EXAMENSARBETE | BACHELOR'S THESIS

Ocean acidification effects on marine organisms: a study of *Littorina littorea* and *Balanus improvisus*

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Abstract

The world's oceans are becoming more acid in a process called ocean acidification. The pH of the ocean have already decreased by 0.1 units from pre-industrial time until today. Scientists predict that by the year of 2100 the pH will decrease by as much as 0.4 units. This is a big potential problem to many marine species, because they have developed in such a stable environment that has not changed for millions of years. It is difficult to predict how they might be affected by such a decrease in pH during a relatively short time period. Several studies have been made on marine species exposed to decreased pH-levels, the results showed changes in their physiology but it is hard to predict how these changes will affect the organism in a long-term scale and if this might change ecosystem dynamics. Our study measured the activity of *Littorina littorea* and *Balanus improvisus* when exposed to lower pH, the results of our study showed an increase in activity for the lower pH (pH 6.0-7.5) when compared to the control (~pH8). The area of ocean acidification is a field that requires further studies to fully understand its effects on the marine ecosystems and the species within it.

Introduction

A rapid increase in atmospheric CO_2 since the pre-industrial revolution (1750) until today, from 280ppm (Widdicombe, 2008) to 390ppm (Tynan, 2010), this rise is equal to a 39 % increase of CO_2. Worst case scenarios predict levels of up to 660-790 ppm in 2100-2150 (Tynan, 2010), a raise of 136-182 % compared with pre-industrial levels. The world's oceans daily take up more than 25 million tons of atmospheric CO_2, most of the CO_2 are anthropogenic and come from the combustion of fossil fuel and deforestation (Porzio, 2011 & Doney et al., 2009). Rich experimental evidence demonstrates that ocean acidification has an impact on marine organism physiology. To survive the organism must fulfill several essential functions including; reproduction, growth, feeding, respiration and motion etc. When disturbed these functions affect the organism's fitness and/or abundance and may lead to significant changes in the marine ecosystems (Turley, 2010). Important marine organism groups such as plankton, corals and other calcifying organisms are likely to be affected by ocean acidification as the ocean pH and carbonate ion concentration changes. This will probably have an impact on how organisms build and maintain their calcium carbonate skeletons (Orr et al. 2005). Longitudinal analyses made 1988-2003 show that the Great Barrier Reef decreased its calcification rate with 21 %, which exceeds what be expected from ocean acidification alone. This is probably an effect of changed environmental conditions (Doney et al. 2009). For our studies we selected the species *Littorina littorea* and *Balanus improvises* because they are common and durable enough for laboratory tests, they also live in an environment that experience variations in temperature and pH (Findley, et al., 2009). Only a few studies exist on how *L. littorea* is affected by low pH and how it will react in the future to ocean acidification. However, some evidence show: how induced defenses are effected by low pH (Bibby et al., 2007) and if ocean warming and acidification has a synergistic or additive effect on the fitness of *L. littorea* (Melatunan et al., 2009). No studies were found on ocean acidification and its influence on the barnacle *B. improvisus*, but a few studies have been made on some of their relatives (Findley, et al., 2009; Findley, et al,. 2009a; Mcdonald, et al., 2009). Most of the researches concerning ocean acidification focus on physiological reactions on single species during short-term periods which make it hard to predict long-term effects (Turley, 2010). Studies which included several species from the same phylum show different sensitivity to CO_2, demonstrating a potential source of error when making assumptions regarding groups of organisms from a single species.

The oceans together with its sediments act as the main buffer and sink for atmospheric CO_2, it is the calcium carbonate system which regulates the ocean´s buffering capacity of CO_2 (Tynan, 2010).When atmospheric CO_2 enters the water it converts to carbonic acid (H_2CO_3) , that produces bicarbonate (HCO_3^-) ions and hydrogen (H^+) ions . If there are no available carbonate ions (CO_3^{2-}) that can react with the free H^+ to convert to more HCO_3^-, the pH will drop. These chemical steps ($CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$) (Widdicombe, 2008) make up the carbonate buffering system, maintaining seawater at a stable pH (Tynan, 2010). Carbon absorbed by marine living organisms and deposits on the ocean floor as sediments when they die (Garpe, 2010). These processes have kept the pH in marine systems in balance for several millennia (Tynan, 2010). Earlier thinking assumed that the increase in atmospheric CO_2 would be compensated by the buffering system, but the view changed in 2003 when Caldeira and Wickett presented their model, predicting that the pH of surface water had dropped by 0.1 units compared to pre-industrial time. They also predicted that in the year of 2100 the pH could have fallen by as much as 0.4 units. Present thinking claim that it is not the amount of CO_2 but the speed of the increase in CO_2 in the oceans which causes that is causing ocean acidification, the increase is too fast for the buffer system to manage (Widdicombe 2008). Such rapid increases in CO_2 as seen today have probably not been seen since the extinction of the dinosaurs, 65 million years ago (Turley, 2010).

The aim of the present is to get a more complete understanding of what effect low pH might have on marine organisms, by examine the organism's activity during a given time. Our predictions are that it will be a distinct difference in behavioral activity between the control group (unaltered seawater, ~pH 8) and the other groups in different pH-concentrations. An interesting result from this study hopefully can support future research and increase awareness of global risks of ocean acidification.

Materials and methods

The samples were collected April 25 at Tylösand and Steninge in the littoral- and sublittoral zone. The sites are located 8 km respectively 21 km north of Halmstad. The common periwinkle were handpicked or collected with a metal sieve from the waterline to a depth of one meter. On each location 50 specimens where sampled and brought back to the lab for species identification. At Steninge beach *Balanus improvisus* attached to *Mytilus edulis* were collected. Seawater and sediment were also collected at Tylösand in plastic bins for the use in the aquarium. The species identification was conducted in a lab visually using the book: A student's guide to the seashore (Fish & Fish, 1996) and Dyreliv i havet (Moen & Svensen, 2003). All of the *L. littorea* were weighed on Denver instrument digital scale with a 0,001g accuracy to receive a mean weight to get equal sized individuals. The mean weight was 3.15 g, and individuals in the span of 3.00-3.40 g were chosen for the study. In total 15 periwinkles were chosen, three individuals for every pH. With *B. improvisus* five mussels were chosen that had at least three barnacles on their shells. The animals were given at least 12 hours to get acclimatized from being collected to be put in the aquarium. Five aquariums of 20 L (40x20x25cm) were filled with 10 L of seawater and a layer of one centimeter of sediment was added on the bottom for the periwinkle. The sand was added to create a more natural surface than the texture of plastic as well as possibly providing some additional food source. For the barnacles five aquariums of 20 L (40x20x25cm) were filled with 10 L of seawater, no sand was added. The seawater was not exchanged and had no circulation during the experiment, no additional food was added. The pH in the tanks were altered using acetic acid 24 % and set to 4 different values; pH 7.5, 7, 6.5, 6. One tank for the barnacles and one tank for the periwinkles were used as a control and only contained seawater at its natural pH (~ pH 8). The pH were measured in each aquarium and were corrected if necessary once a day at 4 PM. Three periwinkles were put in each of the five aquariums, and a *Mytilus edulis* with at least three barnacles on them were put in each of the other five aquariums. The pH levels were measured using HANNA pH-meter and temperature was measured. The study were conducted in a lab without windows so there were no external light sources, daylight were simulated by artificial lights (18W & 58W) from 8 A.M. to 12 P.M., the temperature was kept stabile between 18-20°C. The study was carried out for 11 days over the period of 26 April to 6 of May, each day there were five measurements; at 8, 12 AM and 4, 8, 12 PM. The reason for having several measurements each day was to get a reliable mean value. At each measurement the activity was documented; for the *B. improvisus* the cirral movements were counted on three random individuals during 2 minutes each, with help of a mechanical counter, for the *L. littorea* the stretch that each individual moved during 10 minutes were measured using tape that marked the starting position and a ruler. The shells of *L. littorea* were also marked in each aquarium with three symbols; + - 0 to be able to separate the individuals from another. After every measurement periwinkles that had fallen on their back were turned back around, periwinkles that were above the waterline (escape response) were also documented. The data collected from this study were transferred to Microsoft Excel 2010. The data were used to create an arithmetical mean for the cirral movements of *B. improvisus* and the movements of *L. littorea* for each pH and each day. The mean values were calculated for each group of individuals in each pH for each day. Standard deviations were also calculated for each group of individuals in each pH for each day. These mean values and standard deviations were then put into graphs (Figure 1-5, 7-11). The data from the escape response were made into percentage, calculated for each pH during the 11 days. To ensure that the results are reliable, analysis of variance (ANOVA) was used (Table 1).

Results

The results showed a difference in activity pattern between the individuals in unaltered seawater (Fig.1, 11) and the individuals in the other pH-concentrations (Fig.2-5, 7-10). Increases in activity were displayed for the individuals in the lower pH-concentrations, when compared to the individuals in the higher pH-concentrations. For all periwinkles in all pH-values there were decreases in activity when you compared day 1 of the study with day 2, and there were increases in activity from day 2 to 3. After day 3 the activity for the different pH-values diverged. For pH 8 there were no activity from day 4 to day 6 and some activity at day 7, with a peak at day 9 and a slow decrease until day 11 (Fig.1). The activity in pH 7.5 continued to increase until day 5 when it declined and continue to so until day 8, for the remaining 3 days it fluctuated (Fig.2). The curve for pH 7.0 fluctuated considerably, with peaks on day 4, 6, 9, 11 and low points at day 5, 7-8, 10 (Fig.3). The curve for pH 6.5 also fluctuated but not as much as pH 7.0, it fluctuated with peaks on day 3, 5, 7, 10 and low points at day 4, 6, 8 (Fig.4). The curve for pH 6.0 went up from day 2 and reached its highest value on day 6, after this it declined and the activity dropped to zero after day 9 (Fig.5). We also measured escape response and found that there were a difference between different pH and different escape responses, the highest escape response were in pH 6.0 and pH

6.5, the lowest escape response were in pH 7. The values for pH 7.5 and pH 8.0 were in between the highest and the lowest (Fig.6).

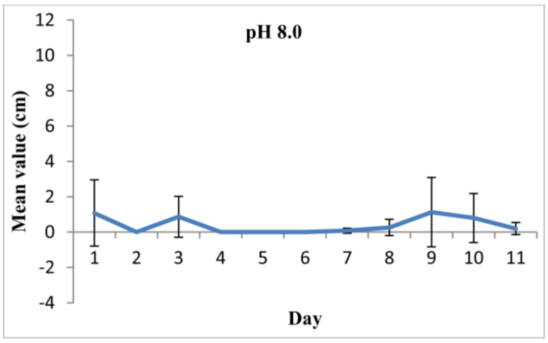

Figure 1. The mean movement in cm for *Littorina litorea* during 10min. Bars represent standard deviation.

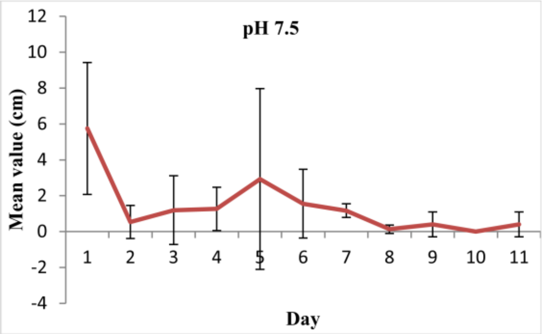

Figure 2. The mean movement in cm for *Littorina litorea* during 10min. Bars represent standard deviation.

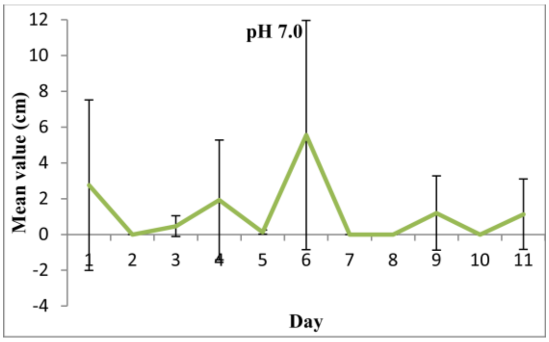

Figure 3. The mean movement in cm for *Littorina litorea* during 10min. Bars represent standard deviation.

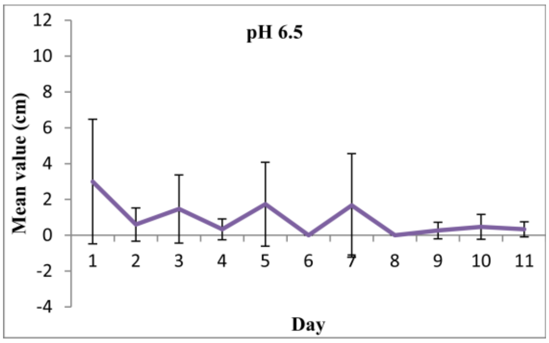

Figure 4. The mean movement in cm for *Littorina litorea* during 10min. Bars represent standard deviation.

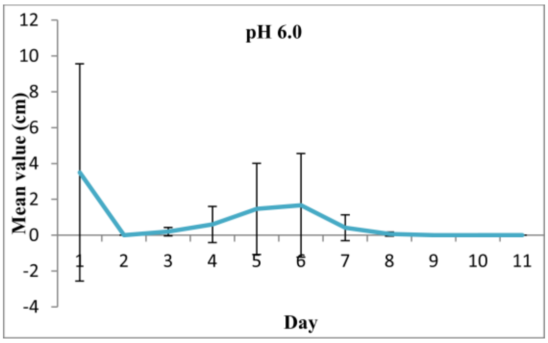

Figure 5. The mean movement in cm for *Littorina litorea* during 10min. Bars represent standard deviation.

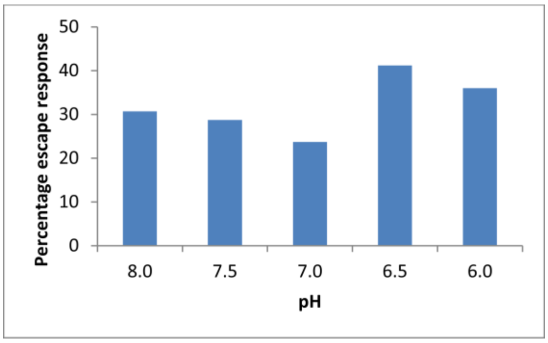

Figure 6. The escape response in percentage for the entire study.

For the barnacles there were increases in activity for the individuals exposed to pH 6.0-7.0 compared to the barnacles exposed to pH 7.5 and pH 8.0. The curves for pH 6.0-7.0 showed a rise in activity after 2-3 days and the curves for pH 7.5 and pH 8 showed a decrease in activity for the same period. The curve for pH 6.0-7.0 reached its peak after day 4 and then declined (Fig.7-9) During the same period the curves for pH 8 and pH 7.5 exhibited a drop in activity and then increased (Fig.10,11) After the peak for pH 6.0 at day 4, the curve continued to decline during the rest of the study (Fig.7). The barnacle in pH 6.5 had a little increase in activity at day 6 which then dropped and flattened out (Fig.8) The decrease in activity for pH 7.0 continued until day 8 and then begun to fluctuate (Fig.9). The slight increase at day 6 for pH 7.5 continued until day 8 and then leveled out (Fig.10). Our control pH 8.0 decreased after an increase on day 6 and then increased again to finally drop on day 10 (Fig.11).

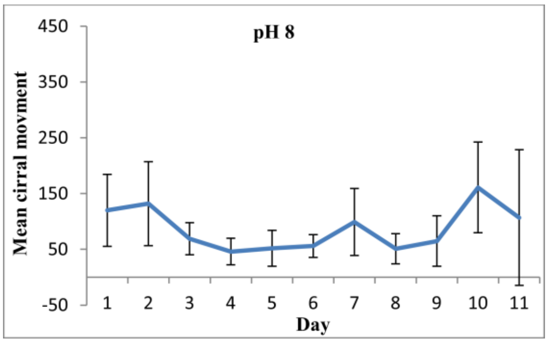

Figure 11. The mean cirral movement of *Balanus improvisus* during 2 min.

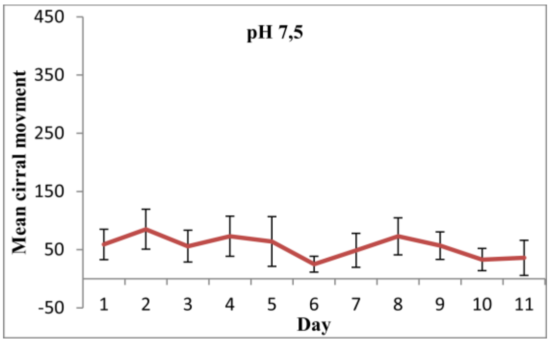

Figure 10. The mean cirral movement of *Balanus improvisus* during 2 min.

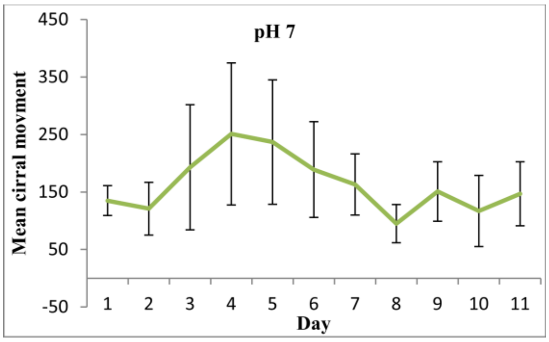

Figure 9. The mean cirral movement of *Balanus improvisus* during 2 min.

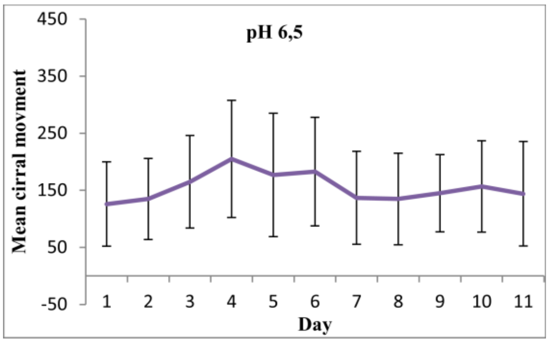

Figure 8. The mean cirral movement of *Balanus improvisus* during 2 min.

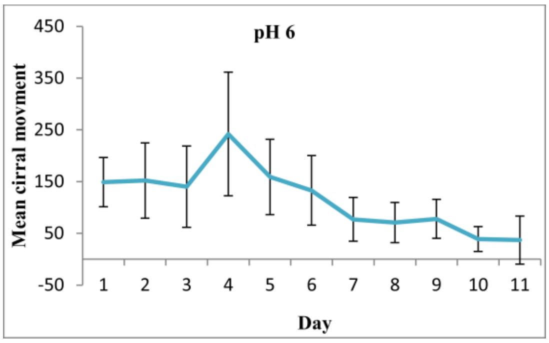

Figure 7. The mean cirral movement of *Balanus improvisus* during 2 min.

Tabel 1. ANOVA results

Littorina litorea	df	F	P
pH 8	1	4,691	0,62
pH7,5	1	0,088	0,775
pH 7.0	1	1,325	0,283
pH 6,5	1	1,448	0,263
pH 6.0	1	0	1
L.litorea escape response			
pH 8	1	16,173	0,004
pH 7,5	1	1,023	0,342
pH 7,0	1	1,998	0,195
pH 6,5	1	6,341	0,036
pH 6,0	1	0,001	0,97
Balanus improvisus			
pH 8	1	9,627	0,015
pH 7,5	1	5,151	0,053
pH 7,0	1	1,272	0,292
pH 6,5	1	0,152	0,706
pH 6,0	1	10,627	0,012

Discussion

Our study showed that there are distinct differences in activity patterns when we compared the animals in pH 8.0 (Fig.1, 11) with the animals in pH 6.0-7.5 (Fig.2-5,7-10). The conclusions indicated that exposure to low pH resulted in increased stress. The results from ANOVA (Tab.1) showed significance in pH 8 for all tests and in pH 7.5 for *Balanus improvises*. This confirms our conclusions. The escape response on *Littorina littorea* showed however a decrease in escape response in pH 7.0 (2.0 %), 7.5 (7.0 %) compared to pH 8. In pH 6.0, 6.5 the escape response increased compared to pH 8 (5.3 % resp. 10.5 %) (Fig.6). This result may indicate that *L. littorea* can manage or might even benefit from a small decrease in pH. But when the pH continue to decline the negative effects are greater than the positive effects. The data showed that the cirral movements of *Balanus improvisus* were more frequent in seawater with lower pH then the ones in unaltered seawater and the tank with pH 7.5. Other studies made on barnicles (*Semibalanus balanoides*, Findley, et al., 2009; *Amphibalanus amphitrite*, Mcdonald, et al., 2009; *Semibalanus balanoides* & *Elminius modestus*, Findley, et al., 2009a) also revealed physiological changes when exposed to lower pH-concentrations. Our theory about why *B. improvisus* are not as effected as much by pH 7.5 as by the lower pH-values are that it is use to fluctuations in pH because it lives in the intertidal zone. Our data also showed that there are increases in activity for the *Littorina littorea* in the lower pH-values when compared to the unaltered seawater at pH 8. Our study indicates that marine organisms are affected by ocean acidification.

A study made by Bibby. et al. (2007) also showed a difference in behavior patterns for *L.littorea* exposed to low pH-values compared to higher pH-values. The increased activity of *L.littorea* and *B. improvisus* will increase the energy consumption, this might affect essential functions as; reproduction, growth, feeding, respiration calcification and motion etc. with reduced fitness as a result (Turley, 2010). Disturbances in these functions may lead to disadvantage for example; difficulties when competing with other organisms, increased susceptibility for pathogens and an increase in mortality due to predation. Other marine species may benefit from *L.littorea* and *B.improvsus*, because of reduced viability due to ocean acidification. This can affect the species composition, food-web dynamics, trophic levels and possibly species ecological niches. The increased energy consumption of *L.littorea* might lead to an increase in grazing to compensate for the energy loss. Since *L.littorea* have an important role in intertidal habits as grazer due to its high abundance, this changed behavior can affect the habitat composition and structure (Bibby. et al. 2007). In the case of *B. improvisus*, the barnacle is sessile and can only compensate for the increase in energy consumption by increasing its feeding activity. If it lives in a nutrient-poor environment, it might not be enough to simply increase its cirral movement. Because there aren`t enough nutrients available in the water, to compensate for the loss of energy. If this is true *B. improvisus* might have difficulties in living in a nutrient-poor environment. This would open up “new” areas for other marine species to colonize and thereby allowing them to spread to other areas or expand. If we could see a difference in behavior patterns for durable and common species such as *L. littorea* and *B. improvisus* (Findley. et al. 2009), how will rarer and more sensitive species respond and how will they be affected by ocean acidification? The earth’s surface is covered by 70 % of oceans (Turley, 2010); a fact that often is disregarded by society when talking about environmental issues. There are studies about rising CO_2-levels and what impacts it may have on the climate and how it affects species composition and diversity. Most of these studies have focused on how the terrestrial organisms are affected and it´s only in recent years beginning at 2003 with Caldeira and Wickett and their model predictions that the science community have begun to understand and do research in the field of ocean acidification. Ocean acidification is a relatively new phenomenon and little has been done in this field. The ocean is the largest coherent ecosystem on the earth and is a vital environment to such a large number of organisms that it is crucial to understand how the organisms in it will be affected by this process. More public attention and research are needed, but we hope that this report will give some insight and understanding to the phenomenon of ocean acidification.

The method of this study had several benefits: it was easy to apply, the methods were easy to understand and it didn't have any complex moments. It was cost-efficient, it didn´t require a lot of equipment, most can be found in a regular lab. The acid used is inexpensive and easy to come by. A downside to this method was that it is very time consuming. The chemical acetum used in the study was effective in lowering the pH in the sea water, but it had to be applied daily. The chemical acetum might affect the test organisms different then CO_2 does in the oceans. For longer studies water circulation is to consider instead of static water. Benefits of circulation are that you get a more steady supply of food and a more continues supply of oxygen.

References

Findley H.S., Kendall. M.A., Spicer. J.I., Widdicombe. S., 2009. Post-larva development of two intertidal barnacles at elevated CO_2 and temperature. Mar. Biol. 157: 725-735

Findley H.S., Kendall M.A., Spicer J.I., Widdicombe S., 2009a. Relative influences of ocean acidification and temperature on intertidal barnacle post-larvae at the northern edge of their geographic distribution. Est. Coast. S. Sci. 86: 675-682

Fish, J.D., Fish S., 1996. A Student´s Guide to the Seashore, Cambridge University Press

McDonald M.R., McClintock J.B., Amsler C.D., Rittschof D., Angus R.A., Orihuela B., Lutostanski K., 2009. Effects of ocean acidification over the life history of the barnacle *Amphibalanus amphitrite*. Mar. Eco. Prog. Ser. Vol. 385: 179-187

Moen F.E., Svensen E., 2003. Dyreliv i havet, KOM Forlag a/s

Porzio L., Buia M.C., Hall-Spencer J.M., 2011. Effects of ocean acidification on macroalgal communities. J. Exp. Mar. Biol. Ecol.

Turley C., Eby M., Ridgewell A.J., Schmidt D.N., Findlay H.S., Brownlee C., Riebesell U., Fabry V.J., Feely R.A., Gattuso J.-P., 2010. The societal challenge of ocean acidification. M. Pol. B. 60: 787-792

Tynan S., Podyke B.N., 2010. Effects of lower surface ocean pH upon the stability of shollow water carbonate sediments. S. Tot. Env. 409: 1082-1086

Widdicombe S., Spicer J.I., 2008. Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us. J.Exp. Mar. Biol. Ecol. 366: 187- 9