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NOTE

OYSTER RECRUITMENT AND GROWTH ON AN ELECTRIFIED ARTIFICIAL REEF STRUCTURE IN GRAND ISLE, LOUISIANA

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Coastal protection remains a global priority as rising sea levels, development, and tropical storms threaten coastal habitat. A common tool used to combat shoreline erosion involves armoring the land/water interface (Yohe and Neumann, 1997; Mimura and Nunn, 1998; Klein et al., 2001). While typical armoring is done with heavy, often non-native materials, recent shoreline protection projects are moving towards promoting the use of native, living materials. One promising method that has been used to restore degraded reef systems and protect shorelines in southeast Asia, is mineral accretion technology which involves the electrochemical deposition of minerals from seawater (Hilbertz, 1979; Hilbertz and Goreau, 1996; Sabater and Yap, 2002, 2004). Essentially, the method involves creating reef units consisting of a rebar structure and passing a weak electrical current through the structure. These artificial reef units use low-voltage direct current that, in the right conditions, results in the precipitation of dissolved minerals in seawater to create a reef structure ten times stronger than concrete (Hilbertz, 1979). In addition to rapidly building mineral substrate, electrified reef structures may also confer survival and growth benefits to coral and mollusks that become attached to the structure (Hilbertz and Goreau, 1996; Sabater and Yap, 2002, 2004). Enhanced growth is suggested to result from the effects of electrolysis of seawater which raises the pH on the mineral precipitate, thus reducing the metabolic energy requirements needed for growth (Goreau and Hilbertz, 2005). It is hypothesized that the electrolysis of seawater creates the high pH conditions for the organisms, hence providing an energy subsidy to the organisms, allowing them to put more energy in growth, reproduction, and disease resistance.

The goal of this project was to explicitly test the effects of this mineral accretion technology (1) in waters off coastal Louisiana, and (2) on growth and recruitment of the native eastern oyster, *Crassostrea virginica* (Gmelin, 1971). Not only might three-dimensional oyster reefs protect shorelines (Piazza et al., 2005), but they can also provide critical ecosystem functions such as nekton habitat and water quality services (e.g., Breitburg, 1999). Specific objectives of this study were to (1) determine the rate of calcium carbonate ($CaCO_3$) accretion of electrified reefs located off the coast of Louisiana, (2) compare oyster spat recruitment and growth of electrified reefs (high, medium, and low DC current) to a control, non-electrified reef and on shell spat collectors, and (3) evaluate juvenile oyster growth on electrified reefs.

Materials and Methods

Study Site

The study was conducted at the Louisiana Sea Grant oyster hatchery in Grand Isle, Louisiana from April 2006–June 2007. During this time period, daily mean (SD) water temperature was 23.6 (6.4) °C (range 7.28–32.7 °C) and daily mean (SD) for salinity was 22.1 (4.1) (range 11.7–31.1) (USGS continuous data recorder 073802515 Barataria Bay Pass E of Grand Isle,

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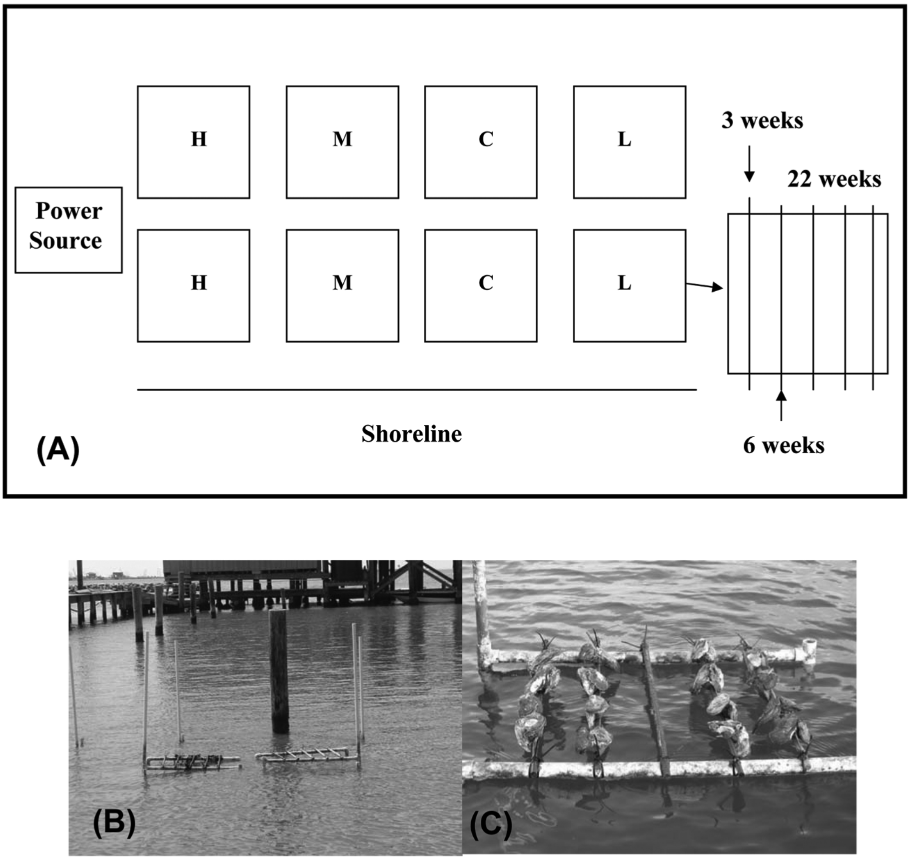

Figure 1. (A) Diagram and (B and C) photos of experimental set up of reefs. Reefs were suspended approximately 0.3 m off the substrate by four PVC poles (reef legs). Letters refer to electrical current level treatments: H = high, M = medium, L = low, C = control (no current). (B) Two of the experimental reefs raised above the water surface. (C) Close-up of reefs with adult oysters cemented onto bars.

LA). These temperature and salinity conditions provide ideal conditions for oyster growth (Galtsoff, 1964) and adequate conditions for the precipitation of calcium carbonate on electrified reef structures (T. Goreau, Global Coral Reef Alliance, pers. comm. April 9, 2006)

Experimental Reef Units

In April 2006, eight experimental units were created. Each unit consisted of two "staples" made with two pieces of 12-foot (3.7 m) PVC attached using a 36" (0.9 m) horizontal piece of PVC and two T-joints. The two staples for each unit were then pushed into the mud bottom parallel to one another, approximately 24" (0.6 m) apart. Five pieces of 30" (0.8 m) rebar were then measured [diameter (mm)] with a caliper (Scienceware, Bel-Art Products, Pequannock, NJ) and secured between the two staples using ties (Fig. 1). The units were organized in two rows of four, parallel to the shoreline. Using a DC power source located on-shore, electrical current was supplied to each individual rebar piece; replicates of a control (no electrical current), low, medium, and high electrical current reefs were established based on distance from the power source (Ohm's Law). Electricity was supplied to each individual rebar piece using Romex 12/2 UF-B electrical cable. Power was supplied to the cable with a Hoefer Scientific Instruments PS 500X DC power supply (500 V, 400 mA, 200 W) that was connected to an onshore AC power source (120 V). The DC power supply was able to maintain a constant low current (~2 A) necessary for optimum precipitation of $CaCO_3$ on the electrified bars (van Treeck and Schuhmacher, 1997). This set-up uses the Biorock™ technique developed for restoration of coral reefs (U.S. Patent # 5,543,043). Traditional oyster shell spat collectors, consisting of 10 oyster shells affixed on a wire hanger, were placed adjacent to each experimental unit to monitor spat availability.

Sampling Design

Experiment 1: Spat Recruitment and Bar Accretion.—Experimental units were sampled every 3 wks from June–November 2006, (June 23, July 12, August 2, August 23, September 13, October 6, November 1, and November 12). One rebar piece from each experimental unit was sampled with replacement of a clean bar every 3 wks and a second one every 6 wks (Fig. 1). The remaining three bars were sampled once on the final day of the experiment. Three oyster shell spat collectors were also collected and replaced with clean spat collectors every 3 wks, 6 wks and at the end of the experiment. Sampled bars and spat collectors were transported to the laboratory (School of Renewable Natural Resources, LSU AgCenter) for processing. In the lab, the diameter of each sampled piece of rebar was measured at five locations. Spat number and spat size (mm) were measured on each bar and on spat collectors using a dissecting microscope.

Experiment 2: Oyster Growth.—From November 12, 2006 through May 2007, oyster growth on the experimental units was measured. Four double (two oysters with attached shells) oysters (3.9 ± 0.03 cm; mean size and SD) were cemented along each piece of rebar with Quikrete Hydraulic Water-Stop Cement (Fig. 1C). The rebar were "saddled" with the double oysters to facilitate attachment without affecting shell hinges or openings of the animals. Oyster size (shell height measured at largest hinge-lip distance, mm) was measured using calipers at the initiation of the experiment, and monthly throughout the experiment (November 2006–May 2007). All oysters for this experiment were obtained from the Louisiana Sea Grant Oyster Hatchery, Grand Isle, Louisiana.

Statistical Analysis

Statistical analyses were performed with SAS software (version 9.1; SAS Institute) and results were considered significant at $\alpha = 0.05$. All data (mineral accretion, number and size of oyster spat, and oyster growth) were tested for normality, by examining model residuals, and homogeneity of variance. Logarithmic [$\log(x + 1)$] transformation was performed for spat number and spat size to satisfy model assumptions.

Reef bars and shell collectors were analyzed separately, and results from the shell collectors were used for comparison purposes only. Two-way analysis of variance (ANOVA, Proc MIXED) was used to examine mineral accretion [electrical current (H, M, L, C), and time interval (3 wks, 6 wks, 22 wks)], and three-way ANOVA was used to test the influence of factors on number and size of oyster spat [factors = electrical current, time interval, date]. Analysis of number and size of spat on traditional shell collectors was conducted with two-way ANOVA [factors = time interval, date]. One-way ANOVA was used for analysis of oyster growth [factor = electrical current]. Least-square means with a Tukey adjustment was used following significant ANOVA results ($P < 0.05$) to examine the differences among treatments.

Results

Experiment 1: Spat Recruitment and Bar Accretion.—Accretion of mineral precipitate resulted in increases in bar diameter ranging from 0–6.95 mm (Table 1). Accretion differed only by current level (ANOVA: $F_{11,92} = 19.8$, $P < 0.001$). Control bars had the lowest accretion, followed by low and medium current bars, with high electrical current bars having highest accretion (LSMeans with Tukey adjustment: $P < 0.05$; Table 1; Fig. 2).

Table 1. Characteristics of mineral accretion and oyster recruitment on experimental reef structures that were exposed to different electrical current levels. Data are mean ± SD (range); N = 26 sampled rebar pieces except for oyster growth where N refers to the number of attached live oysters remaining in March 2007. Electric current levels are high, medium, low, and control (no electrical current).

	Electrical current level			
Response variable	High	Medium	Low	Control
Mineral accretion (mm)	4.4 ± 1.6	3.4 ± 1.0	2.8 ± 0.9	0.4 ± 0.3
	(0.9–6.9)	(1.2–5.4)	(0.9–4.5)	(0–1.0)
Number of oyster spat	3.2 ± 7.1	5.2 ± 12.8	4.6 ± 8.7	10.3 ± 13.8
	(0–30)	(0–53)	(0–28)	(0–54)
Oyster spat size (mm)	4.1 ± 1.9	4.3 ± 1.4	5.0 ± 3.0	5.6 ± 2.0
	(2.0–7.6)	(2.0–5.6)	(2.0–12.5)	(2.5–11.5)
Oyster growth (mm mo^{-1})	1.5 ± 0.9	1.4 ± 0.8	1.5 ± 0.7	1.3 ± 1.1
	(0.2–3.8)	(0.3–2.9)	(0.3–3.6)	(0.02–3.3)
	N = 27	N = 23	N = 20	N = 11

Spat number differed by treatment ($F_{3, 93}$ = 10.73, P < 0.0001, N = 104), with control bars having approximately twice as many spat as compared to any of the electrified bars (LSMeans with Tukey adjustment: P < 0.05; Table 1). Number of spat was also positively affected by time ($F_{2, 93}$ = 30.18, P < 0.0001, N = 104), with the 22-wk samples containing significantly greater numbers of spat than either the 3- or 6-wk samples (LSMeans with Tukey adjustment: P < 0.05; Fig. 3). Number of spat on both bars and shell collectors was significantly affected by date (bars: $F_{5, 93}$ = 9.94, P < 0.0001; N = 104), driven by higher spat recruitment in October and November (LSMeans with Tukey adjustment: P < 0.05).

Spat size on the experimental units did not differ by treatment (Table 1) or sampling time interval. Mean spat size on bars and shell collectors was significantly different by date ($F_{6, 49}$ = 5.33, P = 0.0003, N = 61), and this effect was driven by the higher mean spat sizes (mm) in August and September, that were almost double the

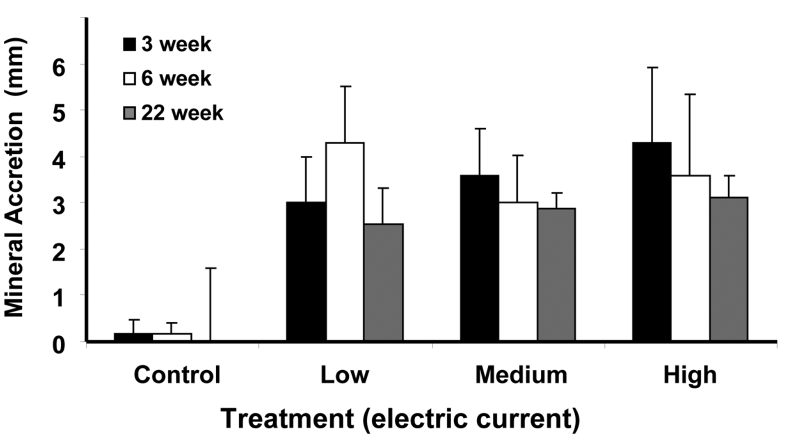

Figure 2. Accretion of mineral precipitate on rebar by electrical current supplied and amount of time electricity was applied to bars. Mean rebar size at initiation of experiment was 13.01 ± 0.36 mm (SD). The 22 wk bars had significantly lower accretion as compared to the bars sampled at 3 and 6 wk time periods. All bars receiving some level of electrical current accreted significantly higher levels than the control bars.

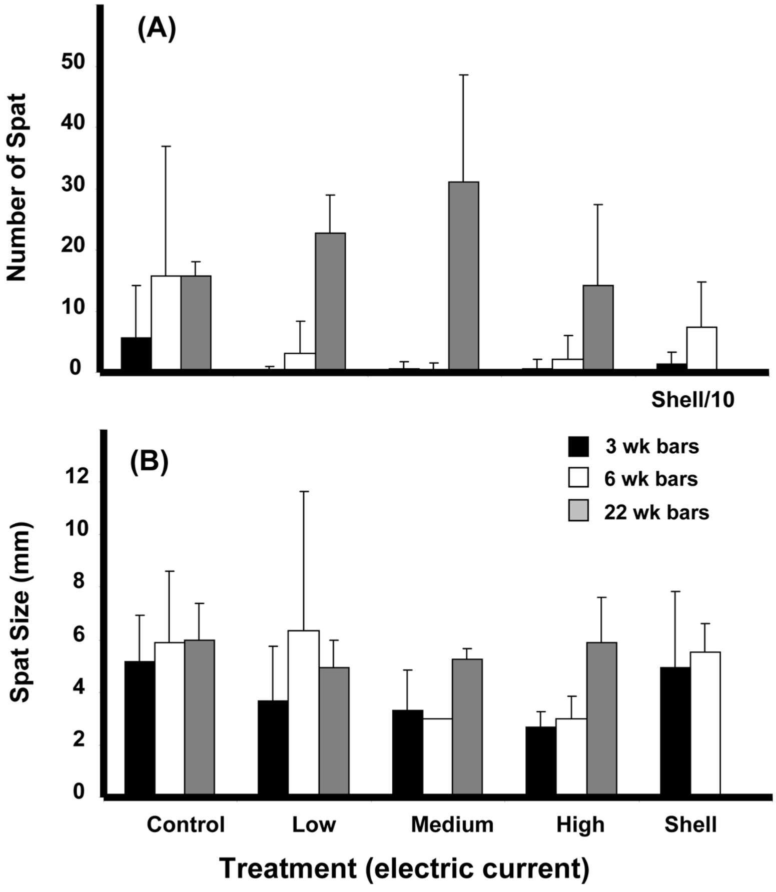

Figure 3. (A) Number (SD) and (B) size of oyster spat recruited to experimental reef structure by electrical current level or shell, and amount of time electricity was supplied to the bars. Shell spat counts were divided by ten. Control bars recruited higher number of spat than bars receiving electric current for 3 and 6 wk samples (LSMeans with Tukey adjustment: $P < 0.05$), but had similar recruitment to bars receiving low, medium and high electrical current at 22 wk spat counts. Spat size was not significantly affected by time or electrical current level, but did increase in size towards the end of the summer (i.e., August and September).

size of spat in June and July. Mean spat size (mm) did not differ significantly between traditional shell collectors or experimental bars over the entire time period (Fig. 3).

Experiment 2: Oyster Growth.—Mean oyster growth did not vary significantly among treatments (Table 1). Mean oyster growth was low (1.4 ± 8.6 mm mo^{-1}; N = 81). The experiment was considerably shorter in duration (4 mo) and sample size was considerably lower than desired as a result of oyster loss from drill [(*Stramonita haemastoma* (Linnaeus, 1758)] predation.

Discussion

While the use of electricity to precipitate minerals from seawater to create strong reef structures and enhance coral reef growth has been shown to be effective in areas around the world (Hilbertz and Goreau, 1996; van Treeck and Schuhmacher, 1997;

Sabater and Yap, 2004), the use of electrical current to enhance bivalve recruitment and growth had not previously been tested in the Gulf of Mexico. We found that electrolysis in seawater off Grand Isle, LA induced cathodic accretion of minerals on rebar, however, no significant positive effects were detected on spat recruitment or size, or oyster growth during this experiment. While the development of a robust reef-like structure using electrolysis of seawater could prove useful in shoreline stabilization and erosion control, this technique does not appear to confer any growth or recruitment advantages on oysters in this region.

Accretion of mineral precipitate occurred on all of the bars, however, it appeared that after 3 wks, accretion slowed significantly. This negative asymptotic relationship has been described for these types of structures, as the growing precipitate decreases the electrical field by insulation (van Treeck and Schuhmacher, 1997). Overgrowth of coral nubbins has been observed in other electrified reef systems (van Treeck and Schuhmacher, 1997), and while we did not observe overgrowth, given that the traditional shell collectors consistently had high spat recruitment, it is possible that the rapid initial accretion found in our study contributed to the lower spat recruitment found on the 3- and 6-wk electrified bars.

At extremely low current levels only, one past study found successful spontaneous settlement of benthic organisms on reef structures exposed to electric current (Schuhmacher and Schillak, 1994). Thus, development of an oyster reef using electrolysis in seawater may involve initially applying electrical current for a set period of time to develop a strong reef framework of precipitated minerals, and then separating the reef from power for a period of time to allow spat to settle and grow to a size where they would not be smothered before reapplying power. This technique has been shown to result in high survival of transplanted coral nubbins in the northern Gulf of Aqaba in the Red Sea (van Treeck and Schuhmacher, 1997), and empirical studies agree that long term survival of organisms is high on these electrified reefs (Schuhmacher and Schillak, 1994; van Treeck and Schuhmacher, 1997; Sabater and Yap, 2002, 2004).

We tested another approach for solving the potential spat overgrowth problem by directly attaching juvenile oysters to the reef backbone. Previous studies with transplanted coral and mollusks have shown that electrified reef structures accelerate the growth of transplanted animals (Sabater and Yap, 2002, 2004). Our results showed no significant growth enhancement for transplanted juvenile oysters, and lower growth rates (1.4 ± 8.6 mm mo^{-1}), compared to other oysters maintained at the Grand Isle hatchery during the same time period (2.2 ± 2.0 mm mo^{-1}). Although localized effects are known to affect oyster growth rates and variable oyster growth rates have been noted around the Grand Isle oyster hatchery previously (J. La Peyre, pers. obs.), it is possible that the use of cement as an attachment medium affected our results. Specifically, it is not clear if the electricity was still transferred to the organisms or whether the cement acted as an insulator that kept current from reaching the oysters. Interestingly, while increased pH in seawater is widely held to increase calcification by marine organisms that make their shells and skeletons from calcium carbonate (Kleypas et al., 1999; Riebesell et al., 2000; Zondervan et al., 2001), recent evidence suggests that this may not always be the case. Calcification of the coccolithophore species *Emiliania huxleyi* (Lohman) Hay and Mohler increased with high CO_2 partial pressures (Iglesias-Rodriguez et al., 2008); increased CO_2 partial pressures in seawater results in formation of carbonic acid, which causes a reduction in pH of the ocean water suggesting that this long-held belief may need to be examined on a more species specific level.

The establishment of oyster reefs using electrolysis of seawater to create a strong framework to support reef development is particularly attractive for shoreline stabilization. Reefs created using this approach have been found to be effective in reducing wave energies and protecting shorelines (Goreau et al., 2000, 2004). The dual spawning seasons in Louisiana ensure that high concentrations of spat are available for settlement (Supan and Wilson, 2001). Our results demonstrate that along the Louisiana coast, the use of electrolysis of seawater to induce cathodic accretion of minerals has the potential for mineral precipitation and growth in high salinity waters. While the data failed to demonstrate enhanced oyster recruitment or growth on the experimental reef structures, there may be ways to manage structures initially developed through electrolysis of seawater, to support long-term development of oyster reefs. Furthermore, as predation by oyster drill may be an issue in high salinity waters, it may be worth investigating the lower salinity ranges and the potential for electrolysis of seawater in areas that either experience only short periods of high salinity, or are maintained at lower salinities such that predation does not decimate the oyster population. In a region such as coastal Louisiana where the landscape is dominated by soft sediments, and there is a need for hard, clean substrate, further investigation of the use of this approach to create a substrate for development and future growth of oyster reefs could be beneficial.

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