BARD

FINAL REPORT

PROJECT NO. I-747-84

The Genetic Improvement of the Freshwater Prawn, *Macrobrachium Rosenbergii*

S.R. Malecha

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Title:
Genetic Improvement of the Freshwater Prawn, Macrobrachium rosenbergii

Investigators Names	Investigator's Institutions
SPENCER MALECHA	University of Hawaii
GIDEON HULATA	Agricultural Research Organization

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Spencer R. Malecha

Signature, Principal investigator
Spencer R. Malecha
University of Hawaii

INTRODUCTION/EXECUTIVE SUMMARY

The main purpose of the Hawaii based research was to: (1) produce and maintain a genetically diverse population of freshwater prawns and, (2) conduct mass selection for individual size using females.

Figure 1, reproduced from the BARD proposal that was funded shows the overall scheme that was proposed.

A synthetic population composed of genotypes from geographical populations was created. This population (ABCDEFGH in figure 1) was constructed. Selection within the synthetic population was not initiated.

Several problems in mating parents in the synthetic population were encountered during the execution of the work. However, facilities for maintaining replicated selected control lines were constructed and are available for future work.

The adult population of prawns representing all geographic genomes (Malaysian, Sarawak, Thailand, Ceylon, Australia, Philippine, Anuenue, Palau) has been constructed. The subsequent generations of this group was also bred and maintained. Randomnly chosen males and females from this population and from a commercial pond population have been shown to mature and mate.

Not all geographic strains nor all crosses performed well. We had had difficulty completing Australian reproductive cycles. While the adults mate easily, many of the females lose their eggs before hatching. Eventually we bred them, and had successful hatches. Also, the cross-bred group containing four geographic genomes which include Australian and Philippine animals seemed to have sterile, non-mating females. The males performed well, however,so all of our final base population animals (ABCDEFGH in figure 1) are from males of this group and females from the bred group comprising the other four geographic strains. The final base population both mated well and grew well.

Shipment to Israel of geographic groups and base population animals was done as the appropriate animals become available. The first shimpment (Spring 1986) consisted of Philippine and base population (F_1) post larve. The second shipment (Summer 1986) was of base population animals (F_2) which did not survive transit. All procedures were standard. The Fall 1986 shipment comprised a base population (F_2) and Philippine animals (multiple parentage). We provided the Israeli group with Australian animals early in 1986.

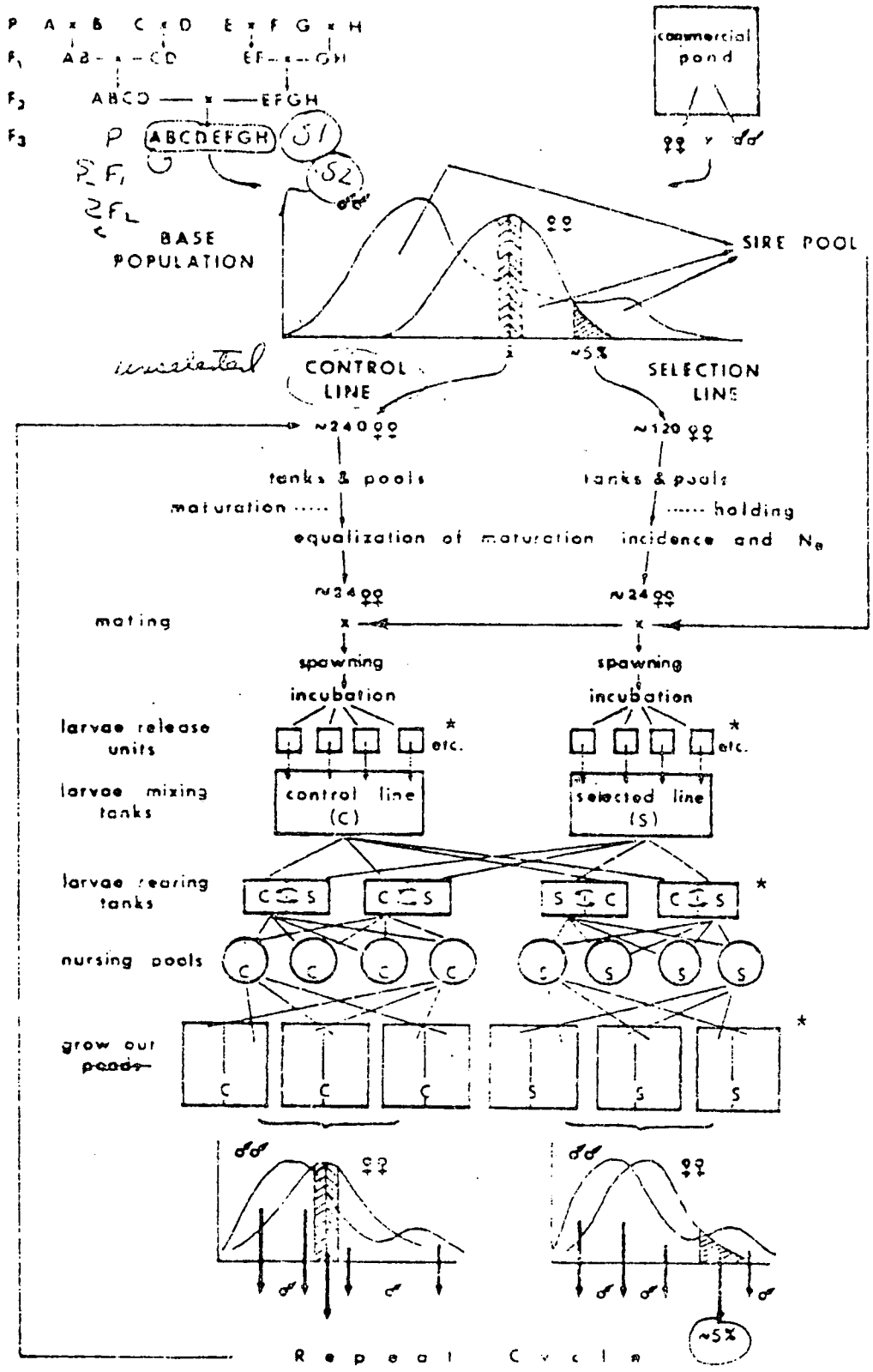


FIGURE 1. General scenario for proposed selection. Selection will be carried out within both a commercial and synthetic population although only one flow diagram is depicted. * = space limitations do not allow depiction of all larval release units, larval rearing tanks and ponds. Also dispersal patterns of larvae to nursery pools and of juveniles from the latter to ponds is shown for only two cases within each line.

ELABORATION OF WORK

Following the generation of the creation of the base population (ABCDEFGH, figure 1) and a subsequent generations within it, work was devoted to the following:

1) Extending the progeny generations in the base population that was to be used in establishing the selection lives.

2) Constructing a experimental facility.

Base population generation

Prior to the 1986-87 research year a base population, called th "F3 generation", composed of ABCDEFGH genomes was produced from a cross representing a "ABCD male" and "EFGH female" parent. (A...H represent the individual prawn geographich stocks). The original design (figure 1 section 5.3.3 of the original proposal) calls for the selection of females from a base population generated as an F4 group directly from an F3 parental population. We changed this design to the selection of females from an F5 generation. We did this to allow least a few generations (between the final combination of all the geographich genomes in the F3) to progress before making the initial selection in order to reduce some of the effects of the linkage disequilibrium that would be present in the parental F3 population.

In addition, the F3 parental population was generated with the cross ABCD males X EFGH females. We attempted to generate the receprocal cross, in order to further reduce linkage disequilibrium. However this was unsuccessful. We therefore emphasized the additional two generations (F4 & F5) of progeny.

Pond broodstock

The base population progeny generation groups were reared to reproductive maturity in ponds, and thinned randomly throughout the grow-out to reduce numbers and promote growth into large (highly fecund) animals. In addition, population growth and size variation was monitored. At the beginnng of the research, approximately 30% of the population was harvested. Twenty females and 15 males which were removed to the hatchery as broodstock; the other 60% was returned to the ponds for additional growth and monitoring. In May 1987 the previous used research ponds were drained and 10 males and 10 females were removed to the hatchery as "back-up" parental generation from which the selection population was to be generated (50:50 males & females). These animals were transported to another research facility, the University of Hawaii's Marine Research Training center (MRTC) (see figures 2, 3, & 4) where they were stocked into larger ponds for additional grow-out.

The population characterization and initial selection of gravid females carrying the F5 base population generation was

completed. All females were between 80 and 100 grams, and approximately 10-20% of the population was gravid.

It is clear from the data collected that prawn population randomly thinned to achieve low density can achieve large average female sizes (> 60 grams) thereby potentially increasing the selection intensity since, the higher ablsolute fecundity (egg number) of the larger female allows the use of smaller number of breeding females. Also the random thinning of the population allows us to possibly eliminate the laboratroy-based sire pool.

Hatchery broodstock

In addition to the continuation of the maintenance of base population pond broodstock generated 7100 additional F4 progeny groups were generated from individuals held in hatchery broodstock tanks. One group was produced from combining the broods of four berried females. In Feb. 1987 portions of the this progeny group was shipped to Israel as part of this program.

Another F4 group was generated from a single gravid female mated to a single individually held male. This F4 group has been combined with the hatches that were used to stock ponds, in effect creating a F4 hatchery broodstock and a pond-reared F4 broodstock.

Shipments to Israel

In 11/86 a shipment of post-larvae was sent to Israel. this consisted of 500 individuals from a progeny group composed of the combined hatches of 3 females, 2 taken from a broodstock pond and one form a hatchery broodstock pool. In addition 100 post-larvae, from the hatches of three separate females were sent to Israel.

In 2/87 500 Australian post-larvae hatched from a single female was shipped to Israel along wit 500 post-larvae from the combined hatches of four individual females representing a F4 group.

New Facilities

The experimental pond complex originally described in the proposal were found to be unsuitable for the conduction of long term growth studies as needed in the project. The facility is some distance from the University Campus and problems of access were beginning to occur along with unsatisfactory maintenance of the ponds by the contractor. Consequently, decision was made to terminate the lease with the private company on whose property the ponds were located. A search was made for an alternative site and one found on the recently acquired University of Hawaii's Marine Research & Training, MRTC, located on the windward side of the island about 45 minutes coloser to the University by car (see figures 2, 3, 4). During the negotiation

for the use of this new facility and the winddown of activities at the previous one, a delay in the research work was incurrred. This, in part, motivated us to maintain the F4 populations, by thinning as described elsewhere. All F4 pond broodstock were transferred to the new facility and staffing & pond management techniques worked out. The new facility has several suitable ponds (ponds 1, 2, 3 in figure 2) to hold low density broodstock populations and to subdivide using mesh screening into smaller units needed for replication of the selected and control lines. We have obtained some non BARD funds to use with re-budgeted BARP funds to sub-divide the existing 1 acre ponds (figures 3 & 4). This construction is completed.

As proposed in the original proposal we estimated a selection differential to be approximately 13 grams, the selection response to be 1.63 grams/cycle or 4.80 gram over 3 cycles. We estimated this to be approximately 2.7 grams above the minimum statistically detectable difference using 8 replicates/line and assuming a heritability of .25 for females. Therefore we have increased the minimum number of units for replicate control and selection lines to 20 in-pond cages (figures 3 & 4). With the flow-through capability, the among-replication variance should be reduced even further, allowing a more precise estimation of the selection response.

The final stages of the research work in the last two years was devoted to establishing the grow-out system for the selected lines and treating disease out-breaks that occured in the broodstock.

Specific Facilities Renovation

A pond at the University of Hawaii's Mariculture Research and Training Center has been dedicated to this project. The pond was drained and all base population broodstock were removed. The pond bottom was then prepared to receive the in-pond-cage walls. The diagrams accompanying this report shows the construction general layout of the cages (figures 2-4). Each cage is constructed of solid corner posts between which is hung heavy duty melting material. Due to weather conditions considerable difficulty was encountered in preparing the pond and constructing the cages but work has been completed.

Broodstock/disease management

The base population broodstock was found to be infected with a epi-zootic algae, Epistylis (sp).

This required immediate action and treatment. The following events were recorded:

1. Drained broodstock ponds and recovered 180 females and 42 males. Twenty males and females died during the process. Animals placed in pens in another pond.

2. Pens were sampled routinely using a cast net to catch a sample of 10 animals, which were observed for health conditions.
3. A freshwater line was put into the pond 3 containing the pens.
4. Water quality continuesd to deteriorate after this procedure.
5. Twenty (20) dead animals floating in pens were removed.
6. Pens were seined and some live animals were removed and put in hatchery tanks; Dead animals were removed.
7. One hatchery tank was treated with 100ppm Formalin as perscribed by Dr. Jim Brock, the veterinarian extension specialist. No mortalities observed subsequently. The other tanks were untreated.
8. No mortalities were observed in treated or untreated tanks. Samples were taken to Dr. Brock for examination, who recommended treeatment at 200ppm. In addition, pond water samples from pond were taken for a water quality examination.
9. Samples were taken from all tanks. Less Epistylis (sp) was observed through time. Animals were kept on a flushing regimen for several days.
10. All animals in hatchery tanks were sampled. Gravid females stripped their eggs. Most of the Epistylis (sp) was gone from all the prawns and they were observed to continue to eat it off their bodies.
11. Daily monitoring of animals was carrried out and we prepared to drain the pond to remove any remaining prawns in pens and any that may have escaped into the pond.
12. The pond was drained and animals removed from pens and transferred to hatchery tanks. No prawns were found outside of pens. There were only a few prawns with a little Episytlis (sp). Animals in hatchery tanks were thinned out and distributed to other tanks.
13. Animals were continued to be observed and tanks kept clean with flushing. Subsequently there was no signs of any Epistylis (sp).
14. We reduced density in hatchery tanks to produce better male to female ratios for mating purposes. A total of 54 females and 12 males were retained.
15. The remaining females, 24, were taken to the hatchery and placed in shallow tanks divided into 8 equal compartments. These females were bred to sibling males held as reserves.

Breeding

Despite the set backs due to the Epistylis (sp) out-break and the unforseen delays in constructing the in-pond cages, the project moved forward.

Mating did progress to a point but repeated attempts to mate certain parents have not been successful. The remaining parents, which are few in number were mated when an appropriate female is in reproductive condition.

Facilities Up Keep

A program of minimal up keep of security of the broodstock facility is ongoing.

Facilities

Figures 2,3, and 4 below refer to the location of the new growout facilities that were constructed for this project.

Figure 2 shows the general location of the research facility and pond no. 1 which was divided with rigid cages to maintain the replications of selected lines. Pond no. 2 was used to grow out some generations of the synthetic base populations.

Figure 3 is a schematic of the pond cages.

Figure 4 contains photographs of the pond cages themselves.

The facility is very well equipped for maintaining separate genetic lines or other experimental groups.

CONCLUSION

Major accomplishments

The major accomplishment of the work done in Hawaii has been three fold:

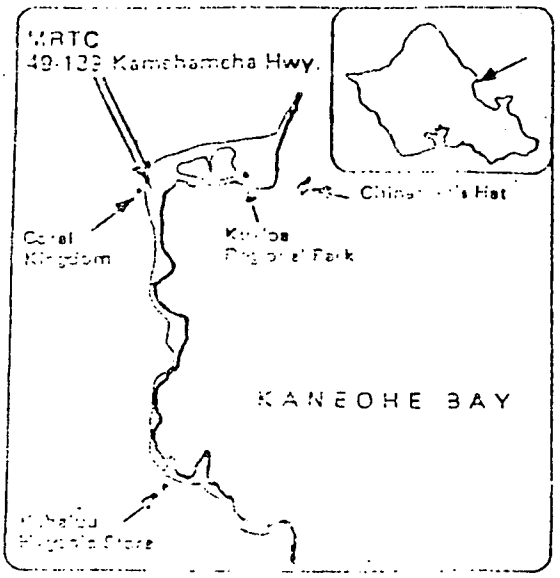
(1) collection and maintanance of geographically diverse prawn genotypes,

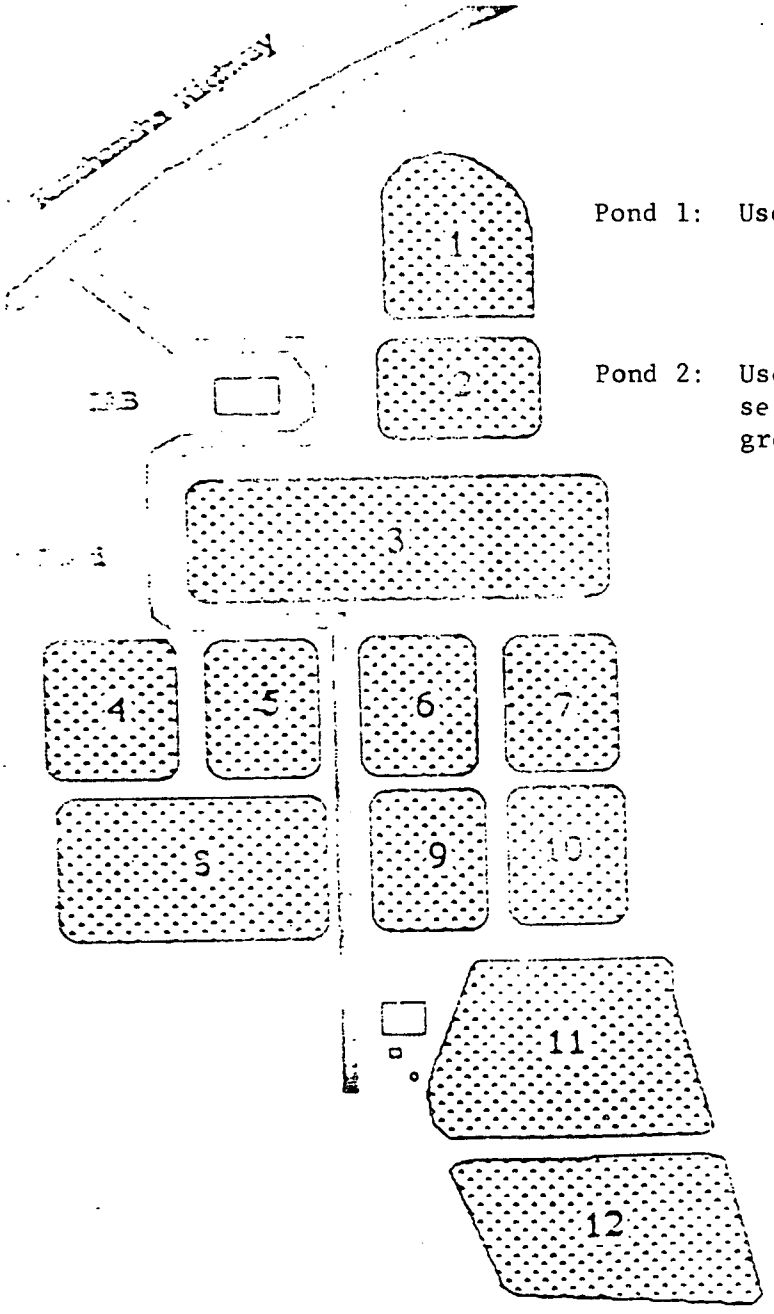
(2) creation of a genetically diverse population composed of all geographic genotypes,

(3) construcition of a suitable facility for maintaining replicate of selected lines.

Recommendations

Problems were incurred in obtaining consistent reproductive performance from some geographic groups and maintaining the base population due to logistic and disease related problems. Because of this the actual selection process within the base population was not accomplished. However, the base population and its parental geographic parental genotypes represent a unique "genetic conservation" resource and should be preserved and utilized. At the moment the numbers in the base populations are dwindling and an effort, either by private industry, the public sector, or genetic conservation organizations should be made to save (preserve) the genetic base population for future use.





Pond 1: Used for cage

Pond 2: Used for selection lir growout.

Pond No.	Size (m²)	
1	.6	2430
2	.4	1620
3	1.5	6070
4	.5	2025
5	.5	2025
6	.5	2025
7	.5	2025
8	1.0	4050
9	.5	2025
10	.5	2025
11	1.5	6070
12	1.0	4050

Figure 2 . Schematic of ponds at the Mariculture Research and Training Center of the Hawaii Institute of marine biology. This map shows the relative size and location of each pond. Ponds 1 and 2 are presently freshwater, while the salinity in ponds 3 through 12 can be varied between freshwater and 30 ppt.

Ocean

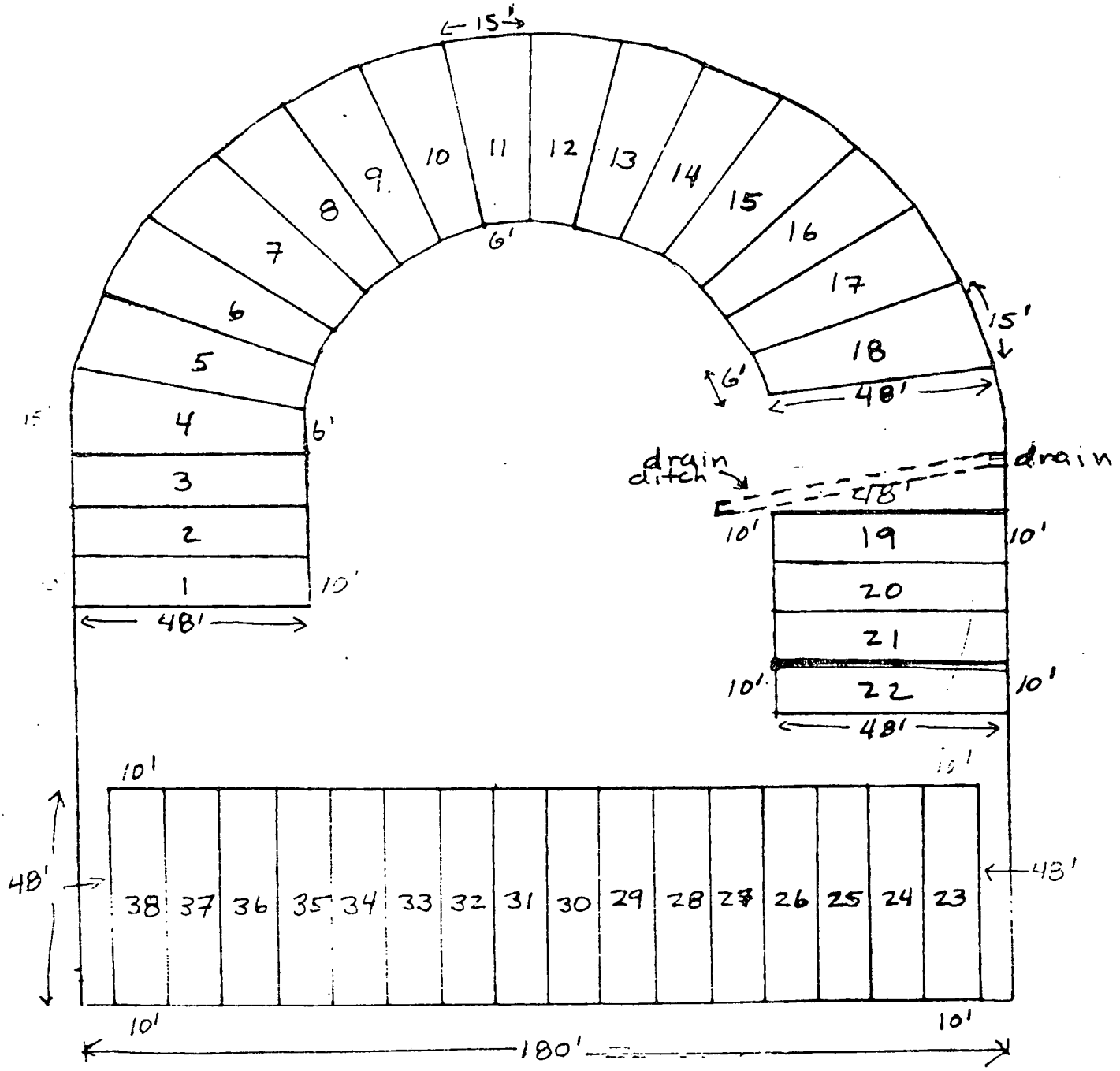


Figure 3. Construction of side-walls to in-pond cage system to be used to house selected lines.

Figure 4 Configuration of 38 in-pond cages to beused to house selected lines. Each cage is approximately 500 ft^2. Twenty-three (23) cages are 10 x 48 x 6 ft, 15 cages are 15, 48 x 6 ft.