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Master Thesis:

Response of the cold-water coral *Desmophyllum dianthus* to future CO_2 concentrations

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Abstract

Future atmospheric carbon dioxide (CO_2) concentrations are considered to increase drastically (up to 500ppm) until the end of the century. Dissolution of CO_2 will cause a reduction of the aragonite saturation state in the oceans, due to ocean acidification. Aragonite is the orthogonal crystal form of calcium carbonate and is formed by coral skeletons. The cold-water coral *Desmophyllum dianthus*, from the Patagonian fjord Comau, was found to thrive at and even below the aragonite saturation horizon and at pH conditions, which are predicted for the future. The aim of the present study was to find out *D. dianthus'* thresholds towards lowered pH by the investigation of corals' physiology and behavior.

Corals' response (i.e. calcification rates (mass increase), respiration rates (oxygen uptake), and polyp extension) was quantified *in vitro* over a time period of 6 months to examine a possible acclimation potential. 32 *D. dianthus* specimens were separated in one control coral group (n = 16), maintained at ambient pH (~8.0) and one treatment coral group (n = 16) maintained at lowered pH (~7.8) in aquaria. Sea water pH was controlled by CO_2 addition.

A lowered pH of ~7.8 did not have a significant effect on *D. dianthus'* growth and respiration rates, as values at ambient pH were equal regarding growth rates ($0.19 \pm 0.13\%$ cm^{-2} d^{-1} (ambient pH), $0.19 \pm 0.10\%$ cm^{-2} d^{-1} (low pH)) and similar regarding respiration rates (22.07 ± 15.07µmol O_2 cm^{-2} d^{-1} and 23.71 ± 7.56µmol O_2 cm^{-2} d^{-1}, respectively). Respiration was even higher at lowered pH conditions, although not significantly. Also corals' behavior was not significantly influenced by lowered pH, whereby polyp extension was higher, although not significantly, at lowered pH ($75.4 \pm 34.6\%$) compared to ambient pH ($68.3 \pm 40.0\%$). However, polyp extension within the last three months of experiment was higher at lowered pH (P=0.035). Corals additionally showed higher growth rates at the end of experiment, compared to the beginning, but not significantly though.

A possible acclimation potential of corals was shown by elevated polyp extension, which might be related to increased oxygen uptake within the last weeks of experiment. *D. dianthus* might have adapted to low pH conditions in the fjord Comau, as corals are able to up-regulate their internal pH, adjusting calcification processes to highly unfavorable conditions for the formation of corals' skeletons. Thus *D. dianthus* may cope with increasing CO_2 concentrations by developing adaptation and acclimation mechanisms, representing optimal requirements to strive against ocean acidification and the thereby induced decreasing precipitation of aragonite.

1. Introduction

1.1 Ocean acidification

Industrial development (combusting of fossil fuels, cement fabrication and land use change (Erez et al, 2011)) led to increasing atmospheric carbon dioxide concentrations ($[CO_2]_{atm}$), which are expected to transcend 500 parts per million (ppm) by the years 2050 to 2100; thereby facilitating ocean acidification and global warming (Hoegh-Guldberg, 2007). Investigations of the Vostok ice core from east Antarctica by Siegenthaler and colleagues (2005) revealed that during the last 650,000 years todays' $[CO_2]_{atm}$ reached highest values. Today water temperatures are higher (+0.7°C) and carbon-ion concentrations (~210µmol kg^{-1}) and pH (-0.1 units) are lower (Feely et al, 2004; Orr et al, 2005) than during the last 420,000 years, pushed by the remarkable magnitude of nowadays $[CO_2]_{atm}$ (Hoegh-Guldberg, 2007). Future pH values are predicted to drop another 0.2 – 0.4 units in water surfaces by the end of the century, which was supposedly not documented over the last 20 million years (Feely et al, 2004; 2008).

About 25% of anthropogenic $[CO_2]_{atm}$ is absorbed by the ocean (Feely et al. 2004; Sabine et al. 2004; Canadell, 2007) producing carbonic acid by the reaction with water. The chemical reaction of water and CO_2 is described via the following equation (Eq. 1) (Hoegh-Guldberg, 2007):

$$\mathbf{CO_2} + H_2O \leftrightarrow HCO_3^- + H^+$$

$$H^+ + CO_3^{2-} \leftrightarrow HCO_3^-$$

$$\mathbf{CaCO_3} \leftrightarrow Ca_2^+ + CO_3^{2-} \qquad \textbf{(Eq. 1)}$$

Carbonic acid (H_2CO_3) dissociates in bicarbonate (HCO_3^-) and protons (H^+), whereby the protons react with carbonate ions (CO_3^{2-}), producing more bicarbonate ions and thus decreasing the availability of carbonate for the formation of calcium carbonate ($CaCO_3$). Aragonite (the orthogonal crystal form of $CaCO_3$) is the basic element of which coral skeletons are formed. Gattuso (1999) described the aragonite saturation state (Ω_{Ar}) of seawater as the product of calcium (Ca^{2+}) and CO_3^{2-} at a given temperature, salinity and pressure, divided by K_{sp} (stoichiometric solubility product of aragonite) = $[CO_3^{2-}]*[H^+]/[HCO_3^-]$ (Eq. 2) (Dickson and Millero, 1987):

$$\Omega_{Ar} = \frac{[Ca^{2+}]*[CO_3^{2-}]}{K_{sp}} \qquad \textbf{(Eq. 2)}$$

An aragonite saturation state (Ω_{Ar}) lower than 0.1 indicates undersaturation which is unfavorable for organisms to build up their skeletons. The latter is called 'calcification'. Values over 1.0 indicate supersaturation which is favorable for calcifying organisms. (Kleypas et al. 1999). With increasing ocean acidification the aragonite saturation horizon (interface between over- and undersaturation of aragonite) rises in shallower waters, as CO_2 dissolves easier at colder temperatures and at higher pressure. Hence deeper waters become unfavorable habitats e.g. for therein thriving cold-water corals, as they barely obtain aragonite for the formation of their skeletons (Kleypas et al. 1999a; Feely et al. 2004; Orr et al. 2005; Guinotte & Fabry 2008; Miller et al. 2011).

As ocean chemistry is very complex due to several interacting oceanographic processes it becomes difficult to evaluate whether ocean acidification is the major driving factor of environmental change and suggested species decline. Ocean acidification is accompanied by CO_2-induced ocean warming, whereby increasing sea water temperatures seem to compensate for lowered calcification from Ω_{Ar} (McCulloch, 2012b). McCulloch and colleagues (2012b) stated that model calculations predict minimal effects on Ω_{Ar} for the combined scenario of ocean acidification and global warming. Warmer waters close to the equator, inhabiting warm-water coral reefs, lead to a solubility reduction of dissolved CO_2 thereby increasing Ω_{Ar} enhancing precipitation of $CaCO_3$ for the formation of coral skeletons.

Ocean acidification not only results in a reduction of dissolved $CaCO_3$ in water (Kleypas et al. 1999a; b), but also leads to a reduction of corals skeletal carbonate cements (Behairy & El-Sayed, 1984). Several field and laboratory studies proof that acidified sea water, entailing a low Ω_{Ar}, reduces coral calcification and growth (Gattuso et al, 1998a; Langdon et al, 2000; Reymond et al, 2013). Further a positive correlation between calcification and Ω_{Ar} has been shown by Smith & Roth (1979) and Gattuso and colleagues (1999). If the Ω_{Ar} drops below 1.0 due to inorganic chemistry control (increase of dissolved CO_2), a dissolution of $CaCO_3$, imbedded in coral skeletons, will be noticeable (see equation (3)) (Erez et al, 2011). Remarkably, dissolution of coral skeletons at $\Omega_{Ar} \geq 1.0$ has been reported by Yates and Halley (2003; 2006), and Silverman and colleagues (2007a). As future CO_2 concentrations are predicted to increase, thereby reducing the Ω_{Ar} in waters, coral reefs thus are expected to decrease

rapidly ((1) due to reduced net calcification rates (N) and (2) due to skeletal dissolution (D), see Eq. 3). In contrast Jury and colleagues (2009) stated that HCO_3^- highly influenced calcification rates, rather than pH and thus aragonite saturation. The primary reason for the sensitivity of corals to ocean acidification is still on debate. It can be due to a lowered CO_3^{2-} ion concentration accompanied with an increased HCO_3^- ion concentration or the lowered (Ω_{Ar}).

$$\text{Gross calcification (G)} = \text{Net calcification (N)} - \text{Dissolution (D)}$$
$$\text{Hence } D = N - G \text{ and when } N = 0;\ D = -G \qquad \textbf{Eq. (3)}$$

The available evidence indicates that at a global scale reefs will go through major changes in response to climate change rather than disappear entirely (Hughes et al. 2003). Nevertheless the rate of changes in ocean chemistry due to higher $[CO_2]_{atm}$ may cause instability of coral skeletons and consequently restrict their regeneration ability (i.e. after hurricane events). The decreased ability of corals to grow and compete could lead to an alternate state, i.e. from a coral dominated to an algae dominated scenario, revealing obvious consequences for the ecosystem (Connell, 1997).

It is challenging for organisms to adapt to new conditions under rapid environmental changes (Guinotte and Fabry, 2008). Thresholds and adaptation mechanisms under varying environmental circumstances seem to differ with high variation among corals. Adaptation mechanisms likely entail energy allocation processes, as corals may maintain skeletal growth rates (apical extension) (Tsounis et al. 2012) and thereby probably reduce skeletal density; or corals may rather grow massive (and thereby probably more dense) than elongated, a common phenomenon observed for the scleractinian cold water coral *Desmophyllum dianthus* (Esper, 1794) (Försterra & Häusermann, 2003). However *D. dianthus* also shows elongated growth depending on its settling site within coral aggregations (Försterra & Häusermann, 2003), probably indicating an adaptation mechanism to the competitive process of food capturing between densely growing corals. Alternatively, corals may maintain growth rates and density by expending more energy for calcification processes (Hoegh-Guldberg et al, 2007). However, the increased energy input for calcification may lead to a reduction of energy for other physiologically important processes like reproduction (Szmant, 2002). Tsounis and colleagues (2012) also stated that corals might procure more energy in the recovery of damaged tissue (caused by anthropogenic disturbances, i.e. SCUBA diving

and fishing) rather than into reproduction. Fine & Tchernov (2007) reported an entire dissolution of coral skeleton of *Oculina patagonica* (Scleractinia) (due to lowered sea water pH= 7.4) thereby leaving behind the intact polyp tissue. Maintaining the coral again (after the lowered pH treatment) under ambient sea water pH resulted in skeletal regrowth. Further, there is evidence that the scleractinian cold-water coral *Lophelia pertusa* (Linnaeus, 1758) showed acclimation mechanisms to CO_2-induced ocean acidification (Form and Riebesell, 2012). The cold-water coral *D. dianthus* occurs within waters (in the Chilean fjord system) featuring pH gradients with concomitant variations in Ω_{Ar} (≤ 1.0), which are predicted for the future (Jantzen et al. 2013a). Nevertheless, scleratinian cold-water corals are considered to be majorly influenced by ocean acidification as they inhabit mainly deep sea regions (Freiwald et al. 2004), with a lower Ω_{Ar} and higher CO_2 concentrations compared to surface waters (Guinotte & Fabry, 2008). Whether scleractinian cold-water corals are able to survive within climate change scenarios depends on their ability to develop adaptation mechanisms and to shift their habitats into shallower regions towards the aragonite saturation horizon.

1.2 *Desmophyllum dianthus*

The scleractinian cold-water coral *D. dianthus* (prior: *Desmophyllum cristagalli* (Milne Edwards & Haime, 1848) is a cosmopolitan deep-sea species inhabiting regions from the Western and Eastern Atlantic over the Indian Ocean and the Western, Eastern and Central Pacific further to the poles of the Subantarctic and Artic (Cairns 1994). There are only two regions where *D. dianthus* does not exist: Continental Antarctica and Northern boreal Pacific (Försterra & Häussermann, 2003). The azooxanthellate coral can be found within a depths range of 8 to 2500m (Grange et al. 1981; Häussermann & Försterra, 2007) thriving at euphotic and aphotic conditions. Mainly growing on seamounts and continental slopes (Miller et al. 2011) *D. dianthus* occurs also in dense aggregations (> 1500 individuals per square meter) under overhangs in Chileans and New Zealand Fjord systems within depths of 45 m (Grange et al. 1981; Häussermann & Försterra, 2003) and 25m in the Fjord Comau (Chile, Patagonia) (Jantzen, personal communication) (Fig. 1). Thus the solitary living coral builds up reef-like banks providing a habitat for the associated community, thereby playing an important role within the ecosystem. In Chilean fjords *D. dianthus* is one of the 23 documented scleractinian cold-water corals and one of the most important habitat builder, forming a major part of the benthic macrofauna (Häussermann & Försterra, 2003, 2007).

Fig. 1 Dense aggregations of *D. dianthus* specimens under an overhang in the fjord Comau, Patagonia (Försterra & Häusermann, 2003).

1.3 Calcification

Calcification, which is the construction of scleractinian skeletons, is also termed as biomineralization (the formation of biologically produced minerals) (Estroff, 2008). $CaCO_3$ is formed by the conversion of dissolved inorganic carbon and calcium. Although, it is still on debate whether biomineralization is biologically induced (control of abiotic kinetics, i.e. ion transport) or controlled (control of extracellular calcifying medium). Allemand and colleagues (2011) concluded on the basis of several studies that calcification may rather be a biologically controlled process.

Calcification takes place within an extracellular calcifying medium (ECM), the interface between corals' skeleton and the tissues basal cell layer (calicoblastic cell layer) (Allemand et al. 2011). To fully understand the mechanisms of calcification the determination of pH and ion concentrations at the site of mineralization is fundamental. First knowledge about chemical processes within the ECM was gained by Al-Horani and colleagues (2003b), who measured higher pH and Ca^{2+} concentrations within the ECM compared to the calicoblastic cell layer and the tissue surface. Thus gaining sea water Ca^{2+} (essential for the formation of $CaCO_3$) to the site of mineralization (Eq. 4) is an energy costing process. Whether transport of ions is an active or passive process is

still on debate (Allemand et al. 2011). However, evidence in favor of active calcification processes is given (Chalker & Taylor, 1975; Chalker, 1976). An active transcellular ion transport through calicoblastic cells into the extracellular calcifying medium is conducted by Ca^{2+}-ATPase (an ion pump) transporting Ca^{2+} in exchange with protons (H^+). During the formation of $CaCO_3$ the net production of protons is either 1:1 (Eq. 4) or 2:1 (Eq. 5) per mole of $CaCO_3$ produced, depending on the source of dissolved inorganic carbon (DIC). Whether seawater derived HCO_3^- or respired (metabolic) CO_2 are transported from the calicoblastic cells to the calcifying medium is still unknown (Allemand et al, 2011).

$$Ca^{2+} + HCO_3^- \leftrightarrow CaCO_3 + H^+ \quad \textbf{(Eq. 4)}$$

$$CO_2 + H_2O + Ca^{2+} \leftrightarrow CaCO_3 + 2H^+ \quad \textbf{(Eq. 5)}$$

Allemand and colleagues (2011) stated that the calcifying medium usually reveals high pH and $CaCO_3$ saturation state compared to seawater. The latter is supported by $\delta^{11}B$ composition investigations of four cold-water coral species (including *D. dianthus*), revealing boron values which lie above the inorganic sea water borate equilibrium curve (McCulloch, 2012). As this curve is pH dependent, this suggests the ability of pH up-regulation of the internal calcifying medium. For *D. dianthus*, McCulloch and colleagues (2012) measured internal pH elevations of 0.6 to 0.8 units (generated by the Ca^{2+}-ATPase) compared to values of the surrounding seawater. Internal pH elevation is thereby accompanied by an Ω_{Ar} increase within the calcifying medium. Nevertheless, deep-sea cold-water corals are at risk as the aragonite saturation horizon rises with increasing CO_2 values leading to a suggested dissolution of the exposed aragonite skeleton. Furthermore, pH up-regulation is an active process which detracts energy for growth, reproduction and other metabolic processes.

Compared to warm-water corals *D. dianthus* shows rather mass increase in density than apical extension (Jantzen et al, 2013b). However *D. dianthus'* growth rates are comparable with massive warm- water corals, e.g. *Porites lutea* (Jantzen et al, 2013b). Corals growth rates are determined as mass increase of $CaCO_3$ over time and can be normalized by either their biomass (in g) or their calyx surface area (in cm^2) (see 2.2.2, Fig. 4). Försterra and Häusermann (2003) described minimum growth of *D. dianthus* of 2.3mm in length and 1.6mm in diameter per year in the Chilean Comau fjord. Relatively

high *in situ* mass increase (0.25 ± 0.18% d-1) of the Chilean *D. dianthus* was measured by Jantzen and colleagues (2013b) by an *in situ* short-term experiment in which mass determination was conducted via buoyant weight technique (Davies, 1989). Under controlled laboratory conditions Mediterranean individuals revealed growth rates of 0.06 ± 0.03% d^{-1} over an experimental period of 8 months (Orejas et al. 2011). Maintaining corals under ambient conditions growth rates were determined with the buoyancy weight technique. Maier and colleagues (2011) showed short-term growth rates of 0.04% d^{-1} determined by buoyancy weight and growths rates of 0.01% d^{-1} via the total alkalinity technique (Smith & Key, 1975).

1.4 The Chilean fjord system – Fjord Comau

A large area of Chile's austral fjord and channel region and the surrounding oceanic waters were unexplored until 1995 in terms of oceanography (Silva, 2008). Until now this area is one of the largest and fewest studied waters on earth (Arntz, 1999; Försterra et al. 2005; Försterra, 2013). The fragmentary knowledge has been filled up step by step but is still in its infancy. With more than 1500km length and numerous channels, fjords and archipelagos, the Chilean fjord system area is highly structured and reveals variable regions inhabiting marine organisms of major diversity.

The fjord Comau is one of the most sampled fjords, due to the 'Huniay Scientific Field Station', currently known for its abundant benthic macrofauna and still revealing new species (Försterra, 2013). Despite varying living conditions like distinctive pH gradients (accompanied by co-varying oxygen variations (Fillinger & Richter, 2013)) with concomitant variations in Ω_{Ar} (Jantzen et al. 2013a), dense and diverse aggregations of marine benthic organisms are thriving within the fjord (Försterra et al. 2005; Försterra, 2013). Along the vertical course of the fjord pH differs within a range of 0.5 units comprising highest values (pH 8.2) at the surface and lowest values (pH 7.4) near the bottom (Jantzen et al. 2013). Thus the fjord Comau represents a fundamental study area to investigate climate induced ocean acidification scenarios which are predicted for the future.

Communities inhabiting the Chilean fjord system could be endangered by human impact in terms of salmon farming which developed increasingly within the past (Försterra, 2013). Thus major habitat forming organisms and their associated community are may be endangered before researchers even get the chance to study them. Heavy impacts on benthic communities were already documented in several

fjords, where complete organism aggregations have been eradicated (Häussermann & Försterra, 2003; Försterra, 2013).

1.5 Objectives and working strategy

The cold-water coral *D. dianthus* thrives along the entire pH gradient of the fjord Comau dealing with pronounced and future predicted values (Jantzen et al. 2013). Even conditions below the aragonite saturation horizon do not restrict corals distribution.

I hypothesize that *D. dianthus* may physiologically and behaviorally acclimate to increased CO_2 concentrations because it is already used to varying environmental conditions in the past. Corals showed remarkable resilience and adaptation mechanisms, like internal pH up-regulation (McCulloch et al. 2012a), towards varying pH gradients in their natural environments. Nevertheless, *D. dianthus* might be at high risk as pH values in the fjord may decrease considerably due to increasing CO_2 concentrations dissolving in sea water, causing a lower Ω_{Ar} resulting in a decreased calcification rate.

To investigate *D. dianthus'* acclimation potential to lowered pH conditions an *in vitro* long-term experiment over 6 months was conducted aiming to answer the question whether *D. dianthus* is able to cope with future CO_2 concentrations by keeping up its natural physiology and behavior within its species-specific tolerance. Due to the lack of knowledge about how corals might react, and possibly acclimate (Form and Riebesell, 2012) to the ongoing process of climate change, long-term investigations are of major interest for the prediction of future scenarios. Filling these gaps of knowledge enhances the chance to develop future orientated management plans supporting sustainability and nature conservation.

As physiological features like growth and respiration rates are indicative for coral fitness, they were quantified as mass increase and oxygen uptake, respectively, via laboratory experiments. As corals extend their polyps to capture nutrients, polyp extension is suggested to be indicative for a coral in 'good condition': "A well-fed coral is a happy coral." (Richter, personal communication), and was therefore quantified. To measure the response to increased CO_2 concentrations, *D. dianthus* specimens which were used to ambient pH values (8.2 – 8.0) in the fjord Comau (at 20m depths) were maintained under lower pH conditions (~7.8) in the aquarium. A control group of *D. dianthus* individuals from the same spot was kept at ambient pH (~8.0), representing corals' natural habitat conditions in the fjord Comau.

2. Material and methods

2.1 Study area: Fjord Comau, Chile

The Fjord Comau, which is located in in the northern part of the fjord system along the 72.3°W longitude, branches off the Gulf of Ancud and measures ~45km in length and 2 - 8.5km in width (Jantzen et al. 2013) (Fig. 2). Surrounded by basaltic steep walls a maximum depth of approximately 500m was measured. The fjord is characterized by its high fresh water influx yield by two rivers Rio Bodudahue and Leptepu, seasonal precipitation and glacial melting; all contributing sediment and organic matter. The surface layer of the water column (0.5 – 10m) is dominated by low-salinity water. Recordings of oxygen concentration, salinity and pH values and aragonite saturation states (Ω_{Ar}) along the course of the fjord revealed a relatively stable stratification of the water profile, showing a gradually pH and aragonite decrease from the surface water (pH 8.2; Ω_{Ar} = 3.5) down to the fjord's bottom (pH 7.4; Ω_{Ar} = 0.5) (Jantzen et al. 2013). Silva (2008) reported the same pattern of a gradually decrease of oxygen concentration and pH for the fjord systems within the northern zone, showing a well-oxygenated surface layer (90 – 130% saturation) and a deep layer with 40 – 50% oxygen saturation. Lowest values were measured among others within the Fjord Comau. Irrespective of these circumstances dense and diverse accumulations of benthic marine organisms, mostly found at rocky walls in the upper regions of the water column, thrive within the fjord (Försterra et al. 2005; Försterra, 2013). The occurrence of benthic life in these regions could additionally be supported by seasonal plankton influxes of the high primary productive waters from the Gulf of Ancud (Iriarte et al. 2007). Generally the surface layers of the northern Chilean fjord zone show lower nutrient values compared to deeper water layers. In the fjord Comau high nutrient levels (> 2.0µM phosphate, > 20µM nitrate) were documented by Silva (2008).

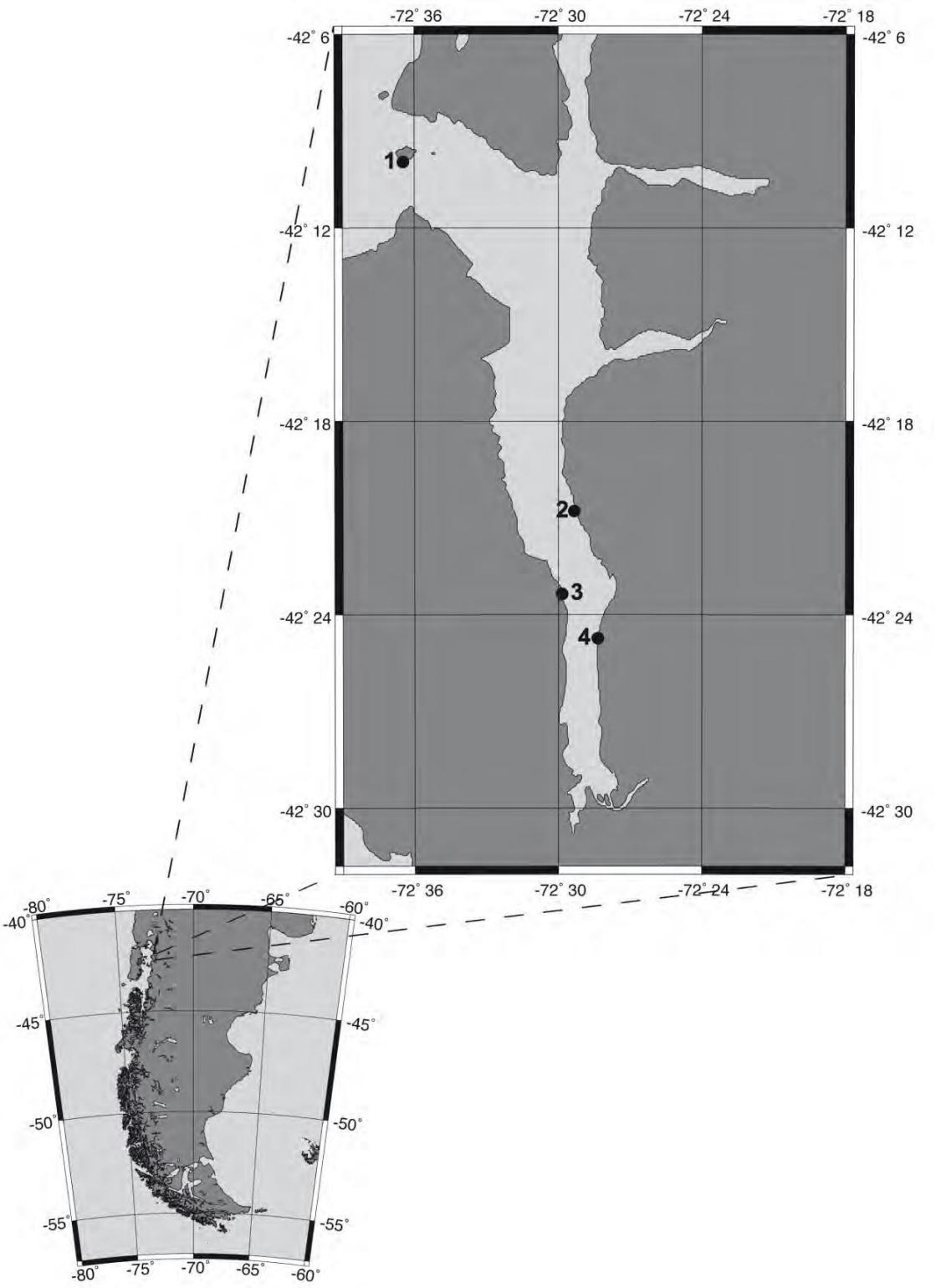

Fig. 2 Map of the Chilean Fjord system (41.47°S – 56.00°S); in detail the Fjord Comau. Numbers 1 to 4 are indicating the sample sites: (1) Liliguapi (2) Swall-Huinay (3) Cross-Huniay (4) Punta Gruesa (after Sokol, 2012).

2.2 Corals

2.2.1 Sampling and preparation of *Desmophyllum dianthus*

Living *D. dianthus* specimens were collected in the years 2010, 2011 and 2012 at four different sites (Liliguapi, Swall- and Cross Huinay and Punta Gruesa) in the fjord Comau in Chile (Fig. 2). Due to protective reasons the number of corals was kept to a minimum. Sampling was carried out at 20m depths via SCUBA diving. Corals were removed gently (with a hammer and a chisel) at their basal skeletal side form the fjords' walls. At the Huinay scientific field station in Chile corals were prepared for transport to the Alfred-Wegener-Institute, Helmholtz Zentrum für Polar- und Meeresforschung in Bremerhaven, Germany. The detailed procedure of coral sampling and transport has been processed during other projects (Sokol 2012; Maier 2013).In the laboratory the fracture zone of corals was cut plainly, sealed with cyano-acrylate gel (UHU Superflex Gel; Jury et al. 2009) and glued to a polyethylene screw. To imitate the natural 'upside down' growth direction, the screw was attached to a coral holder, which was built up in the aquarium (Fig. 3).

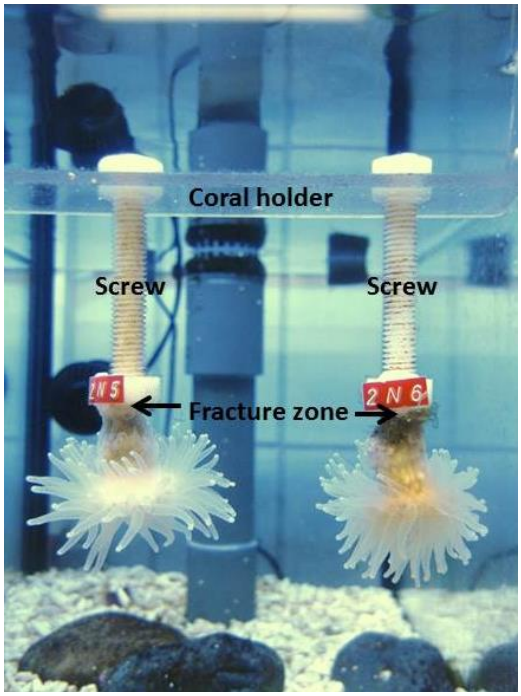

Fig. 3 *Desmophyllum dianthus* specimens were glued to a screw, which was attached to a coral holder to put corals in their natural 'upside down' position.

2.2.2 Cultivation of corals

The cultivation of corals in aquaria is a challenging endeavor due to a highly sensitive organism and has thus to be conducted with accuracy. To maintain corals naturalness, unnatural stress factors have to be kept on a minor level. *D. dianthus* was associated with its native fauna and water parameters. However the *in vitro* peculiarity remained different compared to this *in situ*.

Aquarium system set-up

Corals were maintained for 24 weeks in an aquarium system consisting of two independent circuits (circuit 1 = control coral group; circuit 2 = treatment coral group) (Fig. 6). Each circuit (~130l) was built up of three connected aquaria levels. At Level 1 Macroalgae (*Fucus vesiculosus*) were cultivated for filtration of nutrients. Level 2 maintained *D. dianthus* individuals which were excluded of the experiment due to unfitness. At the third level 4 aquarium tanks were split up in two for the control coral group and two for the treatment coral group. To prevent influence of technical (i.e. tank and circuit artifacts) and biological (i.e. bacteria accumulation and other water parameters) issues within one circuit on experimental corals, resulting in pseudo replicates, circuits were manually reconnected and corals replaced accordingly every 6 weeks (four times within 24 weeks of experiment). Reconnection of aquarium tanks just took place at Level 3 (Fig. 6). Aquarium system was set up in a temperature constant room at 10°C, simulating natural conditions in the fjord. Dysphotic light conditions were chosen similar to the natural conditions in the fjord at 20m depth and were installed for every aquarium Level.

Aquarium tanks

32 experimental corals were divided randomly in two groups of 16 individuals for each circuit, with 8 corals per aquarium tank. Within each aquarium tank hermit crabs (*Propagurus gaudichaudi* (H. Milne Edwards, 1836)), sea urchins (*Arabica dufresnii* (Blainville, 1852), *Pseudechinus magellanicus* (Philippi, 1857)), brachiooda (*Magellania venosa* (Solander, 1789)), hydocorals (*Errina antarctica* (Gray, 1872)), cup corals (*Caryophyllia huinayensis* (Cairns et al. 2005)) and mollusca (sea cradles and snails) were cultivated to keep the corals together with their naturally associated fauna as it was found in the fjord Comau. Bottom of tanks were covered with special aquarium gravel. Aquarium system was provided with artificial sea water (Aqua Medic Reef Salt, Germany). Sea water was pumped via rotodynamic pumps (Eheim 1262210 Universal-Pump 3400l/h) starting from Level 1 up to Level 3. Sea water (90l) was exchanged

minimum two times a week depending on nutrient values. Flow regimes in the water were regulated via dynamic pumps (Tunze Turbelle® nanostream 6015, 1800l/h).

Water parameters

All water parameters except pH were kept equal in both circuits. Circuit 1 was running at ambient pH (8.12 ± 0.13) (in the fjord Comau the ambient pH of corals growing at 20m depths is between 8.0 and 8.2 (Jantzen et al. 2013)) and circuit 2 was running 0.26 ± 0.01 units lower at pH 7.86 ± 0.14 (means ± SD). Sea water pH was controlled by addition of CO_2, which was added to the water via a CO_2 regulation system (Dennerle CO_2 Nachtabschaltung COMFORT). Regular recordings of water parameters (salinity, oxygen concentration, temperature, pH and nutrient concentration) were conducted. Temperature and pH were recorded every 20 minutes, 24 hours a day via a control unit (IKS aquastar controller, *Version 2011*).

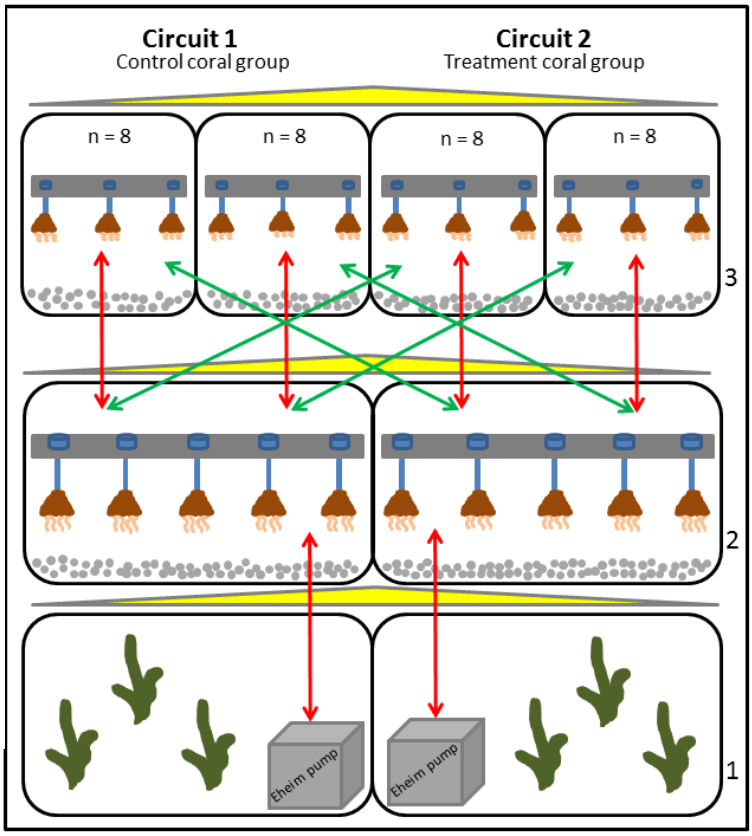

Fig. 4 Cultivation of corals in aquarium system. Circuit 1 contained the control coral group (n = 16) and circuit 2 the treatment coral group (n = 16). Level 1 maintained macroalgae and Eheim pumps, which pumped the water up to Level 2 (excluded corals) and 3 (experimental corals). Red and green arrows indicate water flux between tanks. Green arrows mark reconnection of aquarium tanks. Every six weeks either the red arrow system or the green arrow system was running at Level 3.Yellow triangles indicate light constructions for each level.

2.2.3 Cleaning of corals

As thread algae were growing accidentally in the aquarium system (origin remained unclear) mainly on plastic surfaces and coral skeletons, algae had to be removed form coral surface to secure unconfined movement of polyps. Thus corals were cleaned every two weeks carefully with a toothbrush and foreceps in a separate aquarium. Cleaning was carried out additionally prior every experimental procedure, i.e. estimation of growth rates (to avoid weight effects of algae) and respiration rates (to exclude respiration effects of algae) (see 2.4 and 2.5 respectively).

2.2.4 Feeding of corals

Corals were fed with frozen baby krill, stored at -20°C. Krill was defrosted at room temperature one hour before feeding time. Feeding was conducted once a day at 3pm 5 days a week. Prior feeding procedure current pumps were switched off to ensure that krill was not removed with the flow. The krill was given carefully to corals polys with foreceps. The amount of krill was according to coral size (small coral = 2 pieces krill; medium coral = 4 pieces krill; large coral = 6 pieces krill). Corals were given 2 hours to feed until pumps were switched on again.

2.2.5 Calyx surface area

Calyx surface area of all 32 experimental corals was determined by photographing the projected planar area (Kanwisher & Wainwright 1967). A caliper was used as scale (Fig. 4). Photographs were taken at three different times (at the beginning, in the middle (i.e. after 3 months) and at the end of experiment) to estimate growth of calyx surface area. Photographs were taken with a digital camera (Canon Powershot G10). Calyx surface area calculations were carried out with the image processing program cell^{B}, whereby each coral was measured 3 times. For coral volume calculations (see 2.2.7) the mean value of calyx surface area (A_{calyx}, in cm^2) was taken. As corals did not show significant surface growth (P = 0.794) initial measurements were used for coral volume, growth-and respiration rate calculations.

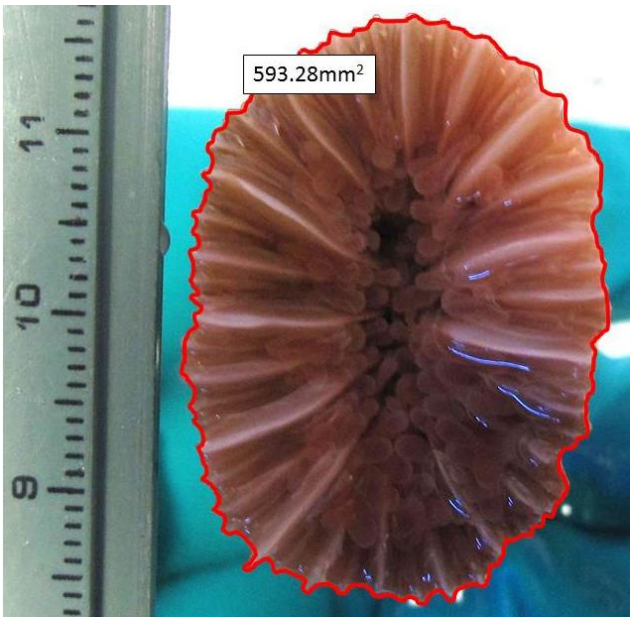

Fig. 5 Example photograph of corals' calyx surface area indicated in red. The area measured 593.28 mm^2.

2.2.6 Coral length

Coral length was determined by photographing the corals from four different angles (Fig. 5). The angle which showed the largest distance from the fracture zone down to the end of the coral was taken for length analysis. Photographs were taken at three different times (at the beginning, in the middle and at the end of experiment) to estimate growth in length. Photographs were taken with a digital camera (Canon Powershot G10). Coral length calculations were carried out with the image processing program CMEIAS-IT 1.28, measuring each coral three times. For coral volume calculations (see 2.2.7) the mean value of coral length (L, in cm) was taken. As corals did not show significant growth in length (P = 0.823) initial measurements were used for coral volume calculations. As a measurement reference the screw, to which every individual coral was attached, was used. The original screw width (0.6cm) was measured with a caliper (precision ± 0.1).

Fig. 6 Coral photographs from 4 different perspectives (A, B, C and D). Photograph A was taken for length analysis indicated by the red arrow.

2.2.7 Coral volume

Coral volume was determined in terms of ascertaining accurate incubation medium volumes for the following respiration experiment (see 3.5). The coral volume (V_{coral}, in cm^3) was calculated with the following equation (Eq. 6) with (A_{calyx}) as the calyx surface area in cm^2 and (L) as coral length in cm:

$$V_{coral} = A_{calyx} * L \qquad \textbf{(Eq. 6)}$$

2.4 Growth rates

To assess skeletal growth rates (mass increase) of *D. dianthus* corals were weighed with use of the buoyant weight technique (Davies, 1989). This technique measures the buoyant weight of a coral in sea water to determine its weight in air. Corals were given two weeks acclimation time before starting the experiments. Assessing long-term growth rates skeletal growth of corals was measured five times within the first three months of experiment and once at the end of experiment. In total 6 measurements were conducted after 1, 3, 6, 8, 10 and 24 weeks. Growth rates of *D. dianthus* can be detected after a time period of 2 weeks (Jantzen et al, 2013b). Weighting experiments

were carried out prior feeding procedure to avoid influences of food on coral's mass. Corals were weighted under water in an aquarium with a high-precision balance (Sartorius CPA 225 D-OCE, Germany, 220g – 1mg ± 0.1mg) using the under floor weighing mechanism. The aquarium was filled with artificial sea water (prepared in a separate tank) using a definite volume of (9.3l). Sea water temperature was kept at 10 ± 1.0°C. Ice packs were used to cool down the water temperature. After a temperature increase of 1°C sea water was replaced by fresh and cool water. Temperature was measured during every single coral weighing with a temperature sensor (WTW ama-digit, -40°C – 120°C). Corals were weighted by tightening the polyethylene screw, which is attached to each coral, to a screw-nut, which was fixed to a cord hanging down from the under floor weighing system. Measurements were conducted till values did not deviate more than 0.005g from each other and repeated three times. Values were averaged to determine the buoyant weight (skeletal weight in water (wt_{water}) in g). Wt_{water} was used to calculate skeletal weight in air (wt_{air}) after Jokiel and colleagues (1978) from equation (7) below; ρ_{water} as seawater density (in g cm^3) and $\rho_{aragonite}$ as coral skeleton density (in g cm^3). Seawater density was calculated after Bialek (1966) by measuring salinity and temperature during weighing process. Aragonite density for *D. dianthus* (2.835g cm^{-3} (mean of n = 8)) was defined after Naumann and colleagues (2011), who derived values from micro-density measurements (Davies, 1989).

$$wt_{air} = \frac{wt_{water}}{\left(1 - \frac{\rho_{water}}{\rho_{aragonite}}\right)} \qquad \textbf{(Eq. 7)}$$

wt_{air} was not corrected for tissue biomass (relationship between ash free dry weight and bulk dry weight of coral), as tissue analysis of corals did not reveal expedient results (see 2.7). Daily growth rates G ($CaCo_3$ cm^{-2} d^{-1}) expressed in % and µmol (1mol $CaCo_3$ = 100.09g = 100.09µg $µmol^{-1}$) were normalized to calyx surface area (cm^2), as polyp diameter is not accurate for *D. dianthus* due to the 'non-circulate calyx top view' (Purser et al, 2010; Jantzen et al, 2013b). Mass increase was calculated for all 6

growth intervals (with M_I and M_{I+1} as skeletal weight in air at the beginning (I_i) and at the end (I_{i+1}) of each growth interval) with the following equations (8 and 9):

$$G\ (\%\ CaCo_3\ cm^{-2}\ d^{-1}) = \frac{(M_{I+1} - M_I)}{(M_I * (I_{i+1} - I_i))} * 100$$

(Eq. 8)

$$G\ (\mu mol\ CaCo_3\ cm^{-2}\ d^{-1}) = \frac{(M_{I+1} - M_I)}{(I_{i+1} - I_i)} * \frac{1000}{100.09}$$

(Eq. 9)

2.5 Respiration rates

Respiration rates of *D. dianthus* were determined via oxygen fluxes (mg L^{-1}) of the water column during closed incubations. Oxygen concentration was measured with an oxygen sensor (HACH, HQ 40d multi, 0.001mg/l – 20.0mg/l ± 1%, Germany) five times within the first three months of experiment and once at the end of experiment. In total 6 measurements were conducted after 1, 3, 6, 8, 10 and 24 weeks. Corals were given two weeks acclimation time before starting the experiments. Incubation experiments were carried out ~24h after feeding procedure to avoid influences of food on corals' metabolic activity (Naumann et al. 2011). The oxygen sensor was calibrated and stabilized prior every experimental run. Sea water was taken directly out of the aquarium system to conduct the incubation under experimental conditions. Coral incubation was carried out at 10°C and kept in a dark room to avoid any oxygen consumption via photosynthetic organisms in the water. For each incubational run sea water blanks were taken as control to test for microbial background oxygen consumption. Hermetic glasses containing a stirring bar were placed on a magnetic stirrer (Varimog Poly, Komet) providing a smooth and constant water flux. Coral incubation within hermetic glasses containing a known sea water volume was limited to 2.5h ($T_{incubation}$, in h) ensuring a minimal oxygen saturation of 80% in sea water (Dodds et al. 2007). At the end of incubation sea water oxygen (mg L^{-1}) was measured directly after opening the incubation glasses to avoid any air oxygen flux into the sea water.

Oxygen consumption (ΔO_2) was calculated by the difference between start (oxygen concentration of aquarium sea water) and end values (oxygen concentration of incubation samples after 2.5h). As incubation corals displace a certain amount of water, coral volume (V_{coral}, in l) was subtracted from incubation volume in the glass (V_{glass}, in l) resulting in the real incubation volume ($V_{incubation}$, in l). Daily respiration rates R (O_2 cm^{-2} d^{-1}) expressed in µmol (1 mol O_2 = 32g = 32µg $µmol^{-1}$) as (means ± SD) were normalized to calyx surface area (A_{calyx}, in cm^2) for each coral and calculated with the following equation (10):

$$R\ (\mu mol\ O_2\ cm^{-2}\ d^{-1}) = \frac{\left(\frac{\Delta O_2 * V_{incubation}}{T_{incubation}}\right) * \frac{1000}{32} * 24}{A_{calyx}} \quad \textbf{(Eq. 10)}$$

2.6 Polyp extension

Fully expanded polyps, signalizing that corals were in a good condition, were mostly observed in the early morning (8:00am) until early afternoon (3:00pm), and especially 1 hour after feeding procedure. Documentations were conducted at 10:00am, when no other activities in the aquarium (e.g. measuring of water parameters or incubation experiments) were conducted to avoid any additional influence on polyp behavior. A certain daytime (i.e. 10:00am) was further chosen to exclude any variations due to endogenous circadian rhythms (Moya et al. 2006).

Polyp extension was documented at 14 randomly chosen days within the first and last 3 months of the whole experiment. For photographical documentation (digital camera (Canon Powershot G10)) corals remained within the aquarium system to minimize stress factors. Measurement of polyp extension was carried out with the image processing program Photoshop. Thus polyp extension was assigned to three different ranks (1 = fully retracted (0% of polyp visible), 2 = half extended (50% of polyp visible), 3 = fully extended (100% of polyp visible) (Fig. 7) and expressed as (means ± SD).

Fig. 7 Polyp extension of *Desmophyllum dianthus* assigned to three different ranks (1 = fully retracted, 2 = half extended, 3 = fully extended).

2.7 Coral tissue analysis

2.7.1 Tissue preparation

Experimental corals were used for tissue analysis after termination of the experiment. Therefore, corals skeleton was opened carefully with a hammer and a chisel. The tissue was removed from the skeleton by air-brushing with filtered sea water. To gain total tissue mass air-brushing took place within a plastic ziplock bag. Afterwards coral tissue samples were shortly homogenized with an ultra-turrax and volume (ml) was noted. Homogenization was stopped immediately when foam was forming as this indicates the denaturation of proteins within the tissue sample. One half of tissue volume (50% of tissue per coral) was shock frozen with liquid nitrogen and stored at -20°C for further protein analysis (see chapter 2.7.3), the other half of tissue sample (50% of tissue per coral) was transferred into aluminium dishes and dried at 40°C in the dry oven. Prior tissue transfer procedure, aluminium dishes were pre-combusted for 5h at 500°C in the muffle oven.

2.7.2 Ash free dry mass

To assess tissue dry mass (M_{dry}, in g) tissue samples were dried in the oven as long as weight of coral tissue remained stable, i.e. moisture was eliminated. Thus tissue samples were maintained within desiccators prior every weighting. M_{dry} was calculated with the following equation (11); with M_{alu} as weight of aluminium dish, in g:

$$M_{dry} = M_{dry+alu} - M_{alu} \quad \textbf{(Eq. 11)}$$

To assess the ash free dry mass of coral tissue ($M_{ash\ free}$, in g) dry tissue samples were combusted in the muffle oven for 4h at 550°C (Widbom, 1984) and again for 5h at 500°C (Laudien, personal communication). Ash free dry mass was calculated with the following equation (12); with M_{ash} as weight of ash after combusting, in g) = $M_{ash+alu}$ – M_{alu}:

$$M_{ash\ free} = (M_{dry} - M_{ash}) * 2 \quad \textbf{(Eq. 12)}$$

Ash free dry mass values did not show any correlation either with corals calyx surface area, or with length, volume or wt_{air}. Additionally single corals revealed tissue contents half of wt_{air} (i.e. 47.49 or 52.19%, derived from the relationship between ash free dry weight and wt_{air}. Averaged values of each coral group (control coral group and treatment coral group, with n = 16 each) showed tissue contents of 14.19 and 12.57% respectively, showing tissue biomass contents differing to those measured by Naumann and colleagues (2011) (i.e. 5.8 ± 2.3%, averaged for 10 individual corals). Possible explanations for these 'expedient' results are discussed in the following: The error rate was higher as laboratory work was conducted by 3 people instead of one person, causing a possible mix up of sample dishes making an assignment of dishes to corals unclear. Further the dry oven heated up 20°C higher than manually adjusted (40°C) resulting in a possible carbonate loss due to high temperatures (carbonate evaporates at temperatures from 40°C on (Jantzen, personal communication)). This could have led to an underestimation of tissue weight. Additionally combusting time of 4h might have been too short so that small, obscured coral skeleton fragments remained in the dish resulting in weight increase (M_{ash} + $M_{skeleton}$; with $M_{skeleton}$ as weight of skeleton fragments). Thus a second run for 5h at 500°C was conducted resulting in comparable ash free dry weights as in the first run. A maximum deviation of 0.958g, which was an exception, as all other values of the second run did not deviate more

than 0.080g from values of the first run, was measured. Additionally, it is to mention that carbonates oxidize from 550°C on (DIN 38414) (the temperature adjustment of the first run), leading to a possible overestimation of carbonate content. The latter might be a possible reason for relatively high tissue weights. However highest tissue values, which represented half of corals total weight probably cannot be explained by carbonate oxidation processes.

Thus corals calyx surface area, which correlated with coral volume ($r^2 = 0.737$) and wt_{air} ($r^2 = 0.746$) (correlation was obtained by linear regression fitting the interception through zero) was taken for normalization of growth and respiration rates.

2.7.3 Protein content of coral tissue

Total protein content of corals could not be determined because samples got defrosted by a technical accident in the laboratory. An electricity cut of the freezer caused retention of tissue samples at room temperature for over two days. Thus proteins of tissue samples were denatured and not expedient for further analysis.

2.8 Total alkalinity

Total alkalinity (TA) was used to calculate for water parameters as: forms of inorganic carbon and calcium solubility using 'co2sys.xls', a calculator for the CO_2 system in seawater for Excel/VBA (Lewis & Wallace 1998) (see Results, Tab. 1A/B). For a detailed description of carbonate system calculations see chapter 2.10. To assess TA filtered experimental sea water was analyzed with potentiometric titration (Gran, 1952) and is determined with the following equation (13):

$$TA \approx [HCO3^-] + 2[CO32^-] + [OH^-] - [H3O^+] + [B(OH)4^-] + 2[PO43^-] + [HPO42^-] - [H3PO4] \quad \textbf{(Eq. 13)}$$

The highest ion concentration is represented by [HCO3-], [CO32-], [OH-], [H3O+] and [B(OH)4-].

One time a week six sea water samples (three for each circuit) were taken directly out of the aquarium. Sea water was filtered with a syringe using glass microfibre filters (Whatman GF/F, Ø 25 mm) which were placed in a syringe filter holder. 50ml aliquots

of filtered sea water were filled air bubble free into Falcon tubes and stored at 4°C until TA was determined. Sea water standards (n = 3), used to account for methodical drifts, were taken out of separate tanks and filtered. Calibration of sea water standards was conducted with Dickson standard batch 120 (24.12.2012; Scripps Institution of Oceanography, San Diego). Standard samples were stored air bubble free within brown glasses at 4°C.To avoid changes in sea water TA, maximum time period until TA was measured has been two weeks.

Potentiometric titration was conducted with a titration unit, connected to an automatic sample changer (Titroline alpha plus, SI Analytics, Germany, pH 0.0 to 14.0 ± 0.02). Both were operated via titration controller software (TitriSoft 2.72). The titration unit consisted of three instruments: a pH electrode, a filling pipe and a stirrer. Prior to every titration run the pH-electrode was two-point calibrated with NIST/PTB buffer (pH 4.006 ± 0.02 and pH 6.865 ± 0.02) and used to measure change in electromotive force (EMF) of the water sample during titration. 25ml sea water and blank samples were placed within the sample changer. The filling pipe, adding titrant (0.05 N HCL) to the samples, was free of air bubbles to avoid oxygen mixing up with samples. The stirrer effectuated consistent mixing of water and titrant. TA, pH, and duration of experimental run were documented by the computer software 'TitriSoft'. TA was calculated from the Gran plot (Gran, 1952) by plotting the total number of protons (assessed from respective pH and total sample volume (start volume (V_0) plus volume of titrant (HCL) added to sample)) against the volume of titrant (HCL) added to samples respectively, with the following equation (14):

$$TA = \frac{(b/a) * c\,(HCL)}{V_0} \qquad \textbf{(Eq. 14)}$$

b = axis intercept of the Gran plot (-1) (mL M^{-1})

a = slope of Gran plot (mL M mL^{-1})

c (HCL) = concentration of hydrochloric acid (mol L^{-1})

V_0 = start volume of sea water (standard) sample (mL)

2.9 Nutrients

Daily nutrient measurements were conducted with quick tests (JBL GmbH & Co KG, Germany) prior sea water exchange and feeding procedure in the early morning. 5mL water samples were taken out of both systems (circuit 1 and 2) and tested for NO_2^- (nitrite), NO_3^- (nitrate), NH_4^+ (ammonium) and PO_4^- (phosphate). NO_2^- values between < 0.01 and 0.025 (mg L^{-1}) revealed an acceptable saturation on a total scale of < 0.01 to 1.0 (mg L^{-1}), whereby values higher than the acceptable limit indicate oversaturation in the water (this valids for all nutrients measured).The scale for NO_3^- lies between < 0.5 and 240 (mg L^{-1}) with a range between < 0.5 and 5.0 (mg L^{-1}) for an acceptable saturation. NH_4^+ values between < 0.05 and 0.1 (mg L^{-1}) showed an acceptable saturation on a total scale of < 0.05 to 5.0 (mg L^{-1}). PO_4^- values between < 0.02 and 0.05 (mg L^{-1}) revealed an acceptable saturation on a total scale of < 0.02 to 1.8 (mg L^{-1}).

2.10 Carbonate chemistry

For the calculation of carbonate chemistry parameters (Total carbon (TC) (µmol kg^{-1}), pCO_2 (µatm), CO_2 (µmol kg^{-1}), HCO_3^- (µmol kg^{-1}), CO_3^{2-} (µmol kg^{-1}), saturation statuses of calcium (Ω_{Ca}), and (Ω_{Ar})) pH and temperature values (obtained from the IKS), TA values, salinity values, and total silicate and phosphate values (TSI, TP respectively) were used. As total silicate and phosphate values were not obtained data were set to 1 (see 'co2sys.xls' sheet for defaults).

2.11 Statistical and graphical analysis

All values are expressed as means ± SD (P, F and df values are expressed as raw data) and graphs were compiled by IBM SPSS Statistics 21. Statistical analysis was performed with SigmaStat 3.5 and IBM SPSS Statistics 21. As measurements were repeated within a time period of six months, a Repeated Measures ANOVA (RM ANOVA) was conducted. Coral length, calyx surface area, growth and respiration rates, and polyp extension were tested for significant differences between the two pH treatments (circuit 1 = control coral group (ambient pH), circuit 2 = treatment coral group (low pH)). Students-Test (T-test) and Mann-Whitney-Test (U-test) (data were not always normally distributed) were conducted to calculate for differences between start

and end values within each treatment and between both treatments for every time measurements were conducted (at the start, middle and end of experiment for coral length and calyx surface area; after 1, 6, 8, 10 and 24 weeks for growth and respiration rates; within the first and last three months of experiment for polyp extension). To test whether water parameters of both circuits were equal T- and U-tests were conducted. For all calculations the significance level was set to 0.05.

3. Results

3.1 Water parameters

3.1.1 pH and temperature

Sea water pH values between both circuits were significantly different from each other (*P* = < 0.001) (Tab. 1(B)). Circuit 1 revealed a total average value of 8.12 ± 0.09 and circuit 2 showed a total average value of 7.84 ± 0.08 over experimental period (Tab. 1(A)). From week 6 to 10 values are lowest with a minimum of 7.71 ± 0.09 in week 9 within circuit 2 (Fig. 8). Highest values were measured between week 14 and 16 showing a maximum of 7.96 ± 0.04 in week 15. Circuit 1 showed lowest values in week 1, 4 and 12 with a minimum of 8.07 ± 0.07 in week 4. Highest values were measured in week 14 and 15 with maximum values of 8.21 ± 0.04 in week 15. The maximum difference between both circuits amounted to 0.4 units from week 7 to 10. The minimum difference came to 0.2 units (see Appendix, Tab. 8).

Sea water pH in aquaria revealed values comparable to those *in situ*, as pH varies between 7.9 and 8.4 in the fjord Comau in the upper 20m (Jantzen et al. 2013a; Fillinger & Richter 2013).

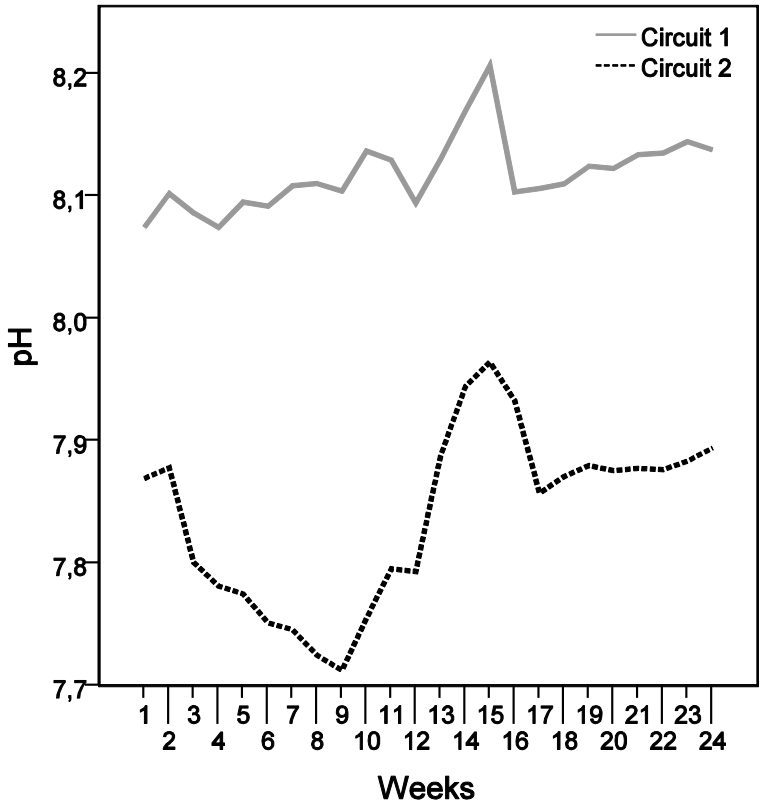

Fig. 8 PH values over experimental time for circuit 1 (ambient pH) and 2 (low pH). Values are expressed as means for every week.

Temperature values between both circuits were not significantly different from each other ($P = 0.222$) and revealed a similar pattern as values are increasing over time (Tab. 1(B), Fig. 9). Circuit 1 showed a total average value of 11.24 ± 0.26°C and circuit 2 came to a total average value of 11.37 ± 0.23°C during experimental period (Tab. 1(A)). Minimum values within circuit 1 amounted to 10.75 ± 0.19°C and maximum values to 11.65 ± 0.31°C (Fig. 9). Circuit 2 revealed minimum values of 10.83 ± 0.31°C and maximum values of 11.78 ± 0.21°C. The maximum difference in temperature amounted to 0.4°C in week 8, 11 and 19 between both circuits; the minimum difference came to 0.1°C (see Appendix, Tab. 8). In the fjord corals are naturally thriving at temperatures between 10.0 and 12.5°C within the upper 20m (Jantzen et al. 2013a; Fillinger & Richter 2013), values which are comparable to those in aquaria.

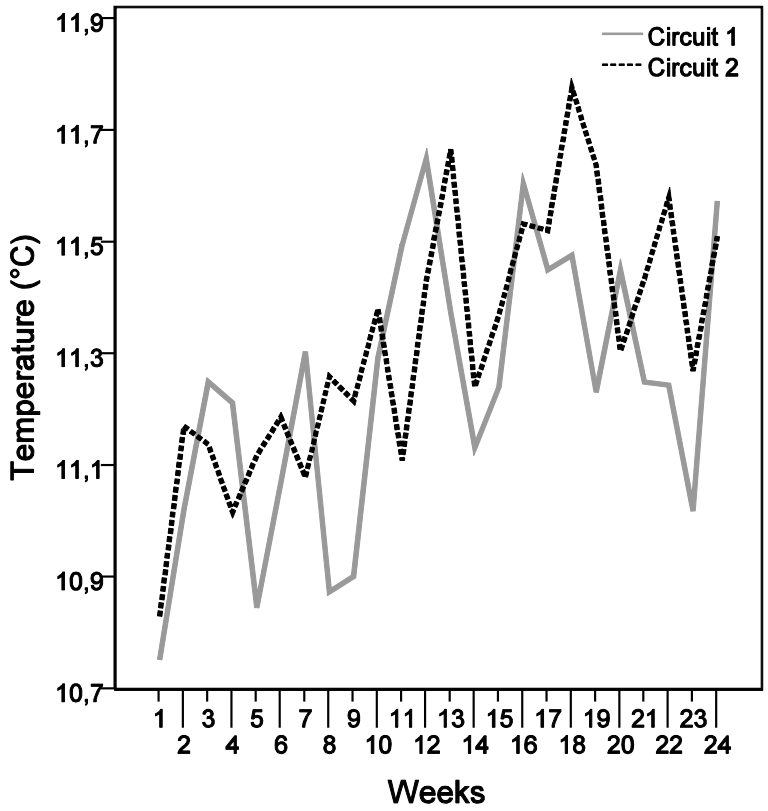

Fig. 9 Temperature values over experimental time for circuit 1 (ambient pH) and 2 (low pH). Values are expressed as means for every week.

3.1.2 Nutrients

Nutrients (NO_3^-, PO_4^-, NO_2^- and NH_4^+ (in mg L^{-1})) do not differ significantly from each other between both circuits (Tab. 2(B)). Nutrient variations between circuit 1 and 2 follow a similar pattern, whereby NH_4^+ shows differing variations between week 4 and 7 and week 17 and 19. However NH_4^+ values reveal the same average for both circuits 0.05 ± 0.00 over time

(Tab. 2(A)). Averaged all nutrient values lie under the marginal value of oversaturation within both circuits, except NO_2^-, which came to 0.005mg l^{-1} (circuit 1) and 0.015mg l^{-1} (circuit 2) above the maximum of 0.025mg l^{-1}.

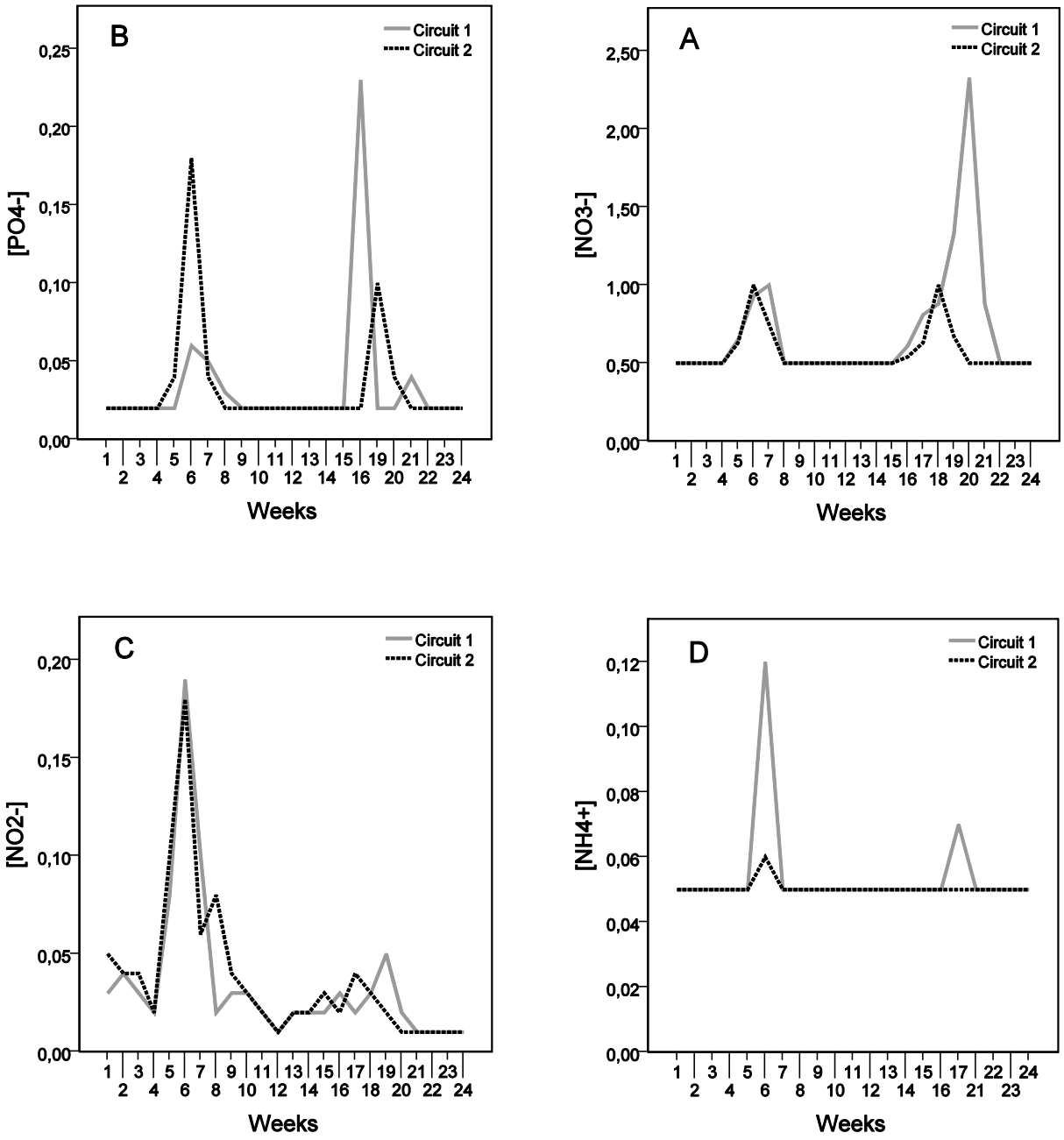

Fig. 10 (A, B, C, D) Nutrient variations over experimental period for NO_3^- (A), PO_4^- (B), NO_2^- (C) and NH_4^+ (D) within circuit 1 (ambient pH) and 2 (low pH).

Tab. 1(A) Overview of water parameters within circuit 1 (ambient pH) and 2 (low pH) during experimental period. Measurements were replicated as follows: TA (one time a week); pH and temperature (every 20 minutes, 24 hours a day), oxygen and salinity (daily). Forms of inorganic carbon and calcium solubility were calculated with 'co2sys.xls', a calculator for the CO_2 system in seawater for Excel/VBA (Lewis and Wallace 1998). Values are expressed as (means ± SD). **(B)** Differences (*P*) between water parameters and numbers (N) of repeated measures within circuit 1 and 2. Significant values are highlighted with (*****).

(A)

	TA	**pH**	**TC**	**pCO_2**	**CO_2**	**HCO_3^-**	**CO_3^{2-}**	**Ω_{Ca}**	**Ω_{Ar}**	**Oxygen**	**Salinity**	**Temp**
	(µmol kg $^{-1}$)	*IKS scale*	*(µmol kg $^{-1}$)*	*(µatm)*	*(µmol kg $^{-1}$)*	*(µmol kg $^{-1}$)*	*(µmol kg $^{-1}$)*			*(µmol kg $^{-1}$)*	*PSU*	*(°C)*
Circuit 1	3637 ± 414	8.12 ± 0.09	3435 ± 406	865 ± 254	37 ± 11	3212 ± 387	186 ± 36	4.50 ± 0.87	2.85 ± 0.55	282.6 ± 4.7	32.33 ± 0.61	11.24 ± 0.26
Circuit 2	3555 ± 371	7.84 ± 0.08	3388 ± 346	982 ± 212	42 ± 9	3184 ± 321	162 ± 39	3.92 ± 0.94	2.49 ± 0.60	281.9 ± 6.6	32.25 ± 0.25	11.37 ± 0.23

(B)

	TA	**pH**	**TC**	**pCO_2**	**CO_2**	**HCO_3^-**	**CO_3^{2-}**	**Ω_{Ca}**	**Ω_{Ar}**	**Oxygen**	**Salinity**	**Temp**
Significance	*P* = 0.318	***P* = < 0.001 ***	*P* = 0.487	*P* = 0.090	*P* = 0.101	*P* = 0.600	***P* = 0.026 ***	***P* = 0.026 ***	***P* = 0.026 ***	*P* = 0.805	*P* = 0.061	*P* = 0.222
N (circuit 1)	24	12096	24	24	24	24	24	24	24	14	125	12096
N (circuit 2)	24	12096	24	24	24	24	24	24	24	11	125	12096

Tab. 2 (A) Overview of inorganic nutrients within circuit 1 (ambient pH) and 2 (low pH) over experimental period. Measurements were carried out minimum 2 times a week with quick tests (JBL GmbH & Co KG, Germany). Values are expressed as (means ± SD). **(B)** Overview of nutrient differences (*P*) and numbers (N) of repeated measures within circuit 1 and 2. Significant values are highlighted with (*****).

(A)

	NO_3^-	**PO_4^-**	**NO_2^-**	**NH_4^+**
	(mg L $^{-1}$)	*(mg L $^{-1}$)*	*(mg L $^{-1}$)*	*(mg L $^{-1}$)*
Circuit 1	0.70 ± 0.07	0.03 ± 0.02	0.03 ± 0.01	0.05 ± 0.00
Circuit 2	0.57 ± 0.08	0.04 ± 0.02	0.04 ± 0.01	0.05 ± 0.00

(B)

	NO_3^-	**PO_4^-**	**NO_2^-**	**NH_4^+**
Significance	*P* = 0.280	*P* = 0.713	*P* = 0.656	*P* = 0.663
N (circuit 1)	114	64	115	61
N (circuit 2)	101	51	101	45

3.1.3 Carbon system and calcium solubility

Measured total alkalinity (TA), which was used to determine inorganic carbons and calcium solubility, showed lower values in circuit 2, compared to circuit 1, although not significantly (P = 0.318) (Tab. 1(A)). Carbonate ions (CO_3^{2-}) and aragonite saturation state (Ω_{Ar}) showed significant differences between both circuits (P = 0.026) whereby total carbon (TC), atmospheric carbon dioxide (pCO_2), carbon dioxide (CO_2), and bicarbonate ions (HCO_3^-) did not reveal significant differences (Tab. 1(B)). Total averaged values of carbonate chemistry over time are listed in Tab. 1(A).

3.1.4 Salinity and Oxygen

Salinity (P = 0.061) and oxygen (P = 0.805) values showed no significant differences during experimental period (Tab. 1(B)). Salinity revealed total average values of 32.33 ± 0.61‰ within circuit 1 and 32.25 ± 0.25‰ within circuit 2. Oxygen measurements came to 282.6 ± 4.7µmol kg^{-1} within circuit 1 and 281.9 ± 6.6µmol kg^{-1} within circuit 2 (Tab. 1(A)). Compared to maximum oxygen values (280µmol l^{-1}) measured in the fjord Comau in the upper 12-20m (Jantzen et al. 2013a; Fillinger & Richter 2013), *in vitro* values are in the same range.

3.2 Corals

3.2.1 Calyx surface area and coral length

Neither length nor calyx surface area of corals showed significant growth over experimental time (P = 0.681, P = 0.077 respectively) and between both pH treatments (P = 0.823, P = 0.794) (Tab. 4). A comparison of start and end data of coral length within each treatment revealed no significantly different values (P = 0.852 (ambient pH), P = 1.000 (low pH)) as well as a comparison of calyx surface area (P = 0.720 (ambient pH), P = 0.955 (low pH)) (Tab. 5).

Tab. 3 Differences (*P*) between the control (ambient pH) and the treatment coral group (low pH) at the beginning, in the middle and at the end of experiment for coral length and calyx surface area; after 1, 3, 6, 8, 10 and 24 weeks for growth and respiration rates; and within the first and last 3 months of experiment for polyp extension. Significant values are highlighted with (*).

	Coral length	**Calyx surface area**
Experimental period	(cm)	(cm^2)
Start	*P* = 0.821	*P* = 0.585
Middle	*P* = 0.898	*P* = 0.611
End	*P* = 0.485	*P* = 0.749
	Growth rates	**Respiration rates**
Week	($\mu mol\ CaCO_3\ cm^{-2}\ d^{-1}$)	($\mu mol\ O_2\ cm^{-2}\ d^{-1}$)
1 (Start)	*P* = 0.457	*P* = 0.559
3	*P* = 0.656	*P* = 0.486
6	*P* = 0.747	***P* = 0.001 ***
8	*P* = 0.459	*P* = 0.357
10	*P* = 0.768	*P* = 0.193
24 (End)	*P* = 0.451	*P* = 0.073
	Polyp Extension	
Month	(%)	
1 to 3	*P* = 0.747	
3 to 6	***P* = 0.035 ***	

Tab. 4 Overview of Repeated Measures ANOVA for coral length, calyx surface area, growth and respiration rates, and polyp extension. Shown are the effect of time and low pH (treatment), F (quotient of variances within and between groups (control coral group (ambient pH) and treatment coral group (low pH)) and df (degrees of freedom). Significant values (*P*) are highlighted with (*).

Measure	**Effect**	**F**	**df**	**Significance (*P*)**
Coral length	Time	0.265	1.355	0.681
	Treatment	0.051	1	0.823
Calyx surface area	Time	2.776	1.780	0.077
	Treatment	0.069	1	0.794
Growth rates	Time	17.229	2.500	**0.000 ***
	Treatment	0.001	1	0.979
Respiration rates	Time	9.246	2.757	**0.000 ***
	Treatment	0.438	1	0.513
Polyp extension	Time	11.377	11.680	**0.000 ***
	Treatment	2.731	1	0.109

Tab. 5 Differences (*P*) within the control coral group (ambient pH) and the treatment coral group (low pH) of start-and end values for coral length, calyx surface area, growth and respiration rates after 1 and 24 weeks; for polyp extension after the first and last 3 months of experimental period. Significant values are highlighted with (*).

Treatment	Coral length (cm)	Calyx surface area (cm^2)	Growth rates (µmol $CaCO_3$ cm^{-2} d^{-1})	Respiration rates (µmol O_2 cm^{-2} d^{-1})	Polyp Extension (%)
Ambient pH	*P* = 0.852	*P* = 0.720	*P* = 0.170	*P* = 0.851	*P* = 0.062
Low pH	*P* = 1.000	*P* = 0.955	*P* = 0.820	***P* = 0.006 ***	***P* = 0.001 ***

2.2.2 Growth rates

Under ambient pH conditions corals showed total growth rates of 0.19 ± 0.13% cm^{-2} d^{-1}, which referrers to 18.70 ± 12.91µmol $CaCO_3$ cm^{-2} d^{-1} (see Appendix, Tab.7). Low pH conditions had no significant effect on growth (*P* = 0.979) as corals showed equal growth rates 0.19 ± 0.10% cm^{-2} d^{-1} (18.62 ± 10.28µmol $CaCO_3$ cm^{-2} d^{-1}) as under ambient conditions (Tab. 4; Fig 11).

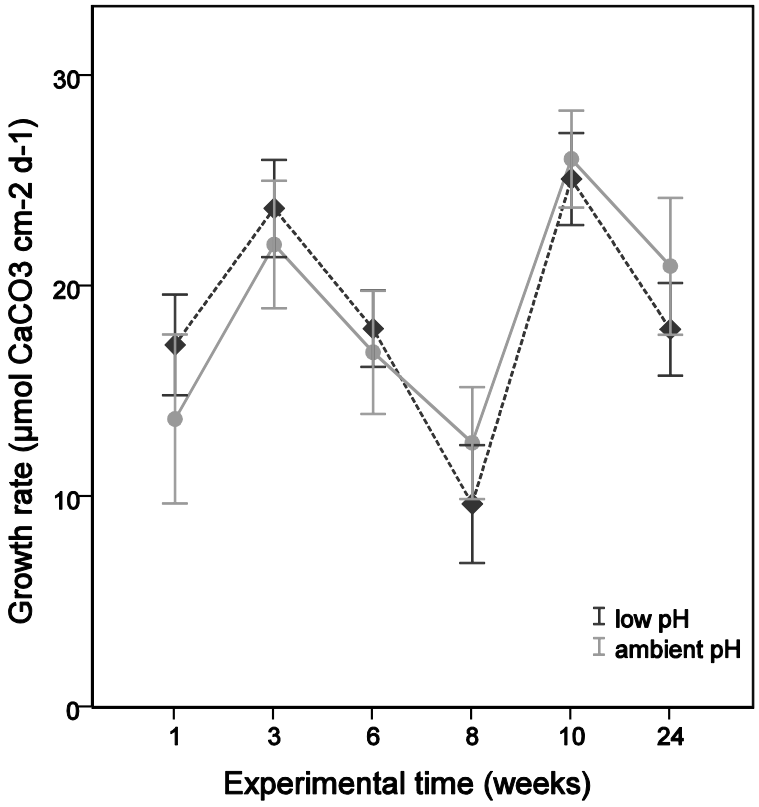

Fig. 11 Growth rates *D. dianthus* under ambient (n = 16) and low pH (n = 16) conditions over time. Values are expressed as means ± SD.

The growth response of corals showed a similar pattern over time within both pH treatments, whereby time had a significant effect on growth (P = 0.000) (Tab. 4). At every time different growth rates were measured within each treatment (P = 0.001(ambient pH), P = 0.001 (low pH)) and between both treatments (Tab. 4). Growth rates increased after three weeks of experiment, decreased until the 8th week and increased again until the 10th week. After 24 weeks of experimental time corals reached higher, but not significantly different (P = 0.170) growths rates (0.21 ± 0.13% cm^{-2} d^{-1}) compared to the beginning (0.16 ± 0.13% cm^{-2} d^{-1}) of experiment under ambient pH conditions (Tab. 5). Corals' response under low pH conditions also revealed higher, but not significantly different growth values (P = 0.820) at the end of experiment (0.18 ± 0.09% cm^{-2} d^{-1}) compared to the beginning (0.17 ± 0.10% cm^{-2} d^{-1}) (Tab. 5).

3.2.3 Respiration rates

Oxygen uptake under ambient pH conditions showed values of 22.07 ± 15.07µmol O_2 cm^{-2} d^{-1}. Low pH conditions had no significant effect on respiration rates (P = 0.513) as corals showed similar values (23.71 ± 7.56µmol O_2 cm^{-2} d^{-1}) as under ambient conditions (Fig. 12; see Appendix Tab. 7).

Experimental corals showed a similar trend over time comparing ambient and low pH conditions, whereby time had a significant effect on respiration (P = 0.000) (Tab. 4). At every time different respiration rates were measured within each treatment (P = 0.001(ambient pH), P = 0.001 (low pH)). Oxygen uptake at the beginning of experiment (ambient pH (29.66 ± 24.18µmol O_2 cm^{-2} d^{-1}), low pH (23.01 ± 5.94µmol O_2 cm^{-2} d^{-1})) and after 3 weeks (ambient pH (29.02 ± 16.98µmol O_2 cm^{-2} d^{-1})), low pH (23.76 ± 5.32µmol O_2 cm^{-2} d^{-1})) is similar within each treatment (see Appendix Tab. 7). Corals response showed a sharp decrease under ambient pH conditions after 6 weeks (9.87 ± 3.90µmol O_2 cm^{-2} d^{-1}) resulting in significantly different values between both treatments (P = 0.001) (Tab. 3). After 8 weeks until the end of experiment oxygen uptake is increasing, revealing a similar pattern for both treatments. After 24 weeks corals reached lower, but not significantly different (P = 0.851) respiration rates (23.75 ± 12.64µmol O_2 cm^{-2} d^{-1}) compared to the beginning (29.66 ± 24.18µmol O_2 cm^{-2} d^{-1}) of experiment under ambient pH conditions (Tab. 3). In contrast corals under low pH conditions revealed lower and significantly different (P = 0.006) respiration rates at the beginning of

experiment (23.01 ± 5.94µmol O_2 cm^{-2} d^{-1}) compared to the end of experiment (30.97 ± 9.09µmol O_2 cm^{-2} d^{-1}).

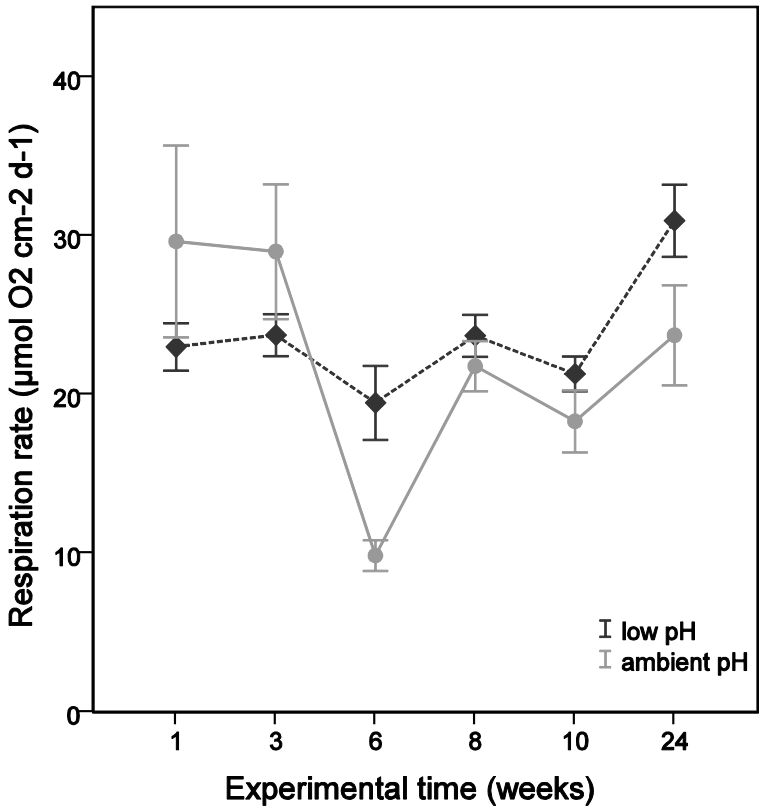

Fig. 12 Respiration rates of *D. dianthus* under ambient (n = 16) and low pH (n = 16) conditions over time. Values are expressed as means ± SD.

3.2.4 Polyp extension

The behavior of corals expressed as polyp extension demonstrated no significant response ($P = 0.109$) as a reaction to low pH conditions over time (Fig. 3). Corals showed polyp extension of 68.3 ± 40.0% under ambient and 75.4 ± 34.6% under low pH conditions (see Appendix, Tab. 7). However, time had a significant effect on the extension of polyps ($P = 0.000$). Polyp extension was increasing over time with 46.88% and 40.63% at the beginning compared to 90.63% and 87.50% (for ambient and low pH conditions respectively) at the end of experiment, whereby polyp extension was significantly different ($P = 0.001$) at low pH (Tab. 5). During the first three months of experiment polyp extension was significantly lower ($P = 0.001$) compared to the last

three months at low pH conditions; values at ambient pH were not significantly different (P = 0.062) (Tab. 5). Within the first three months corals showed 58.93 ± 42.47% polyp extension at ambient pH conditions and 66.29 ± 37.47% at low pH conditions compared to 77.68 ± 35.01% at ambient pH and 84.60 ± 28.75% at low pH conditions within the last three months.

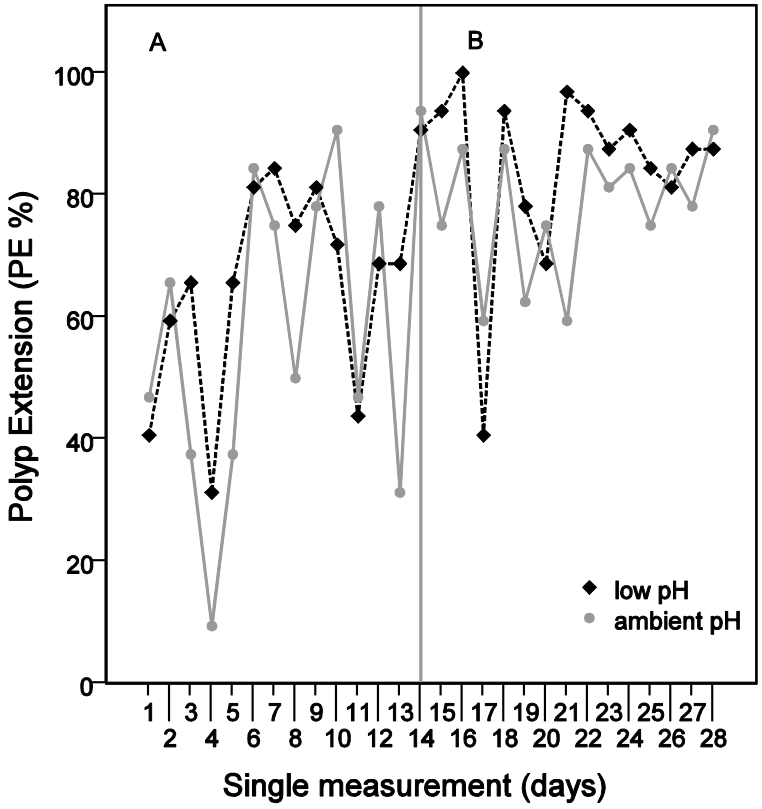

Fig. 13 Polyp extension of *D. dianthus* under ambient (n = 16) and low pH (n = 16) conditions over time (within the first 3 months (A) and the last 3 months of experiment (B). Values are expressed as means ± SD.

3.2.5 Ash free dry weight

Analysis of ash free dry mass ($M_{ash\ free}$) revealed no expedient results which are therefore not taken in account. Single corals revealed tissue contents half of coral weight in air (wt_{air}) (i.e. 47.49 or 52.19%, derived from the relationship between $M_{ash\ free}$ and wt_{air}. Averaged values of the control (ambient pH) and treatment (low pH) coral group (with n = 16 each) showed tissue contents of 14.19 and 12.57% respectively, showing tissue biomass contents differing to those measured by Naumann and colleagues (2011) (i.e. 5.8 ± 2.3%, averaged for 10 individual corals).

4. Discussion

This *in vitro* long-term study investigated the physiological and behavioral response of the cold-water coral *Desmophyllum dianthus* to future CO_2 concentrations. New insights of skeletal mass increase, metabolic oxygen uptake and polyp extension of corals under lowered pH are given.

It was found out that *D. dianthus* might cope with lower pH conditions, as corals' growth rates revealed no significant difference between ambient and low pH treatment. Lowered pH, accompanied by significantly decreased carbonate ions and aragonite saturation status, did not influence calcification, over time. Also respiration rates of *D. dianthus* revealed no significant difference between ambient and low pH conditions, over experimental period, except in week 6, where oxygen uptake of the treatment coral group was significantly higher. Polyp extension of corals was slightly higher, although not significantly, in the treatment coral group, compared to the control coral group, over time. However, within the last three months of experiment polyps of the treatment coral group were significantly extended to a higher degree. In general *D. dianthus* showed a positive response towards increased CO_2 concentrations and may be prepared for future pH values.

4.1 Water chemistry

Carbon dioxide, and thereby pH, mainly influences the oceanic carbonate cycle. The composition of chemicals is determined by equilibrium constants, which are highly affected by temperature, salinity and pressure (Erez et al. 2011). Total alkalinity (TA) is independent of pH and changes in temperature, salinity and pressure (Wolf-Gladrow et al. 2007, also affecting the carbonate system (Erez et al. 2011). The carbonate ion pool in the present study was calculated on the basis of pH and TA, measured at corresponding temperature, salinity and pressure (see Results, Tab. 1(A)).

4.1.1 Aragonite saturation status

Differing pH and TA values caused a significant variation in carbonate ions (CO_3^{2-}), calcium ions (Ca^{2+}) and aragonite saturation states (Ω_{Ar}) between both circuits. A lowered pH, induced by elevated pCO_2, resulted in a decreased CO_3^{2-} and Ca^{2+} concentration, as well as a decreased Ω_{Ar} in circuit 2. The availability of CO_3^{2-} ions is suggested to be the main control of Ω_{Ar} and coral calcification (Erez et al, 2011). A

decrease of Ω_{Ar}, caused by a lowered Ca^{2+} concentration was already described by Gattuso and colleagues (1998). Nevertheless, Ω_{Ar} may not have been low enough to influence calcification rates of corals negatively. An $\Omega_{Ar} > 1$ means that sea water is supersaturated (Hoegh-Guldberg et al. 2007), thus conditions for corals to build up their skeleton were favorable within both treatments (2.85 ± 0.55 (Ω_{Ar} at ambient pH) and 2.46 ± 0.60 (Ω_{Ar} at lowered pH)). As pH and TA influence the carbonate system independently, TA might have become prevalent (as values were relatively high, compared to other studies (Form & Riebesell, 2011; Jantzen et al. (2013b)), causing an elevation of aragonite saturation status, even at coincident lowered pH conditions.

4.1.2 Dissolved inorganic carbon

The total carbon concentration (or dissolved inorganic carbon (DIC)) usually increases with enhanced CO_2-uptake (Gattuso et al.1998). In the present study DIC is lower in CO_2-rich water, although not significantly, compared to ambient CO_2 conditions. The same phenomenon was observed by Form and Riebesell (2011), as they measured a lower DIC amount at increased pCO_2, compared to ambient pCO_2 conditions, during aquarium experiments. Still a comparison between studies remained difficult, as Form and Riebesell (2011) analysed DIC photochemically, compared to a calculation based on TA and pH in the present study.

4.1.3 Variations in CO_2 measurements

Measured pCO_2 concentrations in the present study were higher (865 ± 254µatm (at ambient pH), 982 ± 212µatm (at lowered pH)) compared to Form and Riebesell (2011) (604 ± 105µatm and 778 ± 112µatm, respectively), at similar pH conditions (8.12 ± 0.09, 7.84 ± 0.08, respectively (present study); 7.944 ± 0.064, 7.829 ± 0.053, respectively (Form and Riebesell, 2011)). Hoppe and colleagues (2012) described that pCO_2 values might vary with measurement methods, based on different input data, which could either be TA and pH, or DIC and pH, or TA and DIC. In both studies carbonate chemistry was calculated with co2SYS (Lewis & Wallace, 1998) based on TA and pH measurements, showing that even equal input data could vary pCO_2 values. Inaccuracies of measured pCO_2 concentrations are suggested to lie between 10% (Gattuso et al. 2010; Hydes et al. 2010) and 30% (Hoppe at al. 2012). Thus a direct comparison between studies remained difficult; plus the quantitative data significance

has to be called into question. Hoppe and colleagues (2012) further stated that an underestimation of 30% of calculated pCO_2 values would be accompanied by an overestimation of organisms' sensitivity towards ocean acidification. As corals of the present study did not respond negatively to waters of lower pH, an overestimation of physiological and behavioral sensitivity can be excluded.

4.1.4 Variations in total alkalinity

To determine TA itself, Form and Riebesell (2011) conducted open-cell titration measurements, hence revealing much lower values (1331µmol kg^{-1} (on average) lower) compared to the present study. Jantzen and colleagues (2013b) (who measured TA (of *in situ* data of the fjord Comau) directly) calculated comparable values to those of Form and Riebesell (2011). TA is known to change with biogeochemical processes, like the formation and remineralization of organic matter by microalgae, and the precipitation and dissolution of $CaCO_3$ (Wolf-Gladrow et al. 2007). All further discussed biogeochemical processes were prior described by Wolf-Gladrow and colleagues (2007).

Precipitation and dissolution of CaCO3

In a closed cell the precipitation of one mole $CaCO_3$ is accompanied by an increase in CO_2 (less than one mole), and a decrease of DIC (one mole) and TA (two moles), whereby dissolution of $CaCO_3$ reveals the inverse effect. In the present study Calcification significantly dominated dissolution processes, but one could suggest that $CaCO_3$ precipitation was generally low, resulting in a relatively high TA. However constantly water mixing, and thus altering gas exchange (causing enhanced CO_2 release and a decrease of DIC), had to be considered.

Nitrification, denitrification and remineralization

Photoautotrophic algae take up nitrogen ions in forms of nitrite (NO_2^-), nitrate (NO_3^-) and ammonium (NH_4^+), causing an increase of TA by one mole when nitrite or nitrate is used, and a decrease of TA by one mole when ammonium is used. Thus algae thriving in the aquarium system might have taken up rather nitrite and nitrate than ammonium resulting in an increased TA. Assuming that the amount of microorganisms (e.g. cyanobacteria) in sea water was relatively high, inversely a release of one mole ammonium, due to remineralization of particulate organic matter, could additionally have led to an increase of TA. Nevertheless nitrification under aerobic conditions causes a decrease of TA by two moles per nitrate ion released. Furthermore

denitrification, the conversion of nitrate to nitrite by autothrophic bacteria, results in elevated TA by one mole per mole nitrate converted. Besides nitrogen, the uptake of phosphate by algae also contributes to changes in TA by increasing TA of one mole per mole phosphate gained.
As all the above mentioned nutrients (nitrite, nitrate, ammonium and phosphate) were detected within the aquarium system, nitrification, denitrification and remineralization processes, carried out by algae and cyanobacteria respectively, causing either an increase or a decrease of TA, might have been conducted.

4.2 Corals

4.2.1 Growth rates

The measurement of growth rates (i.e. calcification) in corals is a common method to investigate mass in-and decrease (the latter caused by bioerosion or decalcification) and has already been conducted for cold-water corals (Naumann et al. 2011; Orejas et al. 2011b). Corals show differing growth rates with varying environmental parameters like temperature (Grigg, 1974; Weinbauer & Velimirov, 1995; Matsumoto, 2007; Silverman et al. 2007), light (Chalker & Tailor, 1975; Reynaud-Vaganay et al. 2001), flow speed (Purser et al. 2010; Sokol, 2012), prey abundance (Silverman et al. 2007; Purser et al. 2010; Naumann et al. 2011; Maier, 2013), and aragonite saturation status (Gattuso et al. 1998a; Silverman et al. 2007; Jury et al. 2009; Langdon et al. 2000; Form & Riebesell, 2011).

4.2.1.1 Growth performance

In the present study *D. dianthus* did not show significant length and calyx surface area growth over a time period of six months. Significant growth of the calyx surface area was measured after one year (Jantzen, personal communication). Thus the measurement of linear extension and calyx surface area of *D. dianthus* cannot be taken in account to determine $CaCO_3$ precipitation. To better visualize length growth, corals' skeletons could have been stained (in addition to mass investigations), e.g. with Alizarin Red S fluorescent dye (Form & Riebesell, 2011). To determine coral's growth rates, mass increase, which was detectable after two weeks, was measured over time.

4.2.1.2 A comparison among studies

Calcification rates (0.19 ± 0.13% cm^{-2} d^{-1}) of present corals at ambient pH were in the same range, compared to other *in vitro* studies. As temperatures and food supply varied among studies a direct comparison was difficult. Cultivation experiments of Mediterranean *D. dianthus* specimens, maintained at 12°C (1 - 2°C higher as in the present study) and fed with zooplankton (*Artemia salina*) (five times a week (as in the present study, whereby krill instead of *Artemia salina* was fed)) revealed growth rates of 0.06 ± 0.03% d^{-1} (Orejas et al. 2011b) and 0.1 - 0.3% d^{-1} (fed daily, Naumann et al. 2011). Thus feeding with krill and daily feeding might enhance growth rates, as corals of the present study and those examined by Naumann and colleagues (2011) revealed higher growth rates as corals investigated by Orejas and colleagues (2011b). Enhanced food supply is known to positively influence calcification rates in cold-water corals (Mortensen, 2001; Roberts & Anderson, 2002; Naumann at al. 2011; Maier, 2013). Jantzen and colleagues (2013b) revealed growth rates of 0.09 ± 0.08% d^{-1} of Chilean corals from the fjord Comau, which were maintained at 12°C and fed biweekly with their natural food (i.e. living plankton and an additional continuous supply of nanoplankton, microplankton and dissolved organic matter). *In situ* growth rates showed one third higher mass increase (0.25 ± 0.18% d^{-1}) as measured *in vitro*, probably because corals obtained continuous food supply in the fjord. Maier (2013) measured *in vitro* growth rates of Chilean *D. dianthus* specimens of 0.11 ± 0.06% d^{-1}; corals were maintained at 11°C and fed with zooplankton and additional krill. Concluding, highest growth rates of Chilean *D. dianthus* specimens were measured in the present study, even at lowered pH, and by Jantzen and colleagues (2013b), suggesting that krill and *in situ* food sources represented optimal conditions for corals.

4.2.1.3 Measuring growth rates

The use of different growth measurement techniques among studies led to a variability of calcification rates recorded for *D. dianthus*, causing difficulties in comparisons. The buoyancy weight technique (Jokiel et al. 1978) was conducted in the present study, by Jantzen and colleagues (2013b), Orejas and colleagues (2011b), and Maier (2013). Naumann and colleagues (2011) measured growth rates with the alkalinity anomaly technique (Smith and Key, 1975). Additionally, the use of different peer groups for normalization, i.e. calyx surface area (in cm^{-2}) (Jantzen et al, 2013b; this study); skeletal dry mass (in g) (Naumann et al, 2011) and deviating expression units (i.e. mg; µmol; % CaCO3) made a direct comparison difficult. Hence a standardization of peer

groups and expression units would approve a more precise relation of coral calcification rates.

The alkalinity anomaly technique and the buoyant weight technique allow for repeated calcification documentations, suitable for the experimental set up in the present study. The buoyant weight technique may be more precise than the alkalinity anomaly technique, as it examines the calcifying organism itself. The alkalinity anomaly technique investigates alkalinity changes in the incubation water, which are sensitive to biochemical processes (i.e. nitrification, denitrification and remineralization, see chapter 4.1.4). This disadvantage could be avoided by using pre-filtered sea water to eliminate microorganisms of the incubation water. Conducting the buoyant weight technique might have stressed corals, as they had to be removed from the aquarium into the weighting tank. However, daily observations did not reveal uncommon behavior like increased mucus secretion and/or regular retraction of coral polyps (Jantzen et al. 2013b).

4.2.1.4 Acclimation

At every time measured growth rates of both coral groups were similar, resulting in a steadily pattern over time. Nevertheless, growth rates within both treatments differed significantly at any time measured. A comparison of start and end growth rates led to the suggestion that the treatment coral group showed acclimation towards lowered pH over experimental period, as they were higher at the end, although not significantly different. Evidence for acclimation towards lowered sea water pH was observed for the cold-water coral *Lophelia pertusa*, revealing a decline in growth rates after one week of incubation followed by a slightly increase of growth rates over a time period of six month (Form & Riebesell, 2011). *L. pertusa* showed highest growth rates in waters of lowered pH compared to the control coral group maintained in waters with relatively higher pH. Maier and colleagues (2009) measured declining calcification rates of *L. pertusa* after 24h incubation in acidified sea water (water was acidified by the addition of hydrochloric acid (HCL), which did not influence the carbonate chemistry).

Compared to the beginning of experiment growth rates of *D. dianthus* were slightly higher at any other time measured, except in week 8, where corals growth rates were low. Assuming this growth decrease to be an exception, possibly due to energy allocation processes (i.e. production of gametes (Hassenrück, 2012)), additional evidence for acclimation potential to lowered pH conditions was shown. Maier (2013) stated that reproduction is a highly energetic process, but time-limited. In week 10 of

experiment (i.e. two weeks later) growth rates increased again, possibly entailing a termination of gamete production, however gametes have not been documented for *D. dianthus* yet (Maier, 2013).

4.2.1.5 Adaptation

Besides acclimation potential, *D. dianthus* is featuring adaptation mechanisms, like internal pH up-regulation (McCulloch et al, 2012a; b), allowing for calcification processes within sea water of lowered pH. McCulloch and colleagues (2012a) measured an internal pH of 8.71 for Chilean specimens at a surrounding sea water pH of 7.83. As *D. dianthus* is thus able to elevate its internal pH, about ~0.9 units, a lowered sea water pH of 7.86 in the present study, may not have any impact on corals calcification. However pH elevation is suggested to be accompanied by energetic effort, leading to a reduction of energy for other physiological important processes (i.e. respiration, discussed in chapter 4.2.2).

4.2.1.6 Temperature

Lowest growth rates of the control coral group were measured in week 1 and 8, coincident with lowest temperatures (see Appendix, Tab. 9). Temperatures were 0.49°C (in week 1) and 0.37°C (in week 8) lower compared to the average temperature over experimental time. Water temperature is a parameter influencing marine invertebrates, as growth rates of the gorgonian cold-water coral *Primnoa pacifica*, thriving in cold waters (0.6 – 0.7°C), were lower compared to growth rates of species living at warmer water temperatures (Matsumoto et al. 2007). McCulloch and colleagues (2012b) developed a model (IpHRAC), which measured that enhanced kinetic activities caused by increased temperatures, positively influenced calcification processes. When temperature (in the present study) was elevated (based on the temperature range measured during experimental period), co2SYS calculated increasing CO_3^{2-} concentrations, and Ω_{Ar} and Calcium saturation (Ω_{Ca}) at ambient and lowered pH. Ω_{Ar} was supersaturated revealing maximum values of 2.9 (at ambient pH) and 2.6 (at low pH), favorable for the formation of $CaCO_3$ (Tab. 6) (see chapter 4.1.1). Enhanced temperatures at the end of experiment might have positively influenced growth rates of *D. dianthus* as corals growth rates were higher at the end than at the beginning. As calcification rates may be limited by corals physiology, kinetic processes alone cannot

determine corals thresholds of $CaCO_3$ precipitation (McCulloch et al. 2012b). Countering ocean acidification, calcification rates of azooxanthellate cold-water corals depend on the ability of pH up-regulation, supported by temperature enhanced abiotic kinetics.

Tab. 6 Influence of varying temperatures on the carbonate system of sea water, calculated at ambient and lowered pH conditions.

pH	Te	CO_2	HCO_3^-	CO_3^{2-}	Ω_{Ca}	Ω_{Ar}
	(°C)	*(µmol kg $^{-1}$)*	*(µmol kg $^{-1}$)*	*(µmol kg $^{-1}$)*		
Ambient	10,5	37,8	3224,1	180,9	4,4	2,8
(8.12±0.09)	11,0	37,2	3216,5	184,3	4,5	2,8
	11,5	36,7	3208,8	187,8	4,5	2,9
	12,0	36,2	3201,0	191,4	4,6	2,9
Lowered	10,5	44,0	3272,2	159,9	3,9	2,4
(7.84±0.08)	11,0	43,4	3265,4	163,0	3,9	2,5
	11,5	42,8	3258,5	166,1	4,0	2,5
	12,0	42,2	3251,5	169,3	4,1	2,6

Within actual and future predicted environmental changes warm-water corals may be less robust compared to cold-water corals, as they depend on their algal symbionts, the zooxanthellae. Via photosynthetic processes zooxanthellae provide essential nutrients for the coral host (Holt & Holt, 1967). A temperature increase, exceeding warm-water corals' thresholds, already resulted in a release of symbionts, causing a massive bleaching event in the Indian Ocean in the years 1997 - 1998 (Erez et al. 2011). If ocean temperatures increase, due to climate change, bleaching events might become more frequent, causing mortality of corals in the worst case (Goreau 1964, 1992; Glynn 1993; Hoegh-Guldberg 1999). In contrast, the energy budget of cold-water corals is sustained by active food capturing (see chapter 4.2.1.3). Cold-water corals are thriving at high temperature gradients, as it could be shown for *D. dianthus* (Jantzen et al. 2013a; Fillinger & Richter, 2013). Hence, a future predicted temperature increase, termed as 'global warming', may have a minor impact on cold-water corals, as they show higher thresholds than warm-water corals.

4.2.1.7 Age

The rate of calcification depends on corals' age. Maier and colleagues (2009) measured highest growth rates in youngest polyps of the cold-water coral *Lophelia*

pertusa. They also showed that faster growing, younger polyps were more sensible to acidified sea water than older polyps, with a reduction of calcification rates of 59% and 40%, respectively. Elahi and Edmunds (2007) showed that younger coral fragments calcify faster than older coral fragments, independently of size. As *D. dianthus* shows rather massive (i.e. mass increase) than elongated growth (Försterra & Häusermann, 2003), averaged total mass (in g), instead of size, was related to growth rates, assuming that total mass correlates positively with age. In the present study no correlation between total mass and growth rates could be shown, however, corals revealing highest mass values, showed relatively low growth rates.

In summary, corals of the present study, even those maintained at lowered pH, showed growth rates within the range of values measured in other studies. Besides sea water pH, temperature and abiotic kinetics, corals' age, corals' physiology (internal pH up-regulation) and energy requirement (depending on the availability of nutrients) could have limited calcification processes.

4.2.2 Respiration rates

Respiration may be influenced by changing environmental conditions such as temperature (Dodds et al. 2007) and food regime (Naumann et al. 2011; Maier, 2013). Hence oxygen uptake was measured aiming to represent the metabolic response of *D. dianthus* under lowered pH conditions.

In vitro respiration rates of *D. dianthus* revealed no significant difference between ambient and low pH treatment, except in week 6, with slightly higher oxygen uptake under lowered pH conditions. Maier (2010) documented higher respiration rates of *D. dianthus* specimens incubated at lowered pH of 7.86, comparable to present cultivation conditions. Hence lowered pH might have stressed corals causing enhanced oxygen uptake. Corals are known to respond with increased respiration rates towards stress situations like elevated turbidity (Telesnicki & Goldberg, 1995).

Experimental corals showed a similar trend over time comparing ambient and low pH conditions. At ambient pH respiration rates were lower at the end compared to the beginning of experiments. In contrast respiration rates at lowered pH showed maximum values at the end of experiment, revealing significantly higher values compared to the beginning, and thus showing possible acclimation potential.

Within both coral groups (maintained at ambient and low pH) oxygen uptake was significantly different at any time measured, thus time had a significant effect on respiration of both coral groups. Remarkably, the inner treatment variability was higher

at ambient pH compared to lowered pH. Differing respiration rates within coral groups might also refer to inner-circuit variations of water parameters. In circuit 1 (ambient pH) pH was constantly at ~8.1 during respiration measurements, hence pH could be excluded to cause variations. Temperatures varied between 10.75°C (week 1) and 11.57°C (week 24) during respiration measurements. At ambient pH, maximum respiration rates were measured in week 1, parallel to lowest temperature values. In contrast, Dodds and colleagues (2007) showed that oxygen consumption of the cold-water coral *Lophelia pertusa* increased with higher temperatures, independent of oxygen level in sea water. As temperature could not be correlated with respiration rates, temperature variations might not have influenced present test corals.

Significant inner-individual respiration variations could be related to differing outer tissue growth. However, corals respiration rates did not correlate with the outer tissue amount.

Previous studies showed the high relevance of food, as respiration rates of *D. dianthus* were enhanced with increasing food availability (Naumann et al. 2011; Sokol, 2012; Maier 2013). In the present study, oxygen uptake (0.189 ± 0.056mg O_2 cm^{-2} h^{-1}) of corals maintained at ambient pH was higher, but still in the same range compared to values (0.028 ± 0.053mg O_2 cm^{-2} h^{-1}) measured by Sokol (2012); corals of both studies were fed with krill. Evidence for the enhancing effect of food on oxygen uptake was shown by Maier (2013), as she measured highly increased respiration rates of *D. dianthus* specimens within the first ten hours after feeding. Larsson and colleagues (2013) documented the same response for the cold-water coral *L. pertusa*, revealing a correlation between respiration rates and increased food densities. In summary, food supply in the present study might keep respiration rates on the same level as in other studies, where additional food (fed in higher densities or more regular) led to increased oxygen uptake.

To measure respiration rates *D. dianthus* specimens had to be removed from the aquarium into hermetic Schottglasses. Relocation might have stressed corals, resulting in a possible overestimation of oxygen uptake. To measure oxygen saturation, lids of incubation glasses had to be opened, causing a mixing of air and sea water oxygen. Although oxygen saturation was directly measured after opening the lid, an underestimation of respiration rates cannot be excluded.

After 2.5 hours incubation time, oxygen saturation within control incubation glasses amounted to 333.9 ± 6.1µmol kg^{-1} and 329.4 ± 5.0µmol kg^{-1} for circuit 1 and 2 respectively (15.4 and 14.4% more, although not significantly, than averaged values measured within the aquarium over experimental period). This led to the assumption that rather microbial oxygen production than consumption occurred, although

incubations were carried out in the dark, avoiding photosynthetic processes. Microbial background activities amounted to 0.18µmol kg^{-1} h^{-1}, thus oxygen uptake in incubation glasses could be clearly related to corals.

4.2.3 Polyp extension

Polyp behavior of corals is known to be influenced by varying water parameters like light, current and prey (Sebens et al. 1996; Levy et al. 2001; Levy 2003). In the present study polyp extension was documented to obtain the potential behavioral response of *D. dianthus* towards lowered sea water pH.

During the first three months of experiment lowered pH had no effect on polyp extension. In the last three months polyps were significantly extended to a higher degree, compared to polyps at ambient pH. An increased extension might be related to higher respiration rates of corals at lowered pH in week 10 and 24. Additionally, respiration rates and polyp extension in general were slightly higher at lowered pH compared to ambient pH conditions, although not significantly. A positive relation between polyp extension and respiration was already shown in a previous study by Lasker (1979), as an enlarged tissue surface (i.e. fully extended polyps) increased the amount of oxygen molecules to pass through.

High polyp extension was measured at the end of experiment accompanied by highest respiration rates at lowered pH, leading to the suggestion that corals might compensate the energy loss due to pH up-regulation during calcification processes. Maier (2013) documented maximum polyp extension in the late evening (10:00 - 11:00pm), parallel to high zooplankton densities in the fjord Comau. As polyps have the function to take up oxygen and capture food, the aim of their high extension might be energy generation.

In summary, corals' response towards lowered pH was generally positive, but might be associated with energetic effort. Still, lower pH (e.g. pH 7.7 down to 7.4, values which have been measured in the fjord Comau) may negatively influence corals response. Further studies are needed to investigate corals thresholds towards the degree of ocean acidification that causes a lowered pH and a reduction of $CaCO_3$ in the oceans.

4.2.4 Methodical considerations

4.2.4.1 Corals in aquaria

Maintaining corals in aquaria might have led to an under-or overestimation of corals' response, as *D. dianthus'* growth rates differed between *in situ* and *in vitro* conditions (Jantzen et al. 2013b).
It has to be suggested that corals were exposed to an elevated stress level, possibly causing an underestimation of growth rates. They experienced several site transfers, plus they had to be removed out of the aquarium to conduct experiments and cleaning procedures. However, daily observations did not show any unnatural behavior, like increased excretion of mucus or decreased polyp extension (Jantzen et al. 2013b). Maintaining corals in aquaria reduced inner and outer bioerosion (dissolution of corals' skeleton), caused by endolithic organisms, which might have led to an overestimation of growth rates. *In vitro* corals might have experienced reduced water exchange and flow conditions as in the fjord. Both might have caused a reduced oxygen transport into the diffusion boundary layer and hence decreased corals' metabolic activity, which eventually might have led to an underestimation of respiration rates (Shashar et al. 1996).

4.2.4.2 Ash free dry weight

The analysis of ash free dry mass ($M_{ash\ free}$) did not reveal expedient results which might be due to the following reasons: During $M_{ash\ free}$ analyses the error rate was higher as laboratory work was conducted by 3 people instead of one person, causing a possible mix up of sample dishes making an assignment of dishes to corals unclear. Further the dry oven heated up 20°C higher than manually adjusted (40°C) resulting in a possible carbon loss due to high temperatures (carbon evaporates at temperatures from 40°C on (Jantzen, personal communication)). This could have led to an underestimation of tissue mass. Additionally combusting time of four hours might have been too short so that small, obscured coral skeleton fragments remained in the dish resulting in mass increase. Thus a second run for five hours at 500°C was conducted resulting in a comparable $M_{ash\ free}$ as in the first run. A maximum deviation of 0.958g, which was an exception, as all other values of the second run did not deviate more than 0.080g from values of the first run, was measured. Additionally, it is to mention that carbonates oxidize from 550°C on (DIN 38414) (the temperature adjustment of the first run), leading to a possible overestimation of carbonate content. The latter might be a possible reason for a relatively high tissue mass. However highest tissue mass

values, which represented half of corals total mass probably cannot be explained by carbonate oxidation processes.

5. Conclusion

As hypothesized *Desmophyllum dianthus* responded positively towards lowered pH, showing equal calcification rates as at ambient pH conditions. Corals response might have been promoted by aragonite saturation states, which were supersaturated within both treatments. A sufficient food supply might have stimulated growth rates, which were comparable to those in the fjord Comau. However corals might have been stressed, as they revealed higher, although not significantly, respiration rates at lowered pH. Increased respiration rates were probably supported by elevated temperatures, high oxygen concentrations and increased polyp extension.

D. dianthus may cope with future CO_2 concentrations by showing acclimation potential, as it could be documented in the present study, and developing adaptation mechanisms like internal pH up-regulation (McCulloch et al. 2012a). Nevertheless, sea water pH is predicted to decrease by another 0.4 units and temperatures to rise at least 2°C until the end of the century (Hoegh-Guldberg et al. 2007). This scenario will cause highly lowered aragonite saturation states, endangering cold- and warm-water corals, as they will barely obtain aragonite for the formation of their skeletons. Elevated temperatures already caused bleaching (release of algal symbionts) in warm-water corals (Goreau 1964, 1992; Glynn 1993; Hoegh-Guldberg 1999). If temperatures and CO_2 concentrations increase, warm-water corals will decrease rapidly (Hoegh-Guldberg et al. 2007). Cold-water corals do not rely on algal symbionts and thrive at higher temperature gradients, compared to warm-water corals. The latter provides more suitable requirements to stand global warming. Thus cold-water corals may be more robust towards climate change, than their warm-water counterparts.

As the impact of temperature on *D. dianthus* is not quantified until now, further studies should focus on the combined effects of ocean acidification and global warming, as they are ongoing processes. A better understanding of corals thresholds would contribute to management plans, aiming the protection and sustainability of a peculiar and uncommon organism.

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7. Appendix

Tab. 7 Overview of corals physiological response measured as growth rates, respiration rates and polyp extension for each treatment. Values are given as means ± SD after 1, 3, 6, 8, 10 and 24 weeks for growth and respiration rates; and per day over 28 days for polyp extension.

		Ambient pH	**Low pH**
Growth rates	Time (weeks)		
(µmol $CaCO_3$ cm^{-2} d^{-1})	1	13.71 ± 16.07	17.23 ± 9.57
	3	22.00 ± 12.12	23.72 ± 9.27
	6	16.88 ± 11.72	18.00 ± 7.26
	8	12.57 ± 10.64	9.67 ± 11.19
	10	26.06 ± 9.22	25.12 ± 8.74
	24	20.97 ± 13.01	17.97 ± 8.78
	M_total	**18.70 ± 12.13**	**18.62 ± 9.14**
Respiration rates	Time (weeks)		
(µmol O_2 cm^{-2} d^{-1})	1	29.66 ± 24.18	23.01 ± 5.94
	3	29.02 ± 16.98	23.76 ± 5.32
	6	9.87 ± 3.90	19.49 ± 9.32
	8	21.80 ± 6.29	23.72 ± 5.30
	10	18.32 ± 7.83	21.32 ± 4.41
	24	23.75 ± 12.64	30.97 ± 9.09
	M_total	**22.07 ± 11.97**	**23.71 ± 6.56**
Polyp extension	Time (days)		
(%)	1	46.88 ± 42.70	40.63 ± 37.50
	2	65.63 ± 39.66	59.38 ± 37.50
	3	37.50 ± 34.16	65.63 ± 43.66
	4	9.38 ± 20.16	31.25 ± 35.94
	5	37.50 ± 42.82	65.63 ± 39.66
	6	84.38 ± 35.21	81.25 ± 30.96
	7	75.00 ± 40.82	84.38 ± 23.94
	8	50.00 ± 44.72	75.00 ± 36.51
	9	78.13 ± 36.37	81.25 ± 30.96
	10	90.63 ± 20.16	71.88 ± 25.62
	11	46.88 ± 34.00	43.75 ± 35.94
	12	78.13 ± 40.70	68.75 ± 35.94
	13	31.25 ± 35.94	68.75 ± 35.94
	14	93.75 ± 17.08	66.29 ± 37.47
	15	75.00 ± 36.51	93.75 ± 17.08
	16	87.50 ± 28.87	100.00 ± 0.00
	17	59.38 ± 41.71	40.63 ± 41.71
	18	87.50 ± 28.87	93.75 ± 17.08
	19	62.50 ± 38.73	78.13 ± 31.46
	20	75.00 ± 40.82	68.75 ± 35.94
	21	59.38 ± 49.05	96.88 ± 12.50
	22	87.50 ± 28.87	93.75 ± 17.08
	23	81.25 ± 30.96	87.50 ± 22.36
	24	84.38 ± 30.10	90.63 ± 27.20
	25	75.00 ± 31.62	84.38 ± 30.10
	26	84.38 ± 30.10	81.25 ± 30.96
	27	78.13 ± 31.46	87.50 ± 22.36
	28	90.63 ± 27.20	87.50 ± 22.36
	M_total	**68.3 ± 21.39**	**74.58 ± 18.30**

Tab. 8 PH and-temperature differences between circuit 1 and 2. Values are given in units respectively for pH and temperature (in °C) and represent the difference between mean values measured for each week (1-24)

Time (weeks)	pH	Temperature (°C)
1	0.2	0.1
2	0.2	0.1
3	0.3	0.1
4	0.3	0.2
5	0.3	0.3
6	0.3	0.3
7	0.4	0.2
8	0.4	0.4
9	0.4	0.3
10	0.4	0.1
11	0.3	0.4
12	0.3	0.2
13	0.2	0.3
14	0.2	0.1
15	0.2	0.1
16	0.2	0.1
17	0.2	0.1
18	0.2	0.3
19	0.2	0.4
20	0.2	0.1
21	0.3	0.2
22	0.3	0.3
23	0.3	0.3
24	0.2	0.1
M ± SD	**0.3 ± 0.1**	**0.2 ± 0.1**

Tab. 9 Overview of pH and temperature values within circuit 1 and 2 over experimental period. Values are expressed as means ± SD and averaged for week 1-24.

	Circuit 1	**Circuit 2**	**Circuit 1**	**Circuit 2**
Time (weeks)	pH		Temperature (°C)	
1	8.07 ± 0.10	7.87 ± 0.15	10.75 ± 0.19	10.83 ± 0.31
2	8.10 ± 0.29	7.88 ± 0.31	11.02 ± 0.66	11.17 ± 0.61
3	8.09 ± 0.07	7.80 ± 0.04	11.25 ± 0.13	11.14 ± 0.14
4	8.07 ± 0.07	7.78 ± 0.04	11.21 ± 0.20	11.02 ± 0.16
5	8.10 ± 0.06	7.78 ± 0.04	10.85 ± 0.17	11.12 ± 0.11
6	8.09 ± 0.08	7.75 ± 0.07	11.07 ± 0.43	11.19 ± 0.34
7	8.11 ± 0.09	7.75 ± 0.07	11.30 ± 0.34	11.08 ± 0.50
8	8.11 ± 0.08	7.72 ± 0.08	10.87 ± 0.11	11.26 ± 0.13
9	8.10 ± 0.08	7.71 ± 0.09	10.90 ± 0.11	11.22 ± 0.11
10	8.14 ± 0.53	7.75 ± 0.52	11.29 ± 0.72	11.38 ± 0.74
11	8.13 ± 0.06	7.80 ± 0.06	11.50 ± 0.15	11.11 ± 0.13
12	8.09 ± 0.09	7.80 ± 0.07	11.65 ± 0.31	11.43 ± 0.24
13	8.13 ± 0.04	7.89 ± 0.04	11.37 ± 0.61	11.67 ± 0.15
14	8.17 ± 0.06	7.94 ± 0.04	11.13 ± 0.22	11.24 ± 0.19
15	8.21 ± 0.04	7.96 ± 0.04	11.24 ± 0.16	11.37 ± 0.12
16	8.10 ± 0.06	7.93 ± 0.05	11.60 ± 0.20	11.53 ± 0.17
17	8.11 ± 0.05	7.86 ± 0.03	11.45 ± 0.16	11.52 ± 0.10
18	8.11 ± 0.05	7.87 ± 0.04	11.48 ± 0.12	11.78 ± 0.21
19	8.12 ± 0.05	7.88 ± 0.04	11.23 ± 0.19	11.64 ± 0.21
20	8.12 ± 0.05	7.88 ± 0.04	11.45 ± 0.15	11.31 ± 0.10
21	8.13 ± 0.05	7.88 ± 0.04	11.25 ± 0.18	11.44 ± 0.14
22	8.14 ± 0.05	7.88 ± 0.04	11.24 ± 0.16	11.58 ± 0.13
23	8.14 ± 0.05	7.88 ± 0.04	11.02 ± 0.31	11.27 ± 0.29
24	8.14 ± 0.04	7.89 ± 0.04	11.57 ± 0.21	11.51 ± 0.17
M_total	**8.12 ± 0.09**	**7.84 ± 0.08**	**11.24 ± 0.26**	**11.37 ± 0.23**

Tab. 10 Overview of nutrient values within circuit 1 and 2 over experimental period. Values are expressed as means ± SD and averaged for week 1-24. (n.a.) indicates that no measurements were conducted.

	Circuit 1				Circuit 2			
	NO_3	PO_4^-	NO_2^-	NH_4^+	NO_3	PO_4^-	NO_2^-	NH_4^+
Time (weeks)	*(mg L^{-1})*	*(mg L^{-1})*	*(mg L^{-1})*	*(mg L^{-1})*	*(mg L^{-1})*	*(mg L^{-1})*	*(mg L^{-1})*	*(mg L^{-1})*
1	0.50 ± 0.00	0.02 ± 0.00	0.03 ± 0.02	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.05 ± 0.01	0.05 ± 0.00
2	0.50 ± 0.00	0.02 ± 0.00	0.04 ± 0.02	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.04 ± 0.01	0.05 ± 0.00
3	0.50 ± 0.00	0.02 ± 0.00	0.03 ± 0.02	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
4	0.50 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.02 ± 0.02	0.05 ± 0.00
5	0.65 ± 0.14	0.02 ± 0.00	0.08 ± 0.08	0.05 ± 0.00	0.63 ± 0.14	0.04 ± 0.02	0.10 ± 0.08	0.05 ± 0.03
6	0.93 ± 0.19	0.06 ± 0.02	0.19 ± 0.09	0.12 ± 0.09	1.00 ± 0.00	0.18 ± 0.28	0.18 ± 0.04	0.06 ± 0.02
7	1.00 ± 0.00	0.05 ± 0.00	0.1 ± 0.00	0.05 ± 0.00	0.75 ± 0.35	0.04 ± 0.02	0.06 ± 0.06	0.05 ± 0.00
8	0.50 ± 0.00	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.00	0.50 ± 0.23	0.02 ± 0.00	0.08 ± 0.03	0.05 ± 0.00
9	0.50 ± 0.00	0.02 ± 0.00	0.03 ± 0.02	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.04 ± 0.02	0.05 ± 0.00
10	0.50 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.05 ± 0.00
11	0.50 ± 0.06	0.02 ± 0.00	0.02 ± 0.01	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.05 ± 0.00
12	0.50 ± 0.00	0.02 ± 0.00	0.01 ± 0.01	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.01 ± 0.01	0.05 ± 0.00
13	0.50 ± 0.00	0.02 ± 0.00	0.02 ± 0.02	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.05 ± 0.00
14	0.50 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.05 ± 0.00
15	0.50 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.05 ± 0.00
16	0.61 ± 0.13	0.23 ± 0.37	0.03 ± 0.01	0.05 ± 0.00	0.54 ± 0.10	0.02 ± 0.00	0.02 ± 0.01	0.05 ± 0.00
17	0.81 ± 0.17	0.02 ± 0.00	0.02 ± 0.01	0.07 ± 0.01	0.63 ± 0.14	n.a.	0.04 ± 0.01	0.05 ± 0.00
18	0.88 ± 0.14	n.a.	0.03 ± 0.00	n.a.	1.00 ± 0.00	0.10 ± 0.00	0.03 ± 0.00	n.a.
19	1.33 ± 0.29	0.02 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.67 ± 0.57	0.10 ± 0.00	0.02 ± 0.01	n.a.
20	2.33 ± 0.29	0.02 ± 0.00	0.02 ± 0.01	n.a.	0.50 ± 0.35	0.04 ± 0.02	0.01 ± 0.00	n.a.
21	0.88 ± 0.18	0.04 ± 0.02	0.01 ± 0.00	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.05 ± 0.00
22	0.50 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.05 ± 0.00
23	0.50 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.05 ± 0.00
24	0.50 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.05 ± 0.00	0.50 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.05 ± 0.00
M ± SD	**0.70 ± 0.07**	**0.03 ± 0.02**	**0.03 ± 0.01**	**0.05 ± 0.00**	**0.57 ± 0.08**	**0.04 ± 0.02**	**0.04 ± 0.01**	**0.05 ± 0.00**

Eidesstattliche Erklärung

Hiermit versichere ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die angegeben Quellen und Hilfsmittel benutzt habe. Außerdem versichere ich, dass ich die allgemeinen Prinzipien wissenschaftlicher Arbeit und Veröffentlichung, wie sie in den Leitlinien guter wissenschaftlicher Praxis der Carl von Ossietzky Universität festgelegt sind, befolgt habe.

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