

Regeneration Rates of Porifera as a Function of Morphology Type, Depth,
Spicule Content, and Amount of Damage

Meredith Chase and Kimberly Kylstra

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Dartmouth College
Hanover, N.H.

ABSTRACT

The following study investigates the regeneration rates of sponges with respect to a variety of parameters. We propose that rates will vary between morphology types, and that among three basic types, rope sponges shall regenerate faster than tube sponges, which are in turn faster than encrusting types. Secondly, individuals found in shallower regions will regenerate faster than their conspecifics in deeper areas. Thirdly, species with a greater relative density of spicules will have slower regeneration rates than those with a lower density, due to the energy cost of skeletal structures and resistance to damage. And finally, we predict that the size of the damaged area will have no effect on the rate of regeneration; a larger hole will take a proportionally longer time to regenerate.

Two sites were established at two depths, on the West Fore Reef at Discovery Bay, Jamaica. Six species were examined over a six-day period for regeneration of holes bored into the tissue. Possible trends developed in rate variability as a function of morphology type, depth, and extent of damage inflicted. Structural tissues were also examined, which showed a positive correlation between spongin fibers and regeneration rate. Spicule content did not appear to be related to repair rates in any way.

INTRODUCTION

The regeneration of damaged tissue is crucial to the survival of an organism, but also jeopardizes the fitness of an individual by diverting energy from other metabolic processes. The persistence of an animal depends mainly upon its successful feeding, growth, and reproduction, which suffer from tissue-loss either from direct damage to the system or indirect deprivation of energy.

Sponges on a coral reef incur damage from a variety of sources, including fish predation, turbulence, and sedimentation. Broken portions, or gaps created from fish bites remove part of the canal-circulatory system. Optimal food filtration is impaired by the decreased number of functional flagellated chambers.

The regeneration of tissue is different from normal growth processes in sponges (Jackson & Palumbi, 1978). Growth does not involve budding, as in cheilostomes, but occurs via edge extension, cell proliferation, and immigration. Only later does differentiation into functional units occur (Simpson, 1968). Regeneration may not even involve growth, but rather the immigration of adjacent tissues into the damaged area (Reiswig, 1973).

The rates of regeneration vary widely among species of sponges (Jackson & Palumbi, 1978), and may be dependent upon biochemical processes and aggregation tendencies of sponge cells. A compound identified as 'Aggregation Factor' was demonstrated to induce cells to aggregate at

low temperatures that normally inhibit aggregation (Humphreys, 1970). It also is capable of mimicing a normal cellular reation by causing a mixture of cells to aggregate species specifically.

Rates of regeneration are also dependent upon environmental factors, such as turbulence and sedimentation. Each of these may hinder recovery in addition to creating initial damage. Differential regenerative abilities may determine individual resistance to predation or mechanical damage. Fast regeneration would reduce possibilities of competitive exclusion due to larval recruitment on a newly exposed substrate.

There are several parameters which should have an effect on the individual fate of regeneration of a sponge. Certain morphology types appear more susceptible to damage due to turbulence. Rope sponges ought to be the most prone to mechanical damage, due to their smaller ratio of attachment site to tissue volume. They will be followed by round-tube sponges, with the encrusting sponges hypothetically being most damage resistant. The more ~~more~~ vulnerable structure types are expected to regenerate faster in order to survive the turbulence,

Secondly, exposure to scouring effects of relatively high turbidity and surge at shallower depths might exert selective pressure against vulnerable sponges that cannot regenerate rapidly. The calmer waters at depth would be able to support a population with slower regeneration rates than the conspecifics in shallower regions.

Thirdly, the skeletal structure of a sponge may determine its regenerative capabilities. There are conflicting views on the relationship between spicule content and predation. Bergquist(1978) maintains that there is no predation deterrence related to the presence of spongin fibers. Furthermore, Randall and Hartman(1968) show that there is no general correlation between percentage of spicule content relative to organic matter and the frequency of consumption by fish. They did, however, note that the two species of sponge most widely consumed (results by gut-content analysis) did have extremely low spicule content. The next twenty sponge species showed no such clear relation. In fact, they maintain that fish predation is unimportant in controlling sponge population in the West Indies. Therefore, while predation may exert some selective pressure with respect to spicule content, and in turn, with respect to regenerative ability, the greater ramifications of the support tissues deal with sponge structure and stability.

Spicules are most important in maintaining the gross structure of the sponge, and are necessarily for support. Moreover, spicule secretion requires a large percentage of the sponge energy budget. It would appear that such sponges with a higher density of spicules would be less prone to mechanical damage and therefore would not allocate as much energy to regenerative processes. Hence, sponges with a higher density of spicules would be subjected to less selective pressure for faster regeneration

rates.

Finally, an individual sponge would not be expected to increase its regeneration rate as a result of a larger area of damage. Hence, a larger hole should take longer to regenerate compared to a smaller perforation.

METHODS AND MATERIALS

STUDY SITE. The field manipulations for this investigation were performed at Mooring 1, established by the Marine Laboratory, on the West Fore Reef at Discovery Bay, Jamaica. ^(Fig. 1) The area around the mooring consists of rough reef buttresses divided by sand channels running north-south, and sloping downwards to depths greater than 100 feet. There are many small patch reefs within the channels, as well as piles of rubble from Hurricane Allen, 1980. Much of the coral fauna was obliterated by the storm, leaving a generally depauperate coral population. The sponges, by virtue of their relatively fast growth rates and regeneration rates, were able to re-establish their populations. Significant recovery in the majority of sponges studied was noted within 2-3 weeks after the hurricane (Woodley, Chornesky, et.al., 1981). Thus, at present there exists a rich community of Porifera on the West Fore Reef.

Study sites were established at 40 feet and North-North-West at 90 feet. Sponges considered were located on a variety of substrates, including patch reef, sand, and buttress. The deep area apparently incurred less

hurricane damage, as there is less rubble and more abundant coral at this site. Furthermore, a qualitative assessment with respect to wave surge at the two depths made while SCUBA diving, revealed a much stronger swell at the shallower site.

METHODS. All field work was performed using self-contained-underwater-breath breathing-apparatus(SCUBA). At a depth of 40 ft., immediately adjacent to Mooring 1, six common sponge species were designated. Of the six species, there were three general morphology types--rope, encrusting, and tube/round sponges. We selected two species within each morphology group for study. At the 90 ft. site, one of the species from each shallow morphology group was used to study the effects of depth of regeneration rates within a given species. Three replicates of each species were included.

The sponges were tagged by tying flagging around numbered 75 ml. plastic bottles. These floats were anchored near a given specimen with a string tied to a piece of coral rubble. A hole was bored into each specimen with a 4 mm. diameter cork borer, to a depth of one cm., wherever possible. The following is a list of the species used for the study:

ROPE: (found only on reef substrate)

1. Iotrochota birotulata- green- black with rough, irregular surface. Inconspicuous oscula.
2. Agelas sp.- orange with 1-5mm. oscula scattered over a smooth surface.

ENCrustING: (only on reef substrate)

3. Brown OTU- smooth, dark brown surface with 1mm. oscula.
4. Rust OTU- rust colored, smooth surface with 3mm. oscula.

TUBE/ROUND: (on either sand or coral substrate)

5. Green tube OTU- green-gold color, one large, central osculum (2-4cm.). Rough surface.
6. Ircinia strobilina- gray, spherical or pillow shaped with several dark oscula clustered at the top. Rough surface with conules.

Verongia?

In addition to the 4mm. hole, a larger, 9mm. diameter hole was made in a single individual of the green tube sponge OTU as well as the Agelas sp. at both depths. All sponges were monitored on a daily basis, weather permitting, for regenerative development. The level of regeneration was assessed on a scale from 0-4, from no progress^S to complete regeneration (0=none, 1=regeneration begun, 2= approximately halfway healed, 3= almost complete, 4= completely regenerated). Each specimen was followed for 5-6 days, depending on when the hole was bored.

Samples were also taken from a separate individual of each species to examine the spicule content of the tissue. The investigations were carried out in the DBML wet laboratory. Pieces from each specimen were cut to a size of .125 cm³ and 30 drops of bleach were added to each chunk. The bleach served to partially dissolve the sponge tissue, releasing siliceous spicules into solution. The bleach reaction was allowed to proceed overnight. The following day, 10 drops of water were added to bring the precipitated tissue parts back into solution. As the spicule extraction technique was quantitatively analogous for each species, assessment of relative spicule density was possible. Three drops of sponge solution from a given sample were added to each of two slides. The slides were then examined under a dissecting scope, and a spicule count in five fields of vision was made for every slide.

Qualitative assessment of spongin fiber network was also performed.

Bleach was used to dissolve away some of the soft tissue, and reveal the structural spongin fibers when present.

RESULTS

The results from the regeneration survey are summarized in Figures 2-4. Every species showed some regeneration within 24 hours, except the Agelas sp. which showed no signs of regeneration throughout the length of the study. The other rope sponge, Iotrochota birotulata, showed the fastest full regeneration time. The green tube sponge showed the next fastest rate, followed by Ircinia strobilina, and then the two encrusting sponges. Some sponges displayed an interesting method of regeneration. Most formation of new tissue was from the inside, and grew outward. The Ircinia strobilina produced a dense clot of mucous membranes within the damaged hole. The green tube repaired its osculum wall first, and worked its way outward, the exhalant system first, and then the inhalant.

There was a notable amount of intraspecific variation with respect to regeneration rates. The error bars in Figures 2-4 represent the standard deviations over the three replicate specimens.

In comparing the two species of each morphology type, similar trends appeared for both the encrusting and the round/tube groups. A large difference in regeneration rates was found between the two rope sponges (Fig. 3). One of the species, Agelas sp., showed no regeneration, while Iotrochota birotulata healed the damaged tissue the quickest of the entire

group. The two encrusting sponges showed very similar development up to the third day, when the brown species picked up its rate of regeneration while the rust-colored one remained at the same level. Finally the two round tube type sponges showed very similar rates of regeneration throughout the study, although the initial reaction of the Ircinia strobilina was much more pronounced, and the green-tube sponge regenerated totally by the end of the study.

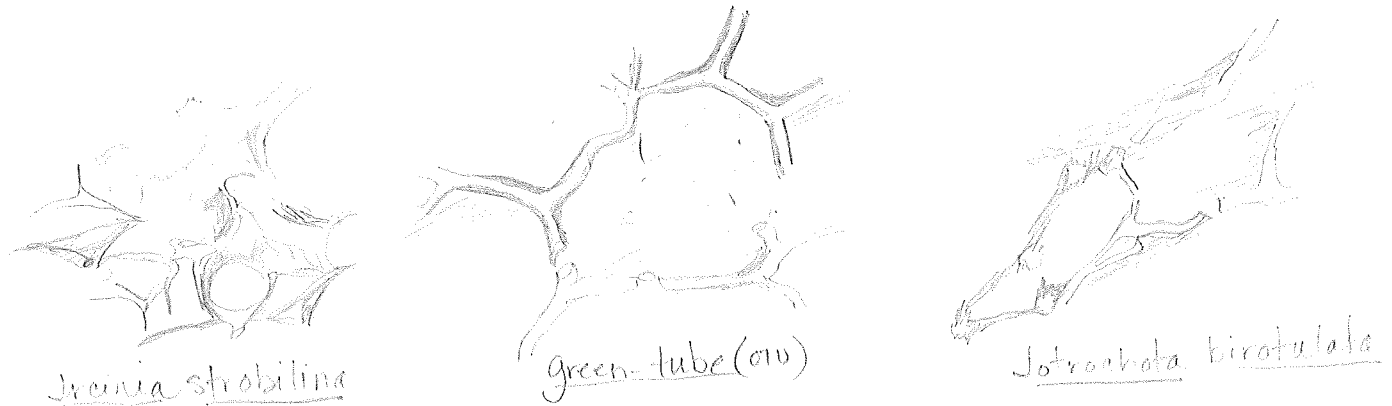
Comparison of conspecific regeneration rates over a depth gradient yielded interesting results. As in shallower water, the Agelas at 90 ft. showed no healing within the experimental interval. The green-tube sponge and the rust encrusting sponge both displayed trends which varied as a result of depth (Fig. 4). The green-tube specimens at the deep site showed no regeneration until the second day, and proceeded more slowly from that point, as compared to the rapid linear increase of the shallow water sample. The rust encrusting sponges displayed a similar lag at depth. These specimens showed no regeneration until the fifth day of observations, whereas the 40 ft. sponges showed an immediate, but gradual response.

There are several missing data points in the field observations, due to inclement weather or unavailability of necessary diving facilities.

Lab analysis of spicule content showed absolutely no correlation between spicule density and regeneration rates (Fig. 5). The scatter of data is apparent, and yielded a correlation coefficient, $r = -.038$. This coefficient is not statistically significant.

Spongin fiber networks were observed in three of the six species

studied: *Ircinia strobilina*, *Iotrochota birotulata*, and the green-tube sponge. These three sponges also exhibited the fastest regeneration rates. Spongin networks appeared as follows:



There were no discernible networks in the other species.

Investigation of regeneration as a function of hole size yielded the following results:

TABLE 1

	1	2	3	4	5	6
<u>Agelas sp.</u> lg.	0	0	0		0	0
sm.	0	0	0		0	0
<u>Green-tube</u> lg.	0	.5	.5		2	2.5
sm.	0	2	2		4	4

As noted earlier, *Agelas* showed no signs of regeneration, regardless of the extent of tissue damage. The green-tube sponge revealed a much slower regeneration time for a larger hole size. It was impossible, however, to quantify or compare the rates for the two hole sizes.

FIG. 1

MAP OF SPONGE STUDY SITES

10

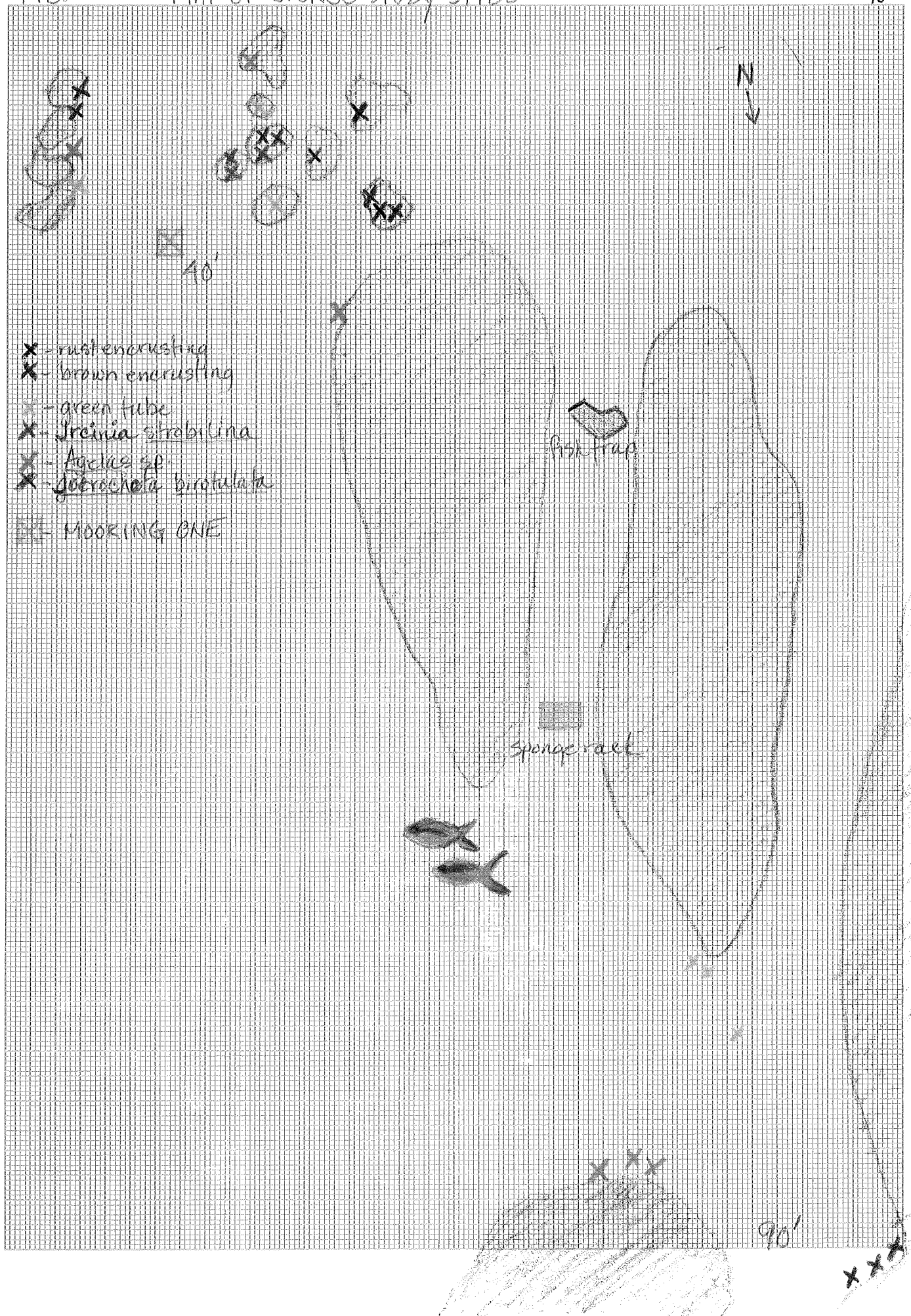


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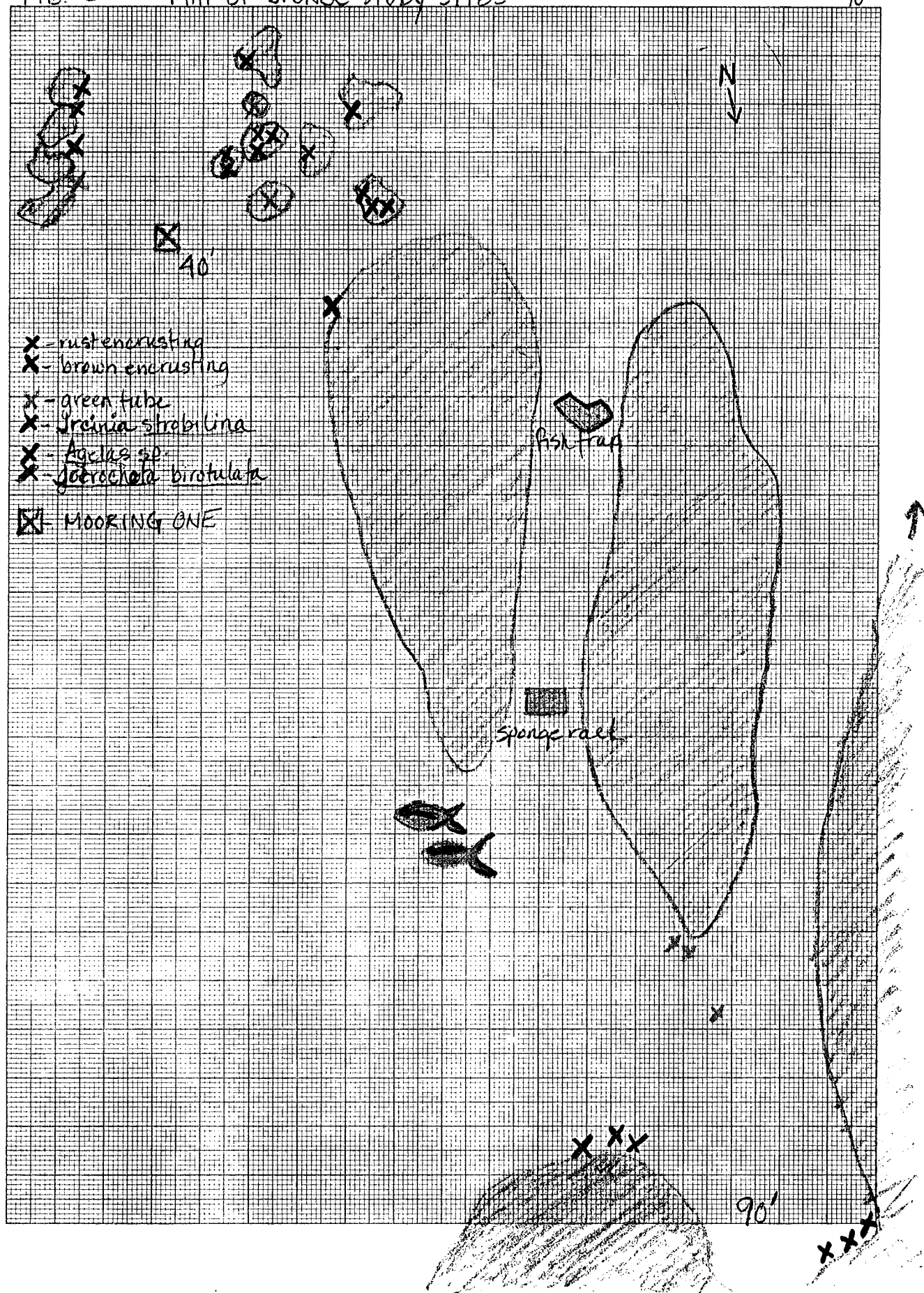


Fig. 2

Regeneration Rates over Time - 40 ft.

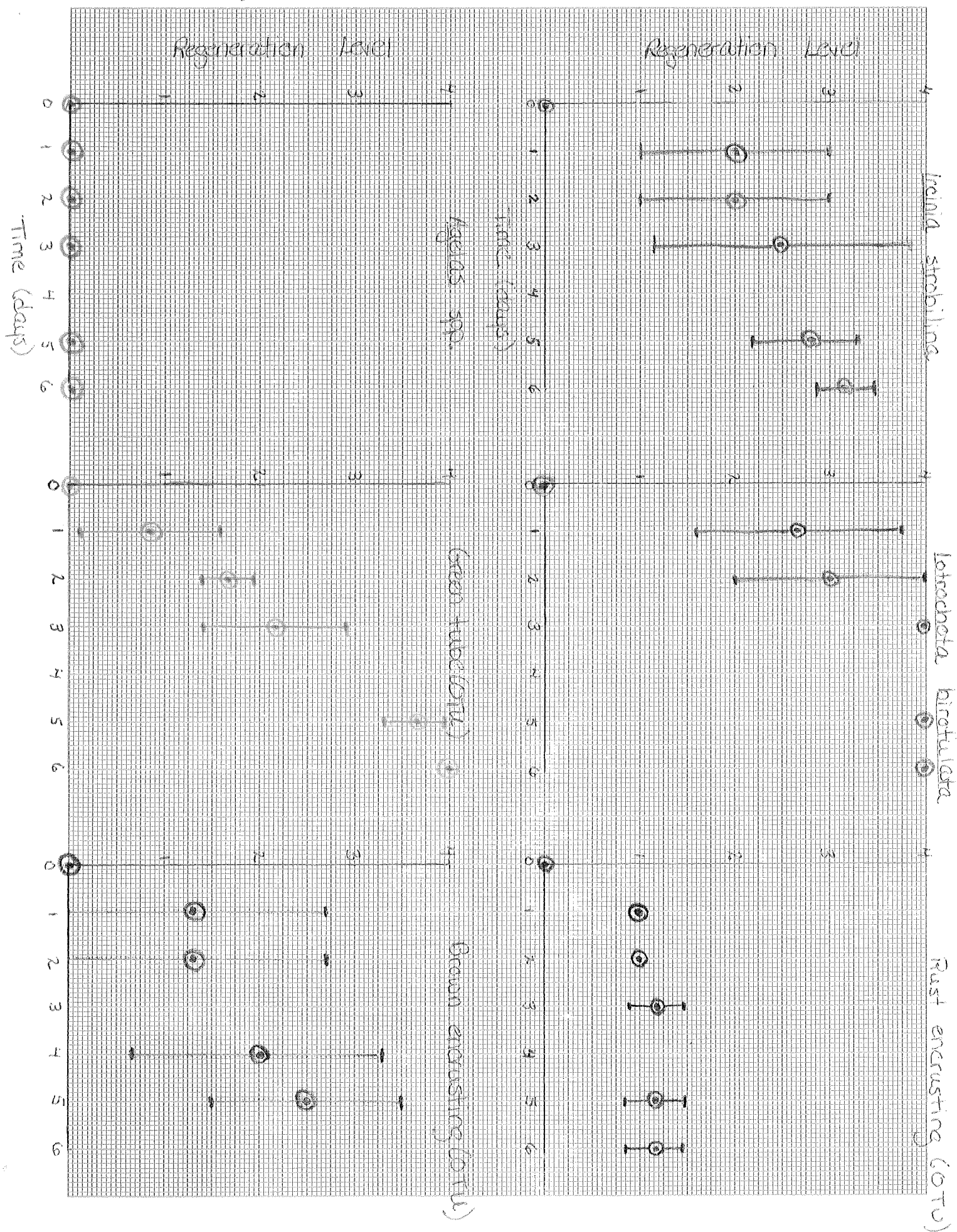


Fig. 3 Regeneration Rates Between Similar Morphology Types-40A

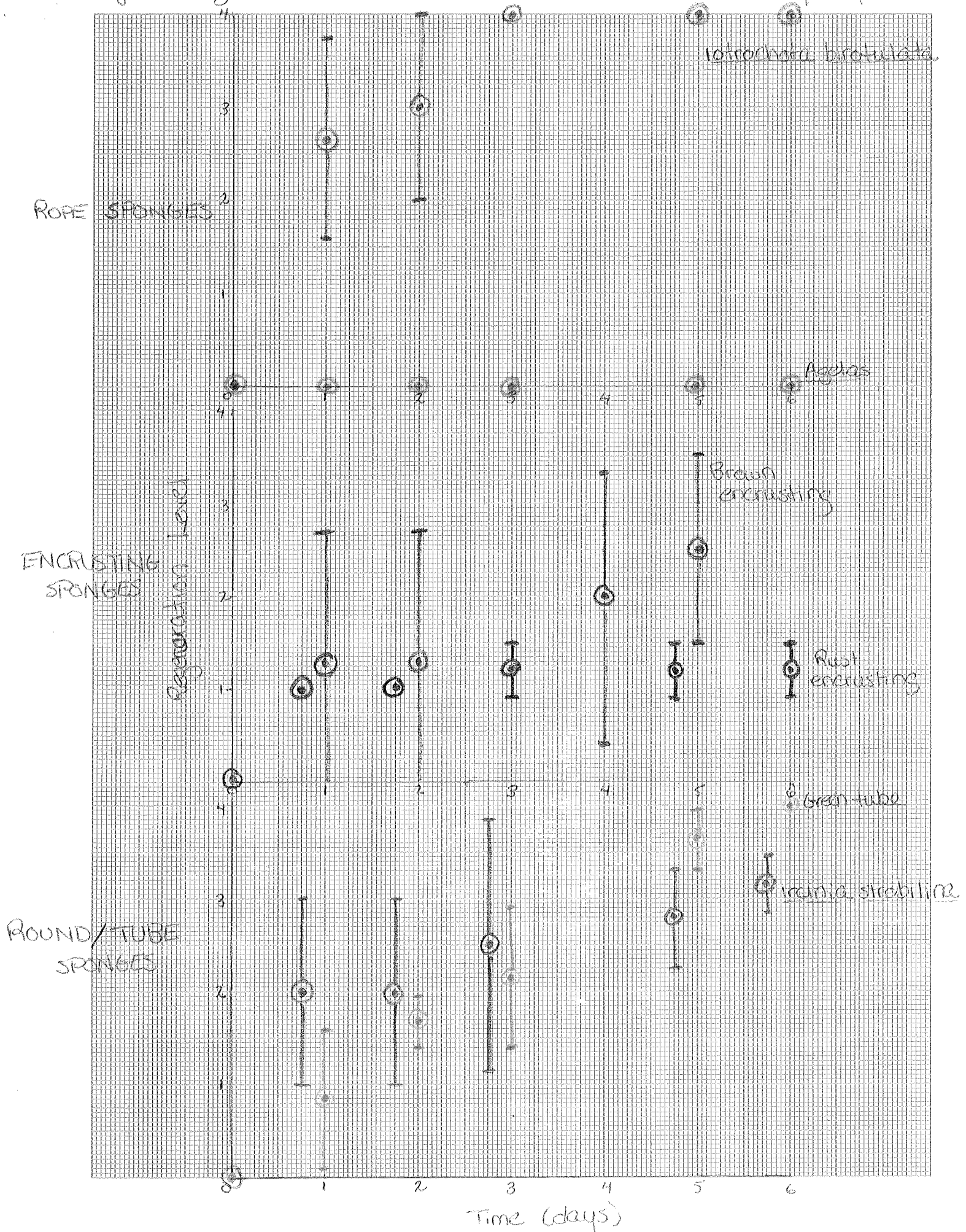
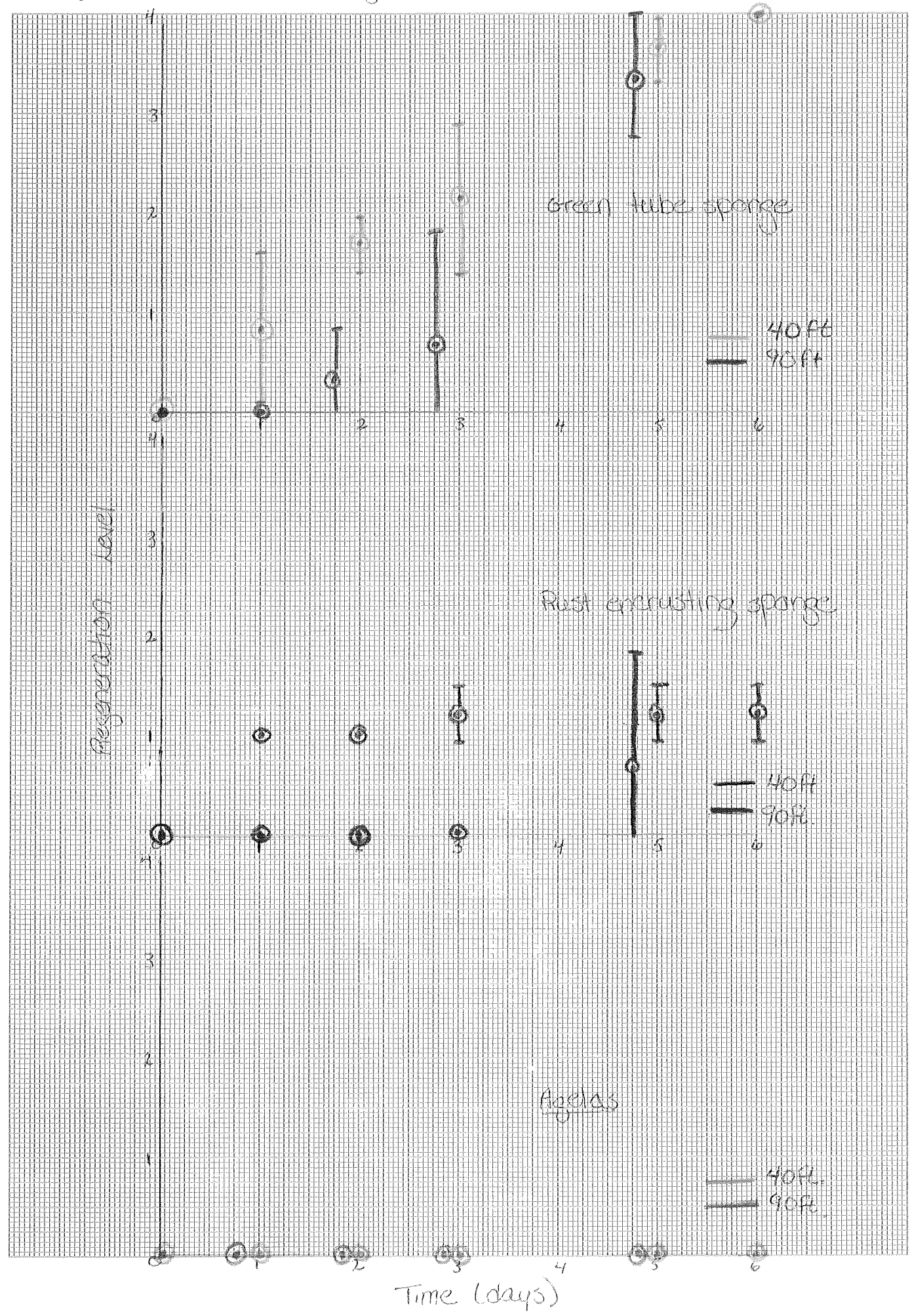
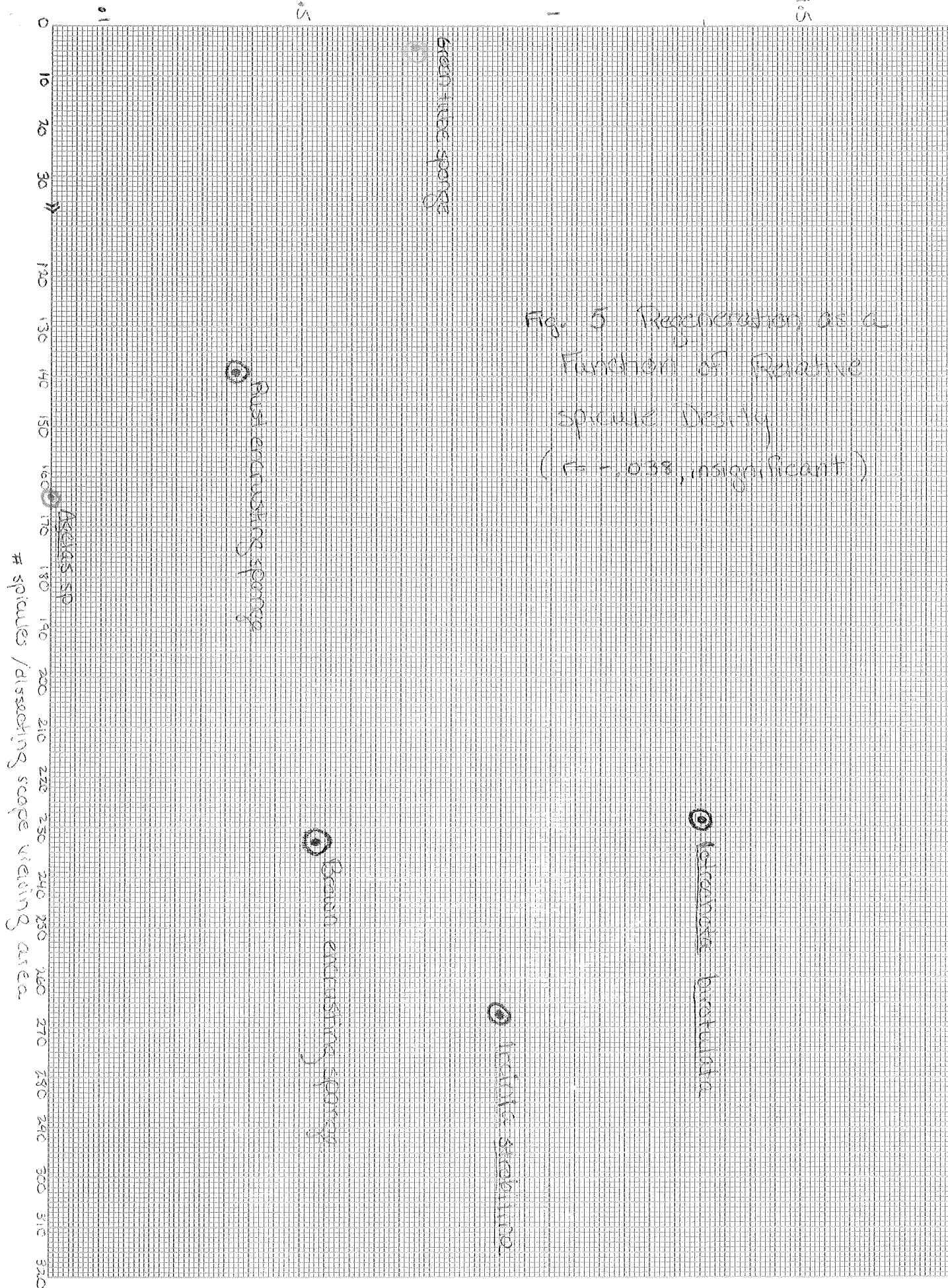


Fig. 4 Variation of Regeneration Rate with Depth





DISCUSSION

This investigation showed several interesting trends and indications in relation to regeneration of sponges. There are several factors, both physiological and environmental, which may play a role in determination of various individual rates. There were intraspecific variations at each site, in addition to differences between species of a similar morphology type. Two of the three groups indicated a similarity between species of a morphology type, but the two rope sponges showed that this pattern is not entirely consistent. The deviation in this trend may be an artifact of a small sample size, and a more in depth study examining a wider range of species would show if either Agelas or I. birotulata were representative of this morphology type.

Regeneration rate variation with depth suggested a trend of slower regeneration in conspecifics occurring at greater depths. There are several reasons for less selective pressure for faster regeneration rates at greater depths. There is less turbulence, less turbidity, and less predation pressure. Each of these factors plays a much smaller role in tissue damage at greater depths, and an individual sponge would be likely to allocate energy to other processes. Furthermore, increased light attenuation may effectively reduce the number of symbiotic algae present in sponge tissue. The decreased energy source may in turn reduce the efforts channelled into regeneration. The reduced light levels may also reduce coral

guidance
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fitness, easing both competition pressure for establishment on a substrate and pressure to regenerate to avoid larval recruitment. A more in depth study of currents, turbidity, and presence of symbiotic algae with respect to regeneration rates at greater depths might reveal some interesting trends.

Contrary to our hypotheses, there was absolutely no correlation between spicule density and regeneration rate (Fig. 5). While spicule content, according to various studies (Randall and Hartman, 1968, and Bergquist, 1978), is not related to fish predation, it would seem that this significant structural component would be correlated with the need to regenerate and/or the energy allocated for this process. A small sample size might explain the lack of any apparent trends in this case, although the extreme scatter of the data points seems to clearly refute the suggested relationships. Perhaps spicule density has a more significant role in actual sponge growth, as opposed to regenerative processes.

Another important structural support tissue, spongin, did appear to be related to regeneration rates. A trend toward the presence of spongin in faster regenerating sponges emerged from qualitative observations. The three species displaying the fastest regeneration rates - Iotrochota biro-
tulata, Ircinia strobilina, and the green tube sponge - were the three sponge types containing a network of spongin fibers. Spongin might allow a

Also, particulate
food may be less
concentrated at depth

damaged individual to retain its structural integrity, providing a tissue framework withⁿis which regeneration may be initiated. The Agelas sp., which exhibited no healing at the lesion site and appeared to actually atroph^y in the area proximal to the hole, has no spongin network to facilitate regeneration. It instead seems to crumble and rot around the damaged area.

Manipulation varying hole size supported the prediction that large areas of damage require a longer interval to repair. This result, however, is based on a very small sample size, since one of the two species examined (Agelas) did not regenerate at all, regardless of hole size. Nevertheless, the result is somewhat intuitive; it is unlikely that a sponge would be able to significantly alter its regeneration rate according to the amount of damage incurred.

In summary, due to limited sample size, it is impossible to conclusively confirm our predictions concerning morphological similarities or depth differences with respect to regeneration rates. Data were decisive however, in rejecting and supporting the spicule and hole size hypotheses respectively. The scope of the results was limited by the duration of the experiment as well as the necessarily small sample sizes.

Improvements in the investigation would include increasing both replicates within species studied, and number of species considered. There is also a need for a more consistent method of damaging the sponges, as several individuals were extremely difficult to penetrate with the cork borer. There was most likely some variation in hole size between individuals. Furthermore, it would be extremely advantageous to quantitatively assess healing progress, rather than using the rather subjective qualitative scale of this investigation.

The study of regeneration along a depth gradient showed some extremely interesting trends. These results would be more conclusive with a much larger sample size. Moreover, multiple sites could be established at several points along the depth continuum.

In conclusion, this study has demonstrated several interesting trends and correlations (or lack thereof) pertaining to the inherent variability of Porifera regeneration rates. Constraints on the experimental scope prevented the formation of conclusive deductions from the results. At worst, however, this investigation suggests a variety of potentially interesting and significant areas for further study.

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Very good report - well-conceived study;
good data collection, presentation, and
analysis; and thoughtful discussion.