may be due to a number of variables such as: 1) lifecycle of the species, 2) canopy height, and 3) epiphyte load. Also, during windy periods, pH in acidified zones may rise, through increased mixing with ambient seawater, leading to a lesser degree of acidification (and therefore the effects of acidification) in months with harsher weather conditions (i.e. March). Most studies on invertebrate populations in response to acidification at the Castello Aragonese have only focussed on a single sampling period (Hall-Spencer et al. 2008; Cigliano et al. 2010; Kroeker et al. 2011b), although Ricevuto et al. (2012) showed that the larval settlement of different groups of invertebrates varied over a temporal time scale. This highlights the importance of temporal studies in examining the impacts of acidification at the community level.

The effect of acidification on the invertebrates found in *P. oceanica* would be expected to be more negative, due to the highly calcified nature of many of them. Very few species appeared to exhibit negative effects, in terms of their abundance, in acidified zones. *P. oceanica* is a net autotrophic system (Barron et al. 2006), and the high levels of photosynthesis may provide a refuge from acidification. pH levels were measured 10 cm above the canopy, so further analysis of pH within the different canopy compartments is suggested. Seagrass diffusion boundary layers can be >2 mm thick, and pH over 1 unit higher within the layer, than the surrounding seawater (Jones et al. 2000). Even if pH levels are only raised within the boundary layer, this may provide an advantage to species tightly associated to the leaf, and explain why heavily calcified organisms (such as gastropods), were able to persist in low pH conditions.

These results suggest that primary consumers (that feed on either plant or detrital material) may be the winners in the case of ocean acidification, as their numbers more than doubled in response to acidification. Whilst assemblage structure changes in acidified stations, no ecological tipping point was observed, and negative impacts of acidification were species specific (Garrard et al. 2013) and only observed for a few species, whilst many thrived in low pH conditions. Results contrast with previous findings from *in situ* studies (Wootton et al. 2008; Cigliano et al. 2010; Kroeker et al. 2011b), reviews (Fabry et al. 2008; Doney et al. 2009b), and meta-analyses (Hendriks et al. 2010; Kroeker et al. 2010) that show that calcifying taxa will generally be more susceptible to OA than less/non-calcified taxa. Surprisingly, heavily calcified groups of taxa showed a positive response to acidification (gastropods and bivalves), whilst less calcified taxa (isopods) exhibited more negative responses. This suggests that indirect effects of OA may be important in structuring marine communities and highlights the fact that the indirect effects could provide advantages or disadvantages to marine organisms.

Previous studies have shown that the response of calcifying species to a decrease in pH are varied (Ries et al. 2009), and life-history strategy may play an important role in determining a species' ability to tolerate the associated changes to carbonate chemistry (Widdecombe and Spicer 2008). This study builds on previous research by suggesting that habitat type may in fact play an important role in determining future invertebrate community structures. The highly productive nature of *P. oceanica* may provide protection from some changes to carbonate chemistry, whilst indirect effects of changes to canopy structure can either positively or negatively affect different taxa, highlighting the importance of elucidating the indirect effects of OA, if we are going to deliver information at the ecosystem level. These results may give some cause for relief that, at least in *P. oceanica* meadows, invertebrate communities will not collapse under future changes in carbonate chemistry, and suggest that this highly productive, nearshore habitat may provide refuge to its associated communities from future OA.

5. Comparison of the effect of acidification on benthic invertebrate communities between rocky reef and *Posidonia oceanica* habitats

5.1 Introduction

Ocean acidification (OA) and associated changes in ocean carbonate chemistry, as a result of increased CO_2 in the atmosphere, are going to affect marine habitats globally (Doney et al. 2009b), although the intensity at which acidification is experienced will vary, dependent on a number of different parameters (Garrard et al. 2013). Polar and sub-polar seas are expected to feel the effects of ocean acidification sooner, as CO_2 dissolves more readily in cold, low salinity waters, plus aragonite saturation horizons are shoaling in high latitude seas (Orr et al. 2005; Fabry et al. 2009). Likewise, areas of seasonal upwelling of high dissolved inorganic carbon (DIC) waters, such as along the western coast of the USA, will be more at risk, due to an increase in spatial extent and decrease in depth of upwelling, as the oceans continue to absorb CO_2 (Feely et al. 2008). Seasonal differences in pH, particularly in high latitude, nearshore waters (Blackford and Gilbert 2007), may exacerbate the impacts of OA (McNeil and Matear 2008).

The regional effects of OA will vary with habitat type (Halpern et al. 2008). For example, although deep oceanic waters should experience fewer changes in pH than surface waters in the near future (Caldeira and Wickett 2003), deep sea organisms may be extremely sensitive to even mild changes in pH (Seibel and Walsh 2001). Similarly, although tropical waters have much higher carbonate saturation levels than high latitude seas (Orr et al. 2005), and should be more favourable to calcification than polar seas, in the face of acidification (Fabry et al. 2008), coral reefs will most likely be one of the most severely impacted habits as the oceans acidify (Hoegh-Guldberg et al. 2007).

This chapter provides the unique opportunity to compare the effect of acidification on benthic invertebrate assemblages in two different habitat types. Effects of OA will not only occur through the direct impact on individual species, but will be caused by indirect effects of changes in environmental and biotic interactions (Russell et al. 2012). Changes in top down and bottom up interactions may favour some species, whilst exacerbating the effects of OA on others (Asnaghi et al. 2013). Natural CO_2 vents can provide useful sites to observe the long term, ecosystem level effects of OA (Barry et al. 2010). At the Castello Aragonese, Ischia, (Tyrrhenian Sea, Italy), two different habitat types, rocky reef and the seagrass *Posidonia oceanica*, can be found exposed to similar changes in pH. Data on invertebrate assemblages in the rocky reef (Kroeker et al. 2011b) can be compared with data on invertebrate assemblages in *P. oceanica* (Chapter 3) in control and acidified zones with similar pH, to determine whether the effect of acidification is more prominent in one habitat than the other.

This is the first time that a comparison of habitat types exposed to similar acidification has occurred, and is particularly pertinent as, due to the close proximity of acidified and control stations in each habitat (Figure 5.1), each habitat is subject to the same environmental conditions (sea temperature, hydrodynamic forces), therefore changes in assemblage dynamics should be solely due to direct and indirect effects of changes in pH.

5.2 Methods

Invertebrate samples were collected from control and acidified stations in *P. oceanica* at the north and south of the Castello Aragonese in March, July and November 2011 (Chapter 4), whilst invertebrate samples were collected from ambient, low pH and extremely low pH stations at the north and south of the Castello Aragonese in the rocky reef habitat in November 2008 (Kroeker et al. 2011b). Comparisons of pH and other carbonate parameters observed in *P. oceanica* in 2011 (Chapter 3) and the rocky reef in 2008 (Kroeker et al. 2011b) showed that carbonate chemistry at control stations in *P. oceanica* was equivalent to that of ambient stations in rocky reef habitat, whilst carbonate chemistry at acidified stations was comparable to values observed in the low pH stations, allowing comparisons to be made between the effects of acidification on invertebrate community composition in the two different habitats (Table 5.1).

Table 5.1 Comparison of mean (± SD) carbonate chemistry parameters between control/ambient and acidified/low pH station in *P. oceanica* in 2011 and in rocky reef habitat in 2010 (Kroeker et al. 2011b).

Site	*Posidonia oceanica*	Rocky reef
North control/ ambient		
TA (µmol/kg)	2,536 ± 15	2,563 ±3
pH	8.1 ± 0.1	8.0 ± 0.1
pCO_2	509 ± 54	567 ± 100
$\Omega_{CALCITE}$	5.04 ± 0.32	4.75 ± 0.53
$\Omega_{ARAGONITE}$	3.30 ± 0.21	3.13 ± 0.35
North acidified/ low		
TA (µmol/kg)	2,567 ± 16	2,560 ±7
pH	7.8 ± 0.3	7.8 ± 0.2
pCO_2	2,158 ± 2,508	1,075 ± 943
$\Omega_{CALCITE}$	3.37 ± 1.81	3.52 ± 1.11
$\Omega_{ARAGONITE}$	2.14 ± 1.18	2.32 ± 0.73
South control/ ambient		
TA (µmol/kg)	2,551 ± 8	2,563 ±2
pH	8.1 ± 0.04	8.1 ± 0.1
pCO_2	504 ± 68	440 ± 192
$\Omega_{CALCITE}$	5.15 ± 0.44	5.11 ± 0.67
$\Omega_{ARAGONITE}$	3.37 ± 0.29	3.33 ± 0.44
South acidified/ low		
TA (µmol/kg)	2,539 ± 12	2,560 ±7
pH	7.8 ± 0.4	7.8 ± 0.3
pCO_2	1,614 ± 1,861	1,581 ± 2,711
$\Omega_{CALCITE}$	3.23 ± 1.51	3.00 ± 1.31
$\Omega_{ARAGONITE}$	2.11 ± 0.99	1.95 ± 0.85

Control/ambient stations and acidified/low pH stations will hereinafter be referred to as control and acidified stations. These stations were located in rocky reef and *P. oceanica* habitat at the north and south of the Castello Aragonese (Fig. 5.1). *P. oceanica* stations were located at a depth of 2.5 – 3.5 m, whilst rocky reef stations were located at a depth of 1 – 1.5 m.

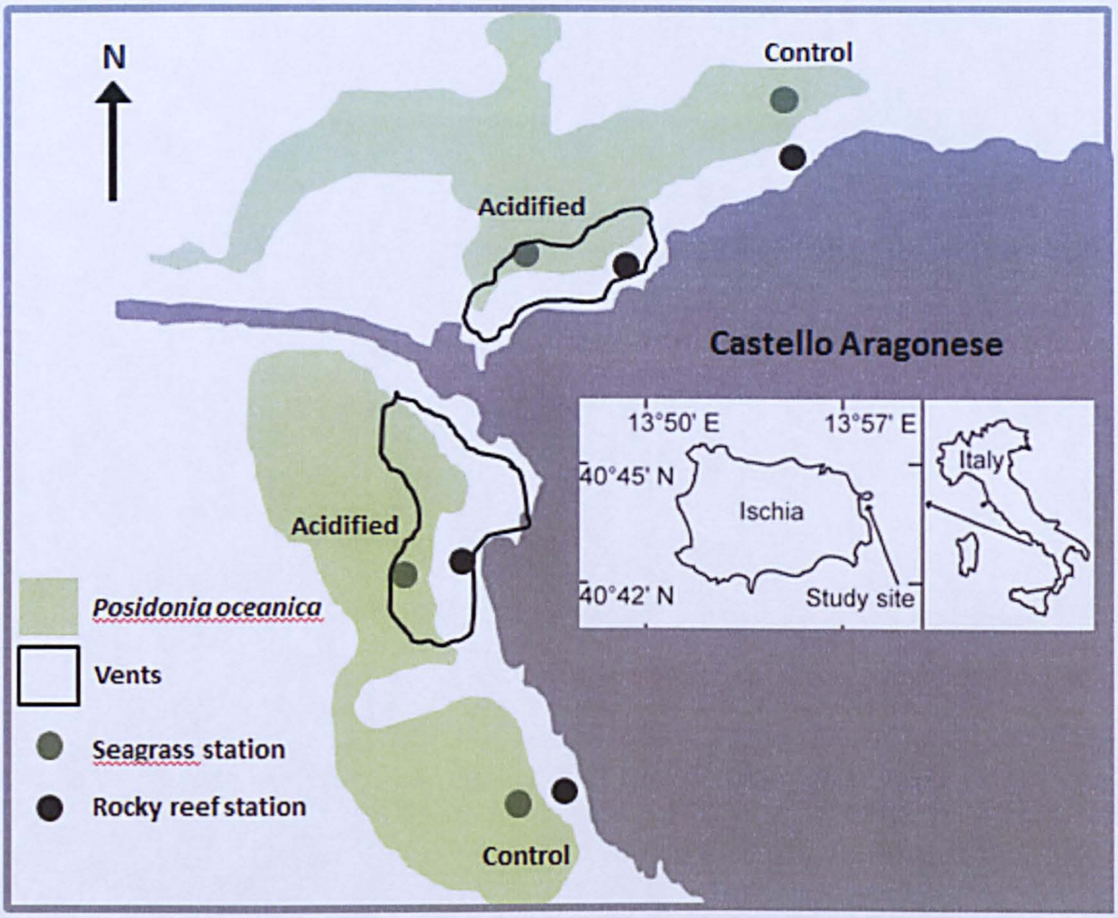


Figure 5.1 Area of the study site (Castello Aragonese), with positions of control and acidified sampling stations, in seagrass and rocky reef habitat, at the north and south of the Castello.

Invertebrate samples were collected by use of an airlift sampler in both habitats (rocky reef and *P. oceanica*), although times and methods of collection varied. In November 2008 invertebrate samples (N = 4) were collected from each station in the rocky reef by means of an airlift sampler, attached to a 150μm collection net, placed over an area of 20 x 20 cm for ~ 30 seconds, and final collection of invertebrates by scraping the algae from the reef with a chisel and hammer (Kroeker et al. 2011b). Invertebrate samples (N = 4) were collected from each station in *P. oceanica* in March, July and November 2011 by means of an airlift sampler attached to a 400µm collection net, placed over an area of 40 x 40 cm for ~ 2 minutes (Chapter 4). In both studies samples were fixed in 4% formalin for preservation and subsequently stored in 70% ethanol prior to sorting and identification. Samples were examined under a dissecting microscope and organisms separated from the sediment, algae and detritus. Gastropods, bivalves, tanaids, isopods, amphipods, decapods and polychaetes were identified by the same specialized taxonomists (researchers at the SZN) to the lowest taxonomical resolution possible.

Data for gastropods, bivalves, decapods, amphipods, tanaids, isopods and polychaetes were compared between habitats, as these were the most abundant taxonomic groups, and were identified to family and lower taxonomic levels. Data was aggregated to family level, as many families were common to both habitats. This also removed any taxonomic discrepancies between species identification and level of taxonomic identification. For example, most amphipods were identified to the species level in the seagrass, but to higher levels for the rocky reef, whilst the reverse was true for polychaetes. As different sampling methods were used, quantitative comparisons of abundances could not be made, although both are considered minimal representative areas for the respective habitat (Bianchi et al. 2004; Buia et al. 2004). Relative (%) change in total abundance and abundance of each taxonomic group between control and acidified zones was calculated for each habitat (seagrass and rocky reef) for November and for seagrass data pooled across all months.

For multivariate analysis, only data from the rocky reef in November 2008 (N = 16) and *P. oceanica* in November 2011 (N = 16) were used, to eliminate any temporal differences. A non-metric multidimensional scaling (nMDS) ordination of Bray-Curtis similarity matrices on square root transformed family abundance data was used to compare differences in community structure between control and acidified stations in each habitat. Data was square root transformed to reduce the influence of common families. Similarly, differences in community compositions were observed through an nMDS ordination of Bray-Curtis similarity matrices on untransformed family presence/absence data. Two-factor PERMANOVAs were used to test for significant differences between site (fixed factor: north and south) and pH (fixed factor: control and acidified) for each habitat. Differences between habitats could not be statistically compared due to unequal sampling methods. PERMANOVA analyses used Bray Curtis similarity matrix applied to square root transformed abundance data (for community structure) and Bray Curtis similarity matrix applied to presence/absence data (for community structure). All PERMANOVA analyses used Type III SS and 9,999 unrestricted permutations. Where significant interactions occurred between site and pH, pairwise t-tests were run in PERMANOVA to test for differences in community structure/composition between pH zones at the north and south sides of the Castello Aragonese.

The response of each family to acidification in November for each habitat was assessed by visual inspection of mean abundances between control and acidified zones using bar graphs. Where standard errors did not overlap, families were either classified as increasing or decreasing in abundance in response to acidification. Rare families were classified as such and excluded from inspection. Statistical significance was not tested for because of low power and the large number of comparisons (Kroeker et al. 2011b). Where families were not rare or absent from one of the habitats, the response in each habitat was evaluated to determine whether acidification lead to similar abundance responses for both habitats.

5.3 Results

5.3.1 Relative changes

Invertebrates from the main benthic taxa (gastropods, bivalves, decapods, amphipods, tanaids, isopods and polychaetes) representing 139 different taxonomic families were collected from control and acidified zones in the rocky reef and *Posidonia oceanica* habitat around the Castello Aragonese in November. Of these, 67 were common to both habitats, 15 were solely collected from the rocky reef and 58 collected solely from *P. oceanica* habitat.

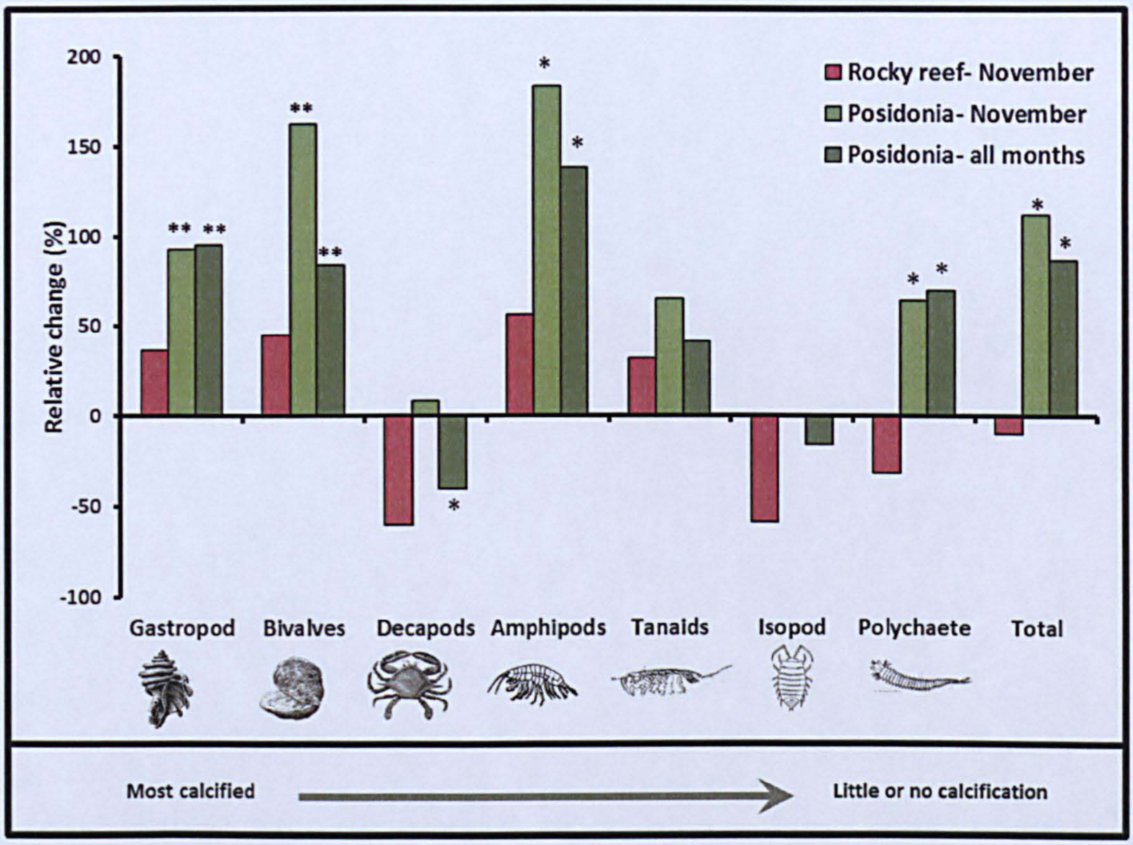


Figure 5.2 Relative change in abundance (%) of each taxonomic group in response to acidification in the rocky reef habitat in November, and in *P. oceanica* habitat in November, and pooled for all months (March, July and November). * = significant difference between pH zones ($p < 0.05$), ** significant 'site x pH' interaction ($p < 0.05$).

In the seagrass, the abundance of amphipods increased both for November ($F_{1,12} = 51.03$, $p < 0.001$) and across all months ($F_{1,36} = 44.87$, $p < 0.001$). A similar increase was observed for polychaetes in November ($F_{1,12} = 6.67$, $p = 0.024$) and across all months ($F_{1,36} = 4.98$, $p = 0.032$). A 'site x pH' interaction was observed for gastropods in November ($F_{1,12} = 13.07$, $p = 0.004$) and for all months ($F_{1,36} = 7.89$, $p = 0.008$), as an increase in gastropod abundance was only observed on the south side (Tukey HSD: $p = 0.009$ and $p < 0.001$, respectively). A similar observation occurred for the abundance of bivalves, as a 'site x pH' interaction was found for November ($F_{1,12} = 9.18$, $p = 0.010$), and across all months ($F_{1,36} = 8.27$, $p = 0.007$) in the seagrass (Tukey HSD: $p = 0.016$ and $p = 0.007$, respectively). The abundance of decapods ($F_{1,12} = 0.14$, $p = 0.714$), tanaids ($F_{1,12} = 3.58$, $p = 0.083$), and isopods ($F_{1,12} = 0.002$, $p = 0.970$) did not differ between pH zones in the seagrass in November, although when data was pooled for all months, decapod abundance decreased in response to acidification ($F_{1,36} = 6.30$, $p = 0.017$). Many of the taxonomic groups that increased in abundance in response to acidification in the *Posidonia oceanica* showed a similar (although non-significant) increase in the rocky reef habitat (Fig. 5.2). Similarly, decapods abundance decreased in response to acidification in the rocky reef (from 271 in control stations to 102 in acidified stations), although this decrease was not significant. No groups of taxa showed a significant difference in abundance between mean pH 8.1 and 7.8 in the rocky reef. Similarly the total invertebrate abundance did not differ between pH zones in the rocky reef ($F_{1,12} = 1.63$, $p = 0.225$), whilst a significant increase in the abundance of *P. oceanica*-associated invertebrates was observed in acidified zones both in November ($F_{1,12} = 39.60$, $p < 0.001$), and across all months ($F_{1,36} = 36.67$, $p < 0.001$).

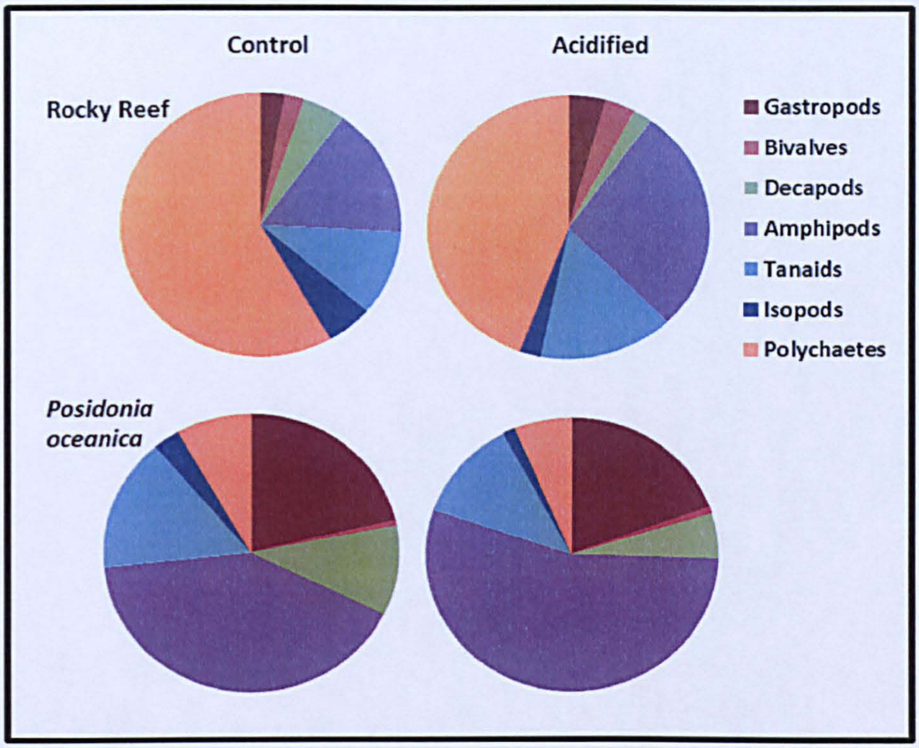


Figure 5.3 Proportion of each taxonomic group collected at control and acidified stations in *Posidonia oceanica* and rocky reef habitat at the Castello Aragonese.

In November the proportions of each group of taxa within the rocky reef and *P. oceanica* habitats were different. The rocky reef was dominated by polychaetes, whilst *P. oceanica* was dominated by amphipods (Fig. 5.3). The proportion of polychaetes decreased in both habitats, from 8 to 6% in *P. oceanica* and from 58 to 44% in rocky reef habitat. The proportion of amphipods increased in each habitat, from 15 to 28% in rocky reef and from 41 to 55% in *Posidonia oceanica*. The proportion of decapods and isopods decreased in both habitats.

5.3.2 Multivariate comparisons

Invertebrate community structure in *Posidonia oceanica* differed between pH zones, although a significant 'site x pH' interaction was observed (Table 5.2). Pair-wise tests showed that differences in community structure between pH zones in *P. oceanica* were significant for both the south (Pair-wise test: t = 2.46, p = 0.023) and the north (Pair-wise test: t = 2.06, p = 0.032) side of the Castello Aragonese, suggesting that this interaction was due to greater differences in structure occurring between control and acidified zones in the south (Fig. 5.4a). Acidification significantly affected invertebrate community structure in the rocky reef (Table 5.2). Invertebrate community composition showed significant differences between control and acidified zones in both *Posidonia oceanica* and the rocky reef (Table 5.2), although a 'site x pH' interaction was observed for community composition in the rocky reef, as significant differences between pH zones were observed for the south (Pair-wise test: t = 1.68, p = 0.042), but not the north (Pair-wise test: t = 1.34, p = 0.09). Multivariate dispersion in community structure and composition was more variable between rocky reef invertebrate samples than between *P. oceanica* invertebrate samples (Fig 5.4).

Table 5.2 Results of community analysis for the effects of acidification on invertebrate populations collected on the rocky reef and in the *Posidonia oceanica* at the Castello Aragonese. Community structure and community composition were analysed using a two-factor PERMANOVA of site (north and south: fixed factor), and pH (control and acidified: fixed factor). Significant results are highlighted in red.

		Rocky reef		***Posidonia oceanica***	
	df	**Pseudo-F**	**P_{PERM}**	**Pseudo-F**	**P_{PERM}**
Community structure					
Site	1	3.65	**0.001**	3.87	**0.001**
pH	1	2.28	**0.026**	8.21	**0.001**
Site x pH	1	1.70	0.058	2.24	**0.006**
Community composition					
Site	1	2.47	**0.009**	2.71	**0.001**
pH	1	2.67	**0.022**	3.28	**0.002**
Site x pH	1	1.78	**0.049**	1.04	0.435

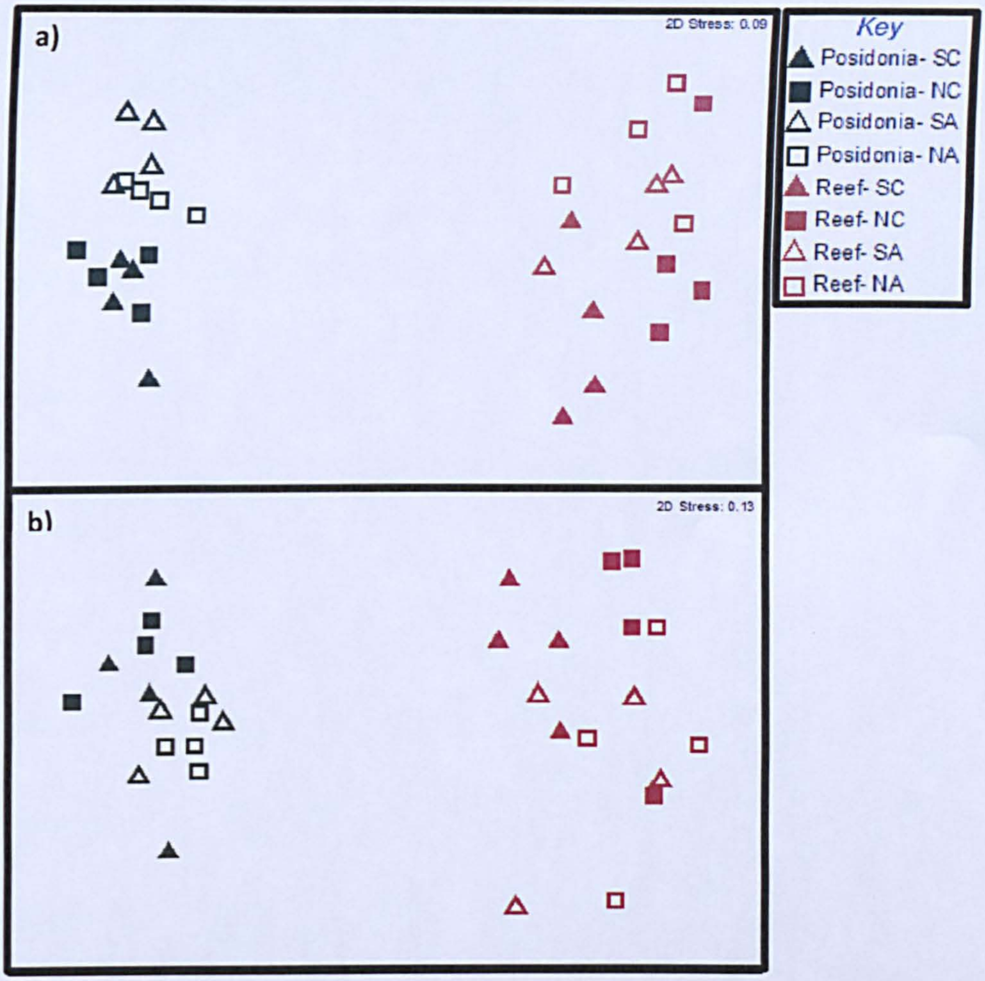


Figure 5.4 MDS ordination of invertebrate a) community structure and b) community composition of invertebrate assemblages in rocky reef and *Posidonia oceanica* habitat at different sites (south and north) and pH zones (control and acidified). SC = south control, NC = north control, SA = south acidified, NA = north acidified.

5.3.3 Comparisons of responses between habitats

The response of different taxonomic groups to acidification was dependant on habitat type. A greater percentage of families decreased in response to acidification in the rocky reef, whilst a greater percentage of families increased in response to acidification in *P. oceanica*, although this was not observed across all taxa (Table 5.3). The abundance of many families of decapods (23.5 and 50%), isopods (18% and 71.5%) and polychaetes (14% and 33%) decreased in both *P. oceanica* and rocky reef habitat in response to acidification, whilst the abundance of amphipod families increased in response to acidification in both habitats (53.5% and 25% respectively).

Table 5.3 The number and percentage (given in brackets) of families that decreased, showed no trend, increased or were rare (present in < 4 samples) *in P. oceanica* (November 2011) and the rocky reef (November 2008) for each taxonomic group. The total number of families for each taxonomic group, collected in each habitat is indicated.

		Decreased	**No trend**	**Increased**	**Rare**	**Total**
Gastropod	*P. oceanica*	5 (19)	8 (31)	3 (11.5)	10 (38.5)	26
	Rocky reef	1 (8.5)	5 (41.5)	1 (8.5)	5 (41.5)	12
Bivalve	*P. oceanica*	2 (16.5)	3 (25)	1 (8.5)	6 (50)	12
	Rocky reef	1 (11)	3 (33.5)	1 (11)	4 (44.5)	9
Decapod	*P. oceanica*	4 (23.5)	3 (17.5)	1 (6)	9 (53)	17
	Rocky reef	5 (50)	1 (10)	0 (0)	4 (40)	10
Amphipod	*P. oceanica*	0 (0)	8 (28.5)	15 (53.5)	5 (18)	28
	Rocky reef	1 (5)	9 (45)	5 (25)	5 (25)	8
Tanaid	*P. oceanica*	0 (0)	1 (25)	2 (50)	1 (25)	4
	Rocky reef	1 (20)	2 40)	1 (20)	1 (20)	5
Isopod	*P. oceanica*	2 (18)	4 (36.5)	2 (18)	3 (27.5)	11
	Rocky reef	5 (71.5)	2 (28.5)	0 (0)	0 (0)	7
Polychaete	*P. oceanica*	4 (14.5)	10 (35.5)	5 (18)	9 (32)	28
	Rocky reef	6 (33.5)	8 (44.5)	0 (0)	4 (22)	18
Total	*P. oceanica*	17 (13.5)	37 (29.5)	29 (23)	43 (34)	126
	Rocky reef	20 (24.5)	30 (36.5)	8 (10)	24 (29)	82

Comparisons of the responses of individual families to acidification revealed that although a majority exhibited the same response in both habitats: 7 increased, 11 showed no trend and 6 decreased. Nine families increased in abundance in *P. oceanica* in response to acidification but showed no trend in reef habitat, whilst only one family increased in abundance on the reef but showed no trend in *P. oceanica*. Six families decreased in abundance in response to acidification on the reef but showed no trend in *P. oceanica* whilst the opposite was observed for only two families. Four families increased in *P. oceanica* in response to acidification, but decreased in the reef habitat (Fig. 5.5). The effect of acidification at both habitats could not be compared for 93 families as they were either rare or absent in either *P.oceanica* or the rocky reef.

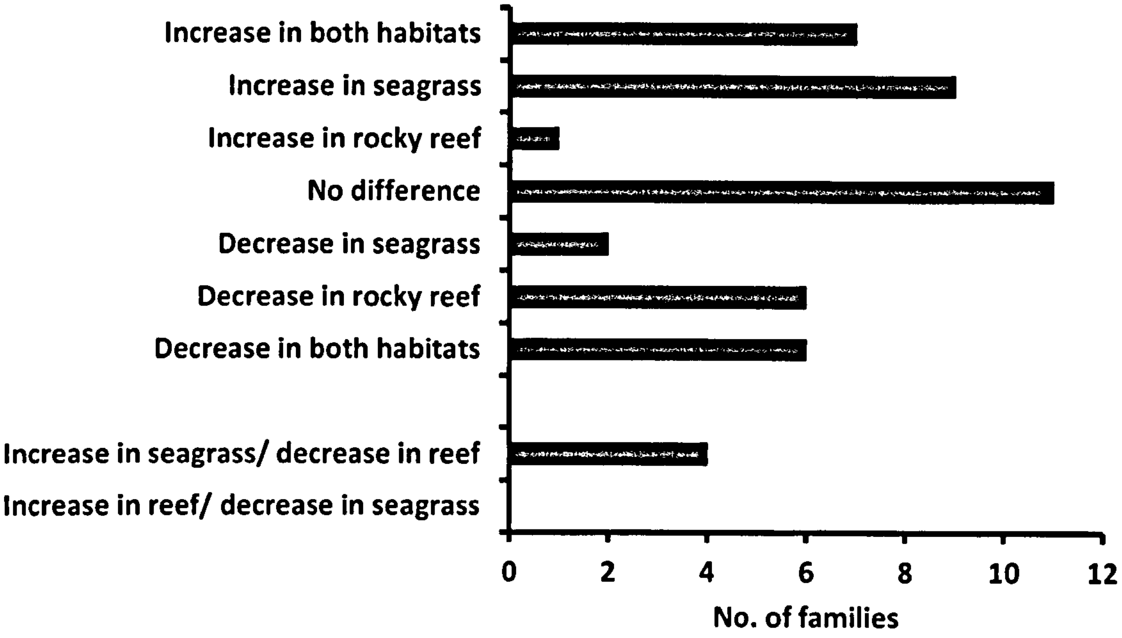


Figure 5.5 Comparison of the individual response of each family to acidification in rocky reef and *P. oceanica* habitats. Families which were either rare or absent in either habitat (93) were excluded.

5.4 Discussion

Comparison of invertebrate communities between control and acidified stations show that acidification lead to similar responses in both rocky reef and *P. oceanica* habitats. The abundance of amphipods, gastropods, bivalves and tanaids were greater, and the abundance of decapods and isopods less in acidified stations in both habitats. The only taxa which showed a differential response between rocky reef and *P. oceanica* habitat was polychaetes. Whilst the number of polychaetes increased in acidified stations in *P. oceanica*, a decrease was observed on the rocky reef in response to acidification.

Though a similar trend was observed between habitats, more negative effects of acidification were apparent in the rocky reef than *P. oceanica*. The abundance of 25% of common families decreased in response to acidification in the rocky reef, although this decrease was not reflected in the number of individuals collected between pH zones (4,688 and 4,506, respectively). This is most likely due to the increase in abundance of a few 'acid-tolerant' taxa (10% of families increased in abundance). In comparison, only 13.5% of families suffered a decrease in response to acidification in *P. oceanica*, whilst 23% experienced an increase in abundance. This lead to more than double the number of invertebrates collected in acidified in comparison to control stations (5,277 and 11,651, respectively) in *P. oceanica*. Some families decreased in response to acidification in the rocky reef, whilst an increase in abundance was observed in *P. oceanica*, although this may be due to both 'tolerant' and 'sensitive' species occurring within the same families.

The effect of short-term reduced pH experiments has shown a high degree of variation in species' abilities to tolerate a drop in pH (Garrard et al. 2013). Meta-analytic methods have provided useful in attempting to show a biological trend, although these have led to debates regarding the extent to which OA may impact marine organisms (Dupont et al. 2010a; Hendriks and Duarte 2010; Hendriks et al. 2010; Kroeker et al. 2010; Andersson and Mackenzie 2011; Kroeker et al. 2011a). It is generally accepted that highly calcified groups of taxa, such as molluscs, more often exhibit negative responses to low pH (Kleypas et al. 2006; Fabry et al. 2008; Doney et al. 2009a; Kroeker et al. 2010), whilst less calcified (e.g. crustaceans) or non-calcified groups of taxa (e.g. anemones), are more resilient to, or even benefit from, a decrease in pH (Kroeker et al. 2010; Suggett et al.

2012). For example, in a multi-species experiment, calcification rates in seven out of nine species of molluscs decreased with a decrease in aragonite saturation state, whilst calcification in all three species of decapods increased (Ries et al. 2009).

At pH levels predicted for the end of this century (~7.8: Caldeira and Wickett 2005), laboratory experiments show many invertebrate species to be negatively impacted (Kurihara 2008). Rather than support this, these results suggest that the majority of benthic invertebrates, including those that are heavily calcified, will be resilient to future levels of OA, at least in these shallow water, biogenic, coastal habitats. Of the 46 families which commonly occurred in both *P. oceanica* and the rocky reef, only five decreased in abundance in acidified zones in both habitats: hermit crabs from the families Diogenidae and Paguridae, isopods from the family Joeropsididae, and polychaetes from the families Dorvilleidae, Ophelidae and Serpulidae. Whilst hermit crabs are heavily calcified, both their exoskeleton, and their salvaged gastropod shell homes, isopods and polychaetes (except for Serpulidea) show a lesser degree, or no calcification, and yet a decrease in their abundance in both habitats in response to acidification, suggests a possible physiological intolerance to acidification. No common families of gastropod or bivalves showed a decrease in abundance in acidified zones in both habitats. It must be taken into consideration that Kroeker et al. (2011b), investigated not only the two pH stations mentioned here (control: pH 8.1, acidified: pH 7.8) but an extremely low pH zone (mean pH 7.2 and 6.6 in the north and south respectively) in the rocky reef habitat. At the extremely low pH both gastropods and decapods suffered an extreme population crash.

Nearshore habitats show strong diurnal pH variability, due to community photosynthesis and respiration (Hofmann et al. 2011). This variation is generally stronger in shallow than deeper waters (Invers et al. 1997; Silva and Santos 2003; Price et al. 2012). pH within *P.*

oceanica meadows can vary between 0.15-0.7 unit diurnally (Frankignoulle and Disteche 1987; Frankignoulle and Bouquegneau 1990; Invers et al. 1997), whilst similar diurnal pH changes (0.1->1.0 unit) have been recorded in other shallow-water subtidal habitats such as benthic macroalgal habitats (Middelboe and Hansen 2007), other species of seagrass (Invers et al. 1997; Silva and Santos 2003; Marbà et al. 2006; Yates et al. 2007), coral reefs (Ohde and van Woesik 1999; Price et al. 2012; Burdett et al. 2013), and kelp forests (Hofmann et al. 2011). For example, if a shallow water habitat has a mean pH of 8.1, pH may vary by as much as between 7.6 and 8.6 throughout a 24 hour period. This high degree of pH variability means that organisms may be used to dealing with low pH, therefore organisms living in these productive coastal habitats may be more robust to changes in pH than previously expected.

pH within seagrass diffusion boundary layers can be over 1 unit higher than the surrounding seawater (Jones et al. 2000). A similar increase in pH has been found at the surface of macroalgae (Cornwall et al. 2013). Increased pH at the boundary layers of macroalgae and seagrass may provide relief from acidification to species of invertebrates which closely associate with photosynthetic material, further increasing a species' ability to cope with OA.

Many groups of taxa showed a significant, and large, increase in their abundance in acidified zones in *P. oceanica*, suggesting that not only will many associated invertebrate species be tolerant of future OA, but indirect effects such as a decrease in predation or an increase in food availability, may be beneficial to some species (discussed in Chapter 4). This increase in abundance was not observed in the rocky reef, showing that the indirect effects of OA (e.g. shoot density), that lead to facilitative interactions, may be seagrass-specific. The increase in *P. oceanica* shoot density observed in acidified stations (Chapter

3), may alter competitor or predator-prey interactions (Orth et al. 1984). Invertebrate abundance has been shown to increase with increasing shoot density (Homziak et al. 1982; Bostrom and Bonsdorff 2000). This may be due to 1) reduced predation efficiency (Coen et al. 1981), 2) increased epiphytic trophic resources (Bologna and Heck 1999), or 3) invertebrate selection for denser meadows (Bell and Westoby 1986a). A second consideration is that the increase in abundance may be partially due to a loss of top down control, as there was a significant decrease in predatory decapods (Chapter 4). Whilst many taxa on the rocky reef showed similar changes in abundance in response to acidification, polychaetes showed a differential response between habitats. They decreased in the rocky reef and increased in *P. oceanica.* On the rocky reef, benthic community structure enters a phase-shift towards a community dominated by fleshy turf algae in response to OA, with a loss of calcifying seaweeds, filter feeders and sponges (Kroeker et al. 2012, Kroeker et al. 2013). This shift in habitat structure may lead to negative impacts for polychaetes.

These results suggest that, in highly productive, shallow-water habitats the majority of benthic invertebrates will be robust to changes in pH expected for the end of this century, although this may only hold true for habitats formed by non-calcifying photoautotrophs, such as seagrass beds, kelp forests and algal turfs. Calcifying ecosystem engineers such as corals are negatively affected by acidification (Fabricius et al. 2011), which is likely to lead to negative impacts on the associated reef community. Tolerance to changes in pH may be due to their daily exposure to pH changes, as photosynthesis/respiration rates change, or due to localised relief from acidification through high photosynthetic activity (Hendriks et al. 2013). An increase in seagrass density in response to low pH may actually provide an advantage to many species of associated invertebrate (Orth et al. 1984).

It is suggested that loss of net autotrophic status of seagrass meadows may lead to a loss of the buffering capacity of seagrass against future acidification. Whilst healthy seagrass beds are generally net autotrophic (Murray and Wetzel 1987; Unsworth et al. 2012), they can become net heterotrophic through anthropogenic and climatic stresses such as fish farming (Apostolaki et al. 2011) or extreme sea surface temperatures (Marsh Jr et al. 1986). Rates of seagrass decline have accelerated to 7% globally, per annum, leading to a loss of 29% of areal extent since seagrass records began in the 1800s (Waycott et al. 2009). Although in some areas seagrass extent is increasing, this increase is small in comparison to the areas lost globally (Waycott et al. 2009). In order to mitigate impacts of future changes in carbonate chemistry, it is important that highly productive coastal habitats such as seagrass beds are conserved.

6. Acidification increases grazing pressure of a key herbivore on *Posidonia oceanica* at natural CO_2 vents

6.1 Introduction

Anthropogenic release of CO_2 has led to the oceans acidifying at a much faster scale than has been recorded over the past 250 million years (Ridgwell and Schmidt 2010), posing possibly one of the greatest anthropogenic threats to marine ecosystems (Halpern et al. 2008). For this reason, a great deal of research has focused on ocean acidification (OA) so that we may gain a comprehensive understanding of this threat. Further general information on OA can be found in Chapter 1. The majority of OA research focuses on the impacts of acidification on physiological processes such as calcification (Ries et al. 2009), growth (Gooding et al. 2009), metabolic rate (Ellis et al. 2009), and larval development (Arnold et al. 2009; Clark et al. 2009) of specific species; however the information on the effect of OA on species interactions, food webs and ecosystems is limited.

There is evidence that predator-prey interactions will be altered under OA through changes in the abundance of predator or prey species (such as a decline in the abundance of the pteropod species *Limacina helicina*, Lischka et al. 2011), or due to changes in defensive ability of prey (such as the loss of induced defences in the mollusc *Littorina littorea*, Bibby et al. 2007) or reduced activity levels of predators (such as the jumbo squid *Dosidicus gigas*, Rosa and Seibel 2008). Juvenile reef fish exposed to OA conditions suffer greater predator-related mortality (Munday et al. 2010; Ferrari et al. 2011a). A loss of their ability to sense predatory olfactory cues has been shown (Munday et al. 2009; Dixson et al. 2010; Munday et al. 2010), which may lead to a loss of predator-avoidance measures.

Alongside impacts on fish and invertebrates, community composition of primary producers will change under future OA conditions (Wootton et al. 2008; Porzio et al. 2011; Kroeker et al. 2013b), through a loss of calcifying species (Kuffner et al. 2008) or a change in competitive dynamics (Kroeker et al. 2013b). This will have knock-on effects for herbivores. Furthermore, there is concern that some species of diatoms, which are an important food source for copepods, show a reduction in fatty acid composition, and therefore food quality, as pH declines (Rossoll et al. 2012).

Posidonia oceanica is an important habitat forming species in the Mediterranean, delivering a number of ecosystem functions (Duarte 2000), whilst providing habitat for many organisms (reviewed in Chapter 2). *P. oceanica* is expected to benefit from increasing CO_2 (Chapter 3, Hall-Spencer et al. 2008), although leaf cover of calcareous epiphytes, particularly coralline algae, will decrease (Martin et al. 2008). *P. oceanica* is nutritionally poor, with a high C:N ratio, due to the large quantity of structural carbohydrates and low protein content (Lawrence et al. 1989). It produces a high quantity of phenolic compounds which deter grazing (Buia et al. 2004). Hence, relatively few species feed on the seagrass directly (Verlaque 1981; Mazzella et al. 1992). The primary consumers of the seagrass are the fish *Sarpa salpa*, the echinoderm *Paracentrotus lividus*, peracarid crustaceans and decapods (Buia et al. 2004). Peracarid crustaceans and decapods generally have a low impact in terms of grazing pressure and most studies focus on *S. salpa* and *P. lividus* (Pinna et al. 2009), which exert the greatest grazing pressure in beds <10m depth (Tomas et al. 2005c).

Although the numbers of herbivores of *P. oceanica* are low, *S. salpa* and *P. lividus* can exert extremely high grazing pressure, in some cases exceeding leaf production (Prado et al. 2008b). This is particularly true for summer months when *S. salpa* migrate to shallow waters to feed (Tomas et al. 2005c). During these months *S. salpa* can be found in high abundances in shallow *P. oceanica* beds (Francour 1997), where they feed on the seagrass and build up reserves for reproduction, but return to deeper waters during the colder months (Peirano et al. 2001). In contrast *P. lividus* reaches its maximum consumption rates during the winter (Peirano et al. 2001). Grazing by *S. salpa* shows high spatial variation and a high grazing pressure from these fish is not experienced in all meadows (Prado et al. 2007b).

Although these species feed on the seagrass itself, there is some speculation on the degree of seagrass carbon assimilation. Some research suggests that diets are primarily epiphyte-based for both *S. salpa* and *P. lividus* (Dauby 1993; Tomas 2004), although direct observations suggest that epiphytic consumption is less important for the fish *Salpa sarpa* (Tomas et al. 2005b).

In the naturally acidified waters surrounding the Castello Aragonese (Ischia, Italy) there appears to be exceedingly high grazing pressure, particularly in summer months (pers. obs.). An experiment was therefore designed to quantify grazing pressure in control and acidified zones to determine whether grazing pressure was enhanced; 1) annually and 2) over a temporal scale incorporating periods of high grazing for both species (*P. lividus* and *S. salpa*).

One of the limitations to working in a naturally acidified system is that it can be difficult to determine whether interactions or changes in community composition are related to the direct or indirect effects of acidification (Barry et al. 2010). This said, it is possible to examine a range of explanatory variables to determine whether these variables are correlated to changes in grazing pressure. Although there is a possibility that increased grazing pressure of *S. salpa* is due to the fish being attracted to areas where the pH is reduced, a more likely explanation is that acidification-induced changes to the plant structural characteristics (Chapter 3) or changes to the associated epiphytic coverage (described in this chapter) increase the palatability of the seagrass blades. Changes in these variables may act as an attractant or repellent to grazing, leading to indirect effects of acidification. If this is the case then significant correlations should be found between these variables and grazing pressure. Constructing a data matrix of grazing pressure and corresponding data on C:N ratio of the leaves, seagrass density, height of the canopy, the % cover of epiphytic algae and the % cover of epiphytic animals on the *P. oceanica* leaves would help to examine the variables that might show the greatest relationship to grazing pressure.

6.2 Methods

This study was conducted at the control and acidified stations at the north and south of the Castello Aragonese (Chapters 3, Fig. 3.1). Control and acidified stations are present in the same meadow, therefore *S. salpa* and *P. lividus* have equal opportunity to graze from both stations and increased grazing should be solely down to preference. pH was measured by collection of 10 samples sporadically between April 2011 and August 2013 ($N = 320$ for southern stations and 300 for northern stations), whilst 10 samples were collected for total alkalinity (TA) as a single point measurement on the 4/11/11. This was considered sufficient, as TA has shown very little variation at sites at the Castello Aragonese between studies conducted at different times of the year (e.g. Hall-Spencer et al. 2008; Cigliano et al. 2010; Rodolfo-Metalpa et al. 2010). Methods of analysis of pH, TA and calculation of carbonate chemistry variables are described in section 3.2.

The control and acidified stations used in this study at the north and south of the Castello Aragonese are reasonably small (~60 m^2). This size of station was defined during preliminary investigation of pH variation along the gradient (Chapter 1.4). Previous studies of macroherbivore grazing pressure have involved quantification of macroherbivore abundance and/or harvesting of shoots to examine them for herbivore bite marks (e.g.

Alcoverro et al. 1997; Tomas et al. 2005b; Tomas et al. 2005c; Prado et al. 2007b). If stations are to be left intact for future research then destructive research practices must be avoided. *Sarpa salpa* and *Paracentrotus lividus* leave conspicuous bite marks on the blade of *P. oceanica* leaves that can be quantified by visual analysis (Fig. 6.1), therefore an *in situ* observational study, using visual census to quantify grazing pressure, leaf and epiphyte characteristics was developed. Surveys of grazing pressure were conducted each year in June between 2011 and 2013 and further surveys in April and August of 2013. Sampling in 2013 was conducted at the beginning of each month, during times of cold water (early spring: April), mid water temperatures (late spring: June) and warm water (summer: August). This timing was adopted to determine if patterns observed for macro-herbivore grazing pressure were long-term trends or responses solely based on the current year, and to examine how grazing pressure changed from cold to warm water months. Abundances of *P. lividus* were also surveyed. Due to the small size of each station and schooling nature of *S. salpa*, it was not possible to obtain enough replication to quantify their abundance, although photographs were taken to show the high abundance of *S. salpa* that could be found grazing in stations.

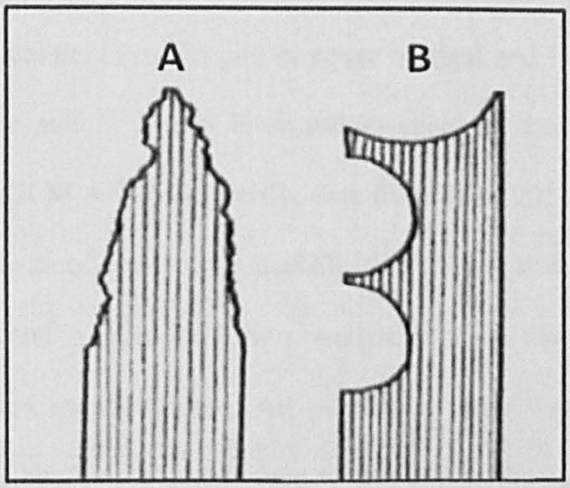


Figure 6.1 Bite marks of a) *Paracentrotus lividus*, and b) *Sarpa salpa* (From Buia et al. 2004).

Each station was located at a depth of 2.5- 3.5 m. Mean density of *P. oceanica* was greater in acidified than control stations (Chapter 3, Fig. 3.3) with mean densities of 372 and 446 for the north and south control respectively, whilst densities of 587 and 815 were found for the north and south acidified stations. Twenty four 40 x 40 cm quadrats were randomly placed at each station. In each quadrat the number of *P. lividus* was recorded. In four of the quadrats the intermediate and adult leaves of 7 shoots were sampled. Each of these four quadrats was located in each quarter of each station to ensure spatial variability within each station was taken into consideration (similar to sampling in Chapter 4). Juvenile and small intermediate leaves (<10 cm) were not grazed (pers. obs.) and therefore excluded from the analysis. The length of the selected leaf was measured, and the % cover of algal and invertebrate epiphytes estimated for both sides of each leaf. The occurrence, on a presence/absence basis, of mechanical damage and bite marks (for each herbivore: *S. salpa*, *P. lividus*) was noted. Mechanical damage (fracture and loss of the leaf tip) occurs through abiotic factors, such as waves and currents (de los Santos et al. 2012). Bite marks from other species were not included as their presence was minimal (<1%).

For each shoot the percentage of leaves with mechanical damage, bite marks of *S. salpa*, and *P. lividus* were calculated. Leaf length, % cover of algal and faunal epiphytes, and % of leaves with *S. salpa* and *P. lividus* bites and mechanical damage were analysed by means of a three-way GLM ANOVA. Firstly, data from June 2011, 2012 and 2013 were analysed to test the effects of site (fixed), acidification (fixed) and year (fixed). Secondly data from April, June and August 2013 were analysed to test the effects of site (fixed), acidification (fixed) and month (fixed). All percentage data was arcsine transformed, whilst leaf length was Log (X + 1) transformed to conform to the assumptions of ANOVA (Underwood 1997). The Kruskal-Wallis non-parametric test was used to analyse the abundance of *P. lividus* as data was highly skewed and variance not homogenous (Underwood 1997). Kruskal-Wallis tests analysed differences between stations, months and years, and post-hoc comparisons were made by using the non-parametric multiple comparison test (MCT).

To analyse relationships between *S. salpa* and *P. lividus* grazing pressure with *P. oceanica* variables, a data matrix was constructed using data collected between May and June 2011 for this thesis. The percentage of leaves with *S. salpa* and *P. lividus* bites were considered against possible explanatory variables of *P. oceanica* condition: C:N ratio of the leaves, seagrass density, height of the canopy (Chapter 3), the % cover of epiphytic algae and the % cover of epiphytic animals on the *P. oceanica* leaves (this Chapter). Height of the canopy was chosen as an explanatory variable rather than leaf length, as the latter is likely to be, at least partially, dependent on the quantity of *S. salpa* bites since they feed from the leaf apex reducing the length of the leaf. Height of the canopy, on the other hand, is the longest leaf length within a 40 x 40cm quadrat, and therefore should be less susceptible to grazing pressure unless all leaves are grazed. High grazing pressure does not occur until the summer months (Tomas et al. 2005c), so this should not be an issue. Pearson's correlation coefficients of the explanatory variables showed multicollinearity between many of the variables, therefore multiple regression was not used, as it can lead to inaccurate model parameterization (Graham 2003). Graphical representation of data for grazing pressure against *P. oceanica* variables showed evidence linear correlations; therefore Pearson's correlation coefficients were carried out between *P. oceanica* variables and the percentage of leaves with *S. salpa* and *P. lividus* bites. Where multicollinearity exists between explanatory variables, principal components analysis (PCA) can be used to identify the linear combination of variables that account for the variations in the observations of explanatory variables (Graham 2003). To test which *P. oceanica* explanatory variables most closely correlated with *S. salpa* grazing, PCA was conducted on significant variables, using component loadings of individual variables. As crossvalidation lead to only two principle components, and, as the first principle component captured the majority of variance we focussed our analysis on this component. To determine the strength of the relationship between *S. salpa* grazing and explanatory variables, linear regression of the proportion of leaves with *S. salpa* bites per shoot and PC1 eigenvectors was conducted (Wootton et al. 2008). Variables were added and deleted in a stepwise fashion for PCA analysis until PC1 linear regression reached maximal values. Statistical analyses were conducted using Statistica 8.

6.3 Results

6.3.1 Carbonate chemistry

Mean pH values showed high degree of fluctuation at acidified stations (Fig. 6.2). Whilst mean pH values observed during 2011 at the southern acidified station were maintained through 2012 and 2013, the degree of acidification at the northern acidified station appeared to decrease over the three years. Mean pH at the southern acidified station was 7.78 in 2011 and maintained a mean pH of 7.77 across the three years, whilst mean pH at the northern station was 7.82 in 2011 but increased to 7.88 across the three years. The southern acidified station fell below a pH of 8.0 (the value considered as being acidified) 67% of sampling periods in 2011 and 71% of sampling periods in 2012-13, whilst the northern acidified station fell below a pH of 8.0 for 55% of sampling periods in 2011 and 43% of sampling periods in 2012-13, leading to higher mean pH values. Carbonate chemistry was highly variable at acidified stations (Table 6.1).

Table 6.1 Mean ± SD seawater carbonate chemistry. Temperature (13-29°C throughout study period, mean = 21.1°C) was continuously recorded between March 2011 and August 2013 using a data logger. Salinity (37.5-38) was a point measurement taken in May 2011. 10 pH samples were collected from each station periodically between April 2011 and August 2013. Total alkalinity (TA) is a point measurement taken on the 04/10/11. The remaining parameters were calculated using CO_2 SYS programme using the constants of Roy et al. (1993) and Dickson (1990) for KSO4. Total alkalinity (TA) is mmol.kg^{-1}; pH_{NBS} was measured using an NBS scale; and pCO_2 is μatm.

		pH_{NBS}	TA	pCO_2	Ω_{CA}	Ω_{AR}
North	Control	8.13 ± 0.05	2.54 ± 0.04	484 ± 69	4.45 ± 0.45	2.87 ± 0.29
	Acidified	7.88 ± 0.36	2.57 ± 0.02	1509 ± 1906	3.27 ± 1.54	2.11 ± 1.00
South	Control	8.10 ± 0.07	2.54 ± 0.01	531 ± 113	4.25 ± 0.53	2.74 ± 0.34
	Acidified	7.77 ± 0.39	2.55 ± 0.01	2149 ± 3060	2.69 ± 1.56	1.73 ± 1.01

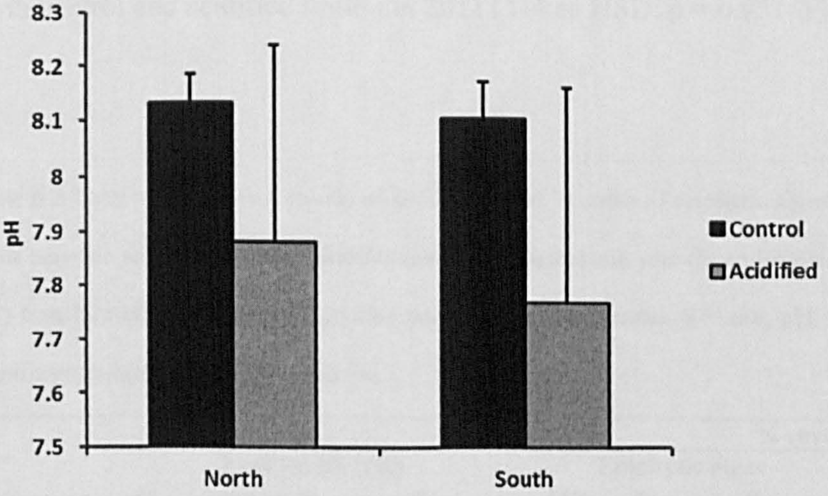


Figure 6.2 Mean pH (± SD) at control and acidified stations at the north and south of the Castello Aragonese.

6.3.2 Comparison of grazing pressure between years.

Epiphytic algal cover significantly decreased under acidified conditions ($F_{1,1545} = 1744.83$, $p < 0.001$), although there was a 'pH x site' interaction (Table 6.2), as although there was a significant decrease in epiphyte cover for both sides (Tukey HSD: p = <0.001); this effect was more pronounced on the south side (Fig. 6.3a). In control stations, the % cover of algae was higher in 2012 than 2011 and 2013 (Tukey HSD: $p < 0.001$ and $p < 0.001$ respectively: 2011 < 2012 > 2013), although this difference was not observed for acidified stations (Fig. 6.3a). The cover of epiphytic fauna was affected by pH ($F_{1,1545} = 43.38$, $p < 0.001$), although there was a 'pH x site' interaction (Table 6.2), as the increase in epiphytic fauna in acidified zones was significant for southern stations but not for northern stations (Tukey HSD: $p < 0.001$ and $p = 0.106$ respectively; Fig. 6.3b). Leaf length was significantly lower in acidified stations ($F_{1,1545} = 197.13$, $p < 0.001$), although there was a 'site x pH x year' interaction as this decrease in leaf length was not observed between the north control and acidified station in 2011 (Tukey HSD: p = 0.951: Fig. 6.3c).

Table 6.2 Three-way ANOVA results of leaf length and % cover of epiphytic algae and % cover of epiphytic fauna between site (fixed factor), acidification (fixed factor) and year (fixed factor). Leaf length was LOG (X + 1) transformed, whilst percentage data was arcsine transformed. S = site, pH = acidification, Y = year. Significant results are highlighted in red.

					% coverage of					
		Leaf length (cm)			Epiphytic algae			Epiphytic fauna		
	df	MS	F	P	MS	F	P	MS	F	P
S	1	0.01	0.17	0.678	5.04	96.33	**<0.001**	0.25	8.33	**0.004**
pH	1	10.09	197.13	**<0.001**	91.21	1744.83	**<0.001**	1.28	43.38	**<0.001**
Y	2	2.44	47.62	**<0.001**	2.51	48.03	**<0.001**	1.89	64.08	**<0.001**
S x pH	1	2.19	42.72	**<0.001**	3.40	64.99	**<0.001**	0.14	4.70	**0.030**
S x Y	2	0.44	8.50	**<0.001**	0.10	1.90	0.151	0.52	17.76	**<0.001**
pH x Y	2	0.45	8.79	**<0.001**	1.31	25.13	**<0.001**	0.07	2.49	0.083
S x pH x Y	2	0.55	10.68	**<0.001**	0.12	2.28	0.102	0.04	1.25	0.287
Err	1,545									

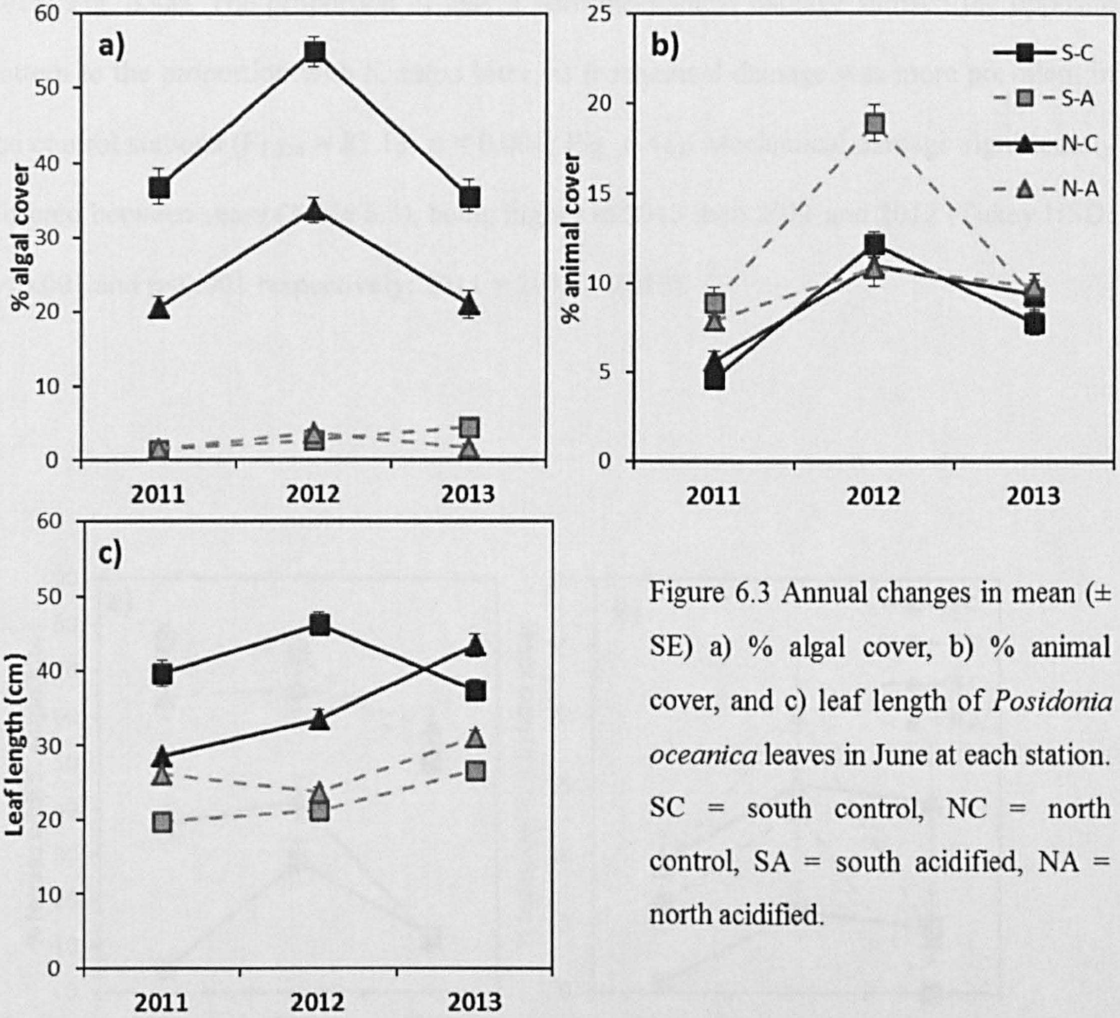


Figure 6.3 Annual changes in mean (± SE) a) % algal cover, b) % animal cover, and c) leaf length of *Posidonia oceanica* leaves in June at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

The number of leaves with *Sarpa salpa* bites was significantly higher in acidified stations ($F_{1,324} = 291.42$, $p < 0.001$) although there was a 'site x pH' interaction (Table 6.3) as, although this effect was significant for both sides (Tukey HSD: $p < 0.001$), the effect of acidification was greater on the south side of the Castello (Fig. 6.4a). In comparison, *Paracentrotus lividus* grazing did not appear to be affected by acidification ($F_{1,324} = 0.46$, $p = 0.497$; Fig. 6.4b), but was higher in the north ($F_{1,324} = 19.30$, $p < 0.001$) and affected by year ($F_{1,324} = 11.89$, $p < 0.001$), with grazing higher in 2012 than 2011 and 2013 (Tukey HSD: p = , 0.001 and $p < 0.001$, respectively; Fig. 6.4b). *S. salpa* grazing was lower in 2013 than other years (Tukey HSD: $p = 0.001$ and $p < 0.001$, respectively; 2011 = 2012 <

2013; Fig. 6.4a). The proportion of leaves with mechanical damage showed the opposite pattern to the proportion with *S. salpa* bites, as mechanical damage was more prevalent in the control stations ($F_{1,324} = 81.13$, $p < 0.001$; Fig. 6.4c). Mechanical damage significantly differed between years (Table 6.3), being higher in 2013 than 2011 and 2012 (Tukey HSD: $p<0.001$ and $p<0.001$ respectively; 2011 = 2012 < 2013).

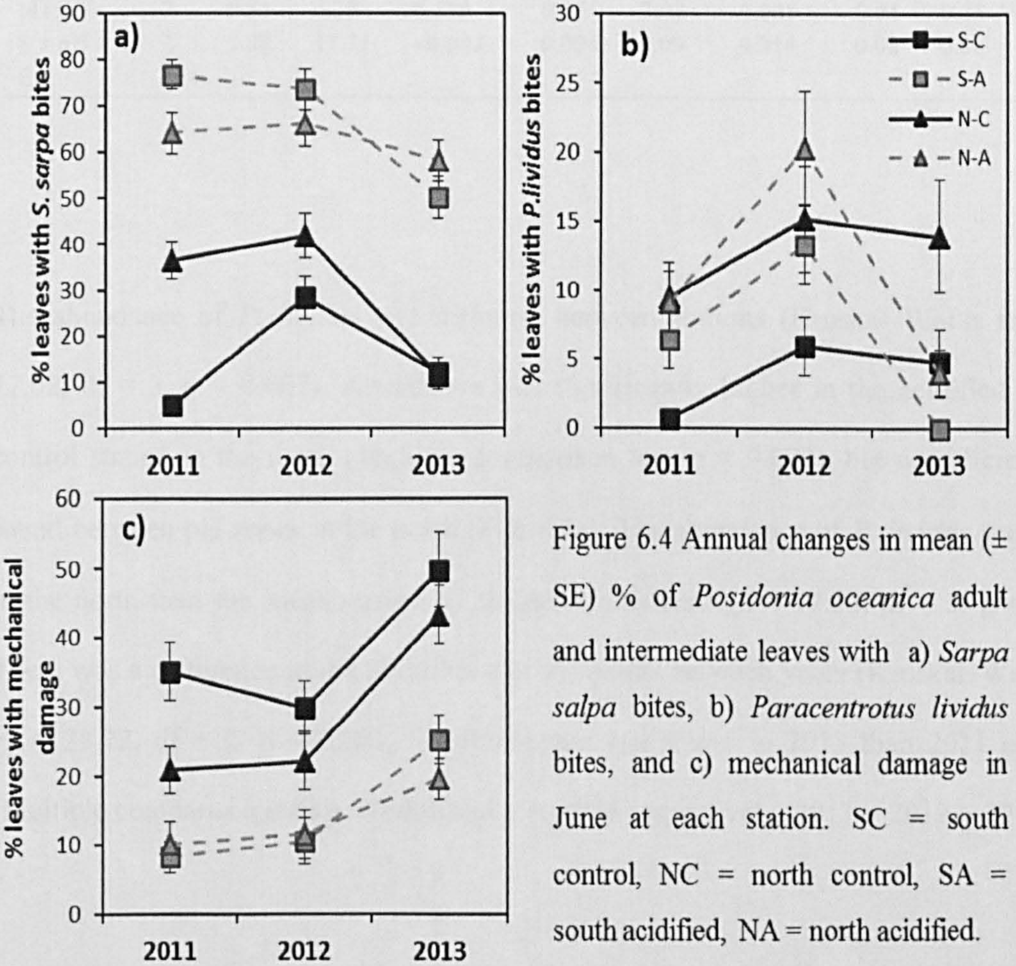


Figure 6.4 Annual changes in mean (± SE) % of *Posidonia oceanica* adult and intermediate leaves with a) *Sarpa salpa* bites, b) *Paracentrotus lividus* bites, and c) mechanical damage in June at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

Table 6.3 Three-way ANOVA results of % of leaves per shoot with: *Sarpa salpa* bites, *Paracentrotus lividus* bites and mechanical damage between site (fixed factor), acidification (fixed factor) and year (fixed factor). All data was arcsine transformed. S = site, pH = acidification, Y = year. Significant results are highlighted in red.

		% leaves with								
		***Sarpa salpa* bites**			***Paracentrotus lividus* bites**			**Mechanical damage**		
	df	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
S	1	0.72	7.56	0.006	1.21	19.30	<0.001	0.42	4.53	0.034
pH	1	27.89	291.42	<0.001	0.03	0.46	0.497	7.49	81.13	<0.001
Y	2	2.51	26.20	<0.001	0.75	11.89	<0.001	2.39	25.93	<0.001
S x pH	1	1.70	17.79	<0.001	0.14	2.23	0.136	0.23	2.44	0.120
S x Y	2	0.13	1.37	0.256	0.008	0.01	0.988	0.001	0.01	0.988
pH x Y	2	0.31	3.28	0.039	0.44	7.02	0.001	0.04	0.45	0.640
S x pH x Y	2	1.08	11.31	<0.001	0.006	0.09	0.914	0.08	0.88	0.417
Err	324									

The abundance of *P. lividus* was different between stations (Kruskal–Wallis test: χ2 = 17.02, df = 3, p = 0.007). Abundance was significantly higher in the acidified than the control station in the south (Multiple comparison test: p = 0.022), but no difference was found between pH zones in the north (Fig. 6.5a). The abundance of *P. lividus* was greater in the north than the south stations (Kruskal–Wallis test: χ2 = 17.33, df = 3, p < 0.001). There was a difference in the abundance of *P. lividus* between years (Kruskal–Wallis test: χ2 = 28.22, df = 2, p < 0.001), as abundance was lower in 2013 than 2011 and 2012 (Multiple comparison test: p < 0.001 and p = 0.036 respectively: 2011 = 2012 > 2013).

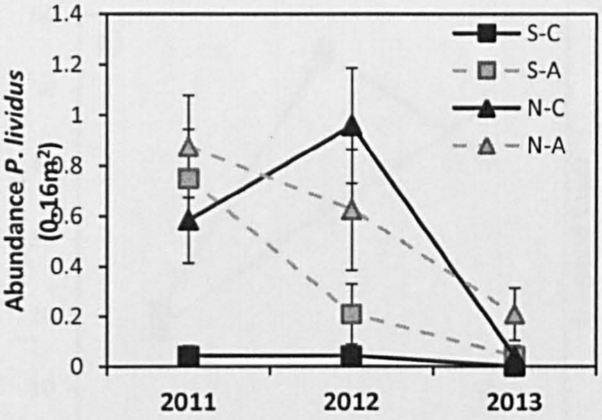


Figure 6.5 Annual changes in mean (± SE) *Paracentrotus lividus* density/0.16 m^2 at each station in June. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

6.3.3 Progression of grazing pressure from cold to warm water months

Epiphytic algal cover was significantly lower in acidified stations (Table 6.4), with a more than 80% reduction in cover found in acidified zones (Fig. 6.6a). This reduction was primarily due to a loss of crustose coralline algae (Fig. 6.7). Algal cover was generally lowest in April and highest in August (Fig. 6.6). The cover of epiphytic fauna was significantly greater in acidified zones (Table 6.4), although there was a 'site x pH x month' interaction, as increased abundance was only observed in certain months, dependent on which site was sampled. Faunal cover was generally highest in June (Fig. 6.6a). Leaf length was highly variable between sites, pH zones and months (Table 6.4). Length was lower in acidified than control zones (Figure 6.6c), although there was a 'site x pH x month' interaction as this difference was not observed in the north side in April (Tukey HSD: 0.988). Leaf length generally increased from April to August in control stations whilst it decreased in length in acidified stations, so that acidified stations appeared as 'mown lawns' in summer (Figure 6.8 - 6.9).

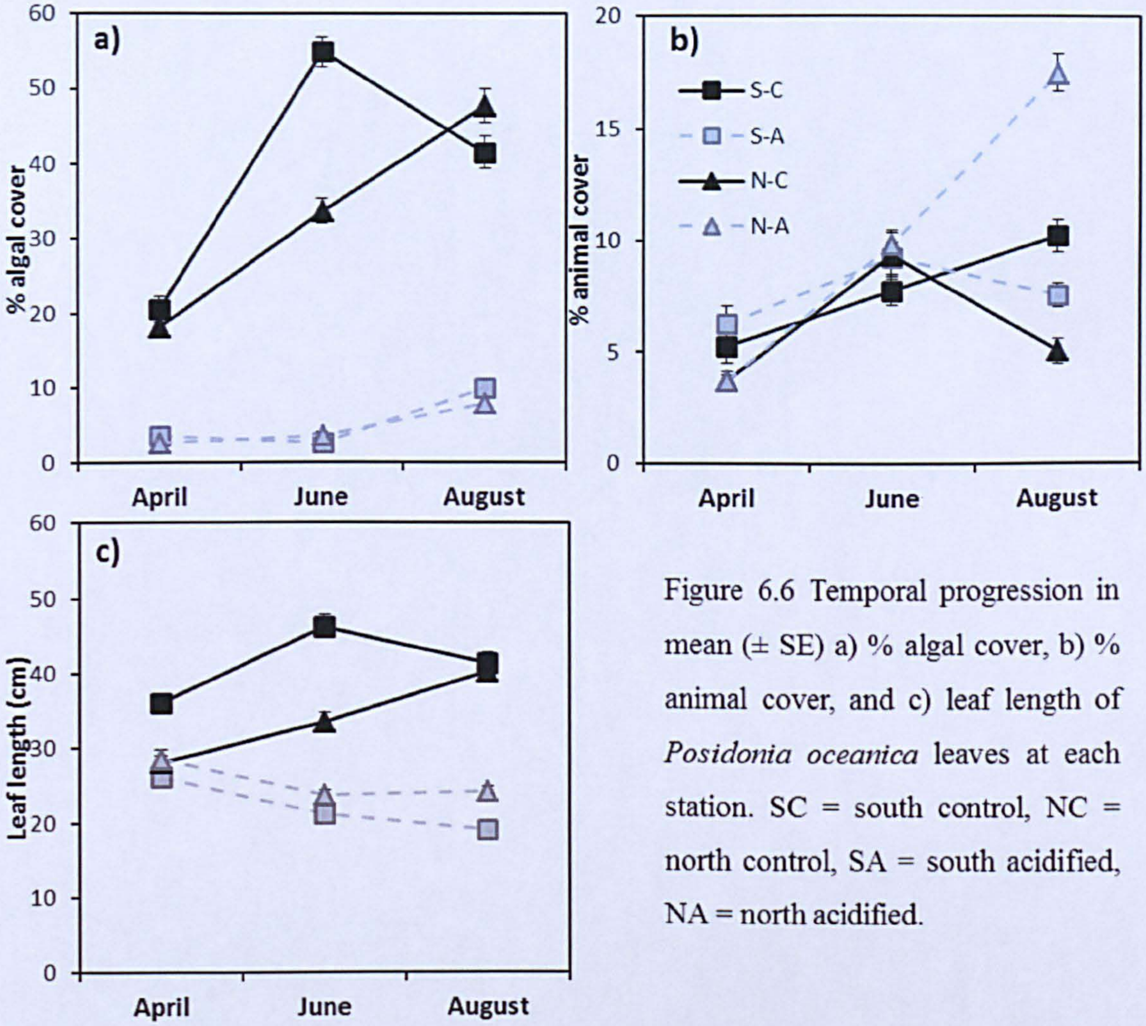


Figure 6.6 Temporal progression in mean (± SE) a) % algal cover, b) % animal cover, and c) leaf length of *Posidonia oceanica* leaves at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

Table 6.4 Three-way ANOVA results of leaf length and % cover of epiphytic algae and % cover of epiphytic fauna between site (fixed factor), acidification (fixed factor) and month (fixed factor). Leaf length was LOG (X + 1) transformed, whilst percentage data was arcsine transformed. S = site, pH = acidification, M = month. Significant results are highlighted in red.

					% coverage of					
		Leaf length (cm)			Epiphytic algae			Epiphytic fauna		
	df	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
S	1	0.12	4.00	0.047	0.77	13.68	<0.001	<0.01	0.01	0.941
pH	1	8.88	289.90	<0.001	59.51	1059.05	<0.001	0.87	30.41	<0.001
M	2	0.72	23.32	<0.001	7.10	126.36	<0.001	3.23	112.64	<0.001
S x pH	1	0.60	19.48	<0.001	0.01	0.25	0.618	0.65	22.62	<0.001
S x M	2	0.50	16.42	<0.001	1.02	18.18	<0.001	0.12	4.18	0.015
pH x M	2	1.14	37.14	<0.001	1.27	22.56	<0.001	0.30	10.35	<0.001
S x pH xM	2	0.12	3.74	0.024	0.33	5.93	0.003	0.89	31.15	<0.001
Err	1,593									

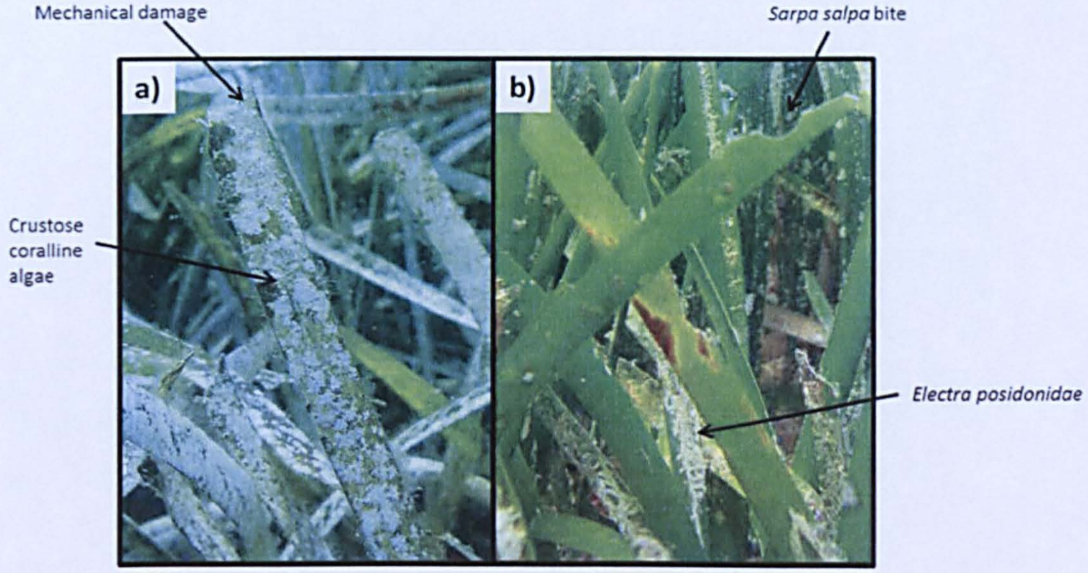


Figure 6.7 *Posidonia oceanica* leaves in a) control and b) acidified stations at the north of the Castello Aragonese, taken the 2nd August 2013.

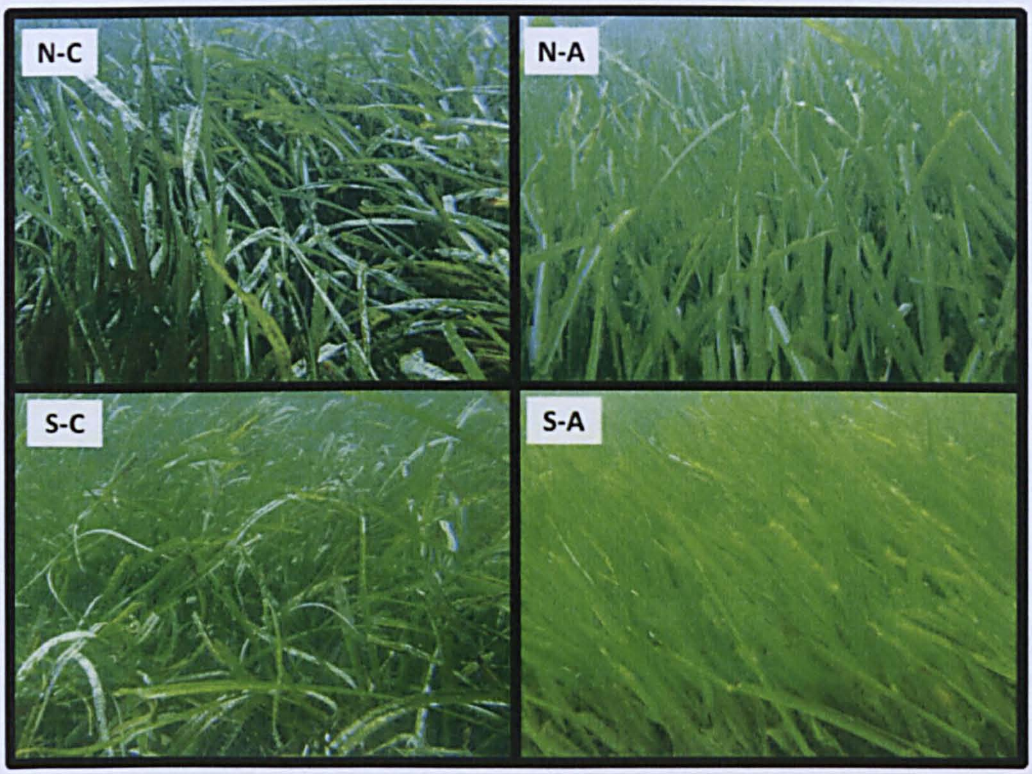


Figure 6.8 Images taken on the 14th May 2013 of control and acidified stations at the north and south of the Castello Aragonese. N-C = north control, N-A = north acidified, S-C = south control, S-A = south acidified.

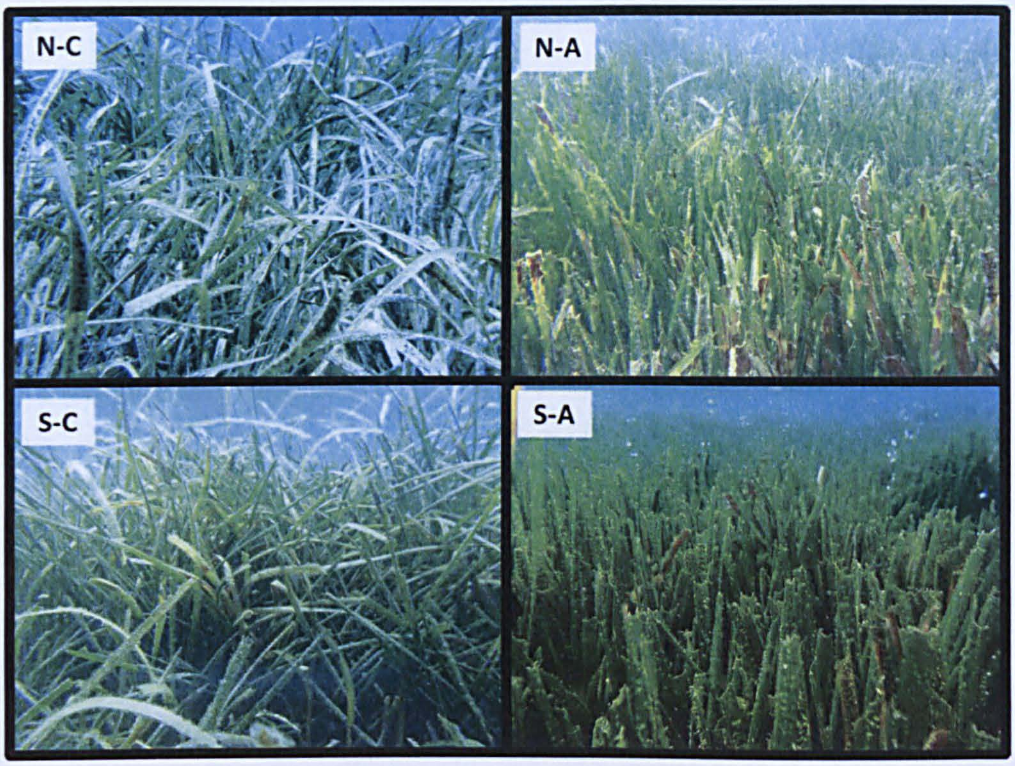


Figure 6.9 Images taken on the 19th July 2013 of control and acidified stations at the north and south of the Castello Aragonese. N-C = north control, N-A = north acidified, S-C = south control, S-A = south acidified.

The proportion of leaves with *S. salpa* bites was more than four times higher in acidified stations (Fig. 6.10a). A 'pH x month' interaction was found (Table 6.5), as, although this difference was significant for all months (Tukey HSD: $p < 0.001$), the difference was greatest in August, as the proportion of leaves with bites increased sharply in acidified stations between April and August (Fig. 6.10a). The proportion of leaves with *Paracentrotus lividus* bites was generally lower in acidified stations (Fig. 6.10b), although no significant decrease was observed. A 'pH x month' interaction was observed (Table 6.5), as in acidified stations the number of *P. lividus* bites was lower in August than April (Tukey HSD: $p = 0.002$). This decrease was not observed in control stations. The proportion of leaves with mechanical damage was significantly higher in control than acidified stations (Fig. 6.10c). There was a 'pH x month' interaction (Table 6.5), as significantly higher proportions of mechanical damage in control stations were only observed in June and August (Tukey HSD: $p < 0.001$). This was due to an increase in mechanical damage between April and August for control stations, whilst the proportion of mechanical damage in acidified stations decreased.

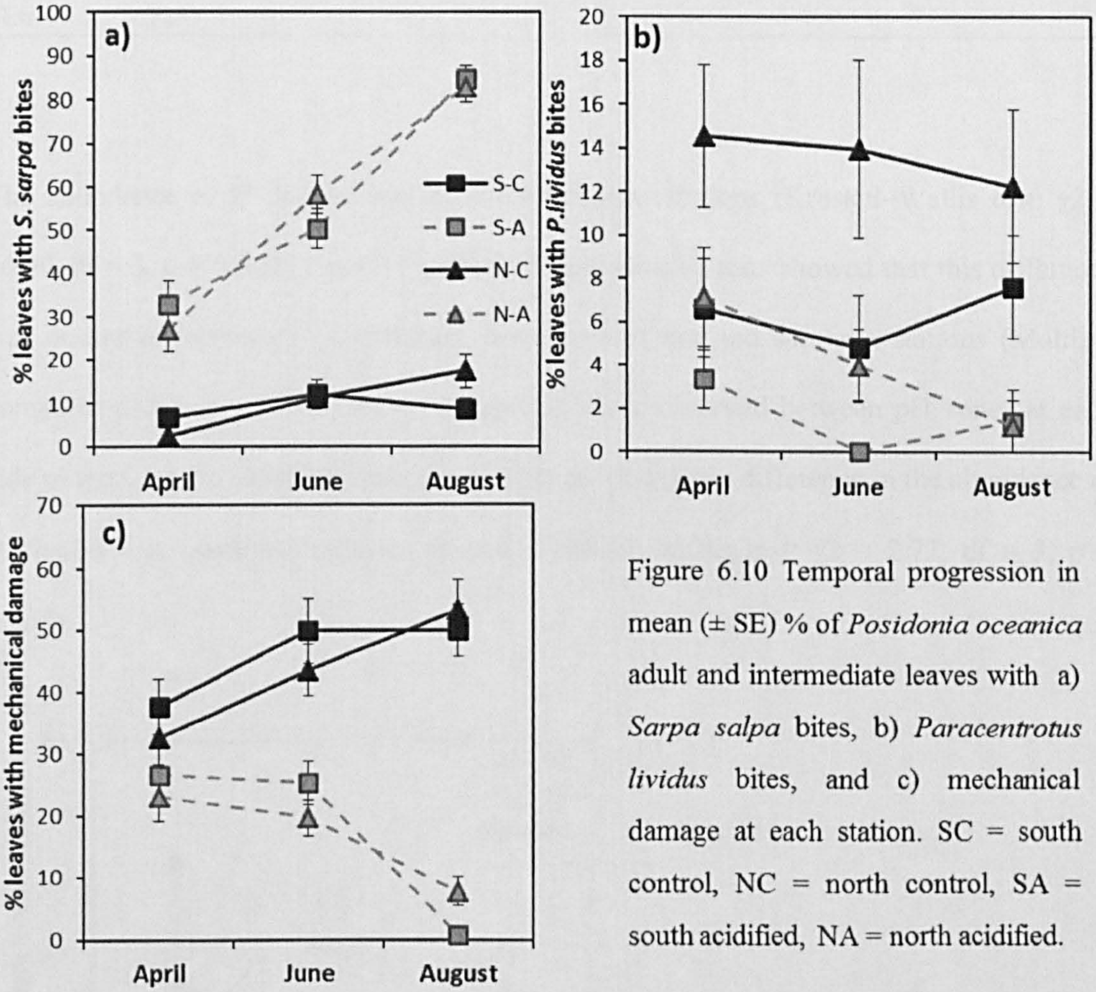


Figure 6.10 Temporal progression in mean (± SE) % of *Posidonia oceanica* adult and intermediate leaves with a) *Sarpa salpa* bites, b) *Paracentrotus lividus* bites, and c) mechanical damage at each station. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

Table 6.5 Three-way ANOVA results of % of leaves per shoot with: *Sarpa salpa* bites, *Paracentrotus lividus* bites and mechanical damage between site (fixed factor), acidification (fixed factor) and month (fixed factor). All data was arcsine transformed. S = site, pH = acidification, month = year. Significant results are highlighted in red.

		% leaves with								
		***Sarpa salpa* bites**			***Paracentrotus lividus* bites**			**Mechanical damage**		
	df	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
S	1	<0.01	0.01	0.906	0.68	10.34	**0.001**	0.04	0.46	0.497
pH	1	31.77	292.08	**<0.001**	0.21	3.18	0.076	11.50	124.23	**<0.001**
M	2	9.35	85.98	**<0.001**	0.65	9.84	**<0.001**	0.98	10.55	**<0.001**
S x pH	1	0.37	3.42	0.065	0.10	1.57	0.212	0.13	1.42	0.234
S x M	2	0.22	1.98	0.140	0.07	1.04	0.356	0.27	2.92	0.056
pH x M	2	3.26	29.94	**<0.001**	0.61	9.24	**<0.001**	2.83	30.59	**<0.001**
S x pH x M	2	0.23	2.14	0.120	<0.01	0.01	0.989	0.09	0.98	0.377
Err	324									

The abundance of *P. lividus* was different between stations (Kruskal–Wallis test: $\chi2$ = 16.60, df = 3, p = 0.009: Fig. 6.11), although comparative tests showed that this difference was due to differences in abundances between northern and southern stations (Multiple comparison test: p = 0.005), as no differences were observed between pH zones at each side of the Castello (Multiple comparison test: p > 0.05). No difference in the abundance of *P. lividus* was observed between months (Kruskal–Wallis test: $\chi2$ = 2.72, df = 3, p = 0.257).

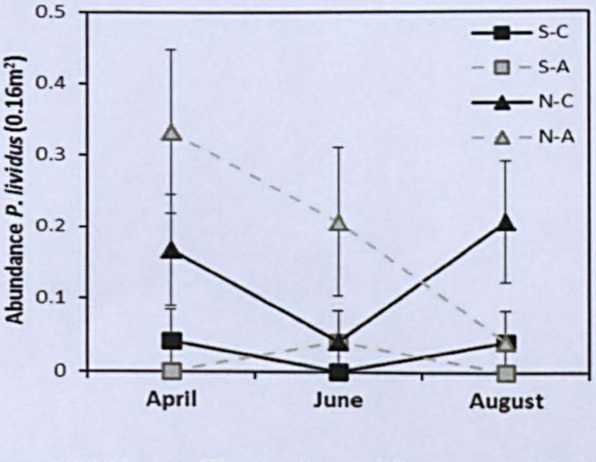


Figure 6.11 Mean (± SE) abundance of *Paracentrotus lividus* / 0.16 m^2 at each station in April, June and August 2013. SC = south control, NC = north control, SA = south acidified, NA = north acidified.

Due to the small station sizes and the schooling behaviour of *S. salpa*, statistical comparisons of the abundance of this species between stations was not possible, although during warm months (July and August) schools of *S. salpa* could be observed in both control and acidified stations at the Castello Aragonese, although sightings of *S. salpa* were infrequent in control stations (Fig. 6.12), whilst observations of *S. salpa* grazing in acidified stations (and particularly the southern acidified station) were common (Fig. 6.13a-b). The abundance of *S. salpa* in each school reached values of >100 individuals, and frequently other shoaling (e.g. Mugilidae gen. sp.) and non-shoaling (e.g *Diplodus sargus*) fish species could be found alongside *S. salpa* in acidified stations, leading a high presence of fish (Fig. 6.13c-d).

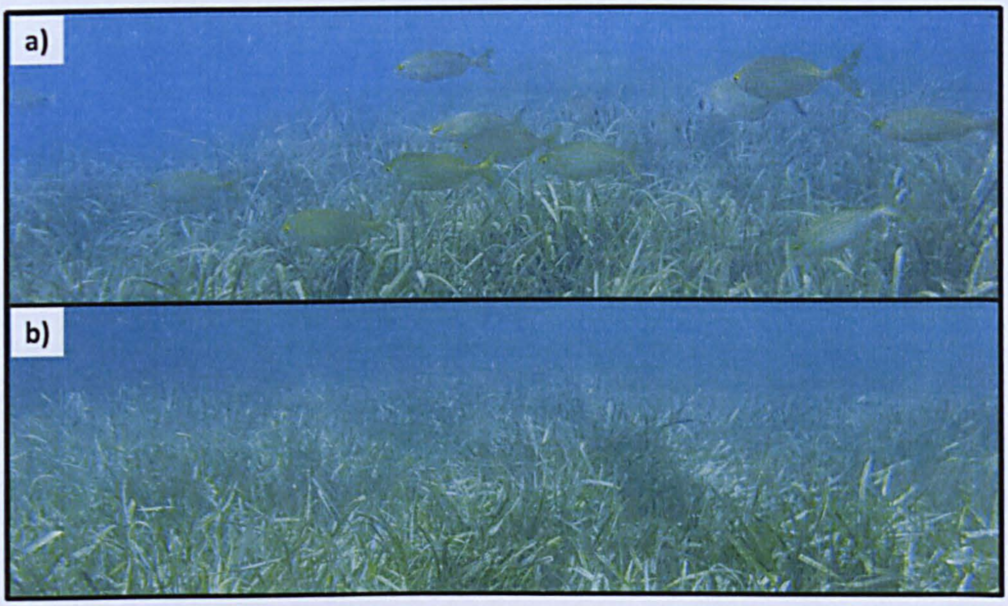


Figure 6.12 a) *S. salpa* school passing through a control station and b) the lack of *S. salpa* commonly observed in control stations.

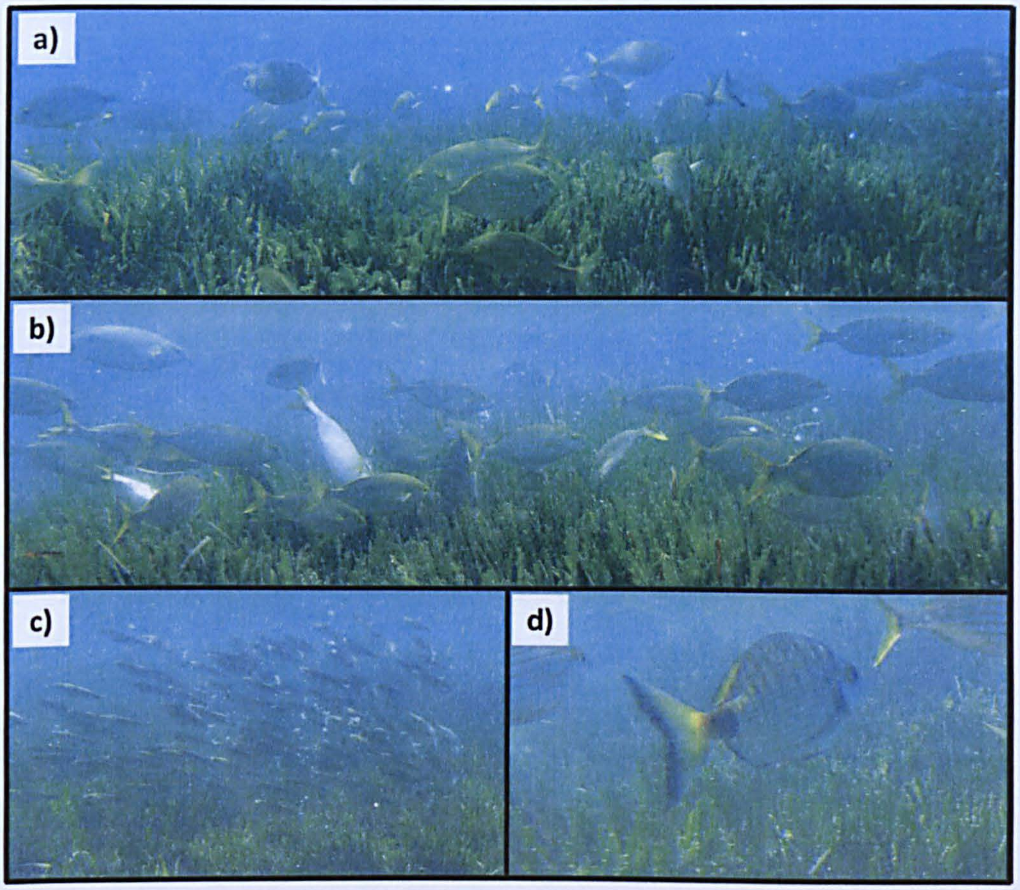


Figure 6.13 a) *S. salpa* feeding in the southern acidified station, b) *S. salpa* feeding in the northern acidified station, c) a school of fish from the family Mugilidae, and d) the fish *Diplodus sargus,* travelling with a *S. salpa* school.

6.3.4 Relationships between grazing pressure and Posidonia oceanica condition variables

Pearson's correlation tests between *P. oceanica* variables revealed collinearity between a number of variables. The % cover of animal and algae on the seagrass leaves were not independent predictors and hence the % coverage of algal epiphytes was negatively correlated to the % cover of animal epiphytes (r = -0.731, p = 0.001). The increase in cover of animal epiphytes is related to the decrease in cover of algal epiphytes through reduced competition for space. Interestingly the % coverage of algal epiphytes is negatively correlated with meadow density (r = -0.613, p = 0.012) and significantly correlated with the C:N content of the leaves (r = 0.660, p = 0.005). The fact that these measures were correlated is not surprising, as all variables, other than height of the canopy, were significantly correlated with pH (Table 6.6).

Table 6.6 Pearson correlation coefficients between *P. oceanica* parameters: C:N content of the leaves, meadow density, height of the canopy, and % epiphytic cover of animals and algae

	C:N	Density	Height	Algae	Animals	pH
C:N	1.00					
Density	-0.47	1.00				
Height	0.41	-0.44	1.00			
Algae	0.66**	-0.61*	0.49	1.00		
Animals	-0.39	0.52*	-0.37	-0.73**	1.00	
pH	0.60*	0.87***	0.49	0.82***	-0.62*	1.00

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Significant correlations were found between *S. salpa* grazing pressure and all described *P. oceanica* condition variables (Fig. 6.14), whilst *P. lividus* grazing was not correlated to any of the variables examined (Table 6.7). The cover of algae was most significantly correlated to *S. salpa* grazing, grazing increased linearly as algal cover decreased. ($r = -0.91$, $t = -8.34$, $p < 0.001$). Similarly grazing increased with a decrease in the C:N content of the leaves ($r = -0.79$, $t = -4.85$, $p < 0.001$). *S. salpa* grazing increased linearly with an increase in shoot density ($r= 0.75$, $t = 4.27$, $p < 0.001$), although some data points were outliers which did not conform to this linear correlation (Fig. 6.14d). These outliers were data points collected from the south control station. Although density was greater in the south control than the north control station, the proportion of leaves with *S. salpa* bites was lowest in this station. This led to a lower r value, and suggests that density is less important in grazing site selection. If the south control is removed from the analysis then density becomes more significantly correlated ($r = 0.86$, $t= 6.27$, $p < 0.001$), although even with removal of data from this station, significance did not reach the levels found for the relationship between algal cover and grazing.

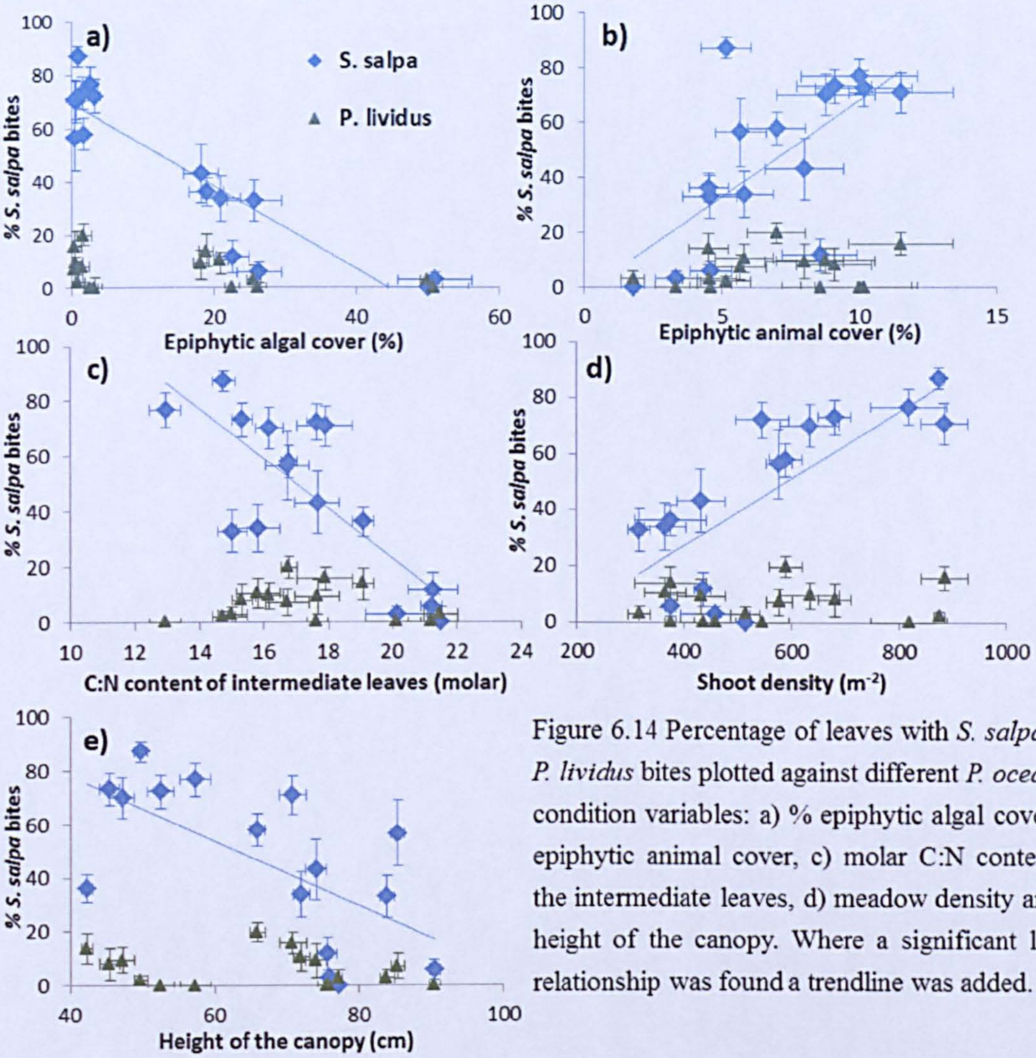


Figure 6.14 Percentage of leaves with *S. salpa* and *P. lividus* bites plotted against different *P. oceanica* condition variables: a) % epiphytic algal cover, b) epiphytic animal cover, c) molar C:N content of the intermediate leaves, d) meadow density and e) height of the canopy. Where a significant linear relationship was found a trendline was added.

Table 6.7 Pearson's correlation coefficients between *S. salpa* or *P. lividus* grazing pressure and *P. oceanica* structural or epiphytic characteristics.

	r	t	p	Equation
Sarpa salpa				
C:N	-0.79	-4.85	**< 0.001**	y = -8.9888x + 202.92
Density	0.75	4.27	**< 0.001**	y = 0.1193x - 20.585
Height of the canopy	-0.64	-3.12	**0.007**	y = -1.2067x + 126
% cover algae	-0.91	-8.34	**< 0.001**	y = -1.5574x + 69.493
% cover of animals	0.67	3.34	**0.005**	y = 6.99x - 1.7496
Paracentrotus lividus				
C:N	-0.16	-0.60	0.559	N/A
Density	0.12	0.44	0.663	N/A
Height of the canopy	0.052	0.76	0.397	N/A
% cover algae	-0.39	-1.56	0.140	N/A
% cover of animals	0.21	0.79	0.444	N/A

The grazing pressure of *S. salpa* showed the most significant correlation with the cover of epiphytic algae, the C:N content of the leaves and meadow density. These three variables were summarized using principal components analysis. The eigenvalues of the dominant principle component (PC1) explained > 97% of the variation in *S. salpa* grazing pressure (Fig. 6.15), showing that *S. salpa* grazing was highest in meadows with low algal cover, high density and low C:N content of the leaves.

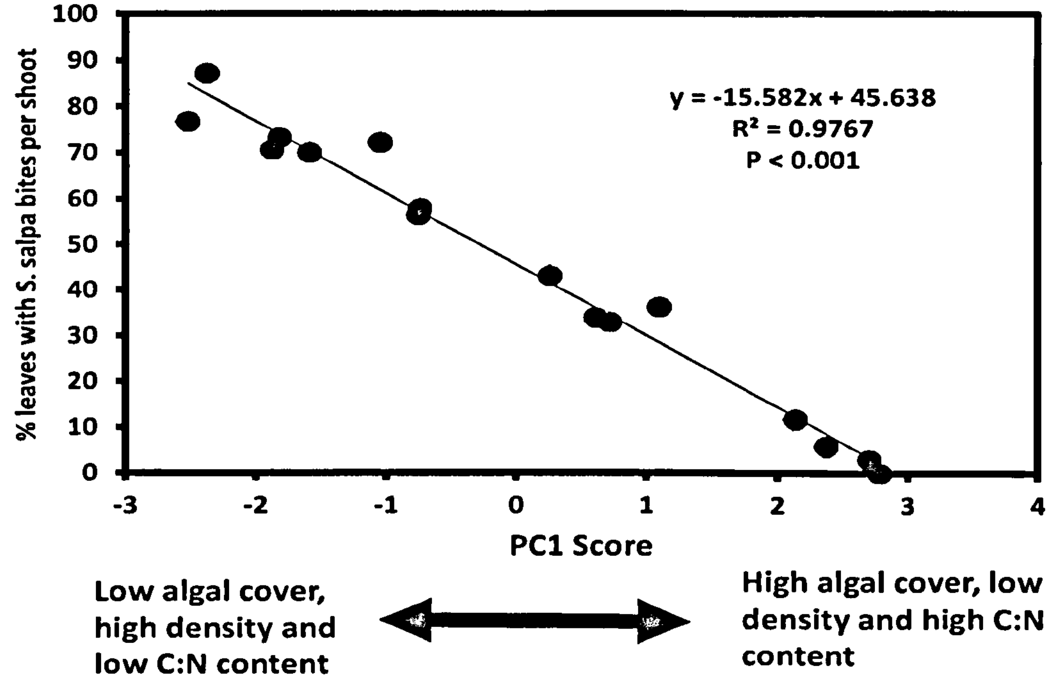


Figure 6.15 Relationship between the first principal component of the most significantly correlated *P. oceanica* condition variables and *S. salpa* grazing pressure.

6.4 Discussion

This is the first study to investigate changes in macroherbivore grazing pressure in response to ocean acidification. These results show that the fish, *Sarpa salpa*, has a strong preference for *Posidonia oceanica* growing in high CO_2 conditions, whilst the sea urchin, *Paracentrotus lividus*, showed no preference for leaves growing in ambient or high CO_2 conditions. Grazing pressure by *S. salpa* sharply increased between April and August in acidified stations, leading to a reduction in leaf length. The increase in grazing in acidified conditions was strongly correlated to a reduction in the cover of coralline algae, increase in C:N content of the leaves and an increase in shoot density.

The difference in *S. salpa* grazing pressure between pH zones was most pronounced in August, when over 80% of leaves had been grazed by *S. salpa* in acidified stations, whilst less than 20% of leaves had been grazed by *S. salpa* in control stations. Whilst *S. salpa* has been shown to have a large home range (up to 200 ha), which covers multiple habitats including macroalgal reefs, seagrass beds and sand (Jadot et al. 2002, Jadot et al. 2006, Pages et al. 2012), the highest grazing pressure on shallow water *P. oceanica* meadows occurs during warm water months when *S. salpa* builds up reserves for reproduction in the autumn (Francour 1997; Peirano et al. 2001). The diet of juvenile *S. salpa* primarily consists of small invertebrates (Dobroslavić et al. 2013). As they mature they become herbivorous, and the contribution of *P. oceanica* to their diet increases (Havelange et al. 1997). In June, across the three years, the percentage of leaves with *S. salpa* grazing scars ranged from between 5-42% in control stations and 50-77% in acidified stations. Values for control stations fit within values observed for similar studies of grazing pressure in shallow water *P. oceanica* beds, whilst the upper values for acidified stations exceeded previous studies. Alcoverro et al. (1997) found that the mean percentage of leaves with *S. salpa* bites in June varied from between 5-70% at five different locations in Spain, whilst Peirano et al. (2001) found that approximately 60% of leaves were grazed by *S. salpa* in La Spezia, Italy in June. *S. salpa* grazing pressure has been shown to be highly variable between locations, and in some meadows the appearance of 'mowed' patches can be observed in summer, where extremely high grazing occurs (Tomas et al. 2005c). Control and acidified stations occur in the same meadow in the north and south sides of the Castello Aragonese, although the appearance of these stations, particularly in the summer, is very different. In the summer, control stations have a high coverage of CCA and leaves are long, whilst in acidified stations leaves are short and the meadow appears 'mowed', plus the presence of CCA is negligible if not completely absent. This difference is striking and can almost show exactly where acidified conditions end and ambient pH occurs.

The cover of epiphytic algae was much lower in acidified than control stations across all months and years. Due to the *in situ* nature of this study we did not measure the cover of different morphological types of algae. Martin et al. (2008) discovered a severe decrease in the epiphytic cover of CCA on *P. oceanica*, leading to complete absence at pH 7.7. Similarly, a study of the algal cover in the rocky reef, adjacent to *P. oceanica* at the Castello Aragonese, found that CCA decreased, whilst cover of other morphological types remains the same or increased in acidified conditions at the Castello Aragonese (Kroeker et al. 2013b). The decrease in algal cover witnessed during this study is due to a loss of CCA, and, as can be seen from the images, CCA was almost completely absent from acidified zones. Although epiphytic algal coverage was low in acidified stations (1-10%), these values were directly related to the cover of non-calcareous encrusting, erect and foliose forms. Loss of CCA may lead to reduced competition for space and an increase in non-calcifying species. A second consideration is that *Myrionema orbiculare*, a dominant species of non-calcifying, encrusting brown algae found on *P. oceanica*, is not visible to the naked eye (Jones 1962), and would not have been included in the visual census, although microscopic examination of leaves confirmed its presence in both control and acidified stations. This species, alongside species of coralline algae, is considered a pioneer species that can be found on both young and mature leaves in all seasons (Jacquemart and Demoulin 2008), so loss of CCA may prompt an increase in the cover of this species.

The epiphytic cover of sessile organisms was not affected by acidification. These results correspond to a study by Martin et al. (2008), who found that the cover of epiphytic bryozoans and hydroids on *P. oceanica* leaves did not vary along a pH gradient. Bryozoans were the most abundant group of taxa, primarily due to the high coverage of the cheilostome bryozoan, *Electra posidonidae*. This species was abundant in both acidified and control stations, suggesting that it is tolerant to low pH. Other species of cheilostome bryozoan have been shown to be tolerant to acidification at the CO_2 vents at the Castello, although changes in resource allocation have been observed (Lombardi et al. 2011a). Any negative impacts of living in a low pH environment may be alleviated by the loss of competition, leading to a similar coverage of bryozoans and hydrozoans between pH zones. Although not quantified during visual census, a decrease in the abundance of calcifying Foraminifera and polychaetes from the subfamily Spirorbinae were observed. A similar decrease in the abundance of Foraminifera was observed in the sediments surrounding the vents at the Castello Aragonese (Dias et al. 2010), and sediments surrounding CO_2 vents in Papua New Guinea (Uthicke et al. 2013) in response to acidification, whilst a corresponding reduction in the number of spirorbid worms were collected by the airlift sampler in acidified stations at the Castello (Chapter 3). Laboratory experiments have shown a reduction in the settlement and growth of spirorbid worms in response to a reduction in pH (Saderne and Wahl 2013). Although these taxa became scarcer in response to acidification, faunal cover was dominated by bryozoans and hydrozoans, so this did not lead to an overall decrease in cover.

Leaf length did not differ between control and acidified stations in April, but in August leaf lengths in control stations were almost double those in acidified stations. This was because the length in control stations increased between April and August, whilst leaf length decreased in acidified stations. Leaf elongation rates were lower in acidified stations (Buia M C, unpublished data), which may account for some of the difference in leaf height, although lengths decreased by approximately 5% between April and August in acidified zones, even though leaves will continue to grow during these months. Cebrian et al. (1996) found that herbivory only accounted for approximately 2% of *P. oceanica* leaf production. Although it is not possible to directly measure the percentage removed by herbivory in the current study, the decrease in mean leaf length between April and August 2013 in the acidified stations shows that *S. salpa* herbivory removes more plant material than is produced during these months, whilst this is not the case for control stations. Leaves of *P. oceanica* are the oldest during the early summer, averaging 300 days (Cebrian et al. 1994) and, generally, during this time they reach their maximum biomass (Buia et al. 1992). Maximum biomass in acidified zones was reached in spring/early summer, due to the loss of biomass through *S. salpa* grazing.

Cebrian et al. (1996) examined the bite marks of *S. salpa* and *P. lividus* herbivory at 25 *P. oceanica* meadows along the Spanish Mediterranean coast between June and July. They quantified the percentage of leaf removed and found that *S. salpa* was responsible for approximately 70% of herbivory and *P. lividus* approximately 30%. This corresponded to data for control stations from June 2011 - June 2013; 73% of bite marks were from *S. salpa* and 27% *P. lividus*. In acidified stations the ratio was much greater; 88% of bite marks were from *S. salpa* and only 12% from *P. lividus*. Whilst quantification of bite marks does not lead to a direct quantification of herbivory, although a significant relationship was observed between the number of bite marks and shoot defoliation for both *S. salpa* and *P. lividus* (Prado et al. 2007).

Although *P. lividus* fertilization success and larval survival were not affected by acidification (Martin et al. 2011), the impact of low pH on adult *P. lividus* has been demonstrated. Asnaghi et al. (2013) found that test robustness decreased in acidified conditions, which may leave this species more vulnerable to predation. *P. lividus* are predated on by sparid and labrid fish, and starfish (Hereu et al. 2005; Bonaviri et al. 2009), all of which are common in the study sites (Bussotti and Guidetti 1999). Calosi et al. (2013) showed this species to be a poor regulator of extracellular acid-base balance in comparison to the co-occurring *Arbacia lixula*. This was further evidenced by the decrease in abundance of *P. lividus* near CO_2 vents in Vulcano, Italy, whilst the abundance of *A. lixula* increased (Calosi et al. 2013). In contrast to this, abundance of *P. lividus* and the proportion of leaves with *P. lividus* grazing scars did not differ between pH zones at the Castello Aragonese during this study. Calcifying groups of invertebrates, which would be expected to decrease in response to acidification, have been shown to maintain numbers or proliferate in a *P. oceanica* meadow (gastropods, bivalves, brittle stars: Chapter 4), suggesting that the negative effects of low pH may be compensated for by biotic factors such as reduced competition or predation. It is suggested that, within *P. oceanica* meadows, the negative effects of acidification on *P. lividus* may be balanced out by positive indirect effects such as an increase in the nutritional value or abundance of food items. It is thought that the diet of *P. lividus* is primarily epiphyte-based (Dauby 1993; Tomas 2004), favouring non-calcareous algae (Privitera et al. 2008; Murillo-Navarro and Jimenez-Guirado 2012). In *P. oceanica* meadows, epiphytic algae are a limiting resource for *P. lividus* (Tomas et al. 2005b), so an increase in non-calcifying algae may provide an advantage to this species in the future.

The proportion of leaves with mechanical damage was significantly higher in control stations in all years, although no difference was observed between pH zones in April.

Mechanical damage decreased between April and August in acidified stations, whilst an increase was observed in control stations. Leaf loss occurs in autumn (Alcoverro et al. 1995b; Gacia and Duarte 2001), although an increase in necrosis and erosion of the leaf tips (mechanical damage) occurs through the year. This was observed for control stations, with the % of leaves with mechanical damage increasing by almost 50% between April and June. A noticeable browning of the leaves in control stations was observed in August, as leaves began to decay, prior to leaf fall. The reduction in the proportion of leaves with mechanical damage in acidified stations was due to the exceedingly high herbivory, although it must be noted that leaves in acidified stations remained green in August (see comparison of leaf colours in Fig. 6.7). Even when herbivores are prevented from grazing (through caging of the canopy) a reduction in necrosis of the leaves is observed in acidified stations (MC Buia, unpublished data). This should be investigated further, as *P. oceanica* is primarily characterised by a detritus-based food web (Mateo and Romero 1997; Pergent et al. 1997; Vizzini et al. 2002). A previous study of two *P. oceanica* beds in Ischia, and Marseille (France) found 3- 10% of primary production was consumed by herbivores, whilst 23-34% was consumed by detritivores, 27-35% exported to other systems and 32-36% stored in the matte (Pergent et al. 1997). This suggests that loss of plant detritus, due to an increase in herbivory or a reduction in the decomposition of the plant, may have negative implications for those animals that feed on plant detritus in the future.

This study shows that the fish *Salpa sarpa* shows a strong preference for consuming *Posidonia oceanica* leaves grown in acidified conditions. Although it is difficult to directly demonstrate causality for this preference, as many of the possible explanatory variables (C:N content of the leaves, density, % cover of sessile invertebrates and % coverage of macroalgae) were all significantly correlated to pH, three of the possible explanatory variables showed the most significant correlations with *S. salpa* grazing. When analysed against the percentage of *S. salpa* bites per shoot, the dominant principle component (PC1) of these three variables gave an r^2 value of over 97%. This suggests that each of these three variables; % algal coverage, C:N content of the leaves, and meadow density may play an important role in shaping the preference of *S. salpa* to feed in acidified zones. Ideas for why these correlations were observed is considered below.

The algal coverage of the shoots was dominated by coralline crustose algae (CCA) in control zones, as can be seen clearly in the images presented. Presence of coralline algae was low to absent in acidified zones, similar to the findings of Martin et al. (2008). In tropical systems the parrotfish *Sparisoma radians* selects for leaves of the seagrass, *Thalassia testudinium,* high in CCA cover. Their stomachs have a pH of 8.4 so the carbonate is not dissolved but may act as a tool for grinding the leaves of the seagrass and releasing the cell contents (Thayer et al. 1984). In contrast, the strong negative relationship observed between *S. salpa* grazing and algal cover suggests that this fish may actively select for areas where CCA cover is low. Although this species is known to feed on a combination of algae and *P. oceanica* (Tomas 2004), the tough, heavily calcified nature of CCA is a deterrent to many herbivores, as it is indigestible and decreases the nutritional value of the algae (Littler et al. 1983; Pennings and Paul 1992; Pitlik and Paul 1997) and often coralline algae barrens are left on rocky reefs where high herbivorous fish grazing occurs, as other forms of algae are removed (Ruitton et al. 2000). This study suggests that CCA may be a deterrent for *S. salpa*, and contrary to findings of Dauby & Coulon (1993) and Tomas (2004) who suggest that *S. salpa* primarily assimilate leaf epiphytes whilst *P. oceanica* plant material passes through the digestive system and is returned to the litter, the low epiphytic cover observed in acidified stations suggests that *P. oceanica* itself may play an important role in the diet of this species.

C:N content of the leaves determines the nutritional value of seagrass (Thayer et al. 1984). C:N content was lowest in the south acidified station, intermediate in the northern control and acidified station and highest in the south control station in May 2011 (see Chapter 3). *S. salpa* grazing pressure in June 2011 followed the same pattern, suggesting that perhaps once *S. salpa* start feeding on plants with a low C:N content they increase feeding activity. Preliminary investigation showed up to a 50% decrease in phenolic content of the leaves of *P. oceanica* in the acidified station at the Castello Aragonese (MC Buia, unpublished data). A similar decrease in phenolic content of the leaves in response to acidification was observed for *Cymodocea nodosa* leaves in Vulcano, Sicily (Arnold et al. 2012). Although the results for *P oceanica* are preliminary and further investigation required, this effect, in conjunction with an increase in nitrogen content will improve the palatability of the leaves, providing a double advantage to the herbivores of this plant.

Optimal foraging theory predicts that an animal will move less in areas where its food source is more abundant in order to maximise their energy intake per unit time (MacArthur and Pianka 1966), suggesting that these fish may be attracted to beds with a high density of shoots. It must be taken into consideration that correlation does not imply causality. There is evidence that increased herbivory by *S. salpa* leads to increased vegetative growth of *P. oceanica* (Planes et al. 2011). Rather than the fish be attracted to dense meadows, it is suggested that increased grazing by the fish leads to increased meadow density, as a response of the plant.

Although the predators of *S. salpa* have not been identified in the Mediterranean, in Eastern Africa it is used as bait for large game fish such as the leerfish, *Lichia amia*, and other predatory finfish, sharks and rays (Otgaar 2013). The leerfish is also common to the Mediterranean, and local sharks and rays may feed on this species. Other possible predators of this *S. salpa* in the Mediterranean include top carnivores, such as the European hake *Merluccius merluccius*, the European barracuda, *Sphyraena sphyraena,* the great amberjack, *Seriola dumerili,* and the common dentex, *Dentex dentex*. These species of fish are commercially important (Froese and Pauly 2006), and if increased nutritional value of the plant leads to positive outcomes for *S. salpa*, this may enhance fisheries in the future.

Acidification appears to provide advantages to *S. salpa*, in terms of food, although the small size of stations, and their connectivity to areas of ambient pH, meant that *S. salpa* may travel in and out of the pH zones with ease. This means that we cannot determine whether ubiquitous acidification, to values predicted for 2100, would lead to a similar advantage. Reef fish have been shown to lose their ability to detect predatory cues (Dixson et al. 2010; Munday et al. 2010), their homing ability (Munday et al. 2009) and their auditory ability (Simpson et al. 2011) in response to a decrease in pH. If ubiquitous acidification leads to similar loss of abilities in *S. salpa*, any advantage of a preferential food supply may be lost.

In conclusion, an increase in *S. salpa* grazing pressure in acidified stations was observed between cold (April) and warm (August) water months, whilst *P. lividus* grazing and abundance did not differ between pH zones or time. This phenomenom of increased *S. salpa* grazing was observed annually, strengthening the conclusion that this is due to preferential feeding of seagrass grown in high CO_2 conditions. Increased palatability of *P. oceanica* to *S. salpa* appears primarily due to a complex mixture of changes in leaf nutritional content and epiphytic coverage of the leaves. In response to increased herbivory, the plant increases vegetative growth, leading to an increase. This increase in density appears to have important knock on effects of its associated invertebrate fauna (Chapter 4).

7. General Discussion

Whilst the number of studies on the biological effects of ocean acidification has grown exponentially since 2005 (Kroeker et al. 2010), our understanding of the ecosystem level effects of OA are still largely unknown, due to the variation in species responses to OA, and the difficulty in predicting changes in biotic interactions (Russell et al. 2012; Garrard et al. 2013). Natural submarine vents, which discharge CO_2 into the water column, lowering the pH, can provide a natural setting in which to observe the effects of chronic acidification. Although there are some drawbacks to this type of study (discussed below), the opportunity to observe community responses within a natural setting can provide valuable information to help understand the effects of OA at the ecosystem level (Hall-Spencer et al. 2008). *Posidonia oceanica* is the most important species of seagrass found in the Mediterranean, forming highly productive meadows, and associated with a high biodiversity of species (Mazzella et al. 1992). Previous studies at the Castello Aragonese have only investigated a small component of the *P. oceanica* system: calcareous epiphytes (Martin et al. 2008), seagrass density, leaf production per m^2, and photosynthetic performance (Hall-Spencer et al. 2008). The aim of this thesis was to investigate how a number of different components of this system change in response to acidification on a temporal scale: 1) the structure and function of the seagrass, 2) invertebrate community dynamics, and 3) the grazing dynamics of key herbivores. This discussion will bring together these different components in an attempt to understand how they interact, providing the first comprehensive overview of the impact of acidification on a *P. oceanica* meadow. It will discuss some of the problems associated with research at CO_2 vents, ideas for future research, and how these results may be important for Mediterranean coastal management. It will conclude with placing these results within a global context.

7.1 The effect of high CO_2 on a *P. oceanica* system

One of the major findings of this thesis is that most species associated with *P. oceanica* were able to tolerate pH levels expected for the end of this century. Although it is thought that calcifying species will be particularly susceptible to OA (Fabry 2008; Fabry et al. 2008; Doney et al. 2009b), the majority of organisms observed during this study were present in both control and acidified stations. Whilst many studies have revealed negative effects of OA on the calcification and growth of benthic invertebrates (e.g. Clark et al. 2009; Comeau et al. 2009; Kurihara et al. 2009; Maier et al. 2009; Brennand et al. 2010; Gazeau et al. 2010; Suwa et al. 2010; Gaylord et al. 2011; Courtney et al. 2013), none of these studies revealed that pH levels expected for the end of this century lead to a decrease in their survival. As physiological condition of the animals was not measured, rates of growth or calcification cannot be commented on. There is a possibility that some organisms, whilst able to survive in low pH conditions, may exhibit negative effects in terms of physiological processes such as calcification and growth, as has been demonstrated in laboratory studies. For example Comeau et al. (2010) found that pteropod larvae became shell-less at low pH (7.5), but were still viable. A second consideration is that many of these laboratory experiments are conducted over a short time period. Form and Riebesell (2012) showed that, whilst the cold water coral, *Lophelia pertusa*, exhibited negative effects to short-term acidification (1 week), it acclimatised, showing no negative effects when exposed to low pH for a period of months. The response of many calcifying species to low pH may be due to organism's ability to acclimatise to chronic acidification. A third and final consideration is that the high levels of photosynthesis during the day may increase pH within the leaf boundary layer and beyond, providing a microclimate of high pH and associated saturation states, giving daytime refuge from acidification for those organisms closely associated with it.

Not all groups of organisms appeared immune to the effect of increased CO_2. Most noticeable was the striking reduction/absence of epiphytic coralline crustose algae (CCA) in acidified zones, as was observed by Martin et al. (2008). Whilst CCA were the dominant epiphyte in control stations, covering as much as 90% of the leaf, it was generally absent in acidified stations, with the occasional occurrence of a small fragment on a leaf. Some species of coral are able to utilise bicarbonate to maintain calcification in low pH conditions (Comeau et al. 2013a). The absence of CCA in acidified stations suggests that no species of CCA can tolerate a reduction in pH, suggesting that this group will be one of the 'losers' in future carbonate conditions. Epiphytic seagrass CCA is thought to be a 'pioneer' epiphyte on the leaves, aiding in the settlement of other epiphytes (Willcocks 1982, Mazzella et al. 1992). The cover of sessile animals did not decrease with a loss of CCA, although a noticeable decrease in the abundance of foraminifera and polychaetes from the subfamily Spirorbinae was observed. It is thought that this is due to their intolerance to low pH, owing to their highly calcified nature. A high cover of bryozoans and hydrozoans were found in acidified stations, suggesting that at least this component may settle in the absence of CCA. Whilst the loss of CCA may have a negative effect on the isopod *Cymodoce hanseni,* which is known to feed on it, the majority of herbivores increased in abundance, suggesting that CCA is not an important food source for many herbivorous invertebrates.

This research found that a decrease in CCA in conjunction with a decrease in the C:N content of the leaves, in response to acidification, were the most likely explanation for enhanced grazing pressure by *S. salpa* in acidified stations. A further possible explanatory variable was the increase in seagrass shoot density, although Planes et al. (2011) found that increased grazing by *S. salpa* has been shown to stimulate vertical growth in *P. oceanica,* leading to increased seagrass density. Therefore, rather than an explanatory variable, the correlation we observed may be due to an increase in density being a response of the plant to enhanced grazing pressure. The abundance of many of the groups of taxa was shown to be positively correlated to shoot density, whilst decapod abundance decreased in summer months when S. salpa grazing reduced canopy height. These results suggest that a suggesting that a cascade of direct to indirect effects will play an important role in influencing the associated invertebrate community dynamics (Fig. 7.1). This shows the importance of whole community observations, as cascading indirect effects such as this would be impossible to predict from experiments based in a laboratory setting.

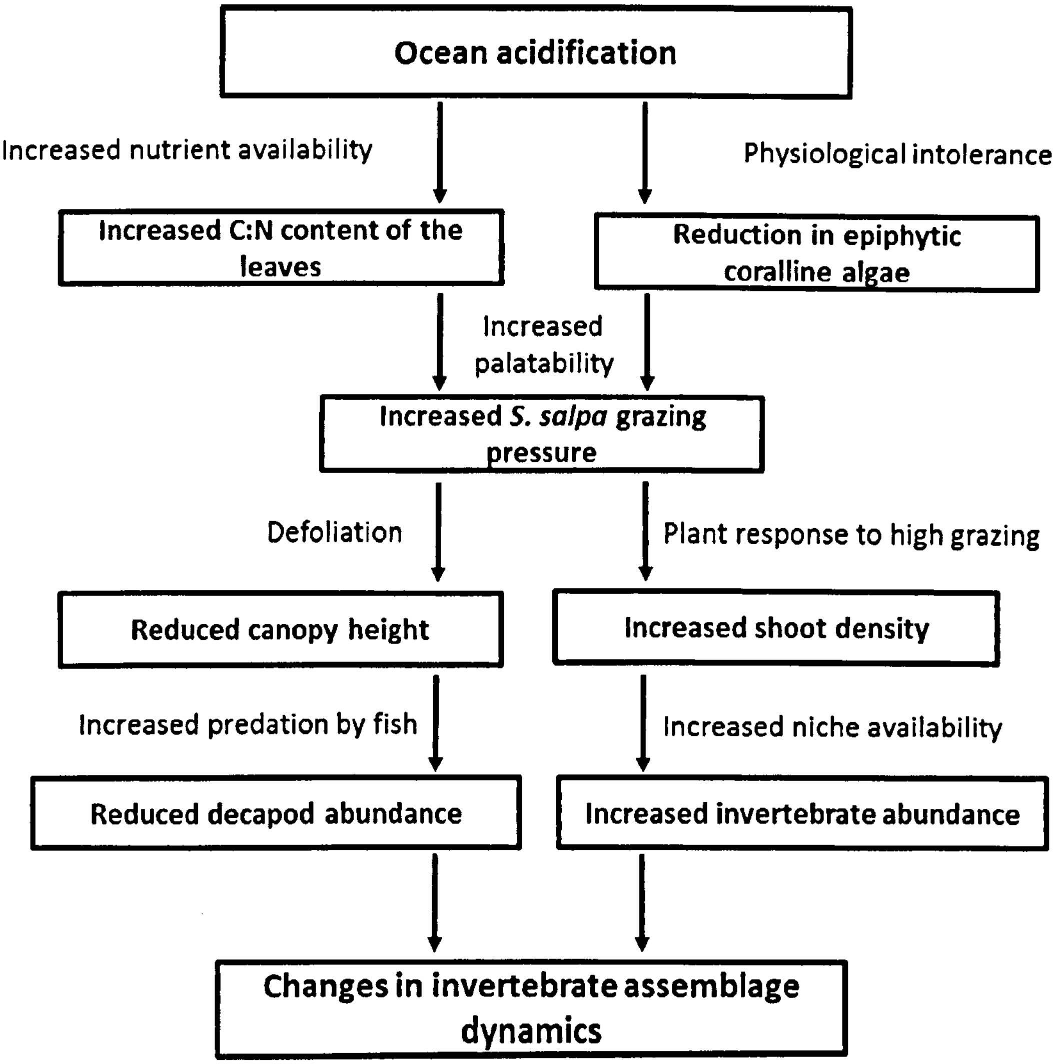


Figure 7.1 Potential cascade of direct to indirect effects of ocean acidification on a *Posidonia oceanica* system, including potential mechanisms and processes.

The food web linkages within a *P. oceanica* system have been demonstrated (Fig. 7.2: Mazzella and Zupo 1995). Although we cannot quantify how detritus and fish respond to a decrease in pH, all other compartments of the food web were investigated during this study. Seagrass density and the abundance of primary consumers increased in response to acidification, whilst the cover of epiphytes and the abundance of carnivorous decapods and polychaetes decreased (Fig. 7.2).

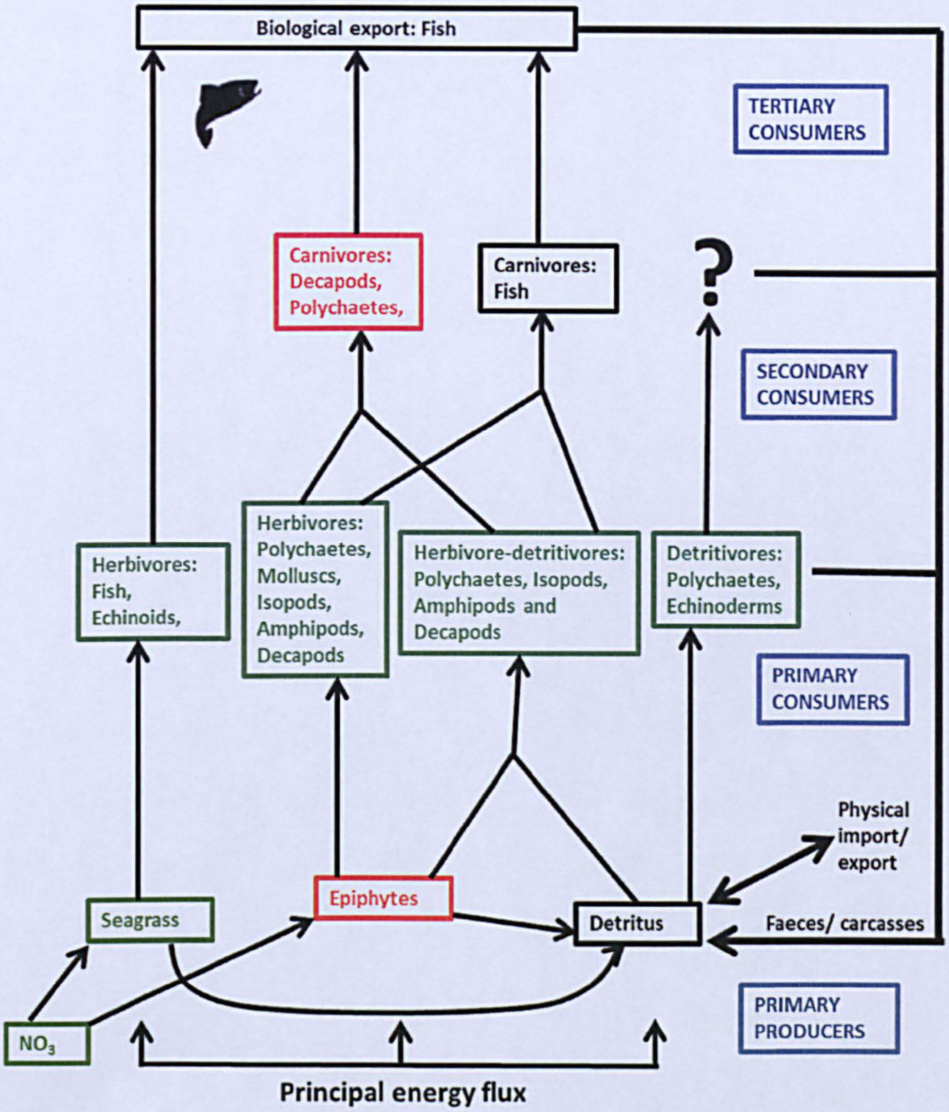


Figure 7.2 Illustration of the trophic energy flux in a *Posidonia oceanica* system (modified from Mazzella and Zupo 1995). Compartments are coloured dependent on their response to acidification. Those in red decreased in density/ quantity, green increased and black are unknown, as they were not measured during this study.

Whilst the consequences of the decrease in carnivorous decapods and polychaetes and the reduction in the cover of epiphytes are uncertain, a loss of ecosystem function was not apparent during this study. Results for measured components of production, suggested that acidified stations showed high levels of both primary (seagrass density) and secondary (invertebrate abundance and species richness) production. The link between species richness and ecosystem function is thought to be strong (Tilman 1996), and secondary production has been linked to fisheries yield (Valentine-Rose et al. 2011). Although the number of fish was not quantified, a generally higher abundance of fish (from families such as Sparidae, Labridae and Mugilidae) were observed foraging in acidified stations.

There has been some suggestion that, in areas of high net productivity, characterised by species of macroalgae, microalgae and seagrasses, OA may be locally ameliorated by the drawdown of CO_2 from the water column leading to an increase in pH, Ω_{arg} and Ω_{cal} (Manzello et al. 2012; Anthony et al. 2013). As it was not possible to quantify the amount of CO_2 released from the vents and the proportion captured by the seagrass, this could not be investigated. Without taking this effect into consideration, we found that cascading indirect effects, leading to facilitative interactions, maintained species richness and evenness, whilst the abundance of invertebrates increased. These results suggest that, in the face of future acidification, seagrass beds may play an important role in facilitating the maintenance of biodiversity in shallow water ecosystems.

7.2 The issues associated with ecological research at CO_2 vents

It is important that the impacts of future acidification can be scaled up to the ecosystem level, in order to provide policy makers with robust scientific evidence of expected environmental impacts. In respect to proposed future acidification, species abilities to adapt or tolerate these changes will be important in determining survival success, but accurate predictions must take into consideration a complex array of both biotic and environmental interactions (Russell et al. 2012).Whilst OA will occur simultaneously with changes in other physical variables such as temperature, salinity and oxygen, the interactions between species may change as well, as the abundance of tolerant and sensitive species changes. Marine organisms will therefore face the challenge of coping with multiple changes at all levels.

Understanding the ecosystem level effects of OA is a priority, and subtidal CO_2 vents can provide a natural laboratory in which to study whole ecosystems level effects (Hall-Spencer et al. 2008). CO_2 vents/seeps can be found in Ischia, Italy (e.g. this thesis; Hall-Spencer et al. 2008), Vulcano, Italy (e.g. Johnson et al. 2011; Calosi et al. 2013), Milne Bay, Papua New Guinea (e.g. Fabricius et al. 2011; Russell et al. 2013), and Puerto Morelos, Mexico (e.g. Crook et al. 2012), providing information on the effect of acidification on seagrass, rocky subtidal and coral reef systems. Attempts have been made to characterize the CO_2 vents at Ischia, through the placement of stationary pH sensors (Kroeker et al. 2011b), and the analysis of gas emissions (Hall-Spencer et al. 2008), and at Papua New Guinea through the analysis of gas emissions (Fabricius et al. 2011). At the CO_2 vents of Vulcano geochemical surveys (Boatta et al. 2013) and analysis of metal concentrations (Kadar et al. 2012) have been conducted, as there was some concern that toxic elements or compounds may be present. Whilst toxic compounds such as H_2S were found in low concentrations at CO_2 study sites (Boatta et al. 2013), there was an enrichment of trace metals (Kadar et al. 2012), which must be taken into consideration when using the Vulcano CO_2 vents for studies of OA. Research at CO_2 vents could be improved by collaborations with physical oceanographers and other disciplines, to gain an in depth of knowledge of the physical and chemical attributes of these vent sites, and also their temporal and spatial variation (e.g. under different atmospheric pressures or hydrodynamic conditions). This would aid in determining their suitability for OA research and potentially allow for the minimization of confounding factors.

Many of these vent sites show a high degree of pH variation (Kroeker et al. 2011b; Johnson et al. 2012), and further effort should be placed into understanding how pH variation, alongside pH reduction, impacts organisms. Some organisms live in areas of high pH variation such as estuarine habitats and areas of upwelling, whilst those in the open ocean deal with very little variation (Hofmann et al. 2011). Are perturbation experiments, whereby pH is maintained at a constant level, truly indicative of future oceans or should pH variations be taken into consideration during experimental periods? At vent sites is it acceptable to note the mean or median value as an indicator of pH or should lowest values be taken into greater consideration? Laboratory experiments should be designed to improve methodology in this field and understand the impact of high or little pH variation on organisms.

A total of between 300 to 320 pH samples were collected and analyzed for each station between April 2011 and August 2013. Only 40 of these were collected during 2012, whilst between 120 and 150 were collected from each station in 2011 and 2013. Whilst the mean pH in the southern acidified station remained the same across the three years (pH 7.8), the mean pH of the northern acidified station was higher in 2012-13 (pH 8.0), than that found in 2011 (pH 7.8). This decrease was because this station was acidified less often during pH monitoring, although it is not certain whether this is a sampling artefact, due to a sporadic sampling regime, or whether acidification events decreased at this station over the three years. Variables affected by acidification (*S. salpa* grazing pressure, leaf length, epiphytic algal cover) did not appear to decline in 2012-13 in the northern stations, suggesting that the effects of acidification did not differ between years. This demonstrates some of the problems associated with sporadic sampling of pH, and highlights the requirement for permanent, stationary *in situ* pH sensors to provide real-time data on pH variation.

A second consideration is that the acidified stations are small, and continuous with much larger areas of ambient pH, allowing for the movement of larvae from control populations into acidified stations. This is unlikely for larvae of small invertebrates with direct development, such as many species of gastropod, tanaid, amphipod and sabellid and syllid polychaetes, which should spend their entire lifecycle in acidified stations (Kroeker et al. 2011b), although species that are lecithotrophic or planktotrophic may migrate into the acidified zones from surrounding areas of ambient pH. It is thought that the larval stage of many invertebrates may be the most susceptible stage for some species (Dupont and Thorndyke 2009; Dupont et al. 2010a), therefore supply of larvae from non-acidified waters could lead to false positives, in terms of survival of certain species. Cigliano et al. (2010) investigated the effect of acidification on juvenile settlement, and many of the species that were found as adults in this study were present as juveniles during the previous study, suggesting that both juvenile and adult stages may be robust to acidification. Subsequent experiments examining the effect of acidification on adult fecundity and larval survival of species that thrived in acidified stations would help to strengthen these results.

Highly mobile organisms, such as fish, can move easily between acidified and control conditions, therefore, although a high density of fish (particularly *Sarpa salpa*), were observed in the acidified stations, we cannot presume that this group of taxa are robust to acidification. *S. salpa* can have a home range extending over thousands of metres (Jadot et al. 2006). Whilst we have provided observations on the preference of *S. salpa* to grazing on leaves in acidified areas, leading to a potential benefit, the chronic effect of acidification on this species remains unknown. In comparison, the home range of the sea urchin, *Paracentrotus lividus*, is generally less than 3 m (Hereu 2005), suggesting that post-settlement, this species remains within the respective stations throughout its whole lifecycle. Larvae from ambient pH can be transported to acidified stations, although *P. lividus* larvae has been shown to be robust to high levels of acidification (Martin et al. 2011). This species can inhabit intertidal pools, which exhibit extreme changes in pH, suggesting that they are able to acclimatize or adapt to pH stress (Moulin et al. 2011).

7.3 Future research into the effects of ocean acidification on a *Posidonia oceanica* system

This thesis provides a broad overview of the effect of acidification on a *Posidonia oceanica* system, although, in order to strengthen these findings, a number of further studies are proposed.

7.3.1 Investigation into P. oceanica pH microgradients

Whilst the negative effects of acidification have been demonstrated for many calcifying species of invertebrates (Kroeker et al. 2010), this research found that some calcifying species were present, and even thrived in the acidified stations. One possible reason for this is that the high levels of photosynthetic activity within the meadow may produce microgradients in pH, close to the photosynthetic tissue or within the rhizomes, providing a refuge from acidification. For this thesis, pH was measured 10 cm above the canopy, so pH within canopy pH is unknown. To understand whether pH microgradients are present, a combination of field and laboratory work should be carried out. Water samples should be syringed from specific niches of the *P. oceanica* habitat (sediment, rhizomes, canopy and above canopy), and at different times of the day, to see how pH differs within the meadow. Laboratory experiments should use microelectrodes (see Jones et al. 2000) to investigate the pH microgradient surrounding the leaves.

7.3.2 Biological traits analysis

Biological traits analysis is a recently developed method of providing a summary of the biological trait composition marine benthic assemblages (Bremner et al. 2006). The processes that occur, and therefore the functioning of a system, is dependent on the biological features of the organisms present (Oug et al. 2012). Therefore changes in patterns of trait expression can be used to investigate how anthropogenic/climatic impacts may lead to changes in ecosystem functioning (Bremner et al. 2006). Biological traits that can be used for marine, macrobenthic taxa include: size, larval type, relative adult mobility, bodyform, degree of attachment, feeding habit, and habitat (Cooper et al. 2008).

To aid in further understanding whether ocean acidification affects ecosystem processes and functioning, it is suggested that a biological traits analysis is performed for data on the invertebrate community collected during this study. Currently, information on the biological traits of marine species is limited, particularly for Mediterranean species (Paganelli et al. 2012); therefore, collation of available biological data for species associated with *P. oceanica* would be required in order to perform this analysis.

7.3.3 The effect of acidification on Sarpa salpa

Sarpa salpa showed a preference for *Posidonia oceanica* in acidified stations, suggesting that OA may provide a benefit to this species, although, due to its highly mobile nature, it may move from low to ambient pH areas at will. To increase our understanding of the effect of OA on this species, laboratory experiments examining the impact of OA on egg and larval stage, and on adult behaviour should be tested, as these have been shown to be impacted in other species of fish in response to OA (e.g. Munday et al. 2009; Frommel et al. 2011).

7.3.4 Herbivore exclusion/inclusion experiments

This study found that macroherbivore grazing pressure increased and C:N content of the leaves decreased in acidified stations, and that these variables showed a correlation, although it is unknown whether a decrease in C:N content lead to an increase in grazing pressure or vice versa. Manipulative experiments could assist in understanding how biochemical properties of the leaves (phenol, C:N, and sugar content) change in response to acidification, and how these changes link to changes in grazing pressure. Whilst the possibility of caging *S. salpa* may be logistically difficult, *P. lividus* numbers may be manipulated through caging experiments. Biochemical analysis of leaves in response to exclusion of both herbivores, *S. salpa* exclusion and no exclusion could be measured on a temporal scale.

7.3.5 Paracentrotus lividus feeding choice experiments

P. lividus abundance and grazing pressure did not vary between control and acidified stations, although at the CO_2 vents in Vulcano, Italy, their abundance decreased in response to acidification, and there is evidence that *P. lividus* may be less tolerant to low pH, due to its poor ability to regulate extracellular pH (Calosi et al. 2013). If this is the case, then could the equal presence of *P. lividus* in acidified stations be due to a preference for *P. oceanica* leaves grown in low pH? Feeding choice experiments, offering seagrass from control and acidified stations (± epiphytes) could assist in understanding this.

7.3.6 Posidonia oceanica primary production, stocks and fluxes in response to acidification

P. oceanica is an important shallow-water carbon sink in the Mediterranean (Apostolaki et al. 2011), due to its ability to store large amounts of carbon belowground. Whilst the shoot density of this plant increased in response to acidification, the effect of acidification on; ratios of below: above ground biomass, detrital dynamics and annual primary production are unknown. The below:above ground biomass of seagrass at CO_2 vents in Papua New Guinea was found to increase fivefold in response to acidification, suggesting that ocean acidification may increase the potential of seagrasses to act as a carbon sink (Russell et al. 2013). It is suggested that future work aims to understand how *P. oceanica* carbon stocks and fluxes change in response to acidification.

Lepidochronological analysis is a technique that can be used to analyse: primary production, growth rate and production of rhizomes, detection of flowering events and leaf renewal cycles (Pergent-martini and Pergent 1994). Leaf litter stocks can be measured by collection with an airlift sampler, whilst litter bag experiments can measure decay (Pergent et al. 1994). These techniques, alongside measurements of above- and belowground biomass would give us a greater understanding of how carbon fluxes in *P. oceanica* may change in the future.

7.4 Implications for management

Posidonia oceanica is particularly susceptible to anthropogenic/climate change impacts, due to its slow growth and infrequent sexual reproduction (Marba et al. 2002). Its cover has declined drastically in some parts of the Mediterranean (Bianchi and Morri 2000). Human pressures such as boat anchoring (Montefalcone et al. 2006), bottom trawling (Gonzalez-Correa et al. 2005), fish farming (Delgado et al. 1999), and water degradation (Fernandez-Torquemada and Sanchez-Lizaso 2005), have been shown to negatively affect *P. oceanica* meadows, leading to a decrease in shoot density, meadow fragmentation or regression. Meadows with low shoot density are then at risk from invasion of exotic species of *Caulerpa* spp. (Ceccherelli et al. 2000). The increased shoot density observed in acidified conditions, may help to ameliorate anthropogenic impacts.

The results of this thesis suggest that this species of seagrass, and its associated community, will be robust to pH levels expected for the end of this century. When mean values were compared to baseline values found for shallow, undisturbed meadows (Chapter 2, Table 2.1), invertebrate species richness was higher in both control and acidified stations than values found in previous studies (Fig. 7.3a). Shoot density was either on the lower margin or below the baseline values in the control stations, and increased in the acidified stations (Fig. 7.3b). Biological indicators such as seagrass shoot density and invertebrate species richness can be useful indicators of the health of a seagrass system (Linton and Warner 2003). This is not to say that there won't be alterations to community structure in response to changes in carbonate chemistry, although no common species disappeared in low pH zones.

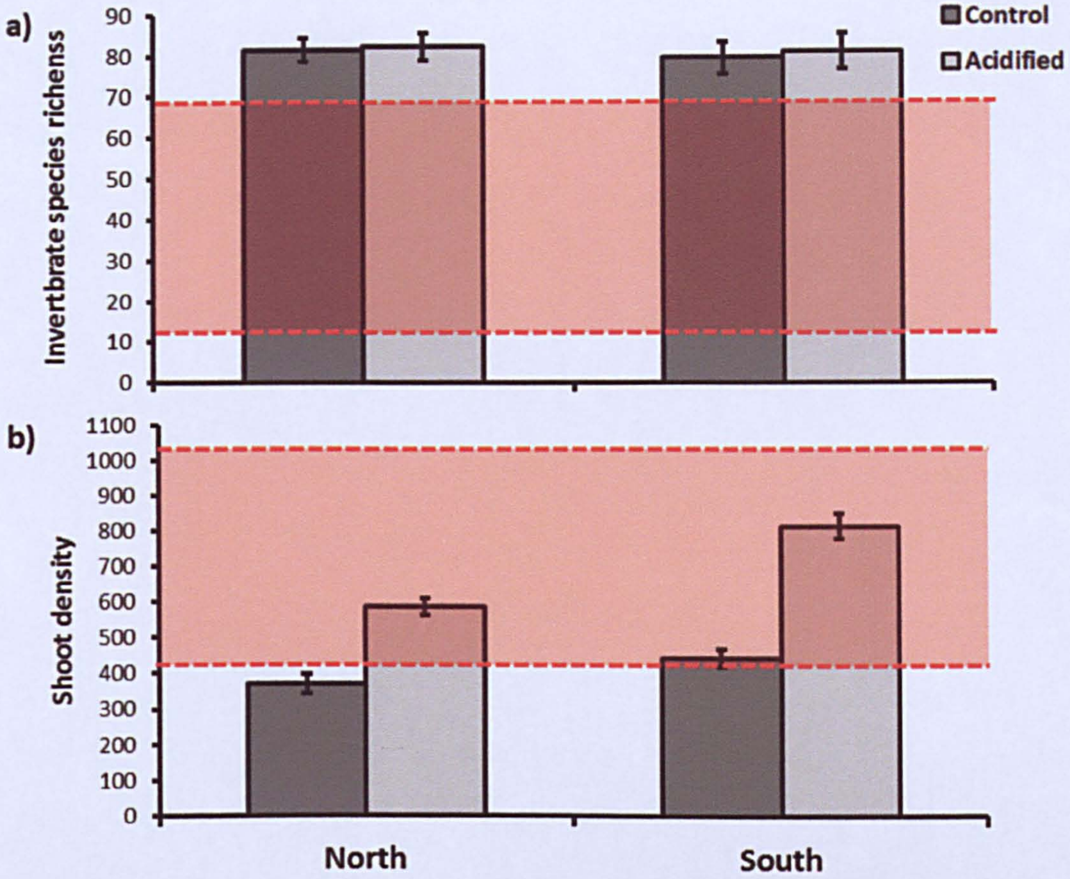


Fig. 7.3 Mean (±SE) a) invertebrate species richness, and b) shoot density at control and acidified stations at the north and south of the Castello Aragonese. Red areas indicate minimum and maximum values found in shallow undisturbed meadows in previous studies (Table 2.1).

Manzello et al. (2012) suggest that seagrass beds have the potential to provide refugia against OA, through the drawdown of CO_2, increasing saturation states of the surrounding seawater. The results of this thesis suggest that, even when pH is low (pH samples were collected 10cm above the canopy), facilitative interactions may play an important role in maintaining richness of the associated community,

P. oceanica is a net autotrophic system, and an important carbon sink in the Mediterranean (Apostolaki et al. 2011). Net primary production (NPP) in *P. oceanica* ranges from 60 – 184 gC m^{-2} yr^{-1} (Pergent et al. 1994). Approximately a third of this production is stored belowground through the burial of roots and rhizomes (Pergent et al. 1997), leading to millennia old biogenic 'mattes' (Mateo and Romero 1997). *P. oceanica* meadows are thought to cover 50,000 km of the Mediterranean seafloor (Bethoux and Copinmontegut 1986). Therefore the potential total carbon storage capacity of *P. oceanica* is:

$$= (60 \text{ or } 184 / 3)^{*}\ 5\text{x}10^{10} = 1 - 3.07 \text{ Tg C yr}^{-1}$$

P. oceanica is protected under the EC Habitats Directive as a priority natural habitat, is listed in the Bern Convention as a species of flora that is strictly protected, and listed as an endangered species under the Barcelona Convention, although despite this there is evidence that cover of this species is still declining (Marba et al. 2005). An increasing number of marine protection areas (MPAs) have been designated in the Mediterranean; however their effectiveness at preventing decline of *P. oceanica* is inconsistent (Marba et al. 2002). The maintenance of associated species richness under acidified conditions, alongside the ability of *P. oceanica* to act as a substantial carbon sink, suggests that more intensive efforts should be placed in conserving this highly productive habitat in the face of future changes in carbonate chemistry.

Whilst this thesis has used the acidification caused by the CO_2 vents to describe possible changes to a *Posidonia oceanica* system in response to global ocean acidification, these results may also be used to consider how small-scale, chronic leakages from carbon capture and storage (CCS) may cause changes to this shallow-water ecosystem. CCS is a relatively new technology, which aims to capture CO_2 from power plants or other industrial facilities and store it in subsea geological formations such as depleted oil and gas reserves or saline aquifers. Leakage from these underground storage sites is a very real possibility (Vendrig et al. 2003), through slow or (less likely) catastrophic release of CO_2 into marine and subsurface ecosystems (Turley et al. 2004). Russell et al. (2013) suggest that seagrass beds may have the potential to partially mitigate this leakage. This research suggests that not only may the leakage be partially mitigated through an increase in seagrass density, but that the majority of invertyebrates associated with this system would be able to withstand small-scale CO_2 leakage.

7.5 Conclusion

These results give some cause for optimism, in terms of the potential effects of ocean acidification or chronic CO_2 leakage from sub-sea storage, in this temperate shallow-water system at the pH levels investigated. No ecological tipping point was observed in response to the reduction in pH observed; although alterations in community dynamics were apparent, suggesting that OA will lead to changes the relative abundances of different species. It is suggested that these changes were caused through cascades of direct and indirect effects. A similar lack of response to a comparable reduction in pH was found for measures of biodiversity (species richness, evenness) and productivity (biomass, abundance) of benthic invertebrates in the rocky subtidal (Kroeker et al. 2011b).

This is not to say that the global reduction in marine pH, expected for the end of this century, will not cause substantial adverse effects to other shallow-water marine habitats. Coral reefs systems are expected to be particularly vulnerable to OA (Hoegh-Guldberg et al. 2007). At similarly acidified CO_2 vents in Papua New Guinea, coral species richness decreased by 39%, and the cover of structurally complex forms decreased threefold (Fabricius et al. 2011). CCA play an important role in coral reefs; suppressing macroalgal growth (Vermeij et al. 2011), inducing coral settlement (Harrington et al. 2004) and providing structural integrity to the reef (Goreau 1963). Whilst loss of this group of algae from the leaves of *P. oceanica* did not appear to negatively impact the associated community, loss from coral reefs may further enhance vulnerability to OA in this system.

One of the most striking findings of this thesis was the ability of calcifying species to persevere, and even flourish in acidified conditions. This may be due to the complex nature of the seagrass, and high levels of photosynthesis, leading to possible pH microclimates. Secondly, the high degree of pH variability that naturally occurs in these shallow water habitats (Hofmann et al. 2011), may result in organisms that are be better equipped to withstand pH stress (Moulin et al. 2011). This research suggests that shallow-water, highly productive, non-calcifying, biogenic habitats, such as seagrass beds, may provide a refuge from OA, and highlights the importance of their conservation.

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