Suitability of Mineral Accretion as a Rehabilitation Method for Cold-Water Coral Reefs

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Abstract

Extensive areas of the cold-water scleractinian *Lophelia pertusa* have been damaged due to the impact of bottom-trawling and natural recovery is slow or absent. Here we evaluate a method for coral reef rehabilitation intended to enhance coral transplant survival and growth, i.e. mineral accretion by electrolysis in seawater. Electrolysis in seawater produces a semi-natural substrate in the form of aragonite ($CaCO_3$). The method has been used in coral reef rehabilitation programmes in tropical coral habitats but has so far not been tested in temperate deep-water habitats. A controlled laboratory experiment was performed to test the effect of the substrate *per se* and different levels of applied current densities (0.00-2.19 A m^{-2}), including galvanic elements (Fe|Zn), on coral fragments attached to the cathodes. The studied responses were; growth rate, budding frequency, mortality, and general health status (degree of polyp activity). We found that the budding frequency differed significantly between treatments, with higher frequencies in low current density treatments. Significant differences were also found in the frequency distribution of calices displaying a growth of ≥2 mm yr^{-1}, with higher frequencies in the lowest applied current density (LI), controls, and galvanic elements. Growth rates were slightly higher in LI, although non-significant. Zero mortality was observed in the control group as well as in LI. The degree of polyp activity was not affected by the treatments. These results are in part congruent with earlier studies and the method is found suitable for *L. pertusa*. The positive effects were mainly restricted to the lowest applied current density treatment (0.06 A m^{-2}). The optimal current density level is hereby found to be considerably lower than levels used in previously published studies and provide new guidelines for what levels to use in rehabilitation programmes with this method.

Keywords: mineral accretion, coral habitat rehabilitation, transplantation, artificial reefs, electrolysis in seawater, calcium carbonate, aragonite, budding frequency, growth rates

1. Introduction

The cold-water scleractinian coral *Lophelia pertusa* (Linneaus, 1758) is the main ecosystem engineer in the northeast Atlantic (Freiwald et al. 2004) building important

habitats for fish and invertebrates (Costello et al. 2005, Mortensen et al. 1995, Reed 2002, Ross 2006). Coral presence increases diversity threefold compared to surrounding soft sediment habitat (Gage and Roberts 2003, Henry and Roberts 2007, Husebø et al. 2002, Jensen and Frederiksen 1992, Jonsson and Lundälv 2006, Jonsson et al. 2004), and loss of coral habitat adversely affects local fisheries (Fosså et al. 2002, Koenig et al. 2000). It is estimated that 30 to 50 percent of the Norwegian reefs have been damaged by bottom-trawling (Fosså et al. 2002). The coral coverage and habitat complexity of the *L. pertusa* reefs in the Skagerrak have likewise been severely reduced. In the Swedish part of Skagerrak only one small live reef consisting of small detached and scattered colonies remains while six hitherto known reefs are extinct and consists only of dead coral rubble. Rubble fields are known to give a poor substrate for recolonization with little or no recovery in both high and low wave-energy environments, and rather than recovery a further deterioration has been the case (Brooke et al. 2006, Clark and Edwards 1994). Furthermore, due to geographic isolation of reef sites in the area the natural recolonization by coral larva from neighbouring reefs is unlikely. For example, dispersion probabilities using a Lagrangian model shows a mere 4-9 ‰ probability of larval recruits from the nearest Norwegian reef (Tisler) reaching Saekken (Ericson and Ljunghager 2006). These results are corroborated by genetic data showing high genetic differentiation between reefs in Skagerrak (Broberg 2006) and high levels of clonality within reefs (Dahl 2006). Hence, it seems that rehabilitation efforts by means of deployment of artificial reefs with coral transplants are necessary to restore cold-water coral cover in the area.

The method of mineral accretion through electrolysis in seawater for the purpose of coral reef rehabilitation was developed by Wolf Hilbertz and Thomas Goreau (1996) and has been experimentally tested by several other workers (Borell et al. 2009, Eisinger 2005, Goreau et al. 2004, Romatzki 2009, Sabater and Yap 2004, Schuhmacher et al. 2000, van Treeck and Schuhmacher 1997). The results have varied widely between experiments and between species of corals and while measurements from rehabilitation projects around the tropics find growth rates two to six times higher than natural coral colonies the scientific reports published so far have more ambiguous results to present. Few of the studies from rehabilitation projects have yet been published except as theses in Indonesian. It has nevertheless been substantiated that there is an increased survival rate of coral transplants growing on cathodes (Sabater and Yap 2002, van Treeck and Schuhmacher 1997). The method has thus far been used in rehabilitation of shallow tropical coral habitats and has never been tested in deeper temperate habitats.
The occurrence of *L. pertusa* on oilrigs (Gass and Roberts 2006) and the use of electrical stimulation in the form of microcurrent electrical therapy (MET) in human and animal medicine with several positive effects observed in different studies (Cheng et al. 1982, Kloth 2005) suggests the possibility of using metal structures and trickle currents to enhance survival and growth in transplanted corals. Gass and Roberts (2006) reported high growth rates of corals growing on oilrigs; trickle currents are used to protect the metal from corrosion and could explain the positive effects on growth. Cheng et al. (1982) observed an increase in ATP production in skin tissue samples exposed to microcurrents ranging from 50 to 1000 μA, thus there is a substantiated positive effect of microcurrents on ATP production. Furthermore, the application mimics the natural mode of mineralisation. Corals produce an alkaline environment in an enclosed space between the calicoblastic cells and the substrate that promote the spontaneous precipitation of aragonite crystals with the deposited

organic matrix working as a primer. Electrodeposition produces the same type of orthorhombic aragonite crystals thus growing a semi-natural substrate on the metal conductor. The proposed benefits for corals (and other calcifying organisms) growing on cathodes are: 1) supersaturation of calcium and carbonate ions in the vicinity of the cathode; 2) increased efficiency of cation uptake and transport due to the availability of electrons; and 3) increased metabolic efficiency since free electrons are available for ATP production (Hilbertz and Goreau 1996).
The aim of this study was to evaluate the potential of mineral accretion for rehabilitation of cold-water coral habitat. More specifically, we tested a pre-deposited mineral layer with no electrical current applied, galvanic elements (~0.4 V) and three levels of applied electrode potential (2.0 V; 2.5 V and 3.0 V) to find the optimal level of current density considering survival and growth of the coral transplants, or if the substrate *per se* without applied electrical current would have a positive effect. In addition, we evaluated the response in polyp behaviour (degree of extension and activity) and budding frequencies.

2. Materials and methods

The experiment was conducted at the marine research station at Tjärnö (Dept. of Marine Ecology, University of Gothenburg) at the Swedish west coast facing the Skagerrak. The research facility has a complete flow-through saltwater system with deep-water of similar composition (chemistry, salinity etc) as the ambient water of the nearby reefs of *L. pertusa*. The experiment was launched September 3, 2008, and ran for 6 months.

2.1. Experimental design

Twenty-four aquarium tanks were set up in a constant temperature room with the in-flowing water temperature set at 8ºC to imitate the *in situ* conditions for local coral populations (natural range: 4-10 ºC). The water intake for the deep-water flow-through system is at 40-45 m depth in the adjacent Koster Fjord. The tanks were assigned either one of the six different treatments to be tested, four replicate tanks per treatment with four coral pieces in each, making a total of 96 coral fragments (mean length ±SD: 47.2 ±12.6 mm; size range: 22.2-85.4 mm). The coral pieces were randomly distributed between tanks. The number of calices on each fragment ranged from 3 to 20. Three different morphological types were recognized; white slender (45 pieces) or compact white corals (31 pieces), and a red morph (20 pieces) with smaller calices and a thin, richly branched skeleton. The red morph was spread out to have not more than one representative in each tank. The corals were collected with a ROV (Sperre Sub-fighter 7500 DC) equipped with a manipulator arm at c. 100 m depth at the Tisler reef (58°59.70’N, 10°58.00’E). Six different treatments were applied:

C:	Controls; corals on plastic mesh
AS:	pre-precipitated Aragonite* Substrate on cathodes, no voltage applied
GE:	Galvanic Elements (Fe|Zn), galvanic potential in seawater ~0.4 V.
LI:	Level 1; 2.0 V and 0.01 A, end value: 0.00 A
LII:	Level 2; 2.5 V and 0.12 A, end value: 0.05 A
LIII:	Level 3; 3.0 V and 0.35 A, end value: 0.21 A

*) Comment: the precipitated minerals were initially very porous and consisted thus mainly of brucite. When the experiment was terminated, however, the minerals had hardened by a gradual deposition of aragonite.

The AS treatment could be viewed both as a procedural control and a zero voltage treatment, testing the substrate *per se.* The galvanic elements with steel cathodes and zinc anodes have a galvanic potential of ~0.4 V in seawater and thereby offers a lower current alternative to the three treatments with applied DC current. The different voltage levels were applied by three EP-613 DC sources with three digit LCD displays (0-30 V and 0-3 A, Manson Engineering Industrial Ltd). Connections were made with the four replicates of each treatment in parallel from one DC source. The voltage was monitored and kept constant throughout the experiment, while the amperage decreased over time. The different treatments were as far as possible randomly distributed between the tanks to avoid artefact effects of position in the room. The cables and connections, however, made it impossible to fully randomize and therefore all the AS treatments were assembled on the same bench together with one control and one GE tank. The current density (A m^{-2}) was calculated for comparisons with other studies where A m^{-2} has been used.

2.2. Tanks and substrates

The tanks were constructed with two separate main chambers; one that would contain the corals and one chamber that would hold the anodes or left empty. Perforated partition walls guaranteed that there were no back-mixing between chambers to avoid exposure to the lower pH or chlorine gas produced at the anodes. All tanks were given the same design to have equal flow regimes, and all anode chambers were covered with plastic and sealed with duct tape. The sealing of the anode chambers were done to retain chlorine gas within the water to purify and neutralize the out-flowing water in filter trays containing activated carbon and oyster and mussel shells prior to release.
The cathodes were cut to 110×160 mm pieces from a steel mesh with a metal surface area calculated to 0.04 m^2. Average weight of the cathodes was 253 g. The anodes were made of a 1×1 mm angular wire mesh of coated titanium (150×200 mm, average weight 12 g). The galvanic elements were produced by attaching zinc anodes to one end of the cathodes; c. 43.8 g of zinc on each galvanic element. In the controls the corals were placed on a plastic mesh of the same size as the cathodes mounted on a small flat stone and affixed with aquarium silicon.
All materials were conditioned in seawater during 5 weeks before mounting the corals. The AS treatment was run on electrolysis (4.0 V) during these weeks to get a mineral cover, however, the deposited minerals consisted mainly of brucite and were very porous. Additional short periods of DC currents (3.0 V) were given on six occasions during the first three weeks of the experiment to counteract corrosion on cathode surfaces that had its mineral layer scraped off while mounting the corals. When the experiment was terminated the mineral layers were measured with a pair of vernier calipers, with five replicate measurements at each end of the cathodes (close or far end relative to the anode).

2.3. Feeding and monitoring

During the experiment the corals were fed with *Artemia salina* nauplii (brine shrimps) on three occasions per week. The *Artemia* were hatched over 48h and reared for

another 48h while fed with micro algae (*Isochrysis* sp.) to increase nutrient value. The food was supplied via the water flow-through system to be evenly distributed. Four plastic containers were used to buffer water so that the water levels in the containers and thereby pressure and flow rate to the tanks could be kept equal. Each container supplied six tanks each with water. Flow rates were measured on three occasions during the experiment with three replicate measurements from each tank.
Water temperature and pH values were monitored weekly during the experiment with a hand held digital pH meter (Waterproof pH Testr 30, accuracy; ±0.001 pH units and ±0.5ºC). Measurements were taken 1 cm above the substrates and randomized between tanks. pH were between 8.06 and 8.10 (see table 1, fig. 1). Water temperature was 7.07ºC ±1.45ºC (mean ºC ±SD), starting around 10.0ºC and levelling off at around 5.5-6.0ºC over several weeks in the second half of the experimental period. During the four last months of 2008 the measured salinity at 30 m depth (close to the depth of water intake) in the Kosterfjord was 32.5-33.8 psu (SMHI Report No. 2009-6).

Table 1
Average pH values (mean ±SD) over the different treatments. The measures from the first five weeks (during the initial larger fluctuations) were excluded.

TREATMENT	pH	SD
C	8.06	±0.024
AS	8.08	±0.030
GE	8.07	±0.029
LI	8.07	±0.021
LII	8.10	±0.032
LIII	8.07	±0.055

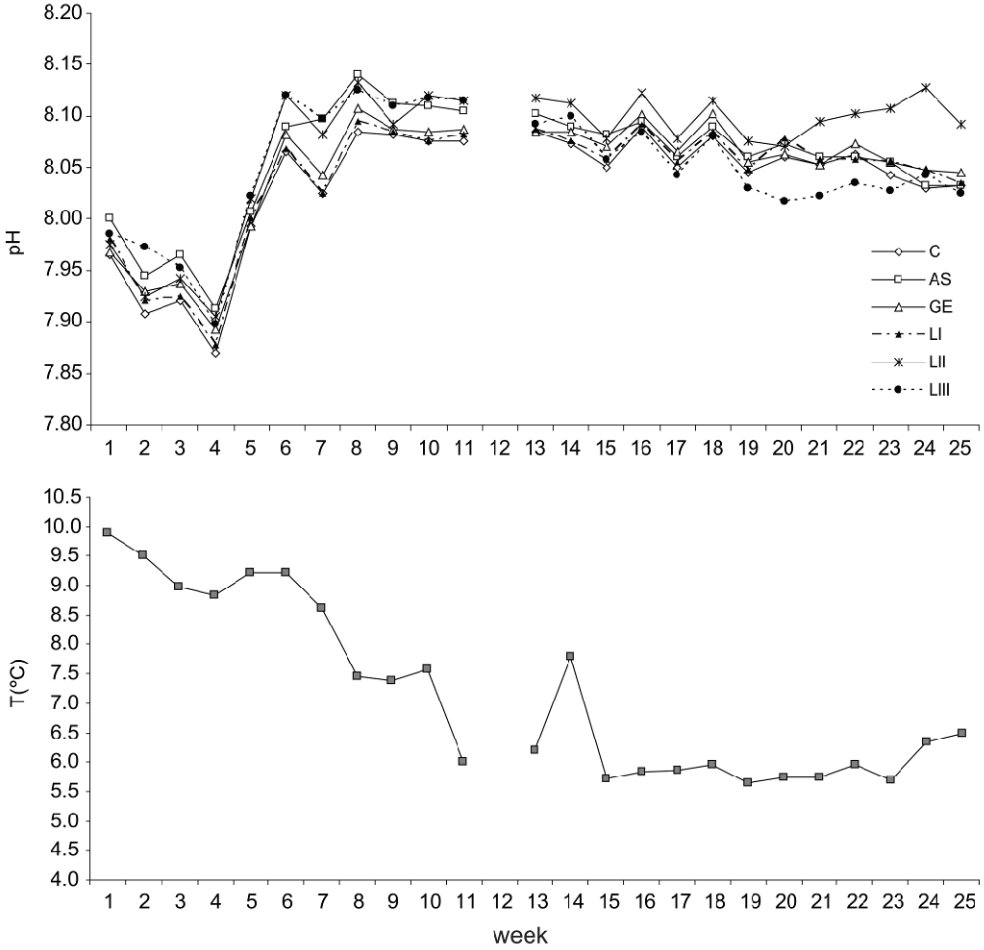

Fig. 1. General conditions; pH differed slightly between treatments, i.e. the AS, LII, and LIII treatments had higher pH levels, and the lowest current density treatment (LI) and the non-charged treatments (C and GE) had lower pH. The pH levels decreased over time in the LIII treatment, probably due to the rapid accretion that filled up the voids in the mesh, thus trapping hydrogen gas underneath the cathode. The overall trend in pH with lower values during the first five weeks is probably caused by early autumn down-mixing of surface waters. The experiment ran from September 2008 to March 2009 and the temperature had its annual maxima (16.8ºC) in September with less pronounced stratification of water masses during the period. Despite the thermo regulation the temperature could not be kept constant and the temperature fluctuated during the experiment as seen in the lower graph.

The health status of the corals was likewise monitored on a weekly basis; the corals were given points based on the degree of extension of tentacles. Fully withdrawn = 0; partially withdrawn = 1; extended but slack = 2; intermediate = 3; extended and vivid (stiff) = 4; and, actively moving tentacles = 5 points. The health status observations were not randomized due to the corals sensitivity to vibrations that caused them to withdraw at slightest disturbance. Instead the observations were made in the same order, from tank one to 24, as swiftly and quiet as possible, and repeated in the opposite direction another day of the week to elucidate if there would be a difference in extension rates due to procedures.

2.4. Growth measurements

Photographs were taken of the coral fragments at the point of start and end of the experiment with the pieces placed on a grey perforated panel to provide a reference for measurements (see fig. 2). Measurements were made in the free software ImageJ (version 1.42a, Wayne Rasband, National Institutes of Health, USA). Several measurements on each coral fragment were made, and mean growth and maximum observed growth were recalculated to growth in mm yr^{-1} for analysis.
Some of the corals had broken calices after the handling during collection, and these had either died or healed to different degrees. The mostly one-sided growth of healing was measured and noted separately in the protocol. Furthermore, the degree of healing was assigned a symbol; one plus sign (+) for a moderate healing ($0.8 \leq x < 2.0$ mm); two plus signs (++) for a good healing (≥ 2.0 mm); a minus (-) for broken calices that did not heal. A zero (0) denotes that there were no broken calices.
The numbers of new buds were counted and partial or complete mortality was noted; i.e. number of dead polyps (not counting those that were already dead at start) divided by the total number of polyps on each fragment.

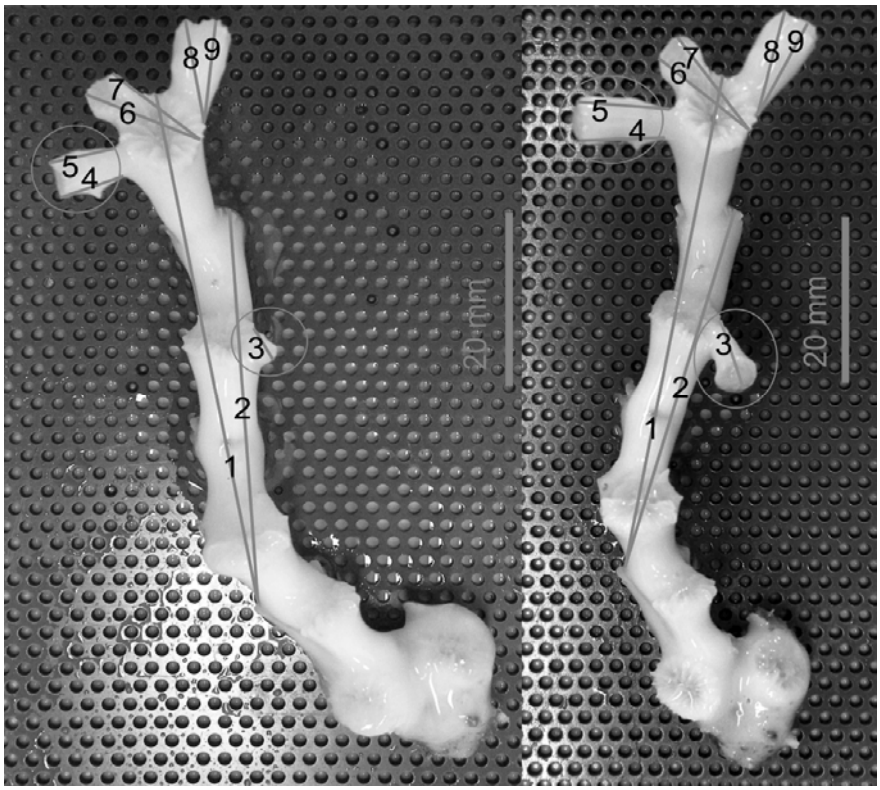

Fig. 2. An example of images taken at the start (left) and at the end (right) of the experiment. Measurements were performed in the free software ImageJ (version 1.42a). This particular coral piece was reared in the lowest applied current density (LI: 2.0V, ≤ 0.06 A m^{-2}). A new bud has developed from a small protrusion into a long calice, and the upper left calice (numbered 4, 5) has grown noticeable.

2.5. Statistics

The frequency distributions of the number of new buds, and calices displaying a growth of $0.8 \leq x < 2.0$ mm yr^{-1} or ≥ 2.0 mm yr^{-1} were analyzed by Chi-square (χ^2) tests. A one-way ANOVA were performed on growth rate data and Pearson Correlations were performed to test if the different water flow rates had an effect on

the variables mean and maximum growth or the frequencies of new buds and calices with a growth of ≥2.0 mm yr^{-1}.
All statistical analyses were performed with the statistical software SPSS 17.0 (2008).

3. Results

3.1. Mineral accretions

The accreted mineral layer varied in thickness between treatments and with distance to the anode. Higher current density produced a thicker layer, and subsequently the end of the cathode that was closer to the anode received a thicker layer of precipitated minerals (table 2). On the LI cathodes the measured layer were 1.53 ±0.74 mm (mean ±SD) closest to the anode and 0.99 ±0.60 mm on the end furthest away. The LII treatment had 5.07 ±0.85 mm and 2.48 ±0.62 mm thick layers on the close and far end relative to the anode, respectively. Both treatments had produced a hard crust. The AS treatment had layers between 7.90 ±2.03 and 1.98 ±0.30 mm. Noteworthy, the rapid deposition of minerals on the AS treatment produced a very porous layer initially, which subsequently was strengthened ending up a hard crust. The efforts to counteract corrosion on the AS cathodes failed. The LIII treatment gave a rapid accretion of porous minerals that crumbled while dismounting the corals and could thus not be properly measured, however, layers were up to 10 mm thick on one side only. There were no visible mineral accretions on the GE cathodes. The mineral layer on LI barely covered the cathode while full accretion was accomplished on the LII cathodes. Coral fragments were firmly attached to the cathode surfaces by the mineral accretions.
The amperage decreased rapidly from the initial values during the first week, and after the initial drop the amperage levelled out and decreased only slightly over the remaining period (table 2). The mineral accretion rates were equal within treatments, indicating an equal distribution of electrical currents over the four replicate electrode pairs.

Table 2
The start and end values, respectively, of current (A) and the calculated current density (A m^{-2}) per metal surface area for the LI, LII, and LIII treatments. The thickness of mineral accretions in AS, LI, and LII are the sum of layers on upper and under sides of the cathodes. The mineral layer on LIII crumbled when dismounting the corals and could not be properly measured, however, layers were up to 10 mm thick on one side only.

	TREATMENT					
	LI		LII		LIII	
	start	end	start	end	start	end
Ampere	0.01	0.00	0.12	0.05	0.35	0.21
Current density (A m^{-2})	0.06	immesurable	0.75	0.31	2.19	1.31

	AS		LI		LII	
	near anode	far end	near anode	far end	near anode	far end
Mineral accretions (mm)	7.90	1.98	1.53	0.99	5.07	2.48
±SD	±2.03	±0.30	±0.74	±0.60	±0.85	±0.62

Table 3
Chi-square output and test statistics. There were significant differences between treatments in the frequency distribution of new buds as well as in calices with a growth of ≥ 2.0 mm yr^{-1}. No cells have

expected frequencies less than 5. The differences in the frequency distribution of calices displaying a growth of $0.8 \leq x < 2.0$ mm yr^{-1} was also tested and found to be non-significant (not shown). $\chi^2_{crit} = 11.07$ at the .05 level.

	Frequency NEW BUDS			Frequency ≥ 2.0 mm yr^{-1}		
TREATMENT	Obs. N	Exp. N	Residual	Obs. N	Exp. N	Residual
C	8	9.7	-1.7	10	6.5	3.5
AS	7	9.7	-2.7	4	6.5	-2.5
GE	13	9.7	3.3	9	6.5	2.5
LI	17	9.7	7.3	11	6.5	4.5
LII	10	9.7	0.3	1	6.5	-5.5
LIII	3	9.7	-6.7	4	6.5	-2.5
Total	58			39		

Test Statistics

	NEW BUDS	≥ 2.0 mm yr^{-1}
Chi-Square	12.345	12.538
df	5	5
Asymp. Sig.	0.030	0.028
Exact Sig.	0.030	0.029
Point Probability	0.002	0.005

Table 4
A table summarizing the observed responses of the corals to the six treatments: mean growth (mm yr^{-1}); maximum observed growth (mm yr^{-1}); heal growth (mm) is the observed one-sided healing growth of broken calices; degree of healing is the frequency of calices displaying the degree of healing within the classification given in brackets, i.e. (+) moderate healing ($0.8 \leq x < 2.0$ mm yr^{-1}), (++) good healing (≥2.0 mm), (-) no healing, and (0) denotes that there were no broken calices; health status, i.e. corals were given a rank based on the degree of extension of the polyps, i.e. 0 = fully withdrawn, 1 = partially withdrawn, 2 = extended but slack, 3 = intermediate, 4 = extended and vivid (stiff), and 5 = active tentacles; No. of new buds is the total sum of newly developed calices in each treatment; No. ≥0.8 and ≥2.0 are the frequencies of calices that displayed a growth rate of $0.8 \leq x < 2.0$ mm yr^{-1} or ≥2.0 mm yr^{-1}, respectively; and mortalities are the observed partial (number of dead polyps/total nr of polyps) or complete mortalities. Values for the mean, minimum, maximum, and standard deviation as headlined in the second column is given for the mean, maximum, heal growth, and for health status. C = controls; AS = pre-precipitated aragonite substrate (no voltage applied); GE = galvanic elements (c. 0.4 V, Fe│Zn); LI = level 1 (2.0 V and 0.01 A); LII = level 2 (2.5 V and 0.12 A); LIII = level 3 (3.0 V and 0.35 A).

TREAT		mean growth (mm yr^{-1})	max growth (mm yr^{-1})	heal growth (mm)	health status (points)	degree of healing	No. of new buds	No. ≥0.8 mm	No. ≥2.0 mm	MORTALITIES
C	MEAN	1.195	3.051	1.292	3.47	8(0)	8	28	10	zero mortality
	MIN	0.005	0.528	0	2.88	5(+)				
	MAX	3.940	6.290	6.031	3.94	3(++)				
	SD	0.967	1.730	1.937	0.41	0(-)				
AS	MEAN	0.843	2.827	0.599	3.07	7(0)	7	32	4	2 cases of partial mortality (0.14-0.43)
	MIN	0.156	0.865	0	0.25	4(+)				
	MAX	1.803	6.426	2.798	3.94	2(++)				
	SD	0.536	1.704	0.939	0.86	3(-)				
GE	MEAN	0.863	2.466	1.158	2.83	3(0)	13	17	9	4 cases of partial mortality (0.29-0.67)
	MIN	0	0.076	0	0.50	8(+)				
	MAX	3.301	10.156	7.337	3.94	2(++)				
	SD	0.898	2.458	1.861	0.99	3(-)				
LI	MEAN	1.269	3.258	0.583	3.10	9(0)	17	26	11	zero mortality
	MIN	0	0.802	0	2.31	3(+)				
	MAX	3.879	10.228	3.709	3.75	2(++)				
	SD	1.220	2.655	1.083	0.43	2(-)				
LII	MEAN	0.579	2.333	0.157	3.07	12(0)	10	27	1	1 case of partial mortality (0.11)
	MIN	0.040	1.192	0	1.94	3(+)				
	MAX	1.914	4.724	1.187	4.00	0(++)				
	SD	0.480	1.066	0.330	0.49	1(-)				
LIII	MEAN	0.539	2.172	0.111	3.05	11(0)	3	15	4	8 cases of partial mortality (0.14-0.29)
	MIN	0	0	0	0	4(+)				2 complete mortalities
	MAX	2.058	6.142	0.967	3.88	0(++)				
	SD	0.640	1.868	0.305	0.93	0(-)				

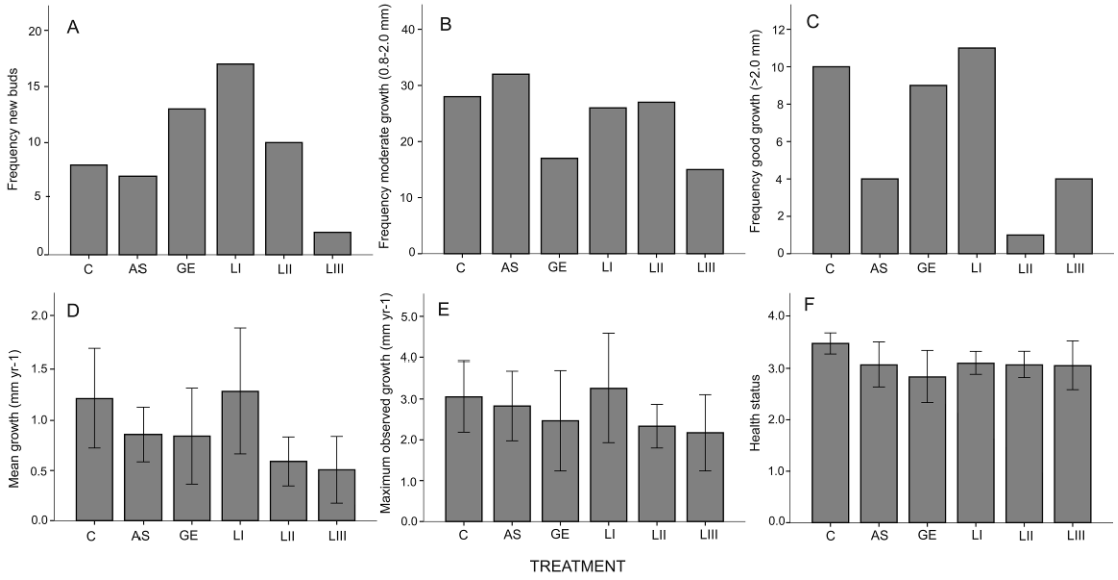

Fig. 3a-f. In diagram **a** all new calices that developed during the experimental period is summarized for each treatment; here a peak occurs in the LI treatment, curtailing laterally over the low current density treatments (GE and LII) while the highest current density (LIII) produced very few new calices and the non-charged treatments (C and AS) had moderate budding frequencies. Diagrams **b-c** shows the frequencies of calices displaying a growth of $0.8 \leq x < 2.0$ mm yr^{-1} and ≥2.0 mm yr^{-1}, respectively. There were significant differences between treatments in the frequency of new buds (fig. **a**; $\chi^2_{(df\ 5)} = 12.3$; exact p = 0.03; point prob. = 0.002; $\chi^2_{crit} = 11.07$ at the .05 level) and in the frequency of calices with growth of ≥2.0 mm yr^{-1} (fig. **c**; $\chi^2_{(df\ 5)} = 12.5$; exact p = 0.03; point prob. = 0.005). The differences were not significant in the frequency of calices in the lower range of growth rate (**b**). Diagram **d-e** shows the trends in observed mean growth (**d**) and maximum observed growth (**e**) with slightly higher growth rates, albeit non-significant, in the lowest current density treatment (LI) compared to the controls for both variables while all other treatments had lower growth rates. Diagram **f** shows that the behaviour (degree of extension) of the polyps was not affected by the different treatments. Data in **a-c** is presented as frequency distributions (total sums) while **d-f** are presented as mean values (4 replicate tanks with 4 coral pieces in each treatment) with standard error bars. C = controls; AS = aragonite substrate, zero voltage; GE = galvanic elements (Fe|Zn) ; LI = 2.0 V, 0.01 A; LII = 2.5 V, 0.12 A; LIII = 3.0 V, 0.35 A.

3.2. Coral response to the treatments

There were significant differences between treatments in the frequency distribution of new buds developing during the experimental period ($\chi^2_{(df\ 5)} = 12.3$; exact p = 0.03; $\chi^2_{crit} = 11.07$ at the .05 level). There were 17 new buds in the LI treatment, 13 new buds in the GE treatment followed by 10 in LII, while the controls had 8 new buds (see table 4, fig. 3a). Pairwise Chi-square tests between controls and the different treatments revealed no significant effects. Testing the substrate *per se* (AS) against the treatments with an electrode potential (GE, LI, LII, LIII) also turned out non-significant, however, the difference between AS vs. LI was close to significant ($\chi^2_{(df\ 1)} = 4.17$; $\chi^2_{(crit)} = 3.84$; exact p = 0.06). The largest difference was found between LI and LIII ($\chi^2_{(df\ 1)} = 9.80$; $\chi^2_{(crit)} = 3.84$; exact p = 0.003).

The number of calices displaying a growth of ≥2.0 mm yr^{-1} (see fig. 3c) differed significantly between treatments and were higher in the controls, LI, and GE with 10, 11, and 9 calices, respectively ($\chi^2_{(df\ 5)} = 12.5$; exact p = 0.03). Pairwise tests were non-significant, except controls vs. LII ($\chi^2_{(df\ 1)} = 7.36$; $\chi^2_{(crit)} = 3.84$; exact p = 0.01). The number of buds displaying a growth rate of $0.8 \geq x < 2.0$ mm yr^{-1} was similar in all

treatments except GE and LIII that had lower numbers, albeit non-significant (fig. 3b).

The one-way ANOVA performed on growth rate data turned out non-significant and only diagrams with means and standard error bars is presented to show the trends in the effects on growth (fig. 3d-f). The observed mean growth was slightly higher in the lowest applied current density treatment (LI) than in the controls, i.e. 1.27 ±1.22 mm yr^{-1} and 1.20 ±0.97 mm yr^{-1}, respectively (mean ±SD), while all other treatments had lower growth rates (see table 4 and fig. 3d). The same pattern was seen in the observed maximum growth where corals in the LI treatment had an average maximum growth of 3.26 ±2.66 mm yr^{-1} compared to 3.05 ±1.73 mm yr^{-1} in the controls (fig. 3e).

All Pearson Correlations testing if there were any effects on growth rates or frequencies due to different water flow rates (mL sec^{-1}) in the tanks were non-significant (table 5). The r^2 value of the maximum growth indicates a lower range medium effect size (Kinnear and Gray 2008), but since there was a non-significant correlation this is interpreted as no effect of water flow rate.

Considering the general health status it seemed as all corals fared as well; the corals in the controls were extending their polyps to a slightly higher degree while the polyps on the galvanic elements (GE) were slightly less extended and vivid, albeit no significant results were found (fig. 3f). Looking at the health status derived from checks in one direction only (tank 1-24) it seemed like the corals on the zero voltage treatment (AS) fared less well, however, this pattern disappeared when pooling the checks from both directions as presented in the diagram.

In the summary table (table 4) there are some additional information presented; heal growth (mm) together with degree of healing (ranks), and mortalities. The healing of broken calices was highest in the controls with an average one-sided growth of healed calices of 1.29 ±1.94 mm followed by GE, AS, and LI. The controls displayed a high degree of healing, while AS, GE, and LI, displayed cases of good healing simultaneous with some cases of no healing. Since the number of broken calices differed among treatments the degree of healing is not entirely comparable, e.g. the higher current density treatments (LII and LIII) had very few broken calices to begin with.

There were zero mortalities in the controls and LI, and a few cases of partial mortality in AS and LII (see table 4). In the GE treatment there were 4 cases of partial mortality ranging from 29% to 67%, two of these are explained by an infestation by bacteria in one of the tanks. The bacteria were not analyzed but red crystalline metabolites on the surface of the cathode indicated chemoautotrophic iron-oxidizing bacteria. The highest voltage treatment (LIII) produced a high number of partial mortality, 8 cases ranging from 14% to 29%, and furthermore, two complete mortalities. These could all be explained by the treatment itself, as the rate of mineral accretion was so high that it covered the calices close to the cathode surface, and entirely overgrew two small coral fragments. In addition, some of the corals in the LIII treatment had so fragile skeletons when the experiment was terminated that they fell apart while dismounting them from the cathodes.

Table 5
The results of the Pearson Correlations show that there were no significant effect of water flow rates on the variables mean and maximum observed growth or the frequencies of new buds or calices with a growth of ≥ 2 mm yr^{-1}.

mLsec^{-1} vs.		
MEAN GROWTH (mm yr^{-1})	Pearson Correlation (r^2)	-0.004
	Sig. (2-tailed)	0.967
	N	96
MAX GROWTH (mm yr^{-1})	Pearson Correlation (r^2)	0.020
	Sig. (2-tailed)	0.843
	N	96
FREQUENCY NEW BUDS	Pearson Correlation (r^2)	0.007
	Sig. (2-tailed)	0.949
	N	96
FREQUENCY ≥ 2 mm yr^{-1}	Pearson Correlation (r^2)	0.101
	Sig. (2-tailed)	0.330
	N	96

4. Discussion

This study is the first to test the method of mineral accretion on cold-water corals. The most striking result of this experiment is the number of new buds that developed in the low current density treatments (table 4, fig. 3a). Although no significant effects were found in the pairwise comparisons between controls and the different treatments there was a significant difference ($p = 0.03$) between treatments in the overall Chi-square test, and testing the substrate *per se* against the charged treatments the difference between AS vs. LI was found to be close to significant ($p = 0.06$). The overall difference between treatments could largely be attributed to the difference between the LI and LIII (lowest vs. highest applied current density treatment, $p = 0.003$). Looking at the frequency distributions of new buds (fig. 3a) the increase lies over the low current density treatments (GE, LI, LII) and this result is congruent with previous studies as discussed below. The highest current density treatment (LIII) appears to be overcharged and detrimental to the corals with very few new buds developing as a result.

Also in the frequency distribution of calices with a growth of ≥2.0 mm yr^{-1} there were significant differences between treatments, although, here the controls displayed an equally good growth as the lower current densities (LI and GE) and this effect could therefore not be linked to trickle currents (fig. 3c). The pairwise comparisons revealed a significantly lower frequency of calices with a growth of ≥2.0 mm yr^{-1} in the intermediate current density treatment (LII) and rather than a positive effect of applied direct current there seems to be mainly a negative impact in AS, LII, and LIII. Also in AS and LIII frequencies were lower albeit non-significant. The low frequencies of calices with a growth of ≥2.0 mm yr^{-1} in AS and LII is somewhat compensated by higher frequencies of calices with the lower range of growth rate ($0.8 \geq x < 2.0$ mm yr^{-1}).

The growth rates observed in this study were below or in the lower range of the reported rates for the species, 4-25 mm yr^{-1} (Freiwald et al. 2004). Only a few calices on the galvanic elements and lowest applied current density (LI) showed growth rates of around 10 mm yr^{-1}, as seen in table 4 where maximum observed growth is presented. Possible causes for this are poor nutritional values of the chosen food (*Artemia*) or effects of stress. The trend in growth rates seen both in mean and maximum observed growth (fig. 3d-e) was a slight increase in growth rate in LI as

compared to controls, while all other treatments had lower rates, the differences being more pronounced in mean growth.
Zero mortality was observed in the lowest applied current density treatment (LI) as well as in the controls, showing that a trickle current has no negative impact on coral transplants if the level is optimized, while overcharging could be detrimental as seen in the higher current density treatments (LII and LIII). The partial mortalities and overall low performance of the corals in the zero voltage treatment (AS) and galvanic elements (GE) could, however, be due to leaking metal ions. Considering the AS treatment the failure of counteracting corrosion on the spots that had the mineral layer scraped off while mounting the coral fragments could have led to detrimental levels of metal oxides. Rust has been seen to be avoided by settling organisms, leaving bare patches on oxidized metal surfaces (Fitzhardinge and Bailey-Brock 1989). Leaking ions could also explain the low performance of corals on the galvanic elements as the zinc anodes were oxidized. Interestingly, the partial mortalities caused by the iron-oxidizing bacteria found in one of the GE tanks did not concur with impaired growth. Noteworthy, three of the corals growing in this specific tank had the highest growth rates observed within the GE treatment. The bacterial growth was restricted to one side of the cathode and thus leaving two corals unaffected by direct bacterial overgrowth. Despite some partial mortality the growth rate of the living calices in one of the affected corals were relatively high. Chemical reactions mediated by the bacteria could have mitigated the effect of leaking zinc ions, thus giving the positive response in growth in this specific tank.
The measured pH differed slightly between treatments with highest pH observed in the LII treatment. pH were initially high also in LIII but dropped after the 12^{th} week (fig. 1). This drop could be driven by the fast accretion that filled the voids in the mesh, thus trapping hydrogen gas underneath the cathodes. The fragility of coral skeletons in the LIII treatment could be explained by the lower pH towards the end of the experiment. An additional explanation of the observed fragility could be that the corals incorporate more porous brucite minerals rather than aragonite.
The observed drop in pH for all treatments during the first weeks was probably caused by an early autumn down-mixing of surface waters as an intense low pressure occurred in August, with winds of storm strength (mean wind velocity 24 m s^{-1}) accompanied by heavy rains (SMHI Report No. 2009-6) that could cause salinity to drop during the following period and affect pH. The water temperature at 30 m depth had its annual maxima in September (temporarily warmer than the surface waters) and the stratification of water masses was less pronounced during the period.

Comparisons with previous studies

The stimulatory effect in bud production seen in this study is congruent with the results of Sabater and Yap (2004) where treated nubbins of the branching coral *Porites cylindrica* had significantly higher densities of corallites. Although the results of the present study were non-significant in the pairwise Chi-square test between controls and LI, the concordance with the results of Sabater's and Yap's study strengthens the present results. The stimulated budding is an interesting effect that could prove valuable in transplantation programmes, as coral transplants that bud off richly initially will have more growing tips and thus a more rapid outgrowth into a complex matrix of coral branches. The morphology of *P. cylindrica* is very different from that of *L. pertusa* and perhaps the latter species is better served by an effect of this nature since budding is directly affecting branching. The mechanism for this,

however, can only be speculated in. While Sabater and Yap (2004) ascribes this effect to the increased mineral ion concentration, an alternative explanation could be an increase in ATP production at an optimal current density.

As shown by Cheng et al. (1982) in a clinical *in vitro* study on the effects of electric currents on skin tissue samples (from rats), currents ranging from 50 to 1000 μA had positive effects on ATP generation with a threefold to fivefold increase in ATP levels. ATP concentrations levelled when applying currents exceeding 1000 μA, and were reduced at currents of 5000 μA. This current density dependent ATP stimulatory effect could explain why positive effects are restricted to low current density treatments while higher levels have a negative impact despite the theoretically higher availability of calcium ions with increasing current densities. ATP is needed for the active transport of calcium ions into the calcifying compartment, as well as removing the hydrogen ions from the same to maintain the alkalinity necessary for precipitation of aragonite within the compartment (Allemand et al. 2004, McConnaughey and Whelan 1997, Tambutté et al. 1996). It is also known that feeding increase calcification rates (Houlbrèque et al. 2004, 2003) and that respiration rates and ATP concentrations are elevated in the growing tips of coral branches thus supporting the higher calcification rates (Fang et al. 1989, Gladfelter et al. 1989). Corals use mainly metabolic CO_2 for calcification (Furla et al. 2000), explaining the positive effect of feeding on calcification rates. ATP is a limiting factor for calcification,and high food availability can support a denser coral colony. We hypothesize that improved coral nutrition and/or energy availability via artificially elevated ATP levels generated by the electrode potential can promote branching.

The two lower current densities used in the present study were low (0.06-0.75 A m^{-2}) in comparison to current densities used in previously published studies, while the highest level (2.19 A m^{-2}) that in this experiment proved to be over-charged were in the range of previously used levels, although the lower values are in the range of most Biorock projects built by Hilbertz, Goreau, and their trained students. The levels used in the referred papers were 2.8 A m^{-2} (Borell et al. 2009), 1.67, 2.38, 3.43, and 2.86 A m^{-2} (Romatzki 2008), 2.8 A m^{-2} (van Treeck and Schuhmacher 1997) and 4.18 A, i.e. 9.5 A m^{-2} (Sabater and Yap 2004). The latter authors did not provide a re-calculation of electrical current to amperes per unit metal surface area and I therefore used the available information to calculate the current density, with possible errors. Noteworthy, Sabater and Yap used solar panels to provide their electrodes with electricity, and thus the given current density is the peak level at noon. van Treek and Schuhmacher (1997) used a constant direct current but turned the electricity off during 6 hours daily around noon to protect the DC source from overheating.

It is clearly seen in both the present study as well as in Romatzki (2008) that the coral response is current density dependent. In the latter study three different experiments were conducted on two coral species (*Acropora pulchra* and *A. yongei*) and in the first experiment where two current density levels were compared slightly increased growth rates on the lower current density treatment (1.67 A m^{-2}) was observed, while the higher current density (2.38 A m^{-2}) had a negative impact. In the second and third experiments the author used 3.43 A m^{-2} and 2.86 A m^{-2}, respectively. Here, without any explanation to why the higher current densities were used, the author found negative impacts of treatment with significantly lower growth rates on cathodes compared to controls.

The study conducted by Sabater and Yap (2004) showed significantly higher density of corallites and longitudinal and girth growth in treated corals than in controls during the first four of the six months of mineral accretion. Growth rates were presented as

cm per two months and the difference between treated and control nubbins were roughly 0.1 cm (controls and treated around 0.5 cm and 0.65 cm, respectively). The corals were reared on a variable current density following a sinusoidal function (0 to 9.5 A m^{-2}). The relatively limited period of peak current density during noon did not seem to impede growth.

Both Sabater and Yap (2004) and van Treeck and Schuhmacher (1997) reported high survival rates of corals on cathodes. The latter workers reported high survival rates of coral transplants of a range of species grown on cathodes, except for *Pocillopora damicornis* that were less robust, however, the species is sensitive to fragmentation and transplantation stress (Yap et al. 1992). Hence, the transplantation routine and not the treatment were likely to cause the increased mortality in this species. In Borell et al. (2009) *Acropora yongei* displayed a pronounced mortality on the cathodes while the mortality of *A. pulchra* was distributed over all treatments; cathodes, electric field and controls. The constantly high current density of Borell et al. compared to the variable current density in Sabater and Yap (2004) and the daily 6 hours resting period in van Treeck and Schuhmacher (1997) could explain the high mortality despite lower current density. In Romatzki (2008) the results regarding survival were ambiguous in the three experiments. Survival was high in the first experiment in both current density treatments (1.67 and 3.38 A m^{-2}) and over all treatments (controls included) while survival were lower in some of the treatments in the 2nd experiment with no apparent pattern. The controls sometimes fared less well than treated corals. The highest survival in the 3rd experiment was observed in corals exposed to an electrical field without direct contact with the cathodes and the author concluded that direct contact with the cathodes diminish growth, however, as suggested by the results of the present study, this was likely due to overcharging.

Summary

Summarizing the results of this study it is apparent that there is a positive effect solely of the lowest current density treatment (LI) while both higher current densities (LII, LIII) and the substrate *per se* and galvanic elements produced poor results. The optimal current density in the present study was found to be as low as 0.06 A m^{-2} or less. These results provide new insight in the optimal current density to use in these installations and indicate that cathodes have been severely overcharged in previously published studies.

The zero mortality and overall performance of the corals in the lowest applied current density (LI) brings to the conclusion that mineral accretion is a suitable method for the target species *Lophelia pertusa*. Although there was no significant gain in growth rate compared to the controls, the increased budding and firm attachment of coral transplants offers sufficient benefits and the method is considered worth testing in a field-study.

There is obviously a contradiction in the optimal current density considering coral survival and growth versus mineral accretion rates, i.e. the optimal level for the corals does not produce a full accretion of the cathodes. Alternating the current density during the day could be a solution to this incompatibility, giving a few hours of higher levels to achieve a thicker layer of minerals. As seen in Sabater and Yap (2004) a variable current density gave significantly higher growth rates and this despite the high peak level of current density at noon.

Considering the rate of mineral accretion it was slower than seen in tropical environments, as expected, and achieving a sufficiently thick accretion on the

cathodes will probably take two years or longer under the local conditions. This could be due to the fact that aragonite is more soluble in cold waters.
The present results suggest that it is the electrical stimulation and increased ATP production that is the main beneficial effect rather than increased mineral ion concentrations, although this needs to be further tested.

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