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THE STATUS OF MICHIGAN'S DOMESTIC SALMONID BROOD STOCK PROGRAM

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SUMMARY

The brood stock program now being implemented at the Harrietta Brood Stock Hatchery is defined and explained.

The selection program presented is based on the maintenance of "families" which are inter-bred to prevent gene deletion. Families of each species have been selected and are now being reared.

The production program presented is based on the utilization of heterosis as achieved by the annual production of F_1 strain hybrids. Brown strain hybrids obtained from the Ha x S.D. cross evidenced superior growth and mortality patterns as compared to either parent strain. The superiority is assumed to be an expression of heterosis. Rainbow strain hybrids produced by crosses of the Man, Wis, and Ha strains did not evidence heterosis i.e. hybrid superiority.

A glossary of technical terms has been included for the benefit of the reader.

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INTRODUCTION

The maintenance of a population of fish for the purpose of predictable propagation of a species dates back through history and has changed little over the centuries. Brood stock is a necessity for the predetermined predictable fisheries program and yet the application of genetic theory to fisheries has lagged far behind its application to other fields of agriculture.

Michigan's salmonid brood stock program, like many others, has therefore received increasing criticism, and well it should. Michigan has maintained a brood stock for the purpose of predictable production of eggs and hence fish, but at that point the program stopped. The program of today has to go further for the fisheries biologist has finally realized that a fish is more than just a fish. However, before a brood stock program can be either criticized or improved, it must be understood.

To understand a brood stock program, one must realize that it is a two phase operation. The first phase is the continued propagation of the species and this phase must protect the species from the disasterous effects of inbreeding while at the same time it must produce an economically feasible product. The second phase is the annual production of a given number of eggs [fish] of a quality which will give the best results possible in the greatest number of environments.

These two phases of the program are not necessarily congruent and most criticism has been directed at this fact. That is; the best product of the hatchery selection may not be the best product for providing survival and hence a fishery in the average lake. The brood stock program now in use in Michigan is designed to come to grips with this incongruency. The program has two distinct parts: Brood stock selection; and Product improvement.

Brood stock selection has traditionally been geared to the selection of phenotypic expressions such as; eggs size, eggs per female, conversion, condition factor, color, etc. In such a selection, little consideration has been given to the genetic changes which have occurred due to the phenotypic selection. The inherent danger of such a program is that phenotypic selection all too frequently has led to increased inbreeding or line breeding and population gene pools have been decreased. The result has been a fish selected for a specific environment [the hatchery] which would have poor or no survival in a different environment.

The brood stock selection program now being applied in Michigan is patterned after the model set forth by Graham A. E. Gall[1], in his work for the State of California. His selection is based on the phenotypic expressions of genotype which are desirable for economic production, however, emphasis is placed on the prevention of gene deletion. This is accomplished by strict maintenance of several families which are inter-bred to maintain the heterozygous alleles which were present in the original population. This approach allows for continued phenotypic selection for more economical production while at the same time it insulates the phenotypic selection from directly affecting population genotype.

A program of this type guarantees the maintenance of the status quo, however, it makes relatively slow progress toward the second phase of our program; that of product improvement. It is in this phase of brood stock program that Michigan has gone beyond the work of Gall.

Geneticists working in other fields of agriculture proposed the theory of "strain hybridization" many years ago, however, the field of fisheries has not pursued the possibilities. The program in use in Michigan has ventured across this gap.

Michigan's product improvement program is based on the predictable production of strain hybrids from pure parent strains which have been chosen on the basis of their evidence of hybrid heterosis. The application of genetic theory to the strain hybrid indicates that the hybrid which evidences "hybrid vigor" does so because of increased heterozygosity. Increased numbers of heterozygous alleles inrich the biochemical and physiological capabilities and should theoretically increase the ability of the fish to survive in different environments. A program of this structure should never reach stalmation since the evaluation of hybrids produced by the current pure strains crossed with new strains continues to allow for product improvement. (?)

Michigan, therefore, is now being programmed to maintain at least two strains of each species, each of which will be selected along pure strain lines. Yearly production quotas will be met with an F_1 strain hybrid which has been chosen on the basis of its expression of heterosis.

METHODS AND RESULTS

Pure Strain Rainbow

In 1970, the Harrietta Brood Stock Hatchery was supplied with two additional strains of rainbow trout (*Salmo gairdneri*). Each strain was received as eyed eggs from the fall spawning of 1969. One was received from the federal brood stock hatchery located at Manchester, Iowa; the other came from the brood stock of the state of Wisconsin. The three strains will henceforth be identified as: 69 Ha (Harrietta's own strain), 69 Man (Manchester strain), and 69 Wis (Wisconsin strain). All three were reared under identical conditions and produced their first eggs in 1972. Comparative growth and egg production is summarized in Tables I and II.

The cost of feed per pound of fish produced reflects not only growth but also mortality and this is responsible for the changes in cost/lb. which occurred between the strains from 1972 to 1973. This type of data now becomes part of the pedrigree history of each pure strain, however, it should no longer be used as a basis for strain selection.

TABLE I

Growth-Cost Summary Rainbow Strain Study 1970-1973

	Pounds Fed	Feed Cost	Pounds Fish Produced	Four Year Conversion	Feed Cost/ Lbs. Produced	Mean Length 11/1/73	Condition Factor Females
69 Ha	13,909	1887.68	6180	2.250	.3054	20.20	5.02
69 Man	17,325	2290.79	6481	2.673	.3535	20.73	5.19
69 Wis	17,312	2220.47	8220	2.106	.2701	20.88	5.36

TABLE II

Egg Production Summary Rainbow Strain Study 1972-1973

	Feed Cost/ Lbs. Produced	Weight/ Female In Lbs.	Cost/ Female	No. Eggs/ Female	Food Cost/ 1000 Green Eggs	Eye-up	Food Cost/ 1000 Eyed Eggs
1972 Spawning							
69 Ha	.2099	2.577	.5409	2123	.2548	61.8	.4123
69 Man	.2176	2.588	.5631	2276	.2474	70.8	.3495
69 Wis	.2188	2.717	.5945	2277	.2611	42.8	.6104
1973 Spawning							
69 Ha	.3054	4.137	1.263	3285	.3845	49.7	.7734
69 Man	.3535	4.623	1.634	3960	.4126	67.3	.6131
69 Wis	.2701	4.879	1.318	3814	.3456	49.3	.7011

During the spawning of 1972, one hundred females of each strain (69 Man & 69 Wis) were mated with one hundred males, one male per female, according to the selection program prepared by Graham A. E. Gall. Each mating was then incubated individually and when the eggs were eyed each mating was scored according to the format of Modification of System Presented by Graham A. E. Gall (see Appendix). The twenty best matings were then reared individually until the first lot reached 500/lb. At that time each mating was scored again according to Modification of System Presented by Graham A. E. Gall, Part B. The ten best matings were given identifying clips according to Part C and were then combined for rearing. Table III compares the top fifty percent of each strain, while Table IV compares the 10 best matings of each strain.

When this generation of brood stock reaches maturity, the selection of the next generation will be accomplished by mating a minimum of ten females from each family with the males of the other nine families. The resulting 100 lots of progeny will be selected via scoring, however, the ten lots finally chosen cannot be composed of more than two lots from any one of the original ten families.

The selection process via scoring is designed to increase the frequency of occurrence of the genes controlling given desirable phenotypes., i.e., large eggs. The inter-family matings and the limits on family representation are designed to prevent inbreeding thereby protecting population gene pools.

Rainbow Strain Hybrids

During the fall spawning of 1972, six lots of reciprocal hybrids were made between the three strains of rainbow. One lot of each hybrid was transported to Wolf Lake Hatchery for rearing and one lot of each was reared at Harrietta. All lots were maintained individually until Wolf Lake was forced to discontinue their experiment. At that time, Harrietta's lots were identified by clip and combined. They were reared as one lot for another nine months; until they were one year old. They were then sorted and individually measured. All data is summarized in Tables V and VI. The lots have now been planted into a pond where they will be allowed to grow under wild conditions for another year.

When they reach the age of two years, the pond will be emptied, and the survivors will be captured so that growth and survival can be determined.

Pure Strain Brown

During 1970, Harrietta Hatchery was supplied with two additional strains of brown trout (*Salmo trutta*). Eyed eggs were received from the 1969 fall spawning of brood stock from the state of South Dakota. Adult browns of the strain selected for furunculosis resistance by Dr. L. Allison were received from the Grayling State Fish Hatchery, Grayling, Michigan. The three strains will henceforth be identified as: S.D. (South Dakota), Ha (Harrietta's strain), and GSF (Grayling select furunculosis resistant).

TABLE III

Scoring Summary Rainbow Pure Strain Selection Program

	No. Females Sampled	No. Eggs/ Female	Percent Eye-Up	No. Eggs/ Ounce	First Score (Mean)
69 Man	55	2342	88.4	600	26
69 Wis	49	2387	70.1	751	15

TABLE IV.

Scoring Summary 10 Best Females Rainbow Pure Strain Selection Program

	No. Eggs/ Female	Percent Eye-Up	No. Eggs Per Ounce	First Score (Mean)	Percent Fry Mortality	No./Lb. At 2nd Scoring	Second Score (Mean)
69 Man	2448	92.6	560	30	26.0	647	39
69 Wis	2544	81.5	674	22	75.2	715	20

TABLE V

Comparative Data - Individual Rainbow Hybrid Lots

	69 Ha F. x 69 Man M.	69 Ha F. x 69 Wis M.	69 Wis F. x 69 Ha M.	69 Wis F. x 69 Man M.	69 Man F. x 69 Ha M.	69 Man F. x 69 Wis M.
% Loss To Eye-Up						
W.L.	29.9	23.2	37.7	47.9	32.8	50.9
Ha	27.7	37.5	45.1	72.9	32.8	50.9
% Loss Eye-Up To End Of 3 Months						
W.L.	15.3	26.5	14.5	26.4	24.9	18.2
Ha	69.7	56.9	46.7	25.2	64.4	48.3
Total % Loss To End Of 3 Months						
W.L.	45.2	49.7	52.2	74.3	57.7	69.1
Ha	97.4	94.4	91.8	98.1	97.2	99.2
Length Gain To End Of 3 Months						
W.L.	1.596	1.612	1.632	1.600	1.544	1.692
Ha	1.481	1.569	1.583	1.434	1.111	1.674
2nd Exp. 3-12 Months (Harrietta Only)						
Length Gain	4.663	4.577	4.697	4.944	4.585	4.693
Clip	R.V.	L.V.	L.P.	R.P.	ANAL	NONE
% Re-covered	44.4	34.8	16.9	49.7	14.1	146.1

TABLE VI

Comparative Data - Reciprocal Rainbow Hybrids

	69 Man x 69 Wis	69 Ha x 69 Wis	69 Ha x 69 Man
% Loss to Eye up			
W.L.	49.4	30.4	31.3
Ha	61.9	41.3	30.2
% Loss Eye-Up To End Of 3 Months			
W.L.	22.3	20.5	20.1
Ha	36.7	51.8	67.1
Total % Loss To End Of 3 Months			
W.L.	71.7	50.9	51.4
Ha	98.6	93.1	97.8
Length Gain To End Of 3 Months			
W.L.	1.646	1.622	1.570
Ha	1.554	1.576	1.296
2nd Exp. 3-12 Months (Harrietta Only)			
Length Gain	4.818	4.637	4.624

Since the three strains of browns were all of different age classes, the comparisons of cost of hatchery production are based on average conversions given an equal cost per pound of feed (Table VII).

During the spawning of 1973, Harrietta strain females were individually spawned for brood stock selection. Selection is being carried out according to the format of Modification of System Presented by Graham A.E. Gall (see Appendix).

Brown Strain Hybrids

During the fall spawning of 1972 reciprocal hybrids were made between the Ha and SD strains. The GSF strain was not used for hybridization purposes because it had originally been selected from the Ha strain and hatchery rearing had indicated that it had not been changed sufficiently enough to be identified as an individual strain.

The brown hybrid lots and a control of each parent strain were reared individually for approximately 3 months. They were then identified by clip and combined for further rearing. When the fish reached one year of age, they were separated and measured. All data is summarized in Table VIII. The hybrids have now been planted into a pond where they will be allowed to grow under wild conditions for another year. When they reach the age of two years, the pond will be emptied and the survivors will be captured so that growth and survival can be measured.

Mechanics of Operation

The greatest expense of a brood stock operation is incurred during the growing of adult brood stock, therefore, Michigan's current program has been designed so that each species is cultured on an every other year rotation. Each species is composed of two strains, therefore, each strain is "selected" every four years (Table IX).

The generation length of four years is so great that improvement via the selection process will be slow. The selection and production data collected from a pure strain will be recorded as the strain pedigree. The pedigree will provide a reference for both measuring improvement and for assuring that pure strains are not accidentally regressing or converging.

If the quality of pure strains is at least maintained at the original level then the quality of a given F_1 hybrid should remain constant from year to year.

DISCUSSION

Rainbow Strain Selection Via Hybrid Data

The early rearing of individual rainbow hybrid lots at Harrietta and Wolf Lake evidenced fluctuating feeding and conversion levels. Therefore, growth data is meaningful only to the extent that all lots were overfed and thus could choose their individual levels of food consumption. The data collected at Harrietta during months 3-12 is more meaningful since all lots were together and were thus forced to compete for food. Theoretically, the more aggressive fish (ones evidencing heterosis) should have consumed more feed and evidenced more growth.

TABLE VII

Egg Production Costs Of Each Strain As Three Year Olds

	Average Conversion	Cost Feed/ Lb. Produced	Weight/ Female	Feed Cost/ Female	Eggs/ Female	Feed Cost/ 1000 Green Eggs
Ha	2.671	.4273	1.538	.6572	1691	.3886
GSF	3.618	.5788	1.559	.9023	1949	.4630
SD	2.602	.4163	1.879	.7882	2773	.2821

TABLE VIII

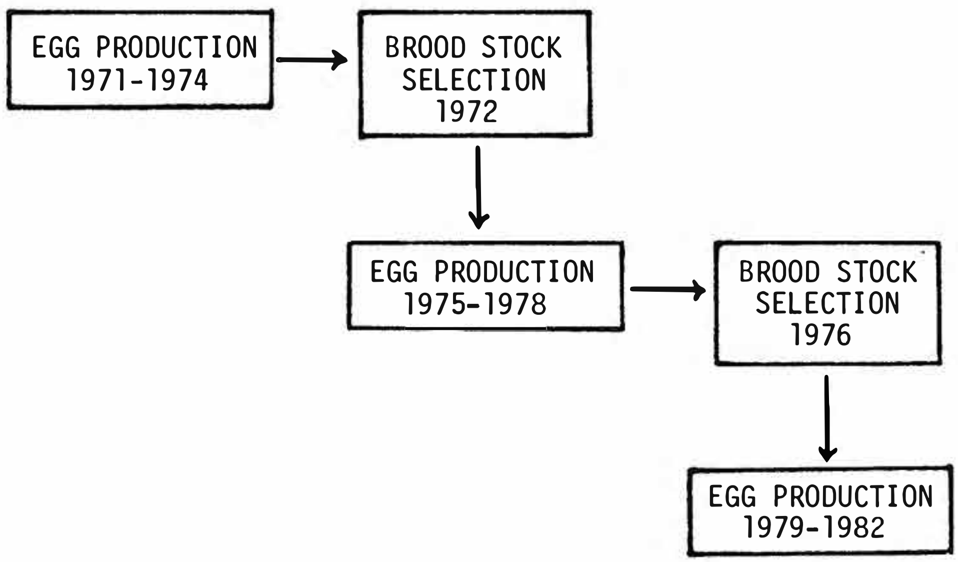
Comparative Data - Brown Hybrids And Control

	Ha x Ha	Ha F x S.D. M	S.D. x S.D.	S.D. F x Ha M
Loss To Eye-Up	31.3	16.9	11.1	16.8
Total Loss to Clip	44.3	42.1	59.1	39.8
Length Gain To Clip	1.470	1.680	1.464	1.527
Clip Given	L.V.	L.P.	ANAL	R.P.
Length Gain 1 Year	3.528	4.076	3.511	4.053
% Recovered	82.7	108.7	51.6	92.3

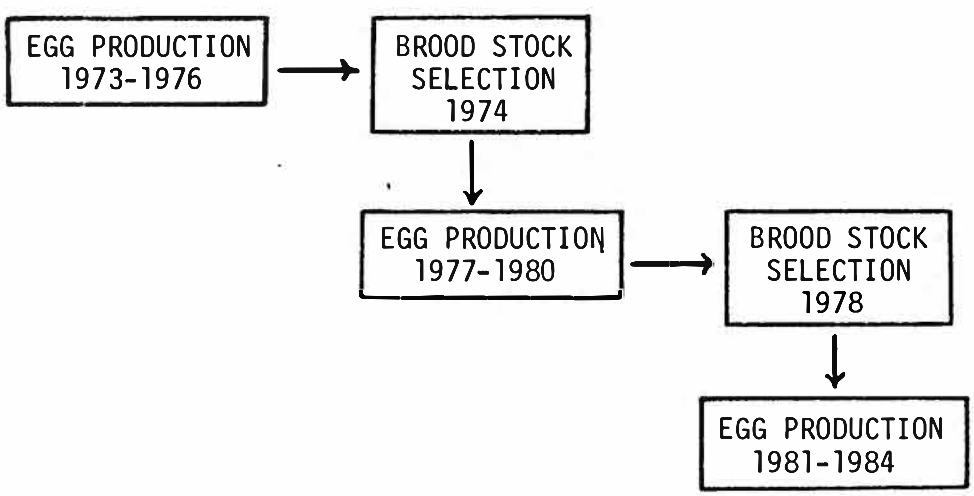
TABLE IX

Graphical Representation of Brood Stock Selection Program
(years are added for clarity)

Strain A.



Strain B.



The Ha x Wis and Ha x Man crosses evidence little difference. The mortality and length gains (Table VI) are practically identical as are the growth patterns and final mean lengths (see Length frequency % of population rainbow hybrids combined reciprocal lots, Appendix).

One hybrid of the Wis x Man cross, the Wis F. x Man M., evidenced a growth of .247 inches more than the other lots (Table V). It also evidenced a more uniform growth pattern as evidenced by length frequency distribution (see Length frequency % of population rainbow hybrids individual lots, Appendix). The reciprocal cross, the Man F. x Wis M. was contaminated when the lot was not clipped at the time the hybrids were combined. All the clips which regenerated were counted as part of the lot and contributed to a recovery rate of 146.% (Table V). If the lot had not been contaminated, it may have shown growth and length frequency data comparable to its reciprocal. This possibility is somewhat substantiated by the fact that the lot evidenced the greatest growth for the first 3 months at both the Harrietta and Wolf Lake hatcheries.

The total percent mortality for the Man x Wis cross (Table VI) indicates a higher loss than evidenced by the other two crosses. However, the mortality to eye up may be more indicative of hatchery techniques than of hybrid lot quality. Therefore, a more accurate picture may be obtained by comparing loss after eye up and this measurement does not evidence a significant mortality difference between the three reciprocal crosses.

The data collected does not seem to indicate the presence of heterosis in any of the existing crosses. However, due to the absence of pure strain controls, the comparisons are only between hybrid lots. The apparent absence of heterosis indicates a high degree of heterogeneity between the original pure strains. These results are comparable with the findings of the Russian investigators M. A. Andrizasheva[2] and G. G. Savost'yanova[3]. That is: the rainbow trout is so heterogenic that it is difficult to evidence heterosis in an intraspecific mating.

The continuation of a program of production of F_1 strain hybrids will help to provide protection for species quality and therefore is advisable in spite of the absence of evidence of heterosis. The six lots of hybrids are now growing wild and it is possible that survival data may determine which hybrid and hence which two pure strains should be cultured. In the interim Harrietta has retained all three strains.

If survival data is not conclusive, the ultimate choice of which strain to eliminate may have to be made on the basis of comparative strain pedigree data.

Brown Strain Selection Via Hybrid Data

The Ha x S.D. cross of brown trout evidenced early superiority over either pure strain. At three months, the reciprocal hybrid crosses averaged 10.7 percent less mortality than the parent strains and demonstrated increased length gains of approximately .14 inches (Table VIII).

At one year of age, the hybrids averaged .54 inches greater length gain and somewhat better survival. Survival as determined by recovery rates may not have been as impressive as the data (Table VIII) would indicate. First the pure S.D. strain was given an anal fin clip and many fish evidenced some degree of regeneration. Of the 3383 fish recovered, 253 did not evidence any type of clip. If these fish were from the S.D. lot, the recovery or survival would have been considerably increased. Secondly, the Ha F x S.D. M cross yielded a recovery rate of 108.7%. This would seem to indicate that either the original count was in error or that some left ventral clips were recorded as left pectoral. In the latter case, the pure Ha strain would have evidenced a higher survival rate than 82.7 percent.

The superior growth of the hybrids is evident in the length frequency data of Length frequency % of population brown hybrids (Appendix). Uniformity of growth seems to follow the tendencies of the maternal parent, however, overall superiority is beyond question.

The increased growth rate of 15 percent plus the tendency toward decreased mortalities would tend to indicate the expression of heterosis by the Ha x S.D. hybrids.

Unless the trends are definitely reversed by the survival and growth in the wild, Harrietta will proceed with a production program for brown trout based on Ha x S.D. hybrids.

RECOMMENDATIONS

The brood stock program as presented is designed for the protection of species quality and the production of a fish which can best fulfill the requirements of both the hatchery and the inland management sections of the Fisheries Division.

These objectives can only be achieved if the program continues to be progressive. Neither the demonstration of heterosis nor the absence of it should be cause for complacency.

The techniques of measuring the biochemical and physiological effects of heterosis are within reality. Tomorrow we may find "the hybrid" and be able to prove its superiority. To do so, we must continue to experiment with different pedigreed strains.

GLOSSARY

<u>Allele</u> — One of a set of alternative functional units located at corresponding sites of homologous chromosomes.

<u>Family</u> — The offspring (progeny) produced by the mating of a single pair of parents.

<u>Gene</u> — The "functional unit" of chromosomal structure responsible for inheritance. Gene proteins are coded in DNA as a linear sequence of nucleotides which can be separated by recombination.

<u>Genotype</u> — The sum total of an individual's or populations', compliment of inheritable determinants.

<u>Heterosis</u> — The expression of hybrid vigor by the offspring. Considered to be due to an increased compliment of dominate favorable alleles.

<u>Heterozygous</u> — The state in which the members of a pair of alleles are unlike.

<u>Heterozygote</u> — An individual in which the members of a pair of alleles are unlike. The individual cannot breed true for the characteristic controlled by the alleles.

<u>Homozygote</u> — An individual in which the members of a pair of alleles are alike. The individual will always breed true for the characteristic controlled by the alleles.

<u>Hybrid</u> — The offspring of the mating of two unlike or unrelated parents.

<u>Inbreed</u> — The mating of individuals, usually closely related, which results in increased levels of homozygosity.

<u>Line breed</u> — Continued inbreeding of the progeny of particular individuals.

<u>Pedigree History</u> — A complete record of ancestory.

<u>Phenotype</u> — The outward appearance of an individual as determined by the interaction of genotype and environment.

<u>Phenotypic Selection</u> — A selection program based on phenotype.

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3. Savost'yanova, G. G., Comparative Fishery Characteristics of Different Groups of Rainbow Trout. Genetics, Selection, and Hybridization of Fish. Translated from Russian for the National Marine Fisheries Service,1972.

APPENDIX

Modification of System Presented by Graham A. E. Gall

I. A. First Selection

1. Size of Eggs 33.3% of Total Score

225 & Larger	20 Points	476 - 500	9 Points
226 - 250	19 "	501 - 525	8 "
251 - 275	18 "	526 - 550	7 "
276 - 300	17 "	551 - 575	6 "
310 - 325	16 "	576 - 600	5 "
326 - 350	15 "	601 - 625	4 "
351 - 375	14 "	626 - 650	3 "
376 - 400	13 "	651 - 675	2 "
401 - 425	12 "	676 - 700	1 "
426 - 450	11 "	701 & Less	0 "
451 - 475	10 "		

2. Number of Eggs 33.3% of Total Score

4681-4840 or more	20 Points	3081 - 3240	10 Points
4521 - 4680	19 "	2921 - 3080	9 "
4361 - 4520	18 "	2761 - 2920	8 "
4201 - 4360	17 "	2601 - 2760	7 "
4041 - 4200	16 "	2441 - 2600	6 "
3881 - 4040	15 "	2281 - 2440	5 "
3721 - 3880	14 "	2121 - 2280	4 "
3561 - 3720	13 "	1961 - 2120	3 "
3401 - 3560	12 "	1801 - 1960	2 "
3241 - 3400	11 "	1641 - 1800	1 "
		1640 and less	0 "

3. Percent eye-up 33.3% of Total Score

95 - 100	20 Points	65 - 69	8 Points
90 - 94	18 "	60 - 64	6 "
85 - 89	16 "	55 - 59	4 "
80 - 84	14 "	50 - 54	2 "
75 - 79	12 "	50% & Less	0 "
70 - 74	10 "		

I. B. Second Selection

1. Size of Fingerlings 50% of Total Score

Largest rated as 100%. All other as a percentage of that lot.

Example:

Lot	Size	Calculation	%
1	25/oz.	-----------	100 %
2	27/oz.	$\frac{25}{27} \times 100$	93 %

Scored via table below

96 - 100 %	40 Points	71 - 75	20 Points
91 - 95	36 "	66 - 70	16 "
86 - 90	32 "	61 - 65	12 "
81 - 85	28 "	56 - 60	8 "
76 - 80	24 "	51 - 55	4 "
		Less than 50%	0 "

2. Percent fingerling mortality 50% of Total Score

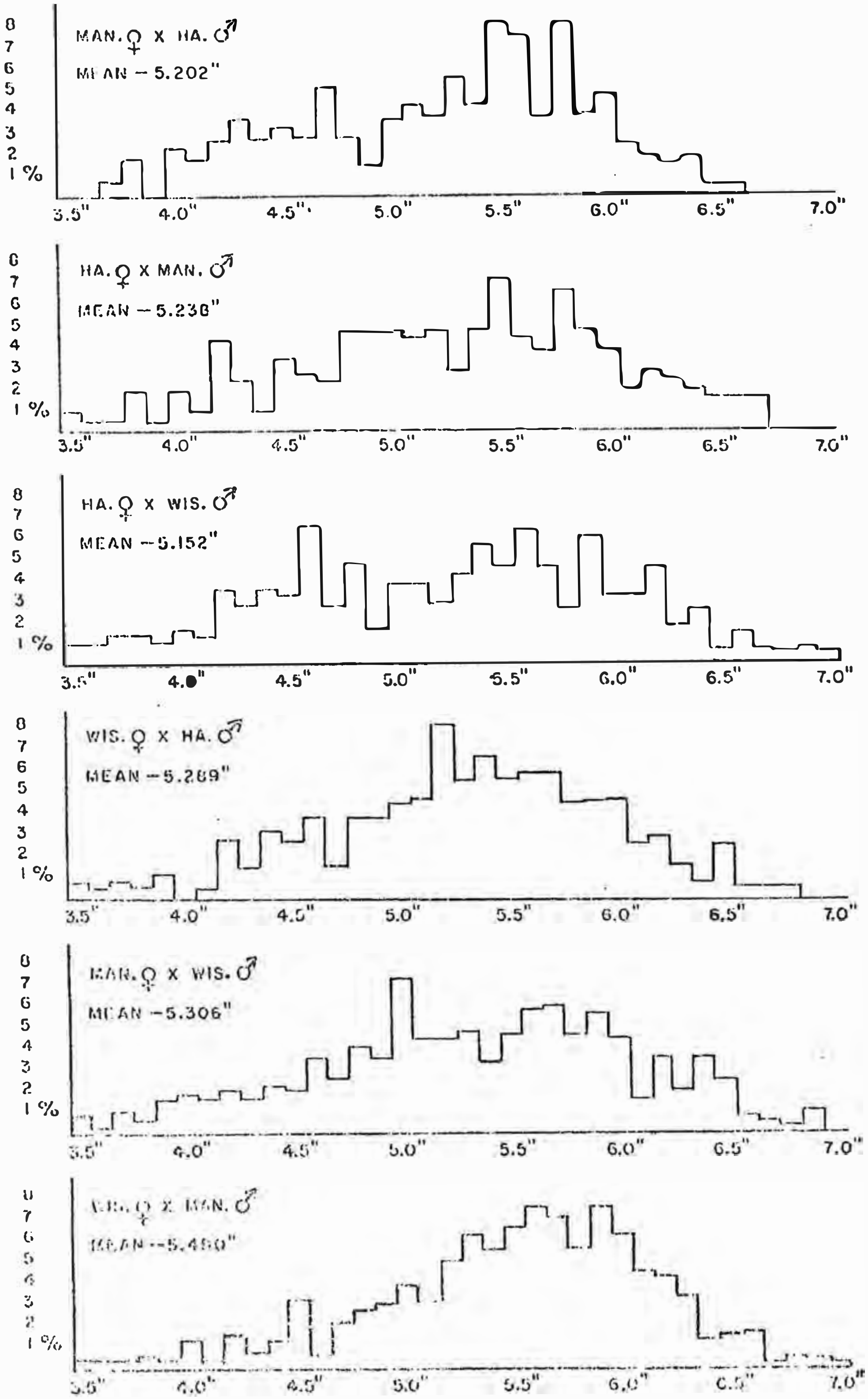
0 - 5	40 Points	31 - 35	10 Points
6 - 10	35 "	36 - 40	5 "
11 -15	30 "	41 - 45	2 "
16 - 20	25 "	46 - 50	1 "
21 - 25	20 "	50% & Over	0 "
26 - 30	15 "		

I. C. Identifing Clips for 10 Best Families

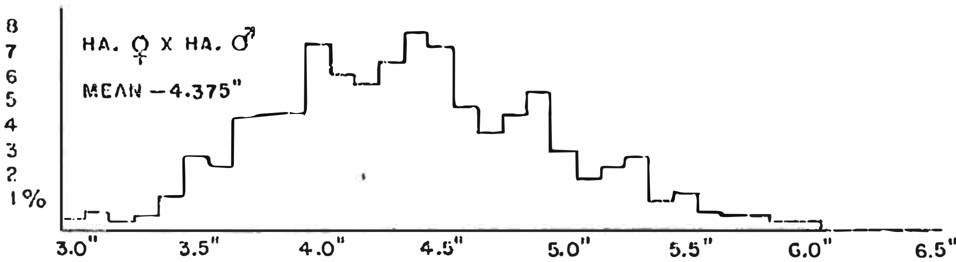
1. L.M.
2. R.M.
3. L.P.
4. R.P.
5. L.V.
6. R.V.
7. L.P. + L.M.
8. R.P. + R.M.
9. L.V. + L.M.
10. R.V. + R.M.

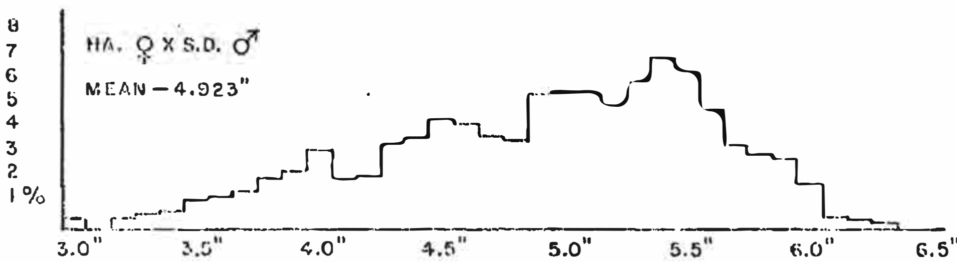
M = Maxillary
P = Pectoral
V = Ventral

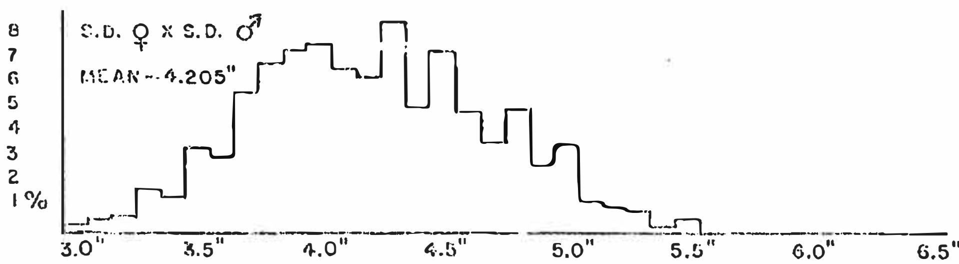
Length Frequency % Of
Population Rainbow Hybrids
Individual Lots

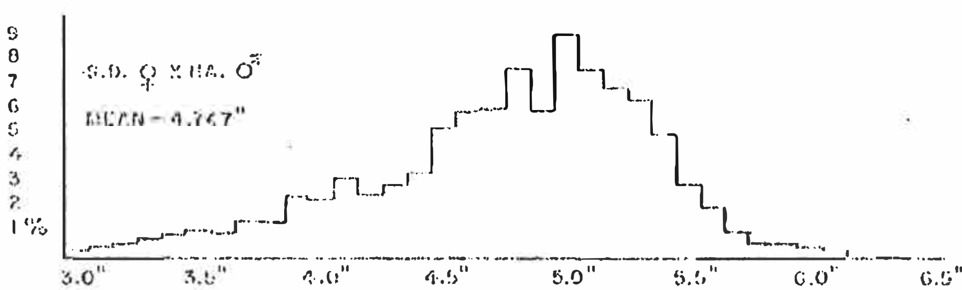


Length Frequency % Of
Population Brown Hybrids









Length Frequency % Of Population
Rainbow Hybrids Combined Reciprocal
Lots