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Scraping and extirpating: two strategies to induce recovery of diseased sea fans *Gorgonia ventalina*

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Abstract

Coral diseases are currently playing a major role in the worldwide decline in coral reef integrity. One of the coral species most afflicted by disease in the Caribbean, and which has been the focus

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of much research is the sea fan *Gorgonia ventalina*. There is, however, very little information regarding the capacity of sea fans to recover after being infected. The aim of this study was to compare the rehabilitation capacity of *G. ventalina* after diseased-induced lesions were eliminated either by scraping or extirpating the affected area. Scraping consisted of removing any organisms overgrowing the axial skeleton from the diseased area as well as the purple tissue bordering these overgrowths using metal bristle brushes. Extirpation consisted of cutting the diseased area, including the surrounding purpled tissue, using scissors. The number of scraped colonies that fully or partially rehabilitated after being manipulated and the rates at which the sea fans whose lesions were cut grew back healthy tissue were compared among: 1) colonies that inhabited two sites with contrasting environmental conditions; 2) colonies of different sizes and 3) colonies with different ratios of lesion to colony areas (LA/CA). Both strategies proved to be very successful in eliminating lesions from sea fans. In the case of scraping, over 51% of the colonies recovered between 80-100% of the lost tissue within sixteen months. The number of colonies that recovered from scraping was similar between sites and among colony sizes, but differed significantly depending on the relative amount of lesion to colony area ratio (LA/CA). When lesions were extirpated, lesions did not reappear in any of the colonies. We conclude that lesion scraping is useful for eliminating relatively small lesions (i.e. LA/CA< 10%), as these are likely to recover in a short period of time, whereas for relatively large lesions (LA/CA≥ 10%) it is more appropriate to extirpate the lesion.

Keywords: sea fans, coral rehabilitation, lesions scraping and extirpation

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Introduction

Coral reef ecosystems provide a diverse array of goods, services and ecological functions vital to human society. Over 400 million individuals across the World's tropical coasts depend on coral reefs for their livelihood or protein intake (Wilkinson 2004; Moberg & Folke 1999; Salvat 1992). Reef-related fishing comprises between 9 and12% of the World total fisheries (Moberg & Folke 1999); and revenues associated to recreational activities are estimated to be hundreds of millions of dollars (Dixon et al. 1993). In the Caribbean, for instance, the estimated economic revenues obtained from coral reef associated activities range from US$350-$850 million annually (Young

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et al. 2012). Reefs are also a major source of carbon sequestration (Remoundou et al. 2009), nitrogen fixation (Shashar et al. 1994), and together with rainforests, are the major centers of the Earth's biological diversity (McIntyre 2010). However, coral reefs are undergoing dramatic declines worldwide (Hoegh-Gulberg et al. 2007). These declines are particularly significant in the Wider Caribbean where nearly 80% of the coral cover has been lost during the past decades (Voss & Richardson 2006). Reasons for these declines are variable and complex, but there is a general consensus that coral diseases have played a major role, being one of – if not – the major cause of partial and total tissue mortality in many coral species in recent years (Efrony et al. 2009).

Coral disease studies have, for the most part, prioritized: 1) the etiology of these afflictions and 2) the ecological impacts of these diseases at the colony, population and ecosystem level (Bruno et al. 2011; Nagelkerken et al. 1997; Smith et al. 1996). Far less attention has been devoted to developing treatment strategies for afflicted colonies, even though field evidence suggests that corals, in general, have relatively low natural recovery (Toledo-Hernández et al. 2009). Two approaches have been proposed to treat diseased colonies: 1) physical removal of the tissue with an active infection and 2) biological controls against pathogen. Hudson (2000) used an underwater suction device to remove the polymicrobial mat typical of black band disease (BBD) from massive scleractian corals, sealing the treated area with modeling clay afterward. Teplitski & Ritchie (2009) used pathogen-specific phages to contain infections produced by the bacteria *Vibrio coralliiyticus* and *Thalassomonas loyana* on the Red Sea corals *Pocillopora damicornis* and *Favia favus* respectively (Efrony et al. 2007). These approaches, however, have not been extensively tested in the field; therefore the applicability of these methodologies as management tools is uncertain.

During the past few decades, Caribbean sea fans (*Gorgonia* spp.) have suffered from several infectious diseases, e.g. protozoan infections (Morse et al. 1981; Goldberg 1984), red band disease (Weil & Hooten 2008; Williams & Bunkley-Williams 2000), skeleton eroding band (Croquer et al. 2006; Winkler et al. 2004) and aspergillosis (Nagelkerken et al. 1997). These diseases induce a macroscopic immune response consisting of an increase of purple sclerites, together with the disappearance of polyps in the afflicted area of the sea fan. As the infection proceeds, partial mortality of tissue occurs creating a lesion and leaving the axial skeleton

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exposed for fouling organisms such as algae and bryozoans to grow over the exposed skeleton (Toledo-Hernández et al. 2009). Lesions maybe contained and become permanent, or may increase in size causing whole colony mortality, depending on the virulence of the pathogen or the strength of the immune response of sea fans (Ellner et al. 2009; Ruiz-Diaz et al. 2013; Toledo-Hernández & Ruiz-Diaz 2014).

The objective of this study was to measure the effectiveness of two strategies, scraping and extirpating lesions, as tools to rehabilitate *Gorgonia ventalina* colonies showing injuries. Scraping consisted of removing, using metal bristle brushes, fouling organisms overgrowing the axial skeleton and the purpled tissue bordering these overgrowths. The success of this strategy was measured by estimating the rates at which the sea fans grow back healthy tissue on the scraped area. Three factors that can potentially affect the rehabilitating process for the scraped colonies were considered: 1) environmental conditions, 2) colony size, and 3) the ratio between the size of the lesion and size of the colony. With respect to environmental conditions, we hypothesized that relatively few colonies would completely rehabilitate and would exhibit slower rate of regrowth of healthy tissue at the sites with poor water quality when compared to colonies at the sites with good water quality. Colonies inhabiting sites with poor water quality have been shown to exhibit higher abundances of lesions than colonies in sites with better water quality (Peters 1997). Turbid waters may induce physiological stress on colonies, ultimately depleting the resources necessary for recovery. With respect to the effect of colony size, we hypothesize that lesion recovery should be independent of colony size, as stated by the localized regeneration hypothesis which state that tissue regeneration is exclusively dependent on the amount of healthy tissue bordering the lesion (Bak & Steward-Van 1980; Meester et al. 1994; Oren et al. 2001). Finally, with respect to the effect of the lesion area/colony area ratio (LA/CA) on the rehab process, we hypothesized that colonies with a higher lesion area/colony area ratio (LA/CA) should exhibit slower recovery than colonies with small lesion area/colony area ratio –colony integration hypothesis (Oren et al. 2001). The colony integration hypothesis argues that the higher the proportion of healthy tissue with respect to the lesion, the more energy available for healing through translocation of energy not just from the tissue bordering the lesion but from areas further away from the lesion.

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Extirpation, on the other hand, consisted of cutting from sea fans, the diseased areas using scissors. The success of this treatment was evaluated based on the reappearance of purpled band tissue at the extirpated edge during the growth process and the amount of new tissue growing where the lesion existed.

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Methodology

Study site

This study was conducted from July 2011 to July 2013 in two nature reserves located along the Northeastern and Eastern coasts of Puerto Rico: The Luis Peña Channel Natural Reserve at Culebra (LPR) and, Isla Verde Urban Natural Reserve at Carolina (IVR, Fig. 1). LPR is characterized by low urban development and no agricultural activities, thus there is lack of impact from runoff or nutrient input. Consequently, there is high water transparency, (average a light intensity of 11673.3 lm/m^2), relatively low suspended particle matter, low sedimentation rate, and low algal cover (Toledo-Hernández et al. 2007; CTH personal observation). The coral assemblage is dominated by small colonies of *Diploria labyrinthiformis, Orbicella (Montastrea) annularis* and *Porites astreoides*. IVR, on the other hand, is impacted by urban runoff and discharges from a nearby estuary that drains into the ocean. There is low water transparency (average a light intensity of 5781.9 lm/m^2), relatively high-suspended particle matter and sedimentation rate year round, high algal cover, and poor low coral cover (<5%) dominated by small-sizes colonies of sediments resistant corals *Porites astreoides* and *Siderastrea radians* (Torres & Morelock 2002).

Lesion scraping experiment

Two criteria were used to select the colonies: 1) the health state of the colony (i.e. diseased or healthy), and 2) the size of the colony (i.e. small, medium, or large). Diseased colonies exhibited lesion overgrown primarily by algae and the purple tissue ring surrounding the lesions. Causes of lesions were unknown to us, as we did not perform any microbiological or histological analyses to identify and diagnose the etiology of the lesions. However, most of the sea fan disease literature use macroscopic features, such as the one used in this study to diagnose colonies as diseased or healthy (Smith et al. 1996; Nagelkerken et al. 1997; Smith & Weil 2004). Healthy

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colonies in contrast, exhibited neither purpling nor lesions with fouling organisms overgrowing the axial skeleton. A total of 32 diseased colonies and 14 healthy colonies were tagged at LPR, whereas 28 diseased and 15 healthy colonies were tagged at IVR. Colonies with a total surface tissue area ranging from 300-500 cm^2 were classified as small, those with a total surface tissue area from 501-1000cm^2 were classified as medium, and colonies bigger 1000cm^2 were classified as large (Toledo-Hernández et al. 2009). To estimate the size of the tagged colonies and their lesions (in the case of the diseased ones), pictures were taken, at an angle approximately perpendicular with respect to the surface of the colony, with a digital submersible camera by placing a calibrated board as background to eliminate noise during the image analysis process.

Lesions from diseased colonies were scraped using metal bristle brushes. Scraping resulted in the elimination of the fouling organisms overgrowing the axial skeleton and the purple tissue surrounding the overgrowth at both sides of the fans. While scraping, caution was taken not to break the axial skeleton. To determine if recovery was affected by the health state of the colony an area equivalent to 10% of the whole surface area of a healthy colony was scraped as explained previously. To document the progression of wound-healing process, close-ups pictures of each lesion (from diseased and control colonies) were taken after scraping at monthly intervals for the following 16 months or until lesions healed completely. Lesions were deemed healed (fully recovered), if the axial skeleton was completely covered by healthy sea fan tissue. Lesions were also deemed healed if the scraped area (axial skeleton) fragmented. As purpling is part of an inflammatory response against infection or injury, we assume that colonies without purpling should significantly reduce the investment of resources into immune response, and therefore should be considered recovered. In order to estimate the percent of tissue that healed or recovered in those colonies that did not fully recover within the experimental time-period we subtracted the area not covered by tissue at the end of the experiment to the initial area (bared axial skeleton) just after scraping the lesion. If the skeleton fragmented during the experimental period, the estimated fragmented area at the end of the experiment was subtracted from the initial bared axial skeleton area. Sigma Scan Pro Image Analysis version 5.0 Software was used to analyze all colony pictures. Measurements obtained from the Sigma Scan software were validated after comparing them with *in situ* measurements.

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Lesion Extirpation experiment

This experiment was conducted in a 500m^2 plot, 1-3m deep in the LPR. For this experiment, 27 not previously manipulated colonies were tagged, 17 were diseased colonies while the remaining 10 were healthy (as defined previously). The area of each lesion was estimated by analyzing the digital images as explained earlier. Extirpation of lesions consisted of cutting with scissors the axial skeleton overgrown by fouling organisms including the purple tissue ring bordering the overgrowth. Cuttings equivalent to 10% in average of total surface area were performed on healthy colonies (to measure the effect of colony health state) as control for the effect of tissue extirpation. Colonies were followed at monthly intervals for one year by means of pictures. Analyses of pictures were performed as explained above. In this experiment, colonies that did not show any signs of disease i.e. tissue purpling or mortality, after lesions were extirpated were considered fully recovered. If disease signs re-appeared these were measured and followed as previously explained.

Statistical analyses

We conducted three χ^2 analyses ($\alpha = 0.05$) comparing the number of colonies that fully recovered after scraping or yielded some level of tissue recovery between 1) study sites; 2) size classes and 3) lesion ratios having ($LA/CA<5$, $5 \leq LA/CA<10$, and $LA/CA \geq 10$). In addition, we compared the rates of tissue recovery after lesions were scraped (change in lesion area through time) between sites, among size classes and among LA/CA ratios using linear regression. To perform these analyses, the rates of recovery per size classes and LA/CA ratios were averaged at each study site and log transformed for data linearization. The slopes from the obtained linear regressions were then compared with an analysis of equality of slopes as described by Sokal & Rohlf (1981). A Student T-test was done to compare the regrowth rate of tissue (defined as the deposition of skeleton and soft tissue /number of days) between diseased and healthy colonies. Statistical analyses were performed using the software R version 3.1 (R Core Team, 2014).

Results

Scraping Technique

Three distinct modes of lesions recovery were observed 1) tissue regeneration *i.e.* growth of healthy tissue over the exposed skeleton from tissue bordering the lesion, 2) partial

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fragmentation of axial skeleton, and 3) a combination of the tissue regeneration and partial fragmentation of exposed axial skeleton (Table 1). Average percent of the initial lesion size with respect to the whole colony area at LPR was 13.61 ± 15.99 SD, while at IVR was 7.59 ± 3.84. For statistical analysis purposes, we created three categories of lesion recovery: colonies that healed between 80-100% of their lesion; colonies that healed between 8-79%, and colonies that did not recover (Table 1). When analyzed based on these classifications, colonies at LPR and IVR showed similar patterns of recovery (χ^2 = 0.0201; df = 1; p = 0.8873), with 22% and 29% colonies reaching full recovery at LPR and IVR respectively. Similarly, 50% of the colonies manipulated at LPR and IVR recovered ≥80% of tissue. Ten percent of colonies at LPR and 7% at IVR showed an increase in lesion area with respect to their initial size.

To further analyze the effect of colony size on lesion recovery, we pooled the data based on colony size i.e. small, medium and large colonies. The statistical analysis showed no significant differences between colony size and recovery success (χ^2 = 0.0357; df = 2; p = 0.9823; Table 1). Tissue regeneration was the most common mechanism of recovery (57%), as recovery exclusively by fragmentation was rare (Table 1).

Finally, recovery success varied significantly with respect to relative lesion ratios (χ^2 = 6.483; df =2; p = 0.039). For instance, 75% of the colonies with relatively smaller lesion ratio (LA/CA<5%), recovered completely whereas the success of colonies with relatively larger lesions ratio, (5≤LA/CA<10% and LA/CA ≥10%), showed 68% and 42% respectively. Most of the healthy colonies exhibited full recovery, i.e. 12 of 14 colonies at LPR, and 13 of 15 at IVR. Of the remaining two healthy colonies that did not recover completely at LPR, one showed between 80-99% of lesion recovery, while the other exhibited between 60-79% of lesion recovery. The remaining two colonies at IVR showed between 60-79% tissue recovery.

Rates of tissue recovery

Rate of recovery for diseased and healthy colonies followed an exponential decrease through time (R^2=0.77, p< 0.05 with Recovery tissue = -0.002t+1.483 for diseased colonies and R^2=0.55, p< 0.05 with Recovery tissue = -0.003t+1.26 for healthy colonies). Rate of recovery of healthy colonies was significantly faster than diseased ones ($F_{s\ (1, 33)}$ = 6.243, p < 0.05, Fig 2A) (Ruiz-

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Diaz et al. 2013). Rate of tissue recovery of diseased colonies did not vary between sites ($F_{s\ (1, 28)}$ = 1.65, $p > 0.05$) nor with respect to colony size ($F_{s\ (2, 21)}$ = 0.027, $p > 0.05$), or relative lesion size ($F_{s\ (2, 21)}$ = 0.080, $p > 0.05$), Fig. 2B-D. Rate of tissue recovery of healthy colonies did not vary between sites ($F_{s\ (2, 21)}$ = 0.09383, $p > 0.05$), or with respect to colony size ($F_{s\ (2, 21)}$ =0.042, $p > 0.05$), Fig. 2 B-C.

Colonies with extirpated lesions

Average size of the extirpated area of diseased and healthy colonies varied from 7.2% to 10.1% with respect to total colony size. None of the 27 colonies (17diseased and 10 healthy) exhibited any physiological stress, i.e. tissue purpling, throughout the monitoring period after lesions were extirpated. By the end of the experiment, diseased colonies had regrown an average of 11% of the original extirpated area, slightly lower than healthy colonies (control), which in average show 19% of regrow. Similarly, the average daily rate at which the diseased (0.093mm d^{-1}; SD =0.087) and healthy (0.138 mm d^{-1}, SD =0.0187) colonies regrew the extirpated area, i.e. was not statistically different (*T-test* = 0.702, N =27, p >0.490).

Discussion

Lesions are small-scale disturbances common to all corals. Yet, the sources, as well as the consequences, of these lesions are variable, i.e. abiotic and biotic (Nagelkerken et al. 1999). For instance, predation-induced lesions for the most part, heal in a relatively short time and leave no permanent scars (CTH, personal observation). Thus, they may have no other consequence than the loss of tissue and the corresponding resource investment in tissue regeneration. However, disease-induced lesions are unlikely to heal in a short time period (Toledo-Hernández et al. 2009). Most likely, a colony will struggle to eradicate the disease and may either contain it or succumb to it, depending on the strength of its immune response (Ruiz-Diaz et al. 2013). In such cases, human intervention is desirable and may contribute towards recovery. To our knowledge, the present study is the first to document the fate of disease-induced lesions after being removed by scraping or extirpation.

Tissue regeneration after scraping

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Three factors with the potential to affect tissue recovery were considered: 1) colony location, i.e. LPR vs. IVR; 2) colonies sizes of colony and, 3) the LA/CA ratio. Contrary to our hypothesis, our results showed that sites, and thus water quality, did not affect the capacity of lesion to recover, or influence the number of the colonies that fully or partially recovered. These results were surprising as we were expecting to observe fewer colonies recovering and a lower rate of lesion recovery at IVR due to stresses caused by lower water quality parameters. Previous studies have linked the capacity of coral to recover their lesions with the level of water degradation (Pastork & Bilyard 1985; Rogers 1990; Fisher et al. 2007; Toledo-Hernández et al. 2007). In fact, Fisher et al. (2007), has proposed to use the abundance of lesions on corals as an indicator of environmental stress. However, our data suggests that sea fans have an impressive resiliency in their regeneration capacity. This might explain why sea fans are one of the most dominant coral across the inshore, degraded reefs in Puerto Rico. Similarly, colony size per se, does not appear to be a good predictor of recovery capacity, as the levels of recovery were not related to size of the colony. These results are in agreement with studies conducted on other gorgonians (Wahle 1983) and scleractinian corals (Fisher et al. 2007; Lirman 2001).

Interestingly, the only factor considered in this study that had a significant impact on lesion regeneration capacity of sea fans was the LA/CA ratio. Between 68-75% of colonies with LA/CA ratio of less than 10%, exhibited 80-100% tissue regeneration, however, only 42% of the colonies with LA/CA≥10% ratio exhibited between 80-100% tissue regeneration. As hypothesized, the smaller the LA/CA ratio, the higher the probability of fully recovering, whereas the larger the LA/CA ratio the lower the probability of fully recovery. In fact, the average tissue recovery increases as the LA/CA ratio decreases (e.g. 80% for LA/CA <5%, 69.9% for colonies with a 5≤ LA/CA<10%, and 58.7% for colonies with LA/CA ≥10%). An explanation for this observed pattern is suggested by Oren et al. (2001), who argued that the higher the amount of healthy tissue to lesion area, the more resources available for healing because resources produced away from the lesion can be translocated to the affected area.

As in previous studies, injury recovery was a time dependent process, being faster at the onset of experiment, and decreasing as time passed (Meester et al. 1994; Lirman 2000). Hence, the longer the time taken to recovery, the more likely the injury will become permanent, either as a consequence of resource depletion, fouling organisms, or both. In this study, lesions that did not

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recovered fully were re-colonized by turf algae and once again surrounded by purpled-tissue, therefore suggesting that the colonies were immunologically active.

Colony recovery after lesion extirpation

Lesion extirpation was successful in that the very small areas with exposed skeleton remaining after cutting, sealed in a very short period of time and thus, no fouling or purpling of the impacted area was observed. Thus, these colonies regained their healthy state rather quickly. This held true in all colonies regardless of the colony size or the lesion area. An interesting fact about this result is that once the lesions are extirpated, the diseased colonies behave like a healthy one in terms of the rate at which the colony tissue regrew. On the other hand, if compared with tissue scraping, extirpation showed a much slower rate of regeneration of tissue. For instance, most of the diseased fans where lesions were scraped exhibited full recovery by the end of the experiment. By contrast, diseased colonies whose lesions were extirpated exhibited only an average of 11% of tissue regeneration. Evidently, it takes more resources and time to regenerate the axis and associated components than to only regenerate scraped tissue. However, as in scraping, the resources necessary to regenerate the lost tissue might have come from the available healthy tissue (Oren et al. 2001).

We are still far from preventing coral diseases as most of the pathogens causing these diseases are unknown to us and the role of environmental factors on diseases etiology are still poorly understood. However, this study shows that scrapping or extirpation lesions are effective techniques for rehabilitating sea fans with lesions. Lesion scraping might be appropriate when LA/CA ratio is < 10% as these are likely to readily recover in relatively short period of time. Moreover, scraping seems to be a safe procedure as, at least in this study, the incidence of lesion across colonies adjacent to the manipulated ones did not increase, suggesting that scraping did not have a negative effect on adjacent sea fans. It is also worth to mention that the state of the colony may have an effect on the recovery rates of lesions, as diseased fans recovered at a slower rate than healthy fans. As these fans were immulogically activated previous to the experiment, they may have depleted part of their resources (i.e. amoebocytes or energy), and consequently have less assets for tissue regeneration.

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Extirpation of the lesion, on the other hand, may be better when lesions LA/CA ≥ 10%, as these seldom recover completely if scraped. The success of these techniques is yet to be tested on other coral species, but if successfully applied they could be implemented as a cost-effective management plan to rehabilitate coral communities. Furthermore, this study demonstrates that sea fans have the capacity to recover with the help of human intervention. Hence, we demonstrate that involvement could help improve coral health state and thus is a desirable strategy for the management and control of sea fan diseases.

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Figure Captions

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Fig. 1 Map of Puerto Rico showing the study sites. La Isla Verde Urban Natural Reserve at Carolina (IVR, box A) and, The Luis Peña Channel Natural Reserve at Culebra (LPR, box B).

Fig. 2 Linear regressions of the rates of tissue recovery through time (A); between control and experimental colonies (B); between sites LPR and IVR (C); among size classes; small, medium and large colonies and (D) among lesion area and colony area ratio LA/CA. LSE lesion size (bare skeleton).

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Tables

Table 1: Number of small, medium and large colonies per locality (LPR or IVR, see Methods) that exhibited different amounts of recovery by different mechanisms: tissue regeneration (R), fragmentation (F) or a combination of both (R-F).

Sites	Size	Percent of tissue recovery (%)																Total
		100-80						79-8										
		Total Recovery			Recovery 80-99			Recovery 60-79			Recovery 40-59			Recovery 8-39			NR*	
		R	F	R-F	R	F	R-F	R	F	R-F	R	F	R-F	R	F	R-F		
LPR	small	1	0	2	0	0	2	0	0	1	2	0	1	0	0	0	2	11
	medium	2	0	2	4	0	0	1	0	1	0	1	1	3	0	0	1	16
	large	0	0	0	2	1	0	1	0	0	1	0	0	0	0	0	0	5
IVR	small	2	0	1	1	0	0	0	0	1	2	0	0	0	0	0	0	7
	medium	2	0	0	1	0	0	0	1	0	0	0	0	2	0	0	0	6
	large	1	0	2	3	0	1	0	0	1	0	0	0	2	1	1	2	14
Total		8	0	7	11	1	3	2	1	4	5	1	2	7	1	1	5	

N R*=No Recovery

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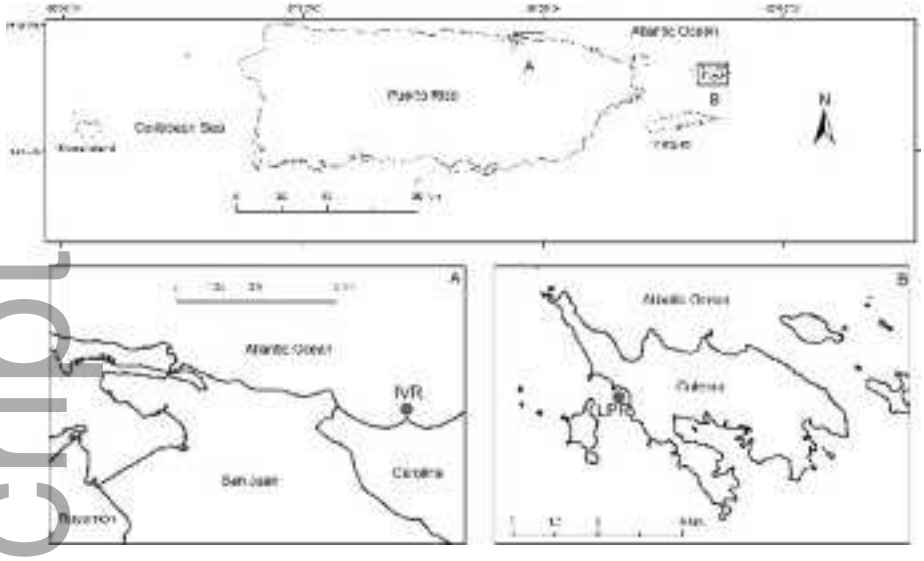

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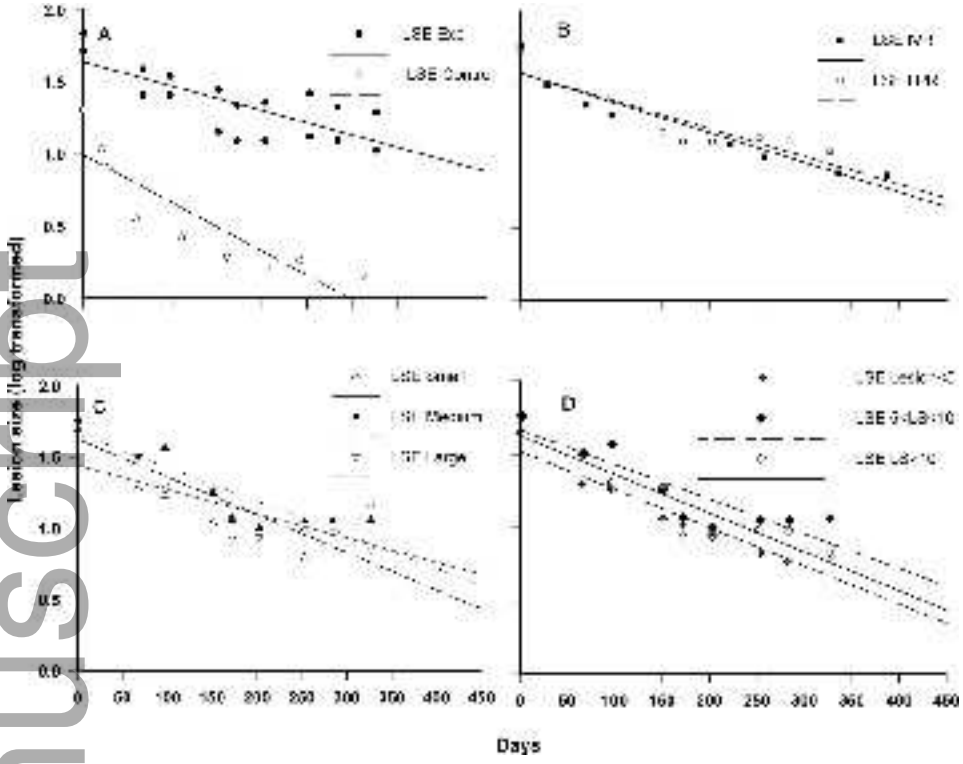

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