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Effect of Ocean acidification on growth, calcification and recruitment of calcifying and non-calcifying epibionts of brown algae

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Abstract

Anthropogenic emissions of CO_2 are leading to an acidification of the oceans by 0.4 pH units in the course of this century according to the more severe model scenarios. The excess of CO_2 could notably affect the benthic communities of calcifiers and macrophytes in different aspects (photosynthesis, respiration and calcification). Seaweeds are key species of nearshore benthic ecosystems of the Baltic Sea. They frequently are the substratum of fouling epibionts like bryozoans and tubeworms. Most of those species secrete calcified structures and could therefore be impacted by the seawater pCO_2. On the other hand, the biological activity of the host may substantially modulate the pH and pCO_2 conditions in the thallus boundary layer where the epibionts live. The aim of the present study was to test the sensitivity of seaweed macrofouling communities to higher pCO_2 concentrations. Fragments of the macroalga *Fucus serratus* bearing the calcifiers *Spirorbis spirorbis* (Annelida) and *Electra pilosa* (Bryozoa) and the non-calcifier *Alcyonidium gelatinosum* (Bryozoa) were maintained for 30 days under three pCO_2 conditions: natural 460 ± 59 µatm and enriched 1193 ± 166 µatm and 3150 ± 446 µatm. Our study showed a significant reduction of growth rates and recruitment of *Spirorbis* individuals only at the highest pCO_2. At a finer temporal resolution, the tubeworm recruits exhibited enhanced calcification of 40 % during irradiation hours compared to dark hours, presumably due to the effect of photosynthetic and respiratory activities of the host alga on the carbonate system. *Electra* colonies showed significantly increased growth rates at 1193 µatm. No effect on *Alcyonidium* colonies growth rates was observed. Those results suggest a remarkable resistance of the algal macro-epibiontic communities to the most elevated pCO_2 foreseen in year 2100 for open ocean (~1000 µatm) conditions possibly due to the modulation of environmental conditions by the biological activities of the host alga.

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1 Introduction

Carbon dioxide is essential to the functioning of the global ecosystem. It is the substrate of photosynthesis for production of organic matter and gaseous oxygen. Atmospheric gas composition is a major driver of the evolution of species. Examples are the appearance and spreading of C4 plants since the cretaceous with the low pCO_2 (Collatz et al., 1998; Prentice and Harrison, 2009) or the giant arthropods in the high pO_2 and pCO_2 late Paleozoic (Graham et al., 1995). That era (−415 to −250 My) has seen the reduction of atmospheric pCO_2 from 5000 to less than 1000 µatm by intense burial into fossil fuels (COPSE model, Bergman et al., 2004). Those stocks became the energy source of human societies in the 20th century, abruptly releasing the buried CO_2 in the atmosphere. The ocean is in gaseous equilibrium with the atmosphere. CO_2 is an acidic gas and its dissolution leads to the release of H^+ ions and reduction of seawater pH. This phenomenon of ocean acidification is already responsible for a drop of 0.1 pH units since the preindustrial time, corresponding to a pCO_2 shift from 280 to 380 µatm (Orr et al., 2005). The most severe scenarios are predicting a peak of atmospheric pCO_2 of 1400 µatm during the 23th century (Ridgwell et al., 2007). This transition will increase the corrosiveness of seawater to the calcium carbonate forming the shells and skeletons of numerous marine species. First impacted will be the calcareous formations under the crystalline form of calcite containing a high percentage of $MgCO_3$ (ex: echinoderms, coralline algae), followed by the ones under the form of aragonite (ex: scleratinian corals), and finally the ones with low $MgCO_3$ (ex: oysters) (Morse et al., 2006; Fabry et al., 2008; Ries, 2011). The corrosiveness of seawater to aragonite and calcite are expressed by the saturation states: Ωa_{rag} and Ω_{calc}. When $\Omega_x \leq 1$ $CaCO_3$ tends to dissolve whereas when >1 it tends to precipitate. Surface waters of the Baltic Sea are naturally more corrosive to $CaCO_3$ than open ocean waters because of the low alkalinity/salinity of the water added to the low temperature in winter and the occasional upwelling of deep hypoxic waters in summer (Thomas and Schneider, 1999). During upwelling events, pCO_2 up to 2500 µatm was recorded (Thomsen et al., 2010;

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Saderne, 2012). Tyrrell et al. (2008) suggested the high pCO_2/low CO_3^{2-} in winter as a cause of the absence of coccolithophores in the Baltic. Fucoids are key species of the Baltic nearshore benthic habitats providing primary producer services, spawning and nursery areas, food source as well as substratum for macrofoulers (Boaden et al., 1975; Kautsky et al., 1992). Macrobial epibionts associated with Western Baltic macrophytes are mostly composed of filamentous algae, barnacles (mostly *Amphibalanus improvisus*), the tubeworm *Spirorbis spirorbis* and several species of Cheilostomes bryozoans (Hagerman et al., 1966). These macrofoulers are very important for/play an important role in seaweed ecology. *Spirorbis spirorbis* can overgrow fucoidblades (O'Connor and Lamont, 1978; Kersen et al., 2011) presumably causing shading and increases in weight and brittleness. The photosynthetic activity of *Fucus serratus* is reduced by 50 and 85 % due to the covering by *Electra pilosa* and *Alcyonidium hirsutum*, respectively (Oswald et al., 1984). Bryozoans contribute to the degeneration of the underlying thallus and enhance the risk of breakage by wave action (Dixon, 1981; Krumhansl and Scheibling, 2011). In kelp forests (*Laminaria* sp.) this can lead to extensive defoliation and severely impact algae reproduction (spore release) (Saier and Chapman, 2004), affecting the entire ecosystems and ultimately stocks of commercially important species such as lobsters (Wharton and Mann, 1981; Scheibling et al., 1999). On the other hand, bryozoans are increasing habitat diversity and improving biodiversity (Cocito, 2004). They directly supply the host algae with ammonium (Hurd et al., 1994) and dissolved inorganic carbon (DIC) (Munoz et al., 1991) and are a food source for grazers such as nudibranchs and urchins (Seed, 1976; Harvell, 1984; Nestler and Harris, 1994).

Within this study we tested the hypothesis that ocean acidification disadvantages calcifying epibionts to the benefit of non-calcifying ones. The serpulid calcifying tubeworm *Spirorbis spirorbis* and two bryozoan species, the calcifying cheilostome *Electra pilosa* and the keratinous Gymnolaete *Alcyonidium hirsutum* were investigated regarding their growth, calcification and recruitment (spirorbis only) during 30 days of incubation under three pCO_2 conditions. In addition, we tested the hypothesis that the diurnally

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fluctuating physiological activity of the host alga will create conditions in the boundary layer which are favorable or disfavorable for calcification of the epibionts during times of net uptake (day) and net release (night) of CO_2 respectively. In this respect, growth rates of spirorbis recruits were assessed under light and dark conditions and the three pCO_2 levels.

2 Materials and methods

2.1 Animal collection

Fucus serratus individuals fouled by *Spirorbis spirorbis*, *Electra pilosa* and *Alcyonidium hirsutum* were collected in less than 2m depth in Eckernförde Bay (Western Baltic Sea, Germany, 54°27′ N, 9°53′ E) on 1 February 2011.

The bryozoans *Electra pilosa* and *Alcyonidium hirsutum* are colonial filter feeders. The colonies are composed of box like subunits called zooids. They are protected by a skeleton of calcium carbonate (*Electra*) or keratin (*Alcyonidium*) pierced of channels connecting the zooids to their neighbors. These connections allow the allocation of energy to the budding edge of the colony making it a single organism with deciduous organs (Lidgard and Jackson, 1989). *Spirorbis spirorbis* is a filter feeding tubeworm of maximum 5 mm length protected by a spiraled calcified tube. The tube is secreted by glands located under a collar between the head and the thorax (Nott and Parkes, 1975). Embryos are brooded within the calcareous tube in an egg bag attached to the parental body (Knight-Jones et al., 1972). Swimming non-feeding larvae are emitted in correlation with environmental factors. Settlement and metamorphosis into a juvenile tubeworm occur within few hours (Knight-Jones, 1951).

2.2 Acclimation and staining

Algae and animals were acclimated under ambient pCO_2 (400 to 500 µatm) during seven days prior to the experiment. Cultivation was made in constant temperature

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room at 15 °C. Light was provided by three Biolux neon tubes (Osram, Germany), delivering 300 µE under 12h day/night cycles. Animals were fed *ad libitum* with microalgae *Rhodomonas* sp. This microalga species is a convenient food source for bryozoans and spirorbis (Hermansen et al., 2001; Lisbjerg and Petersen, 2001). After two days, equivalently fouled sections of one fucus thallus of 1 ± 0.13 g were cut. Mean ± SD numbers of macrofoulers per section were 26.8 ± 16.8 *Spirorbis*, 6.2 ± 6.1 colonies of *Electra pilosa* and 1.9 ± 1.8 colonies of *Alcyonidium hirsutum*. Animals were stained for five days with calcein/seawater at 50 mg l^{-1}. Calcein is a vital fluorescent dye which chelates Ca^{2+} and Mg^{2+} ions, incorporating into growing shells and skeletons and thereby setting a mark for later measurements of their growth rates (see Fig. 1) (Smith et al., 2001; Comeau et al., 2009; Dissard et al., 2009).

2.3 Maintenance system

30 fucus sections of the initial pool were incubated in 650 ml tissue culture flasks (Sarstedt, Germany) randomly distributed between the three pCO_2 treatments. Treated flasks were aerated at 460 µatm CO_2 (ambient), 1200 µatm CO_2 and 3000 µatm CO_2 using an automatic system mixing CO_2 with ambient air (Linde gas and HTK Hamburg, Germany) (see Thomsen et al., 2010 for details). The gas bubbling assured a continuous mixing of the test water. Natural Baltic seawater from the Kiel Bight (Western Baltic Sea, Germany, 54°19′ N, 10°08′ E) was used in the experiment, after storage in a 300 l tank and sterilized by a Microfloat 1 floating UV lamp (Aqua Concept Karlsruhe, Germany) and aerated with ambient air. The stock was regularly renewed. Before use, the seawater was equilibrated with the three pCO_2 treatment levels in three separate tanks for 24 h. The seawater of the flasks was renewed every third day and the animals fed thereafter with *Rhodomonas* sp. to a final concentration of 10 000 cells ml^{-1}. The concentrations of the stock cultures were assessed with a Coulter Counter (Beckman Coulter, USA). After 30 days of incubation, algal fragments were fixed and conserved in borax-formaldehyde-seawater solutions suitable for

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preservation of calcareous structures (see Maybury and Gwynn, 1993, for the detailed recipe) prior to fluorescence microscopy analyses of stained bands and growth rates.

2.4 Seawater chemistry

Seawater processing for carbonate system measurements was made according to the standard operating procedure (SOP) 1 of Dickson and Sabine (2007). Three random flasks in each treatment and the three mixing tanks were sampled for seawater prior to each water exchange. The temperature of the flasks and the tanks were measured with 0.01 °C precision for future in-situ pH recalculation (see below). Samples were analyzed in the laboratory for DIC and pH_{tot}. DIC was measured with an AIRICA (Marianda, Germany), the measurement principle is based on the Infrared measurement of CO_2 (g) purged out of an acidified sample. The system was calibrated on every measurement day with Certified Reference Material (CRM) (Andrew Dickson, Scripps Institution of Oceanography). The pH on total scale was measured as follows. Seawater TRIS pH buffers for 15 psu were made according to the SOP 6a of Dickson and Sabine (2007). A combined reference/measurement electrode Metrohm Ecotrode (Metrohm, Switzerland) was used together with a pH-meter/conductimeter Mettler-Toledo SG 7/8 (Mettler Toledo, Switzerland). For calibration, the TRIS buffer was immersed in a thermostatic bath and the voltage (±0.1 mV) of the pH electrode was measured. The temperature of the buffer was modulated on a range of 1.5 °C. The temperature corresponding to every mV change was recorded with >0.01 °C accuracy with a Fluke 5658 reference thermometer equipped with a 5608 platinum resistance sensor (Fluke, USA). The procedure was repeated by increasing and decreasing the temperature to get an average voltage (mV) versus T (°C) reference curve for the electrode in the buffer. The fitting of the electrode voltage to the curve was assessed on every measurement day to check for the natural depolarization of the electrode. This calibration protocol was repeated at least once a week. The voltage and temperature of the sample was measured at the temperature corresponding to the calibration range. The sample voltage, sample temperature and the theoretical TRIS buffer voltage (corresponding through the calibration

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curve to the sample temperature) were computed through the equation of the SOP 6a to obtain the laboratory pH on total scale of the sample. The temperature of the sample measured the climate room at the time of sampling was used to recalculate the in-situ pH. Preparative work conducted on CRM together with 35 psu buffers demonstrated an accuracy of this method of 0.003 to 0.005 pH units. Salinity of the samples was measured to an accuracy of 0.01 psu with a Mettler Toledo Inlab 738 conductivity probe after calibration at 25 °C with KCl 0.1 mol l^{-1} (Fischer Scientific, USA). The overall in situ-pH and carbonate system was recalculated with the R package Seacarb (Lavigne and Gattuso, 2011) using first and second carbonate system dissociation constants for estuarine systems from Millero (2010) and the dissociations constants of HF and HSO_4^- of Perez and Fraga (1987) and Dickson (1990).

2.5 Measurement of relative growth rates (RGR) of the bryozoans

Colonies were photographed by overlapping fields of vision under epifluorescence microscope (Axio Scope A1, Carl Zeiss, Germany) at the end of the experiment. The partial pictures were reassembled into complete colony pictures. The initial colony surface (SI) (pre-staining) and the final surface (SF) (after 30 days incubation) were measured by image analysis (ImageJ, US National Institutes of Health). The relative growth rate in percent (RGR) was calculated as follows:

$$\mathrm{RGR} = \left[\ln\left(\sum_{j=1}^{n} \mathrm{SF}j \right) - \ln\left(\sum_{j=1}^{n} \mathrm{SI}j \right) \right] \cdot 100 \quad (1)$$

With n the number of colonies in one flask. The logarithmic growth pattern of bryozoans colonies have been assessed by Hermansen et al. (2001). *Alcyonidium hirsutum* colonies (non-stainables), were photographed prior and after incubation. The areas were measured and the RGR calculated as for *Electra pilosa*.

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2.6 Measurement of growth and recruitment of *Spirorbis spirorbis*

Spirorbis tube growth in mm was estimated as the length of the external arc of the coil comprised between the staining front and the tube edge (see Fig. 1a and b). In each flask, the new tube length of all worms was summed and divided by the number of worms. Spirorbis recruitment was quantified for each flask as the number of juveniles found on algae sections at the end of the experiment divided by the number of adults.

2.7 Effect of light on the growth of *Spirorbis spirorbis* juveniles

To test the effect of photosynthetic activity and pCO_2 on the growth of juvenile worms in the thallus boundary layer, calcein was added to all the flasks (final concentration: 20 mg l^{-1}) during the last 24 h of the experiment, half of the flasks were kept in constant light, the other half was darkened with aluminum foils. Growth was measured as the size of the stained newly formed tube (see Fig. 1c and d).

2.8 Statistical analysis

Statistical analyses were conducted with Statistica 7 (Statsoft, USA). Treatments were compared using one way ANOVAs and Tukey's HSD tests. Assumption of normality and homoscedasticity were tested with Shapiro-Wilk's and Levene's tests. In case of abnormality a transformation by natural logarithm was done.

3 Results

3.1 Chemistry

A summary of the variables of the carbonate system measured in the flasks during the 30 days duration of the experiment derived from pH_{tot} and DIC are presented in Table 1. Average (±SD) pCO_2 were 460 ± 59 µatm, 1193 ± 166 µatm and

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3150 ± 445 µatm respectively for the control and the two elevated treatments. Saturation states (mean ± SD) below one, for aragonite (0.82 ± 0.14) and for calcite (0.66 ± 0.14) were reached in the 1200 µatm and in the 3150 µatm pCO_2 treatments, respectively.

3.2 *Spirorbis spirorbis*

The average ± SD of newly formed tube within 30 days were 0.95 ± 0.32, 0.83 ± 0.05 and 0.58 ± 0.15 mm worm^{-1} flsk^{-1} in the control, 1120 µatm and 3150 µatm treatments (Fig. 2a) respectively. The growth of spirorbis tubes was significantly affected by pCO_2 (one way ANOVA, $p \leq 0.01$). Reductions of growth rates were found at 3150 µatm compared to the 460 µatm (Tukey's HSD, $p \leq 0.01$) and, marginally, relative to 1200 µatm (Tukey's HSD, $p \leq 0.1$). While no signs of shell injuries were visible at 460 µatm and 1200 µatm, all spirorbis tubes exhibited substantial shell dissolution at 3150 µatm (see Fig. 1a and b). This loss of integrity affected principally the outer spiral of the tube exposing the worm soft bodies and the embryo bags to the external seawater. Recruitment of spirorbis was significantly affected by pCO_2 (one way ANOVA, $p \leq 0.001$) (Fig. 2b). The numbers of settled juveniles per adults were significantly lower at 3150 than at 1200 µatm (Tukey's HSD, $p \leq 0.01$) and in the control (Tukey's HSD, $p \leq 0.001$) with mean ± SD densities respectively of 1.76 ± 1.5, 7.63 ± 6.7 and 9.34 ± 4.7 juv worm^{-1}. Juvenile tubes growth rates were significantly enhanced under light condition, mean ± SD of 76.8 ± 6.7 µm d^{-1} against 46.5 ± 4.26 µm d^{-1} in the dark, corresponding to a 39.5 % difference (Factorial ANOVA, $p \leq 0.001$; Fig. 3). The pCO_2 treatments produced no significant effect on the juvenile's growth rates but important damages on the early tubes were observed in the 3150 µatm treatment (see Fig. 1c and d).

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3.3 Bryozoans

pCO_2 had a significant effect on growth rates of *Electra pilosa* (one way ANOVA, $p \leq 0.05$) enhancing colony growth at the intermediate acidification level, 1200 µatm (76.5 ± 31.7 %), relative to the highest level, 3150 µatm (43.0 ± 32.2 %) (Tukey's HSD, $p \leq 0.01$) (Fig. 4a) which was similar to the low pCO_2 treatment (50.7 ± 18.1 %). In this highest pCO_2 treatment, the skeleton of uninhabited zooids dissolved completely, resulting in a "ghost" organic matrix of the exact same shape.

Relative growth rates of *Alcyonidium* were similar under all pCO_2 levels: 27.2 ± 25.5 % in the control, 36.5 ± 40.8 % in the 1200 µatm treatment and 19.18 ± 17.9 % in the 3500 µatm treatment (ANOVA, $p > 0.1$; Fig. 4b).

4 Discussion

The present study demonstrated that the calcifying tubeworm *Spirorbis spirorbis* was affected in its growth and recruitment at 3150 µatm CO_2. In this treatment, adults and juveniles shells were exhibiting important damages due to dissolution. The growth rates of the juveniles were not affected directly by pCO_2 but by irradiation, attesting to a protective effect of photosynthesis of *Fucus* on the calcifying organisms living in the boundary layer surrounding the thallus. In the boundary layer (<1 mm off the thallus surface) conditions (O_2, pH) have been shown to fluctuate by orders of magnitude between light and dark phases (De Beer and Larkum, 2001; Beer et al., 2008; Spilling et al., 2010), affecting the physiology of the incrusted animals (Woods and Podolsky, 2007). The bryozoans *Electra pilosa* and *Alcyonidium hirsutum* were marginally affected in their growth by the pCO_2 regardless of the nature of their skeletons. However, the dissolution of the empty skeletons at 3150 µatm CO_2 was noted for *Electra pilosa*.

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4.1 Bryozoans

Our results suggest that the growth in the bryozoan *Electra pilosa* is not negatively affected by very high pCO_2 and even stimulated at 1200 µatm. However, the empty zooids lost their calcified structures at 3150 µatm, when the seawater was undersaturated with regard to calcite. These results could be explained by the known mineralogy of *Electra pilosa*. Their skeleton is exclusively calcitic with 7 to 10 % of $MgCO_3$ (Rucker and Carver, 1969; Smith et al., 2006; Taylor et al., 2009), reminiscent of the appearance of this sub-order in the calcitic seas of the late Jurassic/Cretaceous (Stanley, 2006). In the natural CO_2 vents of Ischia (Italy), Rodolfo-Metalpa et al., (2010) found maintenance of growth and calcification of transplanted colonies of the bryozoan *Myriapora truncata* at 1400 µatm pCO_2, and Ω_{calc} = 3.08 but dissolution of dead zooid skeletons. Dead Baltic *Electra pilosa* skeletons dissolved only when $\Omega_{calc} \leq 1$ (3150 µatm treatment) and therefore seem more resistant to seawater corrosiveness. Nevertheless, both our results corroborate the important role of the zooidal tissues surrounding the skeletons of bryozoans, presumably creating a barrier to corrosive seawater. Such protective effects of external living tissues and organic layers over calcified structures have already been observed in mussels and corals (Rodolfo-Metalpa et al., 2011). Calcification processes in bryozoans are largely unstudied but the functioning of the calcein staining could give a first clue into the process. Bentov et al. (2009) suggest that calcein staining of biomineralized structures implies direct vacuolization of seawater. The classes of calcifiers using this mechanism are presumably the most sensitive to the environmental pH (Weiner and Dove, 2003; Weiner and Addadi, 2011) as they use external seawater at the sites of calcification. Within the order of the Cheilostomata, calcein staining worked with the genera *Electra* (sub-order: Malacosteginae, this study) and *Adeonellopsis*, (sub-order: Ascophorae, Smith et al., 2001) but not with *Flustra* (sub-order: Flustridae, Fortunato and Saderne, unpublished), suggesting different mechanisms of calcification within the cheilostomes. Cheilostome bryozoans evolved calcareous skeletons polyphyletically on at least two distinct occasions and

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as a consequence possess diverse calcified structures that could be based on different calcification mechanisms (Jablonski et al., 1997; Smith et al., 2006; Taylor et al., 2009).

The growth response of *Alcyonidium hirsutum* to pCO_2 followed the same pattern as for *Electra pilosa* but with growth rates 50 % slower. These results are similar to observations in the field. *Electra pilosa* tends to dominate *Fucus* blades by its rapid growth rates (Ryland and Stebbing, 1971) while *Alcyonidium*, less competitive for space, seems more ubiquist in term of habitats colonized (O'Connor and Lamont, 1978).

4.2 Calcification of *Spirorbis spirorbis*

Reduction of growth and shell damages were observed only when seawater reached undersaturation for calcite at the highest level of acidification (3150 µatm). Our findings differ from previous results obtained on the serpulid polychaete *Hydroides crucigera* by Ries et al. (2009). They found a linear decrease of calcification with increasing pCO_2 from 280 to 2800 µatm. This difference might be due to the difference in mineralogy between the two species. *Hydroides crucigera* tubes are bimineralic with 40 % aragonite and 60 % calcite including 20 % Mg calcite (Ries, 2011), while spirorbis tubes are almost exclusively calcitic with 15 % $MgCO_3$ (Bornhold and Milliman, 1973). Generally, serpulids exhibit a wide variety of tube ultrastructure and mineralogy, most of them being bimineralic (Vinn et al., 2008). Their mineralogy seems to be modulated by the saturation state of seawater. In exclusively calcitic serpulids, predominance of low Mg calcite in tubes is observed at high latitude in cold corrosive seawater compared to tropical species (Bornhold and Milliman, 1973). In bimineralic species, no geographic relationship between seawater corrosiveness and low aragonite content in tubes is observed (Bornhold and Milliman, 1973), even if in culture a reduction in aragonite is observed for *Hydroides crucigera* exposed to acidified seawater (Ries, 2011). Therefore, we may expect that tubeworm response to ocean acidification is species-specific

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even if the absence of organic covering protecting their tubes might limit their capacities of adaptation of calcification to corrosive waters.

4.3 recruitment and juvenile growth *of Spirorbis spirorbis*

Breading of Spirorbis larvae in the parental tube varies between 10 to 25 days, depending on seawater temperature (Daly, 1978) and settlement occurs within a few hours (Knight-Jones, 1951). In our experiment the first juveniles appeared after two weeks. Their number was reduced by 80 % in the 3150 µatm treatment relative to the low acidification condition. This was associated with the observation of numerous unspawned eggs bags in the adult tubes. Kurihara (2008) identified five critical early life stages in invertebrates: fertilization, cleavage (embryogenesis), planktonic larva, settlement and metamorphosis. Accidents having happened to those early life stages could explain our results. Exchange of sperm and fertilization of eggs might not have occurred during the experiment since sperm stored during the previous summer in a specific organ of the head could have been used to fertilize the eggs (Potswald, 1967; Daly and Golding, 1977). Likewise, some embryos in dormancy at the beginning of the experiment could have seen their development reactivated. A delaying or interruption of the embryogenesis is a possible explanation for our results, due to the exposition of the embryo bags to external acidified seawater after dissolution of the parental tubes. Such embryogenesis accidents have been noted for gastropods, bivalves and echinoderms (Desrosiers et al., 1996; Kurihara and Shiryama, 2004; Ellis et al., 2009). The planktonic stage is another sensitive life stage for invertebrates on which ocean acidification can be deleterious (Dupont et al., 2008; Kurihara, 2008). In Spirorbis, however, the shortness of the pelagic phase (a few minutes to 3 h) and the non-calcifying nature of the larvae may reduce the impact of acidified seawater. *Spirorbis spirorbis* specifically settles on brown algae and this preference is most likely chemically mediated (De Silva, 1962; Al-Ogily, 1985; Qian, 1999). The necessary sensitivity to cues could be affected by seawater pH and making the larvae incapable of recognizing their hosts. This has been found in clown fish (*Amphiprion percula*) larvae, becoming "blind" to their host anemones cues

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under high pCO_2 (Munday et al., 2009). Reduction of settlement could also be caused by an increase of the algae defenses at elevated pCO_2. Algal metabolites have an inhibiting and/or facilitating effect on foulers. Those effects can be direct (e.g. Wahl et al., 2010), or indirect by the mean of the microbial biofilms (Lau and Qian, 1997; Harder et al., 2002; Nasrolahi et al., 2012). So far no ocean acidification studies on seaweeds defenses have been made.

As a novel aspect of epibiotic associations, our study strongly suggests that juvenile spirorbis benefit from algal photosynthesis which creates temporarily conditions favorable for calcification even under severe ocean acidification. The growth rate of the juveniles was about 40 % faster under light than in the dark. Settlement, metamorphosis and early growth of *Spirorbis spirorbis* takes place in the boundary layer adhering to the thallus surface of Fucus. In this layer of 300 µm to 1 mm thick, pH can be pushed to beyond 9 in daytime by the CO_2 uptake associated with photosynthesis (Fischer and Saderne, unpublished data). Under dark conditions, in contrast, the decrease of pH due to algal respiration seems marginal (De Beer and Larkum, 2001; Beer et al., 2008; Spilling et al., 2010). We may expect that similar phenomena take place in natural stands of marine macrophytes (algae, seagrasses) where the population's combined photosynthesis pushes the habitat's saturation state to favorable conditions and allows calcifiers in the community to produce skeleton material during certain daylight hours.

5 Conclusions

One widespread expectation about ocean acidification is that non-calcified organisms will outcompete calcifying organisms (Fabry et al., 2008) as observed between corals and seaweeds (Diaz-Pulido et al., 2011). A shift to non-calcifying epibionts is observed under high pCO_2 observed in Mediterranean seagrasses due to the disappearance of coralline algae (Kuffner et al., 2007; Martin et al., 2008). Our study does not suggest such an effect. In our experiment, epibionts of *Fucus serratus* resisted future pCO_2 conditions predicted for the open ocean. Part of this resistance may have been caused

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by the alga's photosynthetic activity overriding the simulated acidification in the thallus boundary layer where the epibionts occur. Like in the global ocean, Baltic Sea pCO_2 is predicted to increase, however the amount of this increase remains unknown (Schneider, 2011). This incertitude is caused by the simultaneous but sometimes opposite shift of different parameters: increase of mean sea surface temperature by 2 to 3.5 °C, strengthening of westerly wind, increase of the river run off provoking a decrease of sea surface salinity and increase of inputs of dissolved organic carbon (Schrum, 2001; Gräwe and Burchard, 2011). Locally, in the nearshore ecosystems of the Western Baltic, offshore winds may lead to an upwelling of deoxygenated deeper water increasing the pCO_2 to more than 2500 µatm (Thomsen et al., 2010; Saderne et al., 2012). Strengthening of these winds plus enhanced eutrophication (intensifying anoxic events) might prolong the duration of those hypercapnics events. Consequently, even epibionts in the boundary layer of macroalgae may ultimately be affected by global change directly – and also indirectly by shifts in the physiology and distribution of their host algae. This study illustrates, however, that small scale processes at the habitat or microhabitat scale must be considered when assessing the impact of acidification on species and communities.

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Table 1. Seawater carbonate system in the experimental flasks, derived from DIC and pH_T, data are the means ± SD of three flasks sampled every three days before water exchange, along the 30 days duration of the experiment (n = 10).

Treatment	T° (°C)	Salinity (psu)	pH_T	$p\,CO_2$ (µatm)	DIC (µmol kg^{-1})	A_T (µmol kg^{-1})	Ω_{arag}	Ω_{calc}
Control	16.1 ± 0.7	16.7 ± 0.4	8.105 ± 0.031	460.56 ± 59.42	1966.4 ± 40.4	2089.2 ± 52.5	1.80 ± 0.22	3.02 ± 0.34
1200 µatm	15.7 ± 0.5	16.7 ± 0.4	7.726 ± 0.076	1193.36 ± 166.38	2115.2 ± 62.4	2135.0 ± 71.0	0.82 ± 0.14	1.38 ± 0.23
3150 µatm	15.9 ± 0.8	16.8 ± 0.4	7.334 ± 0.079	3150.40 ± 445.73	2306.8 ± 251.8	2298.9 ± 57.5	0.40 ± 0.09	0.66 ± 0.14

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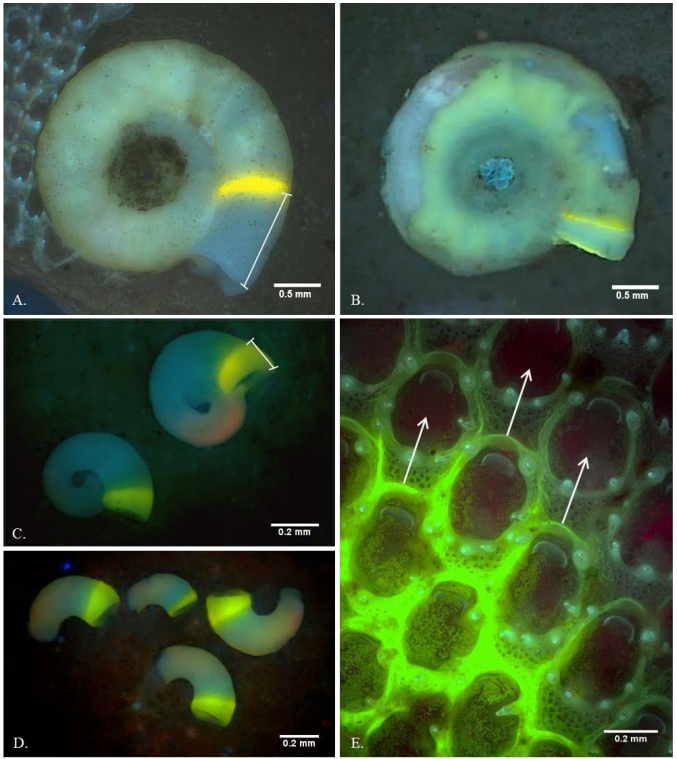

Fig. 1. Illustrative examples of *Spirorbis spirorbis* and *Electra pilosa*. **(A, B)** Adult *Spirorbis spirorbis* tubes after 30 day incubation at 460 µatm and 3150 µatm CO_2 respectively. **(C, D)** Juveniles *Spirorbis spirorbis* tubes after 30 day incubation at 460 µatm and 3150 µatm CO_2 respectively. Note the important dissolution of the shell in B. and the disappearance of part of the tube in **(D)**. **(E)** *Electra pilosa* colony. All photos were taken under epifluorescence microscope, yellow/green: calcein staining. The white lines are showing the considered distances for the growth measurements of spirorbis tubes. Arrows indicate the direction of the growth subsequent to the calcein staining in *Electra*.

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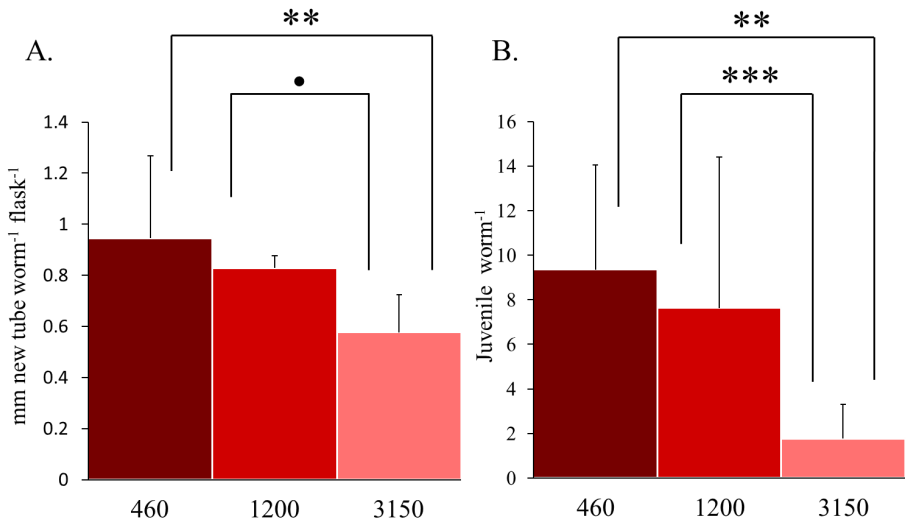

Fig. 2. (A) Growth of the tubes of *Spirorbis spirorbis* during the 30 days incubations at 460 µatm, 1200 µatm and 3150 µatm CO_2, data are the mean ± SD of newly formed tubes sections in mm per worm per flask. **(B)** Recrutement of *Spirorbis spirorbis* during the 30 days incubations at 460 µatm, 1200 µatm and 3150 µatm CO_2, data are the mean ± SD of the number of juveniles settled per adult between flasks. One-way ANOVAs, $n = 10$, statistical significance: ***: $p \leq 0.001$, **: $p \leq 0.01$, *: $p \leq 0.05$, •: $p < 0.1$.

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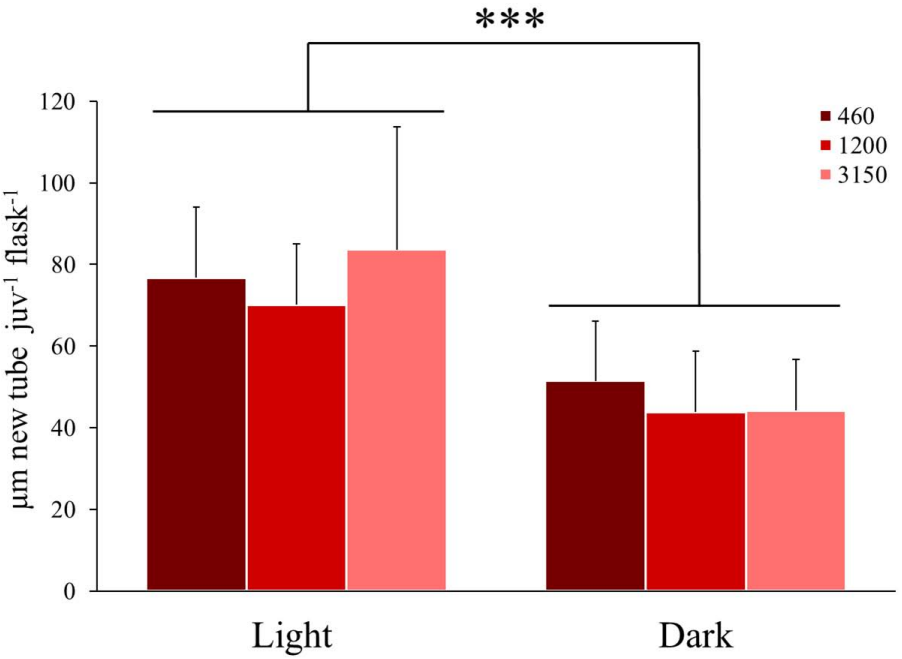

Fig. 3. Growth in µm of the tubes of the juveniles of *Spirorbis spirorbis* during 24 h in light and dark condition exposed to pCO_2 of 460 µatm, 1200 µatm and 3150 µatm. Two-way ANOVA, $n = 5$, statistical significance: ***: $p \leq 0.001$.

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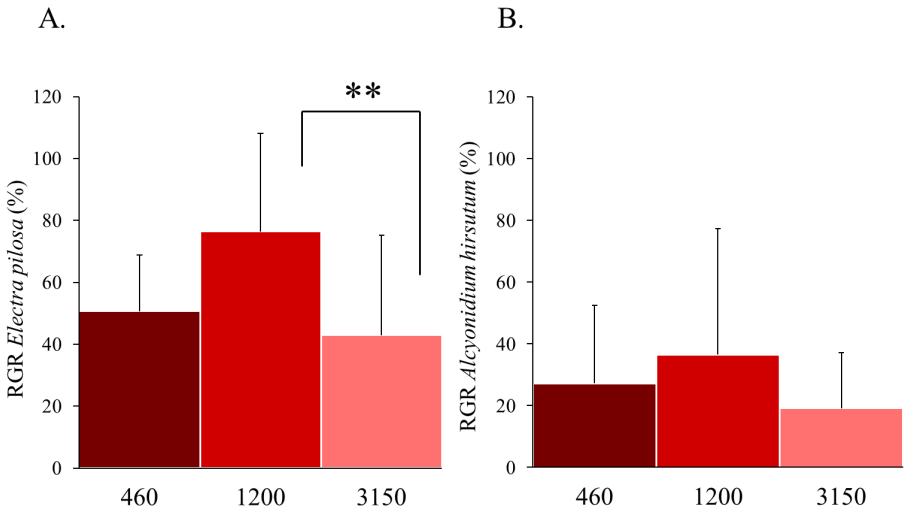

Fig. 4. Relative growth rate (RGR) of **(A)** *Electra pilosa*, **(B)** *Alcyonidium hirsutum* colonies during the 30 days incubations at 460 µatm, 1200 µatm and 3150 µatm CO_2. Data are the mean ± SD of the growth rates in % in $n = 10$ flasks. ANOVA, statistical significance: **: $p \leq 0.01$.

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