Histocompatibility-defined Populations of the Marine Sponge *Iotrochota birotulata* in Discovery Bay, Jamaica

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Dartmouth Biology FSP 1985
March 10, 1985
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Abstract:

There are currently two schools of thought on what graft compatibility means in a population of sponges. Neigel et al. believe that graft acceptance indicates genetic identity whereas Kaye, Ortiz et al. believe that graft acceptance indicates genetic similarity, not necessarily identity. In this histocompatibility study on *Iotrochota birotulata*, we re-examined some aspects of Neigel and Avise's study (1983) and added a depth component. We did not support our hypothesis that there would be a greater proportion of graft acceptances at larger distances from a parent donor in the shallow, more turbulent water. We did support our hypothesis that there would be a greater proportion of acceptances at all three depths for grafts done within patches than for those done between patches. Finally, we found that the proportion of within patch acceptances was significantly higher at the intermediate depth than at the shallow, more turbulent depth.

Introduction:

Members of the phylum Porifera are known to have both a sexual and an asexual mode of reproduction. Sponges produce motile larvae sexually which may live in the open water for a long time and travel some distance before finding a suitable substrate to settle on. Asexual reproduction (in the form of vegetative propagation) is an alternate mode of reproduction. Storms and other disturbances can tear off fragments from branching sponges which can settle and adhere to a new substrate. Still, a great deal is unknown about the relative importance of these two different modes of reproduction. Individual sponges that have been derived vegetatively from fragments are morphologically indistinguishable from those derived from larvae. (Neigel and Avise, 1983) Therefore, when studying an established population of sponges, it is impossible to know the relative proportions of sexually and asexually recruited individuals.

hours Days only

Many histocompatibility studies have been done on invertebrate populations with the belief that self-recognition can help determine the genetic structure of a population. In the past it was believed that invertebrates (unlike vertebrates) would always accept tissue from individuals of the same species. (Kaye and Ortiz, 1981) It is now known that sponges and other invertebrates will exhibit incompatibility responses from some allografts (tissue transplanted between individuals of the same species) as well as xenografts (tissue transplanted between individuals of different species)

There are basically two schools of thought on what graft compatibility illustrates in a specific invertebrate population. Neigel and Avise (1983) examined histocompatibility in the coral Acropora cervicornis and found that graft acceptances decreased with increasing distances between colonies and that histocompatibility relationships were all reproducible and transitive. They concluded, that in this invertebrate, graft compatibility indicates genetic identity. Jokiel et al. (1982) examined histocompatibility in the marine sponge Callyspongia diffusa and found no graft acceptances over long distances or across physical barriers such as sand channels. Thus, like Neigel et al., they concluded that a graft acceptance between two individuals indicates clonal identity.

There is still, however, a great deal of controversy about what graft compatibility in a population indicates. Some invertebrates, such as colonial ascidians or hydroids, are known to be histocompatible with members of the same species without being genetically identical. (Neigel, 1984) Kaye and Ortiz examined histocompatibility in Verongia longissima and found graft acceptances up to 10m away from the donor. They found groups of individuals which only accepted grafts from individuals within their own group, and designated these as strains. Thus Kaye and Ortiz believe that acceptances indicate genetic similarity but not necessarily genetic identity. Janie Wulff found quite similar results for Iotrochota birotulata. (Janie Wulff, personal communication) She used the term fusion group to designate individuals which would only graft with eachother and (unlike Kaye and Ortiz) even found some morphological differences between these groups. Curtis et al. (1982) believe that it is impossible to draw conclusions about the genetic structure of a sponge population by grafting alone. They performed gel electrophoresis on Ectyoplasia ferox sponges and found that graft-accepting sponges can have dissimilar plasmalemmal proteins. Thus Curtis et al. follow Kaye's school of thought; they believe that graft compatibility does not necessarily mean genetic identity.

Iotrochota birotulata is a branching sponge which is often found in patches or aggregations of individuals. The sponge is purplish to black but is usually covered with layers of green cells. It is also sometimes covered with the orange Zoanthid, Parazoanthus swifti. I. birotulata is an excellent sponge to use in grafting experiments because a clear graft acceptance or rejection is apparent in 3-5 days.

Neigel and Avise (in 1983) examined the histocompatibility of I. birotulata in Discovery Bay, Jamaica. They found that almost all graft acceptances occurred within patches of I. birotulata and that 2.7m was the farthest distance of acceptance between patches. In addition, (as in their study of A. cervicornis) they conclude that a graft acceptance indicates that two individuals are isografts or genetically identical. We performed a similar histo-compatibility study on I. birotulata on the East Back Reef and the West Fore Reef in Discovery Bay. Unlike, Neigel and Avise, we added a depth component to our study. Our goal is to examine graft compatibility at three different depths which vary in turbulence. One hypothesis of our study is that there will be a greater proportion of acceptances at larger distances from a designated center patch in the shallow, turbulent water. This would occur if more fragments were generated and dispersed farther by the greater wave action in these areas. Our second hypothesis states that there will be a greater proportion of acceptances (at all three depths) for grafts done within patches than for those done between patches. Because there is still a great deal of controversy about what a graft acceptance means, we can only assume that graft acceptances indicate genetic similarity, not necessarily identity. By grafting at varying distances from a center (donor) individual we will be able to map the spatial distribution of genetically related individuals at all three depths.

Study Site:

All grafting was done *in situ* in Discovery Bay, Jamaica. A total of 6 sites were chosen that varied in depth and/or turbulence.

The first 2 sites (sites 1 and 2, EBR) were located at the East Back Reef at depths ranging from 10 ft. to 17 ft. Clumps of *Iotrochota birotulata* were found mostly on solid coral rock promontories (and patches) separated by sand channels. Both sites were located parallel to and approximately 50 meters away from the reef crest. Site 1 and Site 2 were separated by a 10 meter wide area of sea grass beds. The water in this area was very turbulent (even at a depth of 20 ft.) presumably from the wave action off of the reef crest.

The next 3 sites were located on the West Fore Reef off of Mooring 1 at a depth of 45 ft. (sites 1, 2 and 3, Mooring 1). Site 1 was located in a sand channel directly beneath the mooring line. Site 2 was located in a sand channel east of the mooring, separated from Site 1 by a large patch reef. In both Site 1 and site 2, clumps of *Iotrochota* were found on coral outcroppings separated by areas of sand. Site 3 was located about 30 meters east of site 2 on a large patch reef. Here *I. birotulata* was found as single individuals or small clumps attached to coral substrate. At all 45 ft. sites, turbulence was much less than at the shallower EBR sites.

The final site was also located off of Mooring 1 at a depth range of 70 to 80 ft. This site occurred on a downward sloping sand channel at the edge of a large patch reef. Again *I. birotulata* was found in small clumps attached to hard coral substrate separated by sand. The turbulence here was not as great as that at the 45' sites, but was not markedly different.

Methods:

All sponge-grafting was done in situ with the use of SCUBA equipment. Segments of branches of Iotrochota birotulata 5 to 10 cm long were cut with razor blades and attached to live, marked sponges by thin cotton thread and twine. Sponge segments were placed firmly in direct contact with the donor sponge at one interface. Grafts were checked after three and four days and finally scored for graft acceptance or rejection after 5 days. In most cases, graft acceptance was apparent within 3 days and was characterized by complete fusion of the sponge segment to the host sponge branch at the interface. Tissue flowed across the interface leaving no definite cuticle. Also the fused segment could not be separated from the host by gentle pulling. A graft was scored as a rejection if a cuticle formed at the interface. In some cases, the attached sponge fragment stuck to the host sponge at the interface but after the thread was cut, the fragment either fell off independently or could be separated from the host by gentle pulling (in all cases). In some grafts there was also some apparent tissue damage in both host and recipient sponge segments; some original tissue had sloughed off leaving a black discolored area of soft tissue.

In the shallow area at the East Back Reef, two sites were chosen arbitrarily since a large number of dense patches of I. birotulata were found there. At both intermediate and deep depths (Sites 1-4, Mooring 1) sites were chosen more on the basis of the pre-existing spatial distribution of I. birotulata clumps. The Iotrochota patches were not as dense at these sites and seemed to occur in well-defined areas separated from other groups of patches by sand channels or patch reefs. Each site consisted of a center patch (or donor) around which other recipient patches (from 3 to 10) were chosen. Patches varied from 1m to 21.5 m in distance away from the center patch and contained 1 or more individuals. An attempt was made to graft in all directions from the center although we were limited by the existing spatial structure of the Iotrochota population at each site (see Figures 1-6; Maps of all 6 sites). Patches at each site were marked by fluorescent orange ribbon and with a wax pencil.

Three types of grafting experiments were conducted at each site. A total of 99 grafts were done at all six sites (see Table 1). Of these, 26 were autografts in which segments were cut off of and grafted to the same host sponge. In addition, 36 within-patch allografts were done. In these experiments a center individual was designated for each patch from which fragments were cut and grafted to a different individual from the same patch (on the same substratum). Finally, 37 between-patch allografts were also done in which fragments were cut from the center individual of the center (donor) patch and grafted onto the center individual of each surrounding patch. Long distances were measured using a marked spool of twine while short distances (i.e. distance between a within-patch allograft and center individual) were measured with a metric ruler.

No attempt was made to graft all possible pairwise combinations between individuals because for 99 individuals, 9,801 (99x99) operations would be required. Since this was not feasible we were only able to do arbitrary within-patch and between-patch allografts.

Results:

All 26 autografts performed at each of the three depths (10-17 ft., 45 ft. and 70-80 ft.) exhibited acceptance responses. (See Table 1) Acceptance responses for within patch allografts were significantly higher in the 45 ft. site than they were in the 10-17 ft. site. (2x2 Chi Square, $\alpha < .01$) No Chi Square analysis was performed comparing the deep site to either the shallow or intermediate site due to a very small number of within patch allografts at the deep site.

On the East Back Reef there were significantly fewer rejections in the 0.1-1m distance group than in the 1-10m distance group. (2x2 Chi Square, $\alpha < .05$) (See Figure 7) No Chi Square analysis was performed on the 0.1-1m distance group and the 10-100m distance group in the EBR because there were so few individuals in the 10-100m group. On Mooring 1 at 45 ft. there were significantly fewer graft rejections in the shortest distance groups. (4x2 Chi Square, $\alpha < .025$) (See Figure 8) Due to small sample sizes, pairwise analyses were not performed on these four distance groups. However, visual inspection of Figure 8 shows that allografts in short distance groups have fewer rejections than those in long distance groups. Seven allografts were performed near Mooring 1 at 70-80 ft; graft rejections are lower in the shorter distance groups. (See Figure 9)

There were only rejection responses for between-patch allografts on the EBR. (See Figure 10) The greatest number of individuals exhibiting acceptance responses within patches were .3m from the center individual (or donor). The only between-patch allograft acceptance occurred at Mooring 1 at 45 ft. with a donor-recipient distance of 6.0m. (Figure 11) Almost all individuals at this depth (except one) exhibited within-patch allograft acceptances. The largest number of individuals with acceptance responses were .2m from the donor. There were very few individuals tested at Mooring 1 at 70-80 ft. but all graft responses between patches were rejections. The only within-patch allograft done at this site, .1m from the donor, showed an acceptance response.

TABLE 1 : Numbers, Types, and Percentages of Acceptances for All Grafts

RAW DATA	EBR: 10-17'	Mooring 1: 45'	Mooring 1: (70-80 ft)	Totals
# of autografts	9	14	3	26
# of within-patch allografts	25	10	1	36
# of between-patch allografts	18	13	6	37
totals	52	37	10	99

% GRAFT ACCEPTANCE	EBR: 10-17'	Mooring 1: 45'	Mooring 1: (70-80 ft)
autografts	100 %	100	100
within-patch allografts	40	90	100
between-patch allografts	0	7.7	0

KEY for FIGURES 1-6

⬭ = patch

■ = center individual of patch

▲ = within-patch allograft acceptance

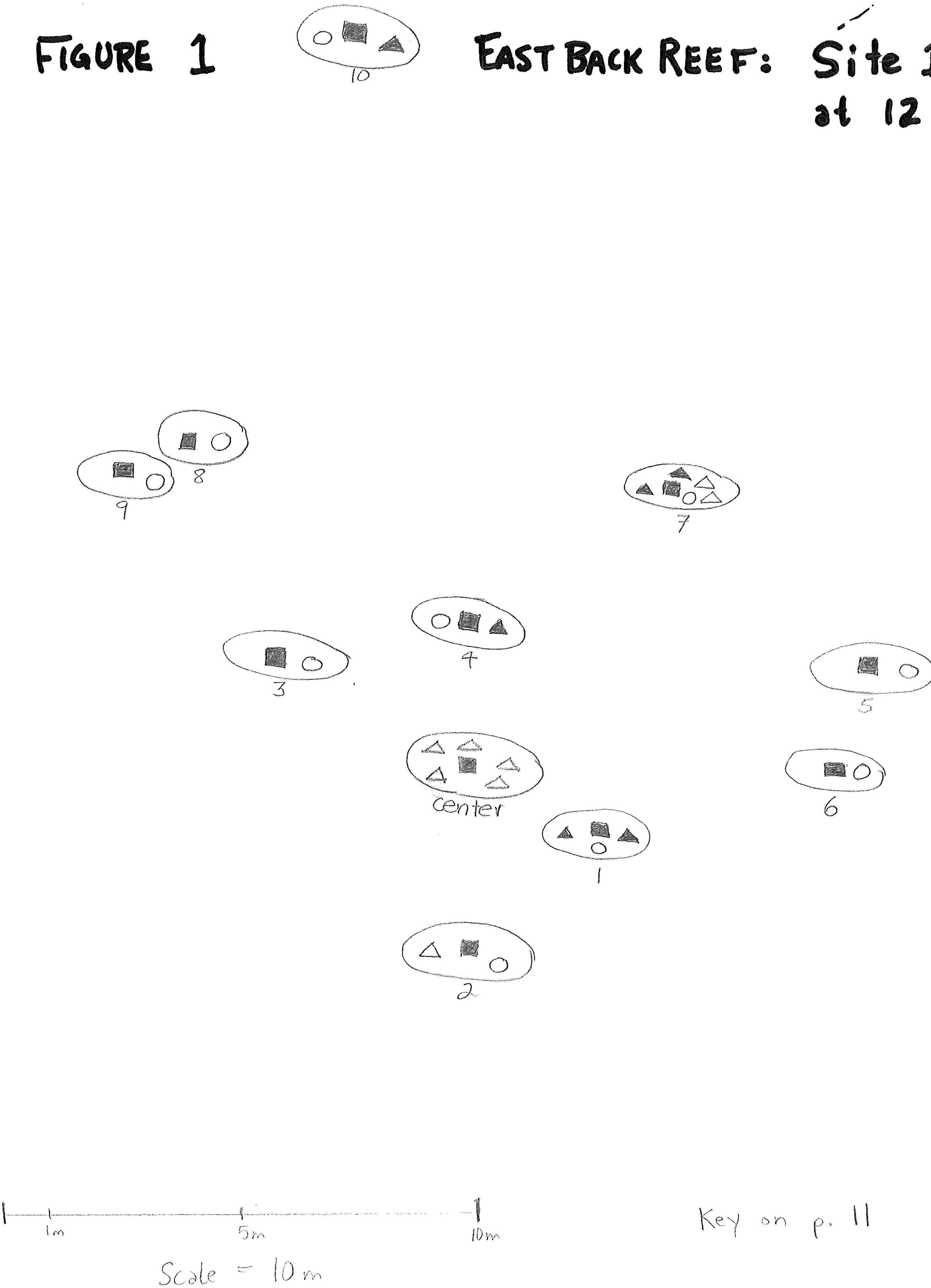
△ = within-patch allograft rejection

● = between-patch allograft acceptance

○ = between-patch allograft rejection

NOTE: Distance between individuals at each patch and size of patch were not drawn to scale. Also, distance between an individual patch and the center patch was measured using the center individuals as reference points.

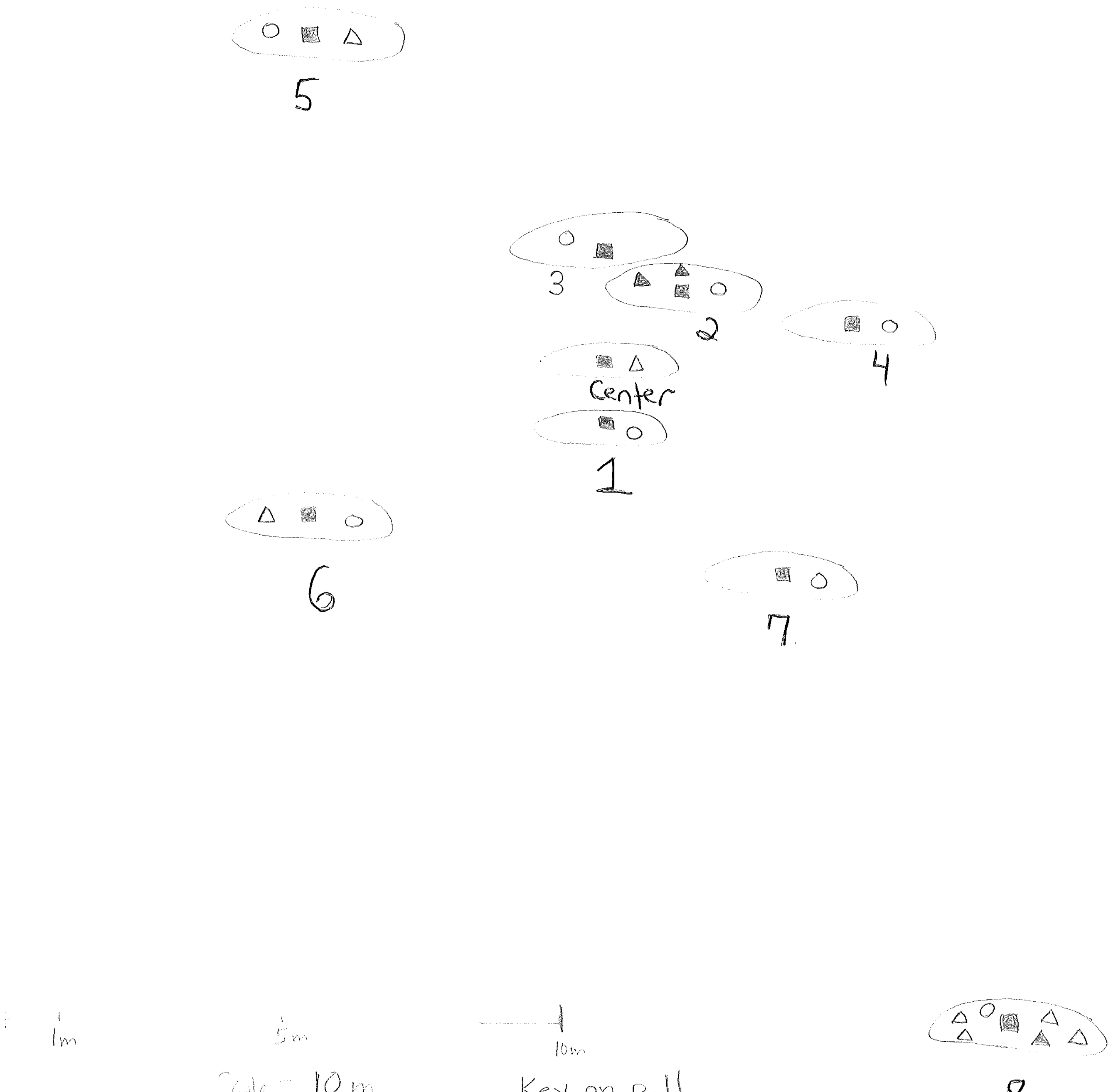
FIGURE 1 EAST BACK REEF: Site 1 at 12 ft.



Key on p. 11

FIGURE 2

East Back Reef: Site 2 at 17 ft.



Key on p. 11

Figure 3
Mooring 1: Site 1 at 45 ft.

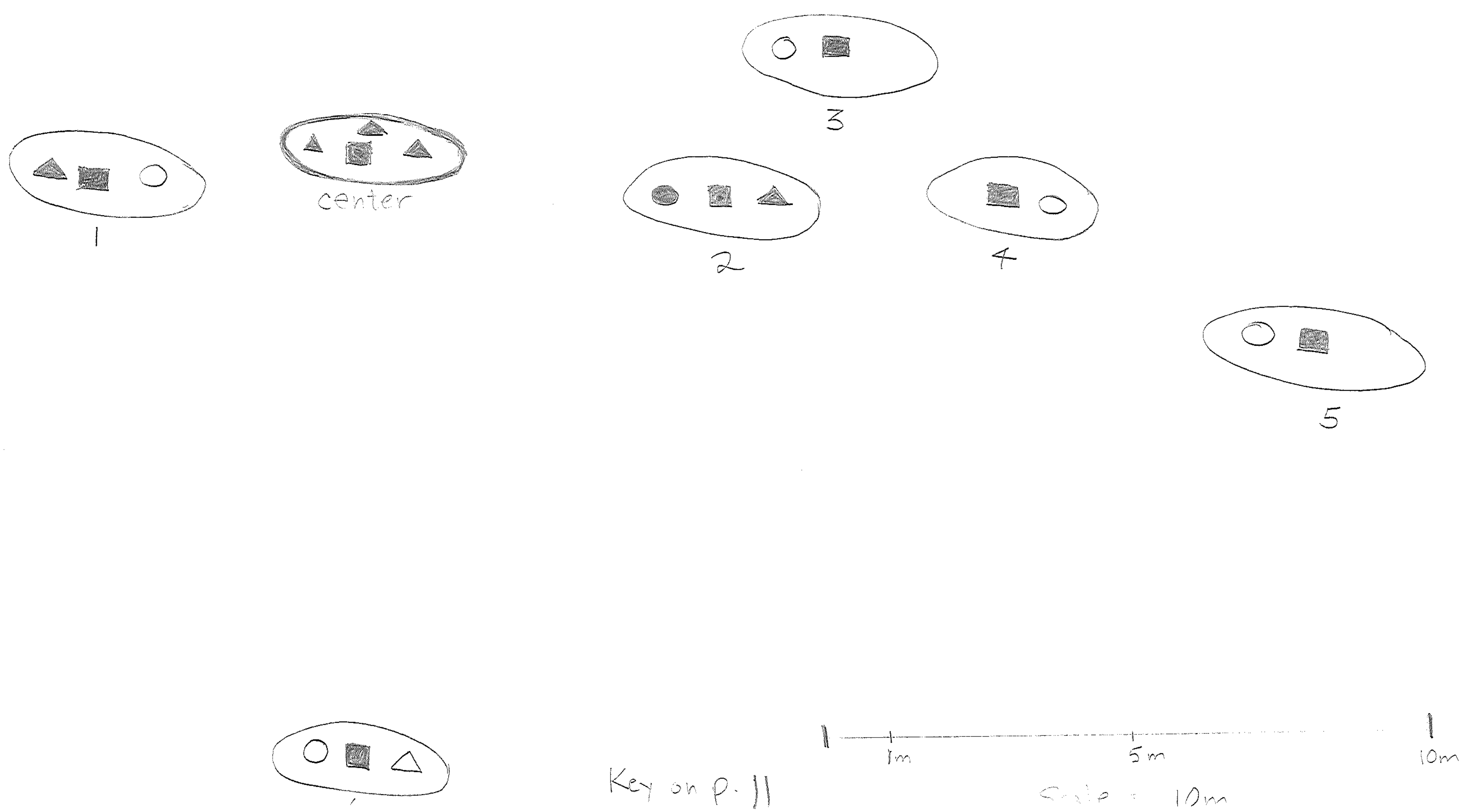
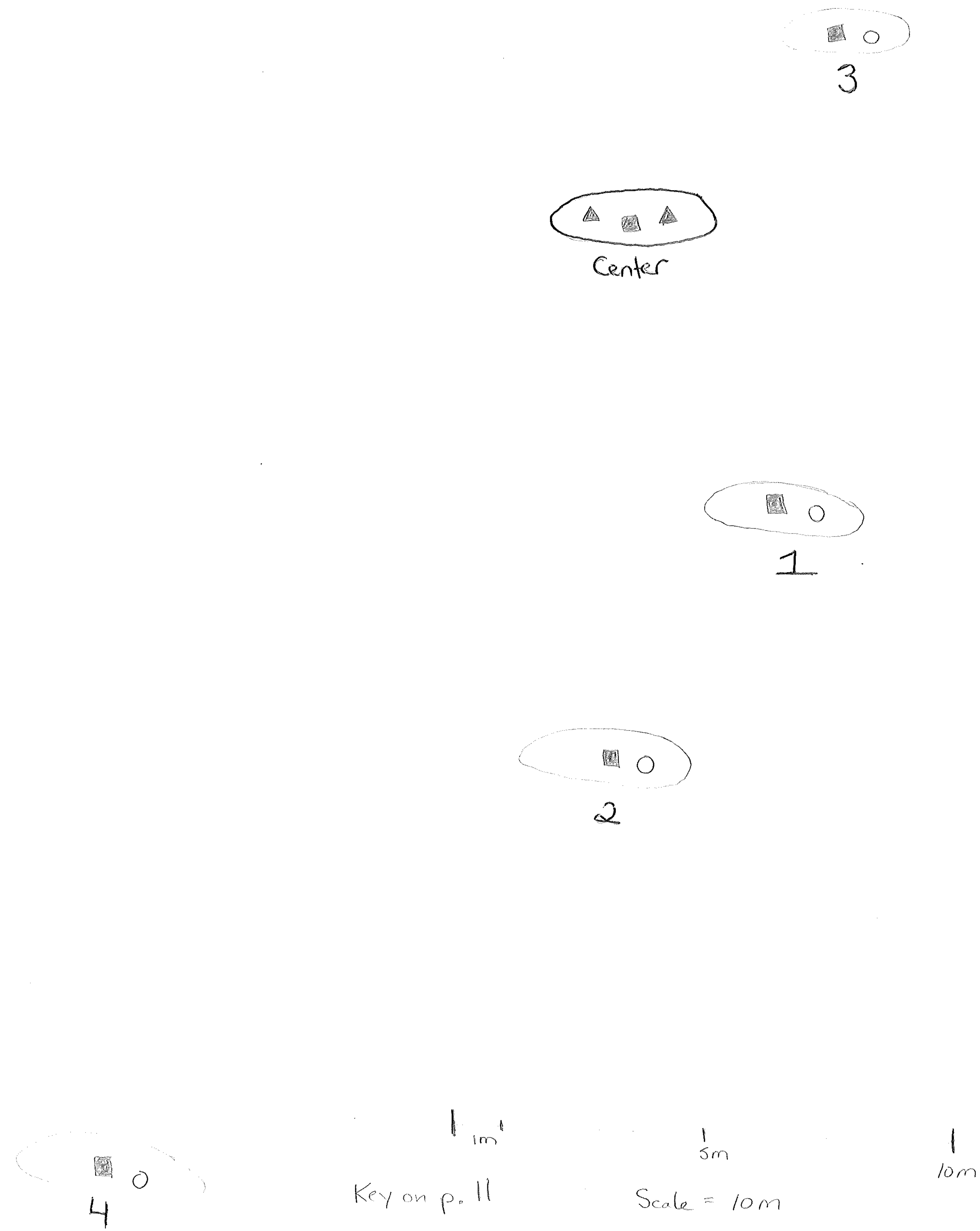


FIGURE 4

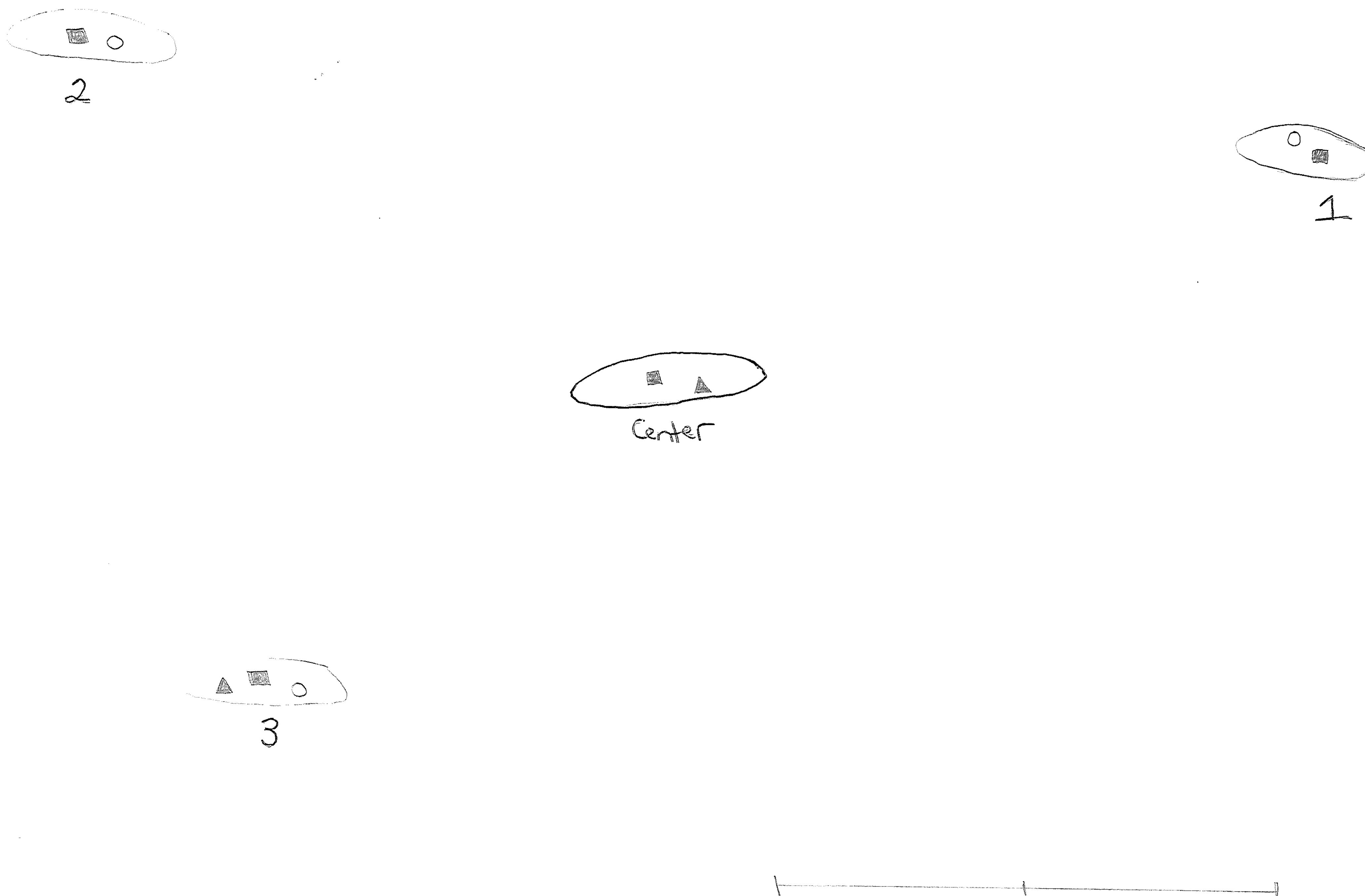
Mooring 1 : Site 2 at 45 ft.



Key on p. 11 Scale = 10m

FIGURE 5

Mooring 1: Site 3 at 45ft.



Key on p.11

FIGURE 6

MOORING 1 : Site 4 from 70 - 80 ft.

Key on pill

Scale = 10m

Figure 7: Percent Graft Rejection on East Back Reef (10-17ft.)

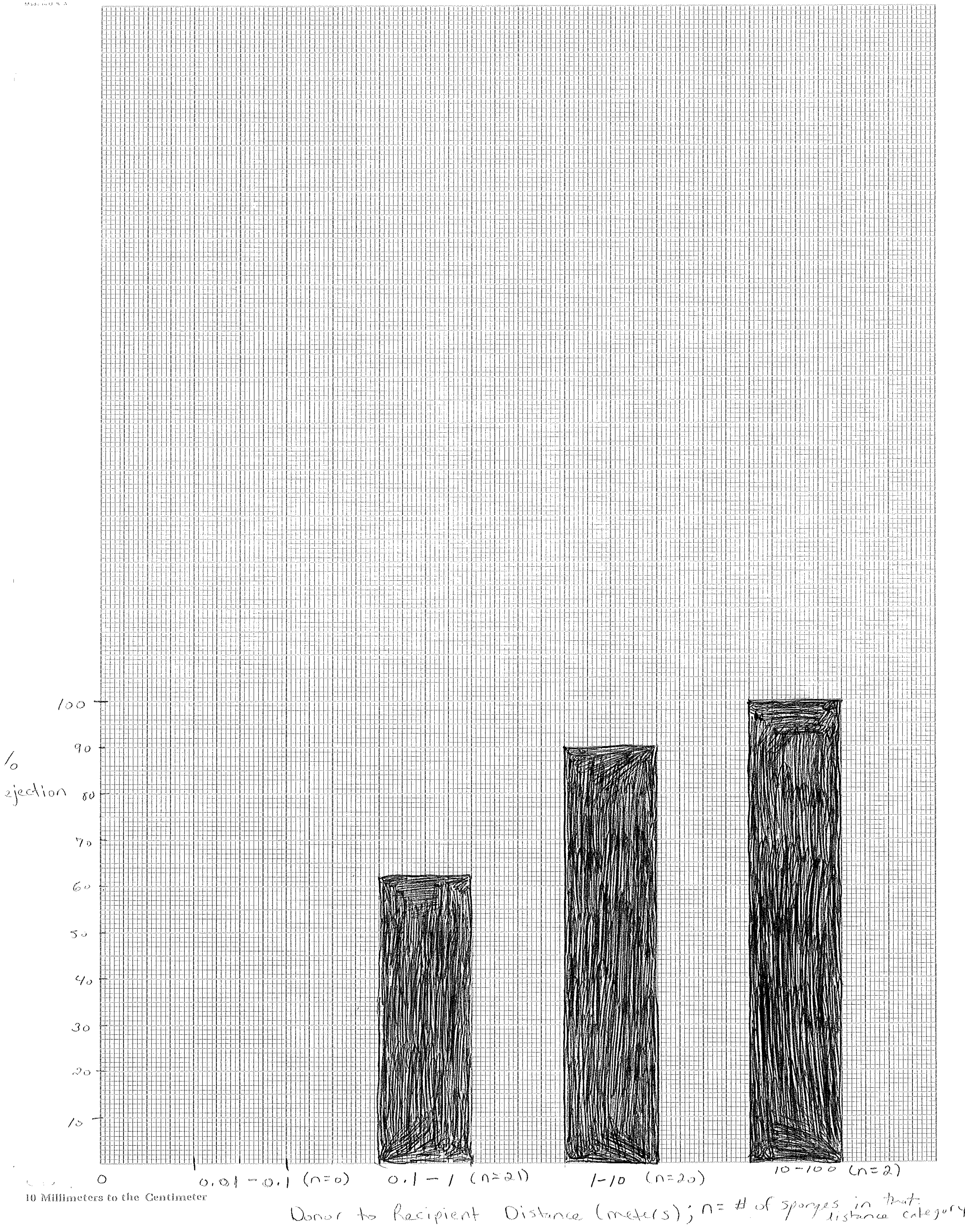


Figure 8: Percent Graft Rejection on Mooring 1 (45 ft.)

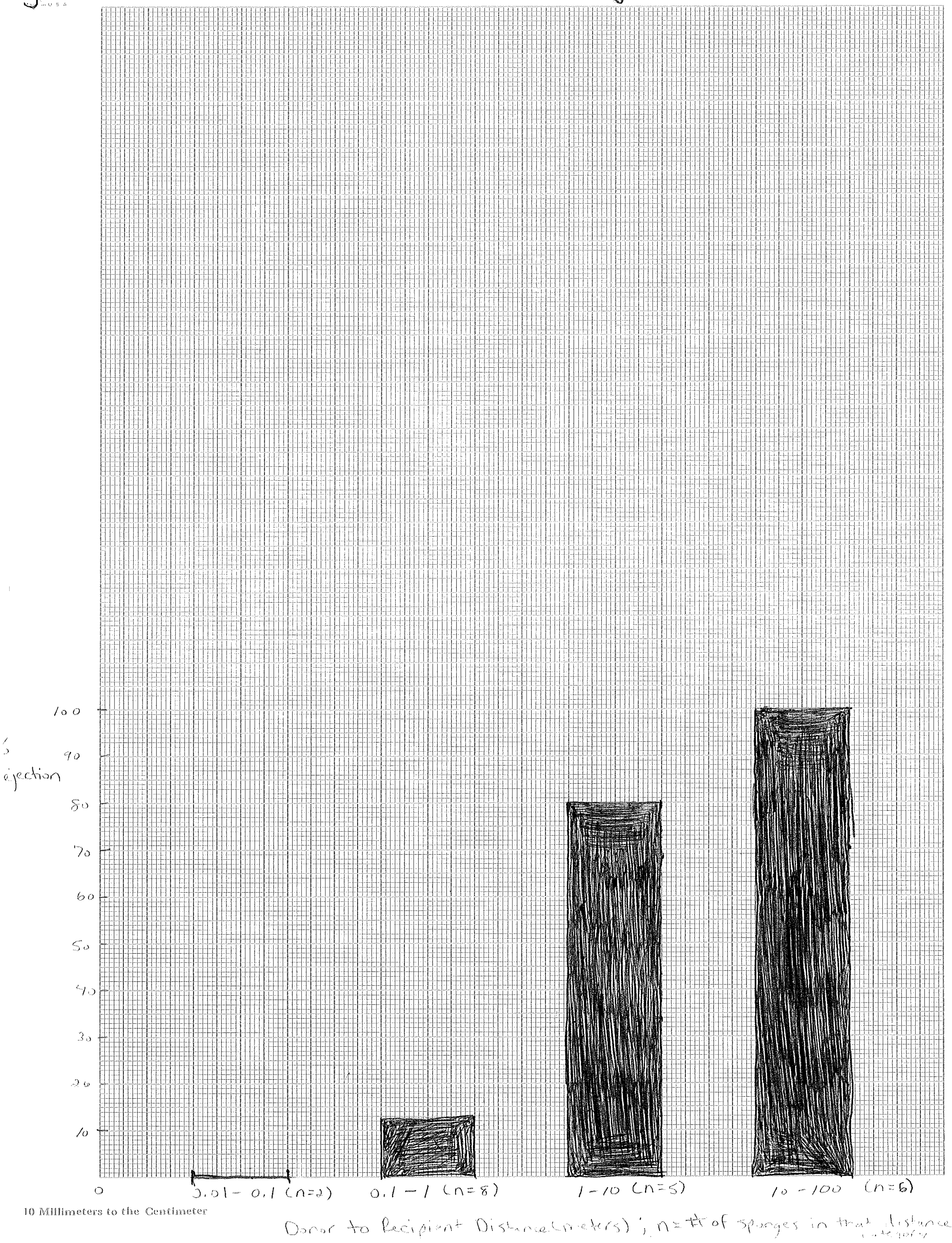


Figure 9: Percent Graft Rejection on Mooring 1 (70-80 ft.)

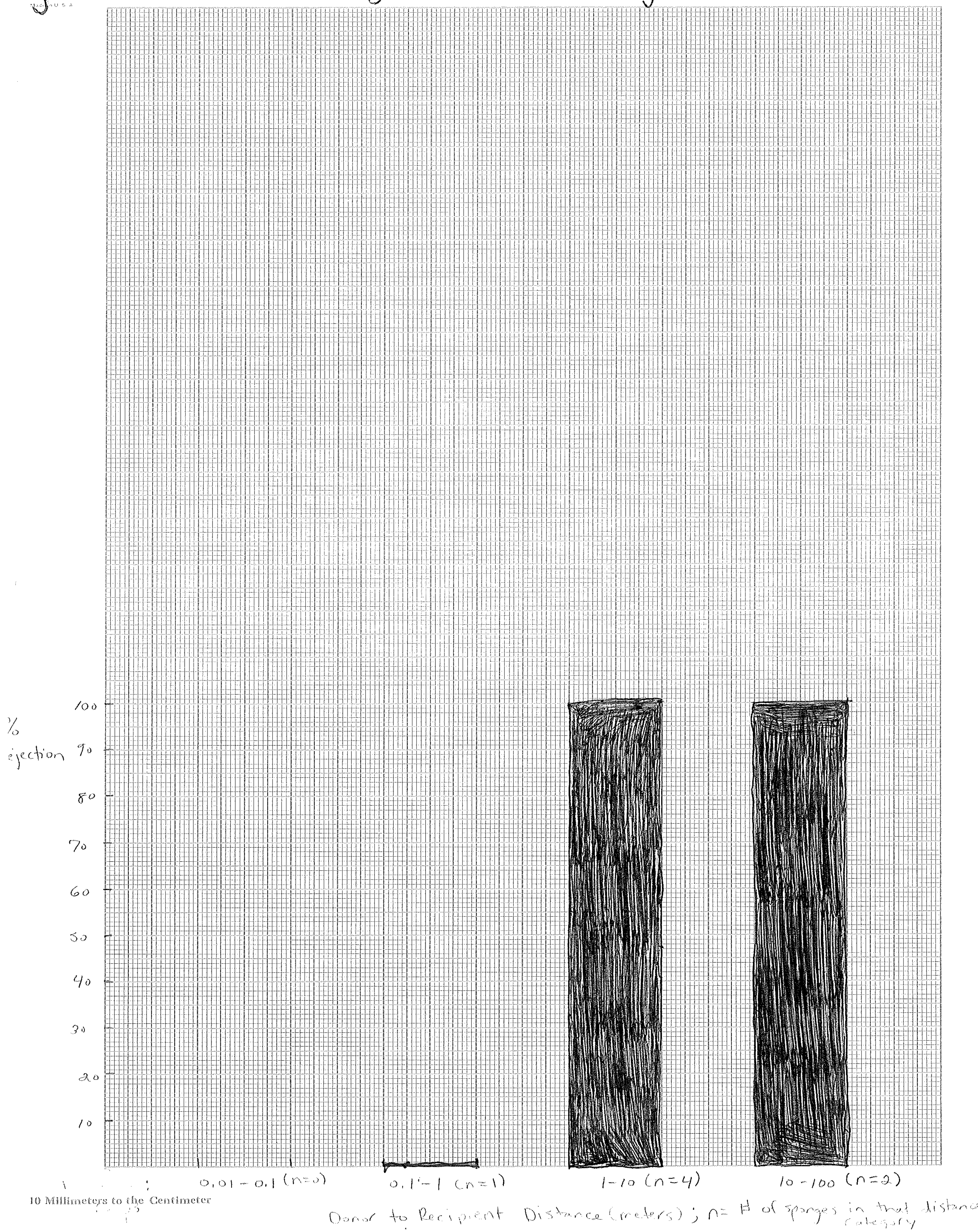


FIGURE 10 : Graft Responses of Individuals over Distance (log) at East Back Reef : 10-17 ft.

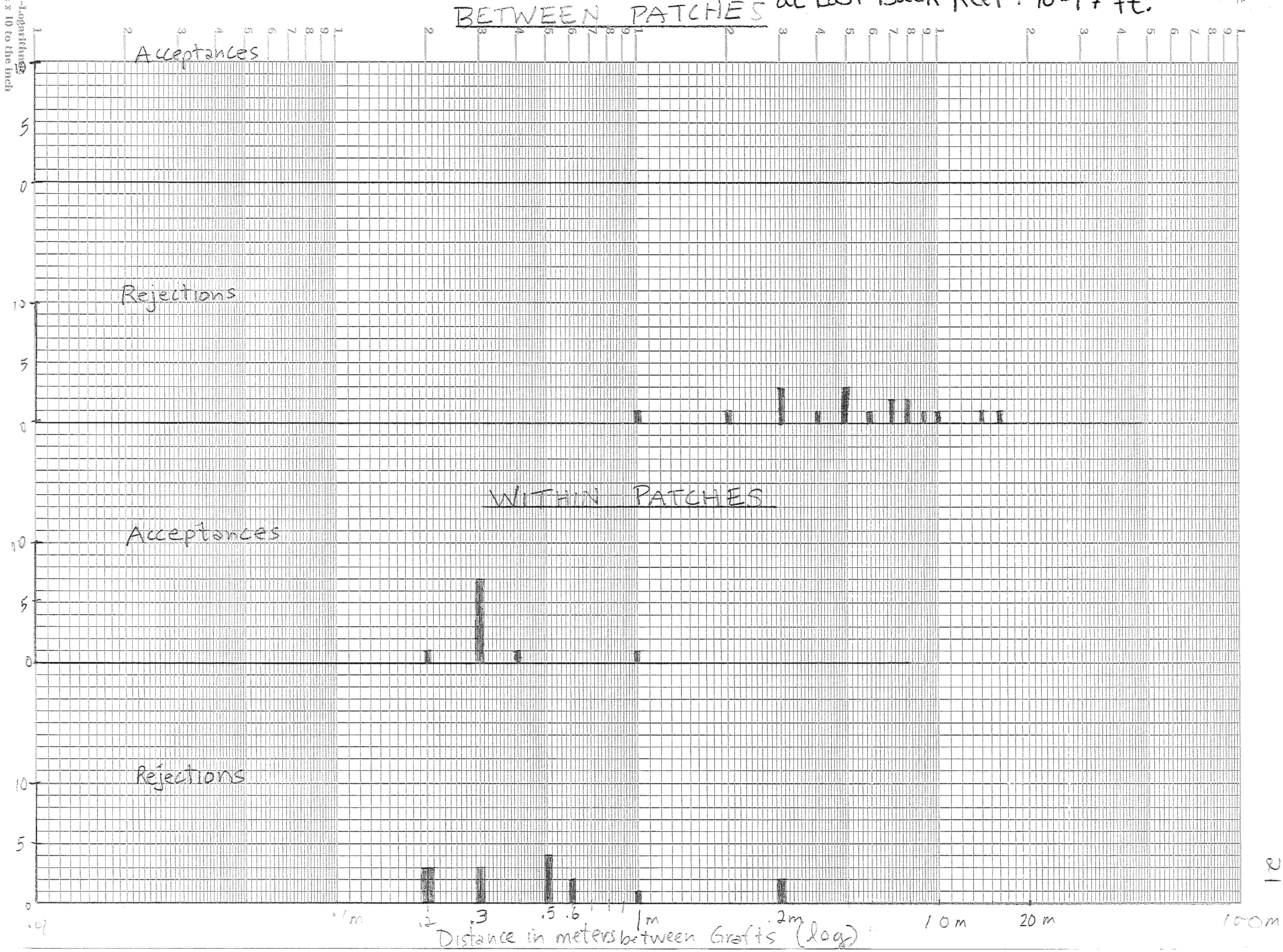


FIGURE 11 : Graft Responses of Individuals over Distance (log) at Mooring 1 : 45 ft.

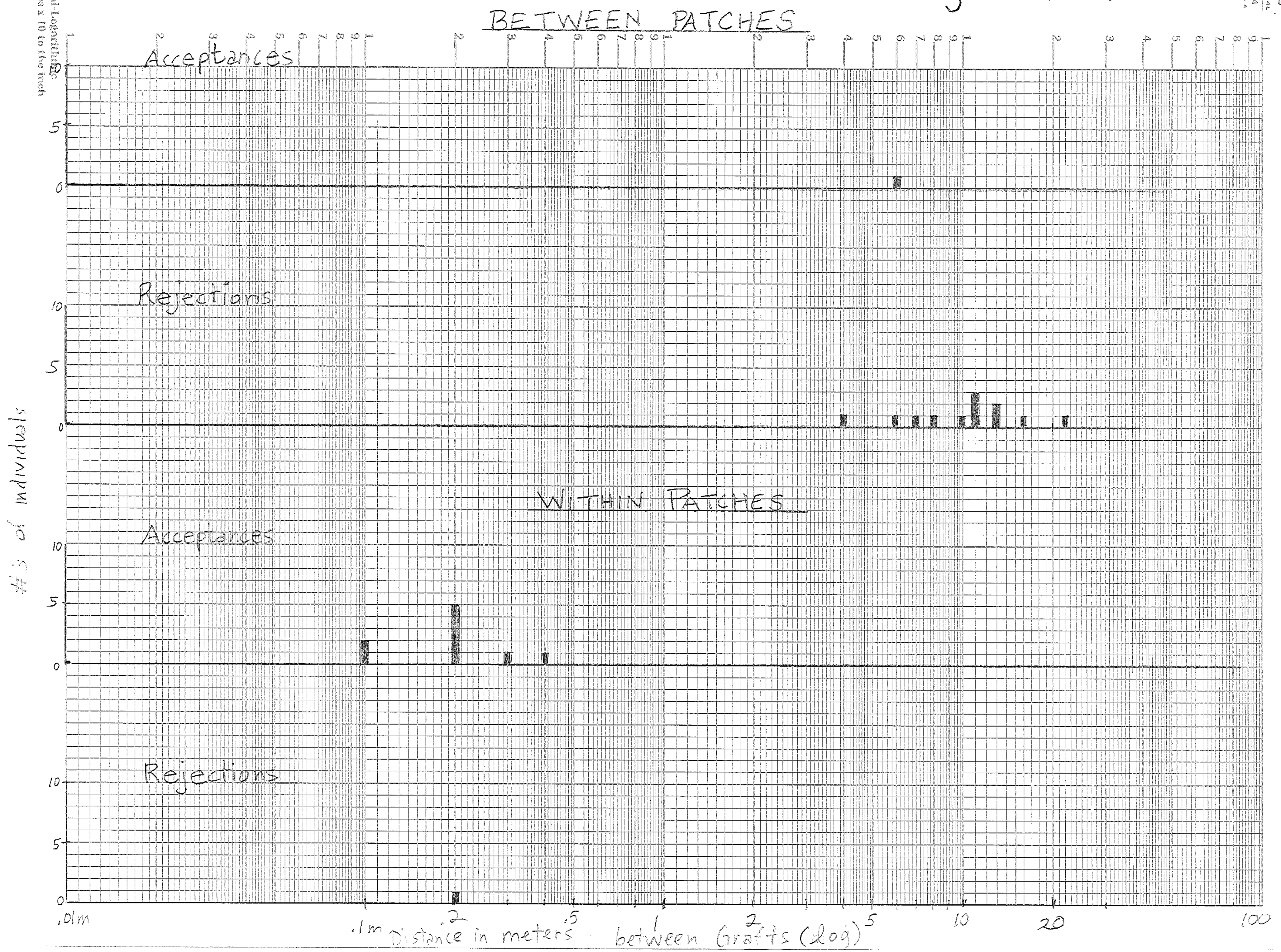
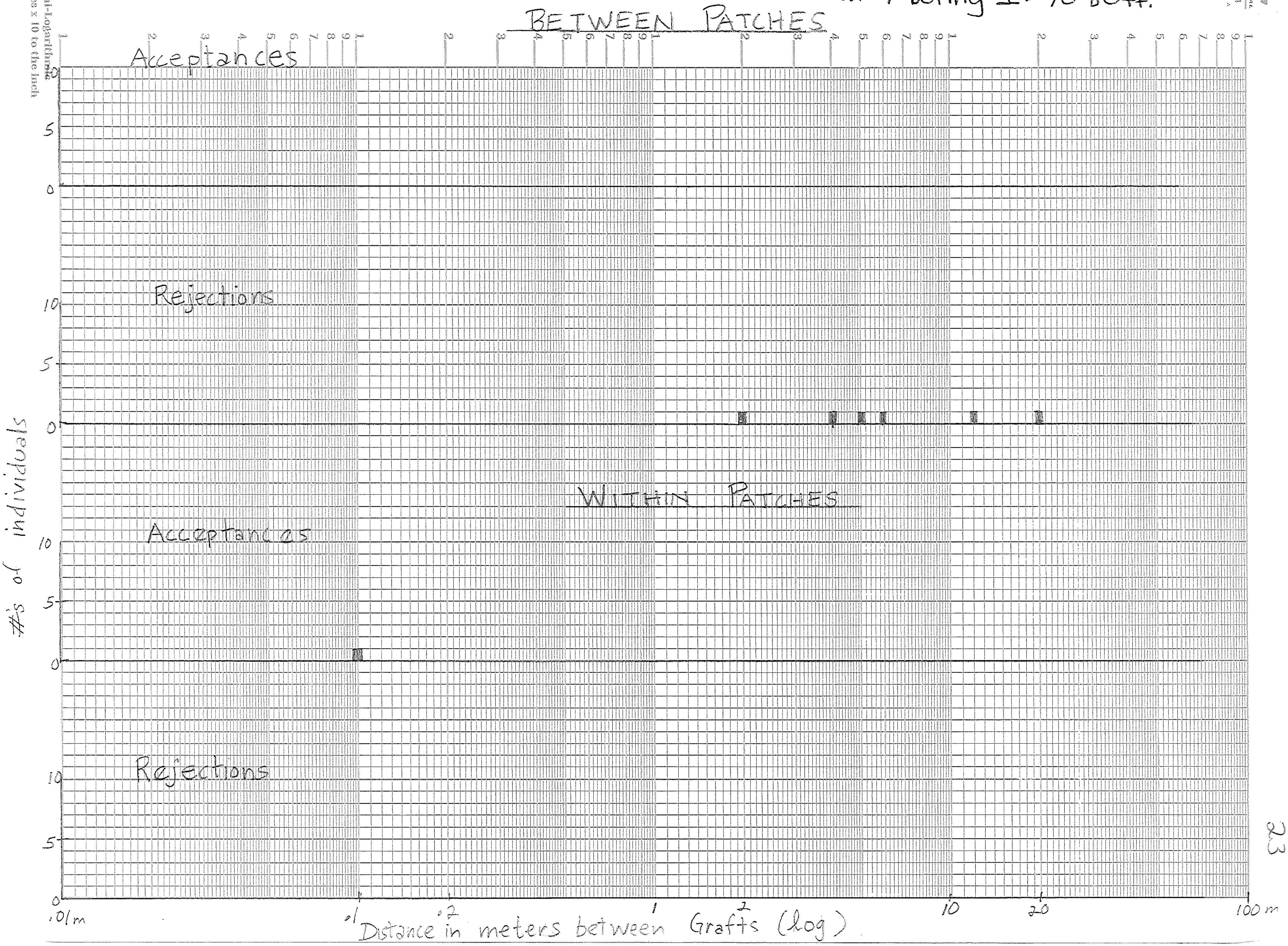


FIGURE 12: Graft Responses of Individuals over Distance (log) at Mooring 1: 70-80ft.



Discussion:

Results of these tissue grafting experiments on populations of the sponge Iotrochota birotulata do not support our first hypothesis that there will be more between-patch allograft acceptances at greater distances from the parent (or donor) at the shallow, more turbulent sites. Our results show no between-patch graft acceptances at the shallow EBR (10-17ft) site while one between-patch acceptance was found at the 45ft. site. Variation in turbulence at different depths could possibly explain this result. Turbulence may be so great at the shallow site that it sweeps any potential clonal fragments far away from the parent (and therefore away from our grafting radius). By the same reasoning, decreased turbulence at a depth of 45 ft. could allow more between-patch acceptances since fragments would be less likely to disperse out of our grafting radius. A more probable explanation for these results is that it is unlikely that many arbitrarily chosen individuals from different patches will be genetically related to a particular parent donor; hence, the predominant trend of between-patch graft rejection. Thus the one between-patch acceptance probably occurred by chance. More pairwise combinations of grafting between individuals from different patches would probably result in more between-patch acceptances, revealing more about the genetic population structure at each site.

As expected, our data show that, at all sites, there is a greater proportion of within-patch acceptances than between-patch acceptances. One might expect this pattern to occur if fragmentation is the predominant means of dispersal for this sponge. Fragments generated from a parent sponge have the best chance of survival if they attach immediately to the same substrate as the parent. If fragments are carried greater distances, it is possible that they will be dispersed to completely unsuitable environments. Our results support this possibility since, with only one exception, all allograft acceptances were within patches. In addition, the greatest numbers of individuals exhibiting acceptance responses had very short donor to recipient distances. (0.1m - 0.3m). Our results also show a general trend of increased graft rejection with increasing distance between individuals. Jokiel et al. have concluded that asexual reproduction is the primary cause of this pattern. Not only do fragments have a lower chance of survival at greater distances from the parent substrate, but fragments are also physically limited in their ability to disperse. Unlike motile larvae which can travel great distances, fragments, sometimes negatively buoyant, rely completely on environmental factors such as wave action for dispersal.

The population structure of sponges at each of the sites could also be explained by vegetative propagation (. fragmentation). Figures 1-6 are maps of the spatial distribution of genetically related individuals as defined by histocompatibility responses. From them, one can see that _I. birotulata_ is found in clumps of genetically similar, if not genetically identical, individuals. This is consistent with Neigel's findings about the population structure of _I. birotulata_ in Discovery Bay (in 1983) Neigel explained this clumping pattern of the sponges by the fact that _Iotrochota_ cannot successfully colonize loose coral rubble or sand; thus, these substrata act as barriers to vegetative propagation. Like Neigel, we found that clumps of _Iotrochota_ were confined to solid coral outcroppings separated by sand. This lends further support to the idea that fragmentation is an important means of dispersal for this sponge.

However, sexual reproduction could conceivably produce this clumping of histocompatible individuals observed in the population structure. For example, if gamete and larval dispersal are limited enough to restrict gene flow within a population, closely related individuals with the same genetic determinants of histocompatibility might occur together in clumps. Unfortunately little is known about larval dispersal, the genetic composition of histocompatible individuals or the relative importance of sexual and asexual reproduction in these sponges.

Our study also reveals a new (unexpected) finding concerning within-patch graft acceptances at each depth. We found a significantly greater within-patch acceptance response at the 45' site (90%) than at the shallow site (40%). Again, this can be explained by the greater turbulence in the shallow waters on the EBR. When fragments are generated in the turbulent water, they are more likely to be swept away from the parent before they are able to attach. In the same manner, at the less turbulent 45' site, fragments have a better chance of settling on the same substrate as the parent donor.

In conclusion by studying graft responses of the sponge Iotrochota birotulata at different depths, we showed that turbulence probably does have a great effect on its population structure. Also, as did Neigel in his study (1983), we found a clumped distribution of genetically similar individuals in Iotrochota populations. Unfortunately a major drawback of our study is that we did not include more pairwise combinations in our grafting experiments. It is possible that many more individuals within and between patches could be histocompatible than were actually tested. Still, our grafting techniques proved to be sound as shown by the 100% autograft acceptances. Also, _Iotrochota_ was a particularly easy sponge to work with because of its clear-cut acceptance and rejection responses within a short period of time.

Other improvements in a future study are beyond the scope of our abilities. Ideally, in order to resolve the controversy between the two afore-mentioned schools of thought, one would need to isolate the DNA of sponges and compare the genetic composition of histocompatible individuals. If this were done successfully one would be able to conclusively determine the spatial and genetic structure of these invertebrate populations. One would also be able to better understand the relative importance of sexual versus asexual reproduction in these organisms.

Excellent study and report!

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