

THE GENETIC AND ENVIRONMENTAL MECHANISMS CONTROLLING
THE LAKEWARD MIGRATION OF YOUNG RAINBOW TROUT
(Salmo gairdneri) FROM OUTLET AND INLET REARING STREAMS

by

BRYAN WILLIAM KELSO

B.Sc. University of British Columbia, 1970

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

in the Department
of
Zoology

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
1972

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study.

I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of

zoology

The University of British Columbia
Vancouver 8, Canada

Date

October 5, 1972

ABSTRACT

The upstream-downstream response to water current exhibited by rainbow trout fry in inlet and outlet streams of Loon and Pennask Lake stream systems was studied in experimental laboratory performance channels. Analysis of diallel tables, developed by crossing seven different inlet and outlet spawning stocks from the two stream systems, demonstrates additive genetic differences between the two stocks with respect to current response.

Tests performed in daylight showed a net upstream movement for all stocks, but far greater for outlet compared to inlet fry. At night, inlet fry showed a very strong downstream movement while outlet fry showed very little movement, similar to their behavior in the field.

Further analysis of the diallel table, when all the stocks were tested at three temperatures (low: 5C, medium: 10C, high: 17.5C), showed that temperature both in daylight and darkness tended only to change the degree of upstream or downstream movement of the fish, rather than the direction of movement. In daylight, upstream movement for all stocks was greatest at low temperature and least at high temperature. In darkness the greatest downstream response was at high temperature. However, at high temperature outlet fry moved farthest upstream in daylight while in darkness inlet fry moved farthest downstream.

Other possible controlling mechanisms (sudden temperature rises in the outlet creek, water source, abundance of food, genetic differences in liver lactate dehydrogenase) are considered.

The diallel analysis suggests that there are genetic differences in the current response between the inlet and outlet stocks and that water temperature plays only a minor role in the migration of rainbow trout fry to the lake.

TABLE OF CONTENTS

	Page
TITLE PAGE	i
ABSTRACT	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	v
LIST OF FIGURES	vi
ACKNOWLEDGEMENTS	viii
I INTRODUCTION	1
II MATERIALS AND METHODS	4
A. Collecting and Holding Adults	4
B. Crossing Procedure	6
a) <u>Diallel cross</u>	6
b) <u>Within population crosses</u>	9
C. Performance Channel	11
D. Method for Behavioral Tests	13
III GENETICAL ANALYSES AND STATISTICAL METHODS	14
IV SCORING SYSTEM	17
V RESULTS	18
A. Behavioral Responses of Fry in the Experimental Channels	18
B. Survival	19
C. Performance Tests	19
a) <u>Pure stocks</u>	19
b) <u>Diallel crosses</u>	26
i) <u>Hayman's analysis of the diallel tables</u>	26
ii) <u>The factorial analysis of variance</u>	34
VI DISCUSSION	43
A. Genetic Effects	44
B. Maternal Effects	50
C. Temperature Effects	50
D. Other Possible Effects	52
a) <u>Water sources</u>	52
b) <u>Other environmental effects</u>	53

VII CONCLUSIONS	53
BIBLIOGRAPHY	55
APPENDICES	57

LIST OF TABLES

- Table I The mean and range in fork length (mm) for parents of the 1971 diallel cross.
- Table II The mean and range in fork length (mm) for parents of the 1971 self-crosses.
- Table III Analysis of variance on the net scores of the behavioral tests on the pure stocks using the three factors where A = stocks, B = light and C = temperature.
- Table IV The expected mean squares and the variance components for the three-factor analysis of variance on the pure stocks. $n = 12$, $a = 6$, $b = 2$, $c = 2$.
- Table V Model for expectations of mean squares of a n^2 diallel cross, when the parental lines are fixed, for Hayman's analysis of variance.
- Table VI Levels of significance for Hayman's analysis of variance of the diallel crosses for the net scores. "P" means the item was tested against the pooled interaction mean square.
- Table VII Percent of the variance components for Hayman's analysis of the diallel tables of the net scores. Pooled mean square used as error variance.
- Table VIII Three-factor analysis of variance table on the net scores where A = males, B = females, C = temperature, for the daylight and darkness runs for both Cross I and Cross II.
- Table IX Expected mean squares for three-factor analysis of variance, fixed model, and the variance components for the four analysis of Cross I and II for daylight and darkness. $n = 2$, $a = 7$, $b = 7$, $c = 3$.
- Table X The percent of the adults used in the diallel cross which contain three types of liver lactate dehydrogenase (L.D.H.). See text for explanation of the three types of L.D.H.

LIST OF FIGURES

- Fig. 1 Loon Lake and Pennask Lake stream systems showing major rainbow trout spawning areas (~~~~~).
- Fig. 2 The arrangement of the 1971 diallel cross. The leading diagonal (X) represents the self-crosses while the rest are interpopulation crosses.
- Fig. 3 The standard 25-compartment performance channel (entrance and exit of each compartment staggered). The circle in the upper plan view shows the compartment used to release fry.
- Fig. 4 Net scores for the behavioural tests for the current response of the pure stocks. Solid circles and bars represent means and ranges, respectively of 12 replicas.
- Fig. 5 First order interactions of the analysis of variance (Table III) on the pure stocks; significance indicated by the degree of crossing between lines (see Sokal and Rohlf, 1969 p.355).
- Fig. 6 Upstream-downstream preference tests on three stocks of Loon Lake fry conducted in "Brannon-type" (Brannon, 1967) performance troughs during the summer of 1969. Water temperatures = 9.4C, velocity = 6.7 cm/sec., water source = Loon Creek Hatchery spring water.
- Fig. 7 The mean scores of both crosses for the "leading diagonal" (see Fig.2) and some of the hybrids for the daylight and darkness tests conducted at the high temperature ($17.5 \pm 1.5C$). Single symbols represent the self-crosses, paired symbols represent the hybrids.
- Fig. 8 The mean scores of both crosses for the "leading diagonal" and some of the hybrids for the daylight and darkness tests conducted at the medium temperature ($9.7 \pm .5C$). Single symbols represent the self-crosses, paired symbols represent the hybrids.
- Fig. 9 First order interactions of male (A) by female (B) in the factorial analysis of males by females by temperature.
- Fig. 10 First order interactions of male (A) by temperature (C) in the factorial analyses of males by females by temperature.
- Fig. 11 First order interactions of female (B) by temperature (C) in the factorial analyses of males by females by temperature.

- Fig. 12 The mean scores of both crosses for the "leading diagonal" in a linear regression of high temperature and low temperature for daylight and for darkness. Question marks means it is uncertain if the stock should be called an upstream or a downstream stock.
- Fig. 13 Net score of the current response of Loon Inlet fry which contain two separate phenotypic forms of liver lactate dehydrogenase. Tests conducted in 25-compartment performance channels. Circle and bar represent mean and ranges, respectively of 12 replicas. Data provided by T.G. Northcote; to be published elsewhere.

ACKNOWLEDGMENTS

The Fish and Wildlife Branch of British Columbia and the National Research Council of Canada provided financial support for this study.

Dr. J.R. Calaprice of the Fisheries Research Board at Nanaimo, B.C. granted the use of their facilities at their genetics hatchery at Rosewall Creek on Vancouver Island. Also, Mr. R.A.H. Sparrow, Fish and Wildlife Branch biologist in charge of Fish Culture, granted permission to utilize Look Creek Hatchery facilities. This cooperation was greatly appreciated.

Numerous associates, including G.R. Peterson, R. Norman, R. Land, D. Smith, G. Card, M. Flynn and G. Ennis aided in the collection of the adult fish and in the performing of the diallel cross, and their assistance was greatly appreciated. Much assistance was also given during the laboratory tests by A. Solmie of the Fisheries Research Board.

A great deal of aid was given in the computer programming of the statistical analysis by Mrs. D. Lauriente of the U.B.C. Computer Centre and Messrs. J. Thompson, F. Nash and Mrs. A. Sandres of the Computer Centre at the Fisheries Research Board in Nanaimo.

Dr. C.F. Wehrhahn read the manuscript and made valuable suggestions during the course of the study.

The support, advice and patience of Dr. J.R. Calaprice has been greatly appreciated throughout all phases of this study.

I wish to express my gratitude to my supervisor, Dr. T.G. Northcote, for his guidance and constructive criticisms throughout all phases of the

study and the preparation of the manuscript.

Finally, I wish to express my gratefulness to my wife, Verna, for her understanding, patience and assistance throughout all phases of my studies.

INTRODUCTION

Migration of animals from one region to another for breeding or feeding has been the object of study of many zoologists and naturalists for some time. One group of migrating animals are the many species of freshwater and marine fish that migrate to spawn during their life cycle. Some of these fishes, such as eels, herring, cod, plaice and tuna, travel hundreds of miles (Harden-Jones 1968). Of most interest on the North American Pacific Coast are the anadromous Salmonidae and the mechanisms that control their migrations.

Hoar (1953) states that chum, pink, and sockeye fry migrations downstream are a passive movement. He believes that this is brought about when they lose visual contact with the bottom due to low light intensity at night. However, the adult sockeye not only migrate "up" inlet streams to spawn, but also move "down" outlet streams to spawn. Thus, the young, must either migrate "downstream" or "upstream" from their incubation stream to the rearing lake.

The occurrence of inlet and outlet spawning is not only associated with sockeye salmon, but also with brown trout, Salmo trutta; grayling, Thymallus articus; white and longnose suckers, Catostomus commersoni and C. catostomus; as well as rainbow trout, Salmo gairdneri (Northcote, 1962).

Because the fry of the outlet spawning adults must move upstream, their movement cannot be passive but must be a deliberate, controlled movement. Therefore, a fundamental problem in migratory behaviour was to determine the mechanism that controls these marked behavioural differences between fry of the same species.

Northcote (1962) made two postulates as to the control of lakeward

migration, (1) genetically distinct outlet and inlet stocks of which each had an appropriate innate behavioural response which resulted in the movement of young into the lake, (2) genetically similar outlet and inlet stocks responding to environmental differences between streams which produced the appropriate current responses and other behaviour characteristics of the young as they move into the lake. Raleigh (1967) added a third postulate which stated that there were genetically distinct outlet and inlet demes maintaining innate behavioural responses which may be modified by the environment.

In Northcote's original study (1962) on the inlet and outlet progeny of rainbow trout, he concluded that the mechanisms controlling migration were associated with environmental differences between streams rather than genetic differences between spawning stocks. The main environmental factors were temperature and photoperiod. Cool water temperature, and long day lengths apparently induced downstream movement while short day lengths and warm water temperatures were associated with upstream movement of fry. However, he noted in his 1962 paper and again in his later studies (1969), that different migratory traits apparently have developed within a few years from "single stocks" of trout. He stated that in another inlet-outlet stream system, Pothole Lake, there could be a selectivity for a genetically controlled upstream-downstream migration. Furthermore, in 1969 he found that there were marked differences in migratory behaviour between "above falls" and "below falls" populations which were not controlled by obvious environmental factors. From this, he concluded that there might be genetically controlled, as well as, environmentally-induced behavioural mechanisms operating in the Loon Lake system.

Brannon (1967), in a study of upstream and downstream movement of sockeye fry, concluded that there was a deliberate, genetically controlled

movement from incubation area to the nursery lake. Also, Raleigh (1967, 1971) and Raleigh and Chapman (1971) concluded from their studies of sockeye salmon, cutthroat trout, and rainbow trout that the migratory behaviour of salmonid fry from natal to rearing areas is under innate control subject to modifications by the environment.

In 1969 a preliminary study suggested and directed by T.G. Northcote (Kelso, MS, 1970), showed that there was some genetic control in the migratory behaviour of stocks of rainbow trout of Loon Lake, B.C. Recently, Calaprice (1972a and 1972b) designed an experiment using biometrical genetics to describe differences in heritable factors that occur among populations of sockeye salmon. He found that there were additive genetic differences and maternal effects that influenced the survival of the young (1972a) as well as heritable differences in current response among the progeny from adults collected in different streams (1972b).

Previous studies on rainbow trout of the Loon Lake system had shown both environmental and genetic controls in migration of the fry. Also Raleigh's (1967) and Brannon's (1967) work on young sockeye salmon migration showed a genotype-environmental interaction. The diallel analysis used by Calaprice (1972b) partitions the total variation between populations into genetic and environmental components; the genetic component consisting of additive and dominant effects, while the environmental component is maternal (or paternal) effects and those effects brought about by the surrounding environment. This method was used to test Northcote's (1962) hypothesis for genetic differences in current response between stocks. If there are no significant differences in response between families, then any variation that exists must be environmental and thus tests his second hypothesis. The analysis should also show if there are significant interactions between

the genetic and environmental components, testing Raleigh's (1967) hypothesis. To increase the number of populations, a second lake, Pennask Lake, was also used in the study. In all, five spawning populations from Loon Lake plus two spawning populations from Pennask Lake were used to set up a 7 x 7 diallel cross to test for genetic and environmental mechanisms that might control the current response of young rainbow trout fry.

II

MATERIALS AND METHODS

A. Collecting and Holding Adults

Nearly mature spawning rainbow trout were collected from five different spawning areas on the Loon Lake stream system (Fig. 1), during May and early June, 1971. These areas were (1) Loon Inlet Creek, (2) Thunder Creek, (3) Loon Outlet (Outlet Trap 1), (4) Hihium Creek (from 50 to 100 meters upstream from the confluence of Hihium and Loon Outlet Creek), and (5) 50 to 200 meters downstream from the confluence of Hihium and Loon Outlet Creeks. Also, an inlet and an outlet stock ¹ was obtained from Pennask Inlet at the B.C. Fish and Wildlife Branch hatchery trap situated approximately 100 meters upstream from the lake and from Spahomin Creek at a trap situated at the mouth of the Creek (Fig. 1).

The adults were held in eight fiberglass tanks (approximately 1.2m x .6m x .45m), and in two wooden ponds (approximately 5m x 1.2m x 1m) at the B.C. Fish and Wildlife Branch Hatchery about 5 miles southwest

¹ For purposes of this study each group of fish from these areas has been called a separate "stock".

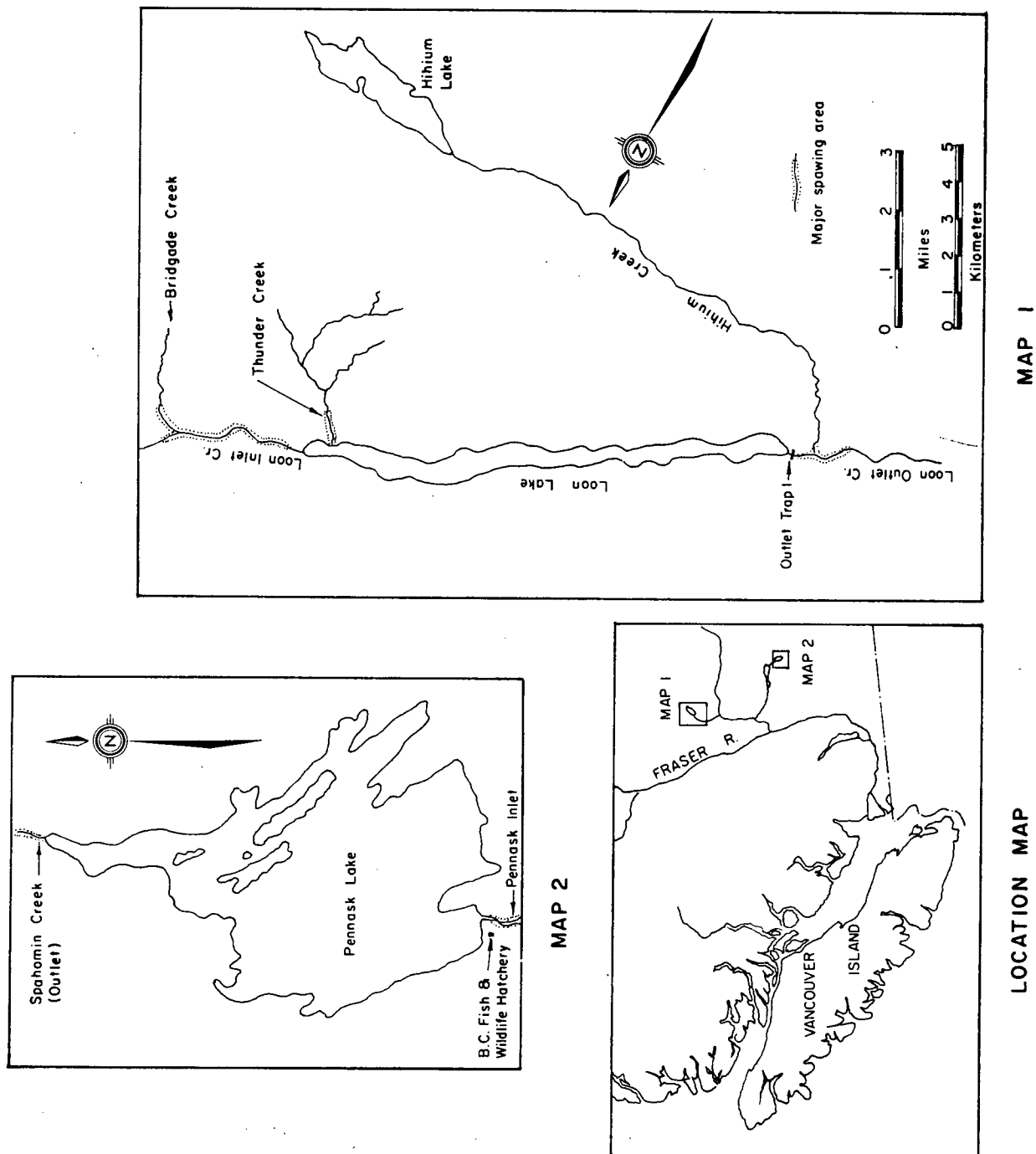


Fig. 1 Loon Lake and Pennask Lake stream systems showing major rainbow trout spawning areas (.....).

of Loon Lake. A minimum of twenty pairs of adults were collected from each stock.

B. Crossing Procedure

a) Diallel cross

The diallel cross consists of making all possible matings between n pairs of adults. In 1971 two such crosses were performed separately and the eggs and sperm of each stock were pooled. For each cross, five adult pairs were used and the eggs were divided into two replicas. In some cases it was necessary to use more than five males to obtain enough sperm and in other cases fewer than five females were mature enough to provide ripe eggs. The number of adults used and their mean lengths are shown in Table I.

Crosses between the seven areas were performed as illustrated in Fig. 2. Each stock of eggs were placed in separate 2 liter plastic freezer cartons and were gently stirred so that the pooled eggs were as equally distributed as possible. The pooled sperm of each stock was placed in separate styrofoam cups and also stirred. Two trays 1.2m x 1.2m were prepared so that each tray held forty-nine plastic cartons. The rows and columns of the trays were colour-coded with the rows designated for males and the columns designated for the females.

The pooled eggs of each stock were first divided into two replicas. Each replica of eggs in turn was then divided into seven approximately equal lots and placed in their appropriate cartons in the tray (columns Fig. 2). After all seven stocks of eggs were placed in the tray, they were then fertilized with the aid of seven different plastic disposable hypodermic syringes, one for each stock (rows, Fig. 2). The tray was then placed under the egg washer (water temperature 10C) and the eggs

Table I. The mean and range in fork length (mm) for parents of the 1971 diallel cross.

Stock	Cross I					
	Female			Male		
	no.	mean	range	no.	mean	range
Pennask Inlet	6	313	280 - 342	8	322	236 - 350
Loon Inlet	6	338	317 - 364	6	347	263 - 404
Thunder Creek	6	344	330 - 365	6	339	320 - 360
Pennask Outlet	5	288	260 - 306	6	301	295 - 313
Loon Outlet	4	310	285 - 331	6	285	201 - 337
Below Hihium	3	288	280 - 295	7	231	192 - 300
Hihium Creek	6	291	260 - 320	6	229	165 - 360
				Cross II		
Pennask Inlet	6	306	287 - 340	6	285	261 - 297
Loon Inlet	5	361	338 - 405	4	250	195 - 351
Thunder Creek	6	307	277 - 343	6	280	240 - 300
Pennask Outlet	5	307	282 - 383	6	306	258 - 330
Loon Outlet	5	310	290 - 332	6	286	232 - 379
Below Hihium	4	278	210 - 342	15	235	191 - 284
Hihium Creek	7	316	260 - 355	15	235	168 - 360

Fig. 2 The arrangement of the 1971 diallel cross. The leading diagonal (X) represents the self-crosses while the rest are interpopulation crosses.

		<u>FEMALE</u>						
		Pennask Inlet	Loon Inlet	Thunder Inlet	Pennask Outlet	Loon Outlet	Below Hihium	Hihium
<u>MALE</u>	Pennask Inlet	X						
	Loon Inlet		X					
	Thunder Inlet			X				
	Pennask Outlet				X			
	Loon Outlet					X		
	Below Hihium						X	
	Hihium							X

were water hardened. The next replica was then begun. After approximately ten minutes of washing, the egg cartons were capped and placed in styrofoam boxes and packed in ice. The total number of egg cartons was 196 (2 (7 x 7 x 2)). Egg taking and crossing took approximately four hours.

The eggs were then transported by truck 525 km to the Fisheries Research Board's genetic hatchery at Rosewall Creek on Vancouver Island. Transportation time was approximately ten hours. The eggs were placed at random in 196 separate rearing tanks. One liter of water per minute circulated through each tank. The eggs were treated twice weekly with malachite green until just before hatching. Up to swim up, ie. the time when fry emerge from the gravel, water temperature ranged from 6.4° to 11.8°C with a mean of 9.2°C . After swim up (476 CTU²) the incubation baskets were removed and outside standpipes were installed. Fry were fed frozen commercial hatchery mash supplemented with frozen brine shrimp.

b) Within population crosses

As well as the diallel cross, males and females from each stock were mated and for purposes of this study, are called the "pure stocks". The eggs for each stock were pooled and fertilized with the pooled sperm of the same stock. The number and lengths of the adults are given in Table II. The eggs were held at the Loon Creek Hatchery (water temperature 10°C) until they were eyed or, in some cases, had already reached the alevin stage and were then transported to the Rosewall Creek hatchery.

² Degrees centigrade temperature units (sum of the degrees centigrade per day the water was in the rearing tanks above zero degrees centigrade).

Table II. The mean and range in fork length (mm) for parents of the 1971 self-crosses.

Stock	Female			Male		
	No.	Mean	range	No.	Mean	range
Pennask Inlet	5	316	299 - 349	5	305	278 - 320
Loon Inlet	4	334	302 - 353	5	348	315 - 367
Thunder Creek	5	304	283 - 324	3	351	334 - 366
Pennask Outlet	2	293	280 - 306	2	321	310 - 332
Loon Outlet	4	289	284 - 292	4	294	228 - 343
Below Hihium	6	294	274 - 308	4	294	222 - 331
Hihium Creek	2	314	300 - 328	2	255	205 - 305

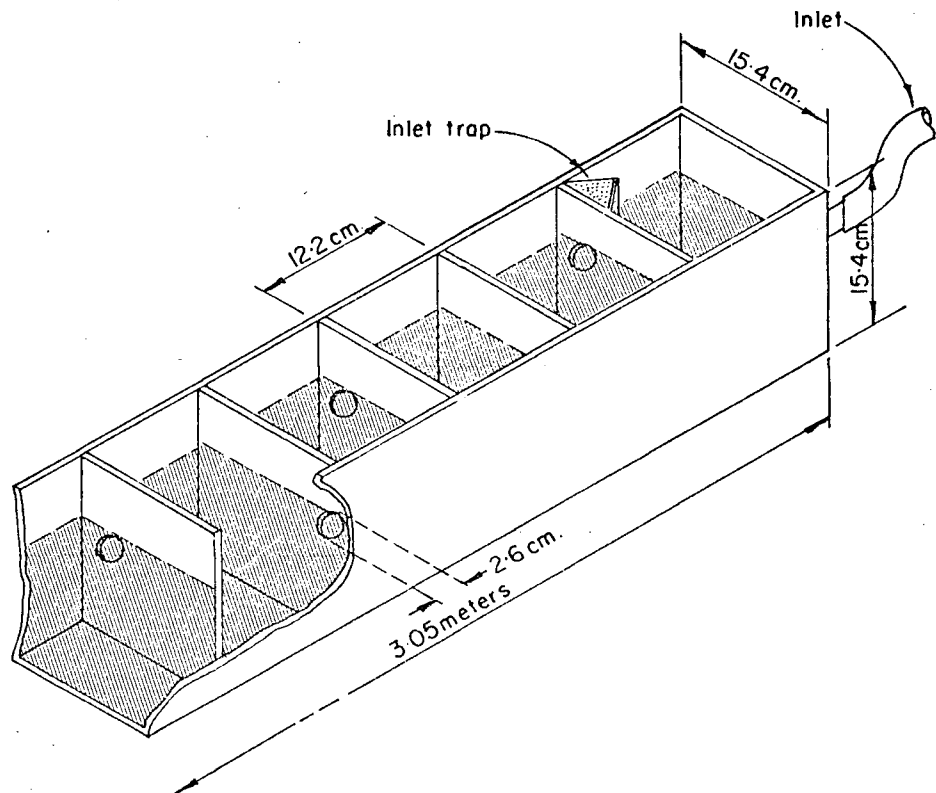
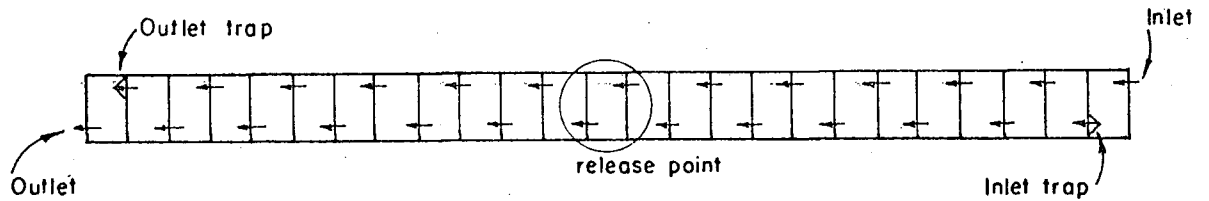
C. Performance Channel

The experimental apparatus used (Fig. 3) was designed by J.R. Calaprice (1972b). It consisted of a wooden trough, 15 cm wide by 3.05 m long and 15 cm high; divided into twenty-five compartments. A 2.5 cm hole lead from one compartment to the next in a staggered position. This prevented a direct flow of water from one end of the trough to the other end, as the water would "swirl" around in a compartment before running into the next one. Thus, a fish had to "seek out" the entrance into the next opposing compartment whether it was moving upstream or downstream. Twelve such performance channels were used in two banks of six.

A dark room, approximately 3.7 m x 3.7 m x 2.7 m was constructed by covering a wooden framed area with 4 mil black polyethylene plastic. This was divided into two rooms by a polyethylene partition, each having a separate entrance and containing a bank of six performance troughs. All twelve channels were fed by the same headtank which protruded through the centre partition into each room. Controlled flow (1250 ml/min) was maintained to each channel giving a velocity of $7.4 \pm .5$ cm/sec. through the compartment openings.

The lighting used for each bank of performance troughs consisted of five 150 watt, 125 volt, projector flood lamps. These were hung on the centre partition wall and directed upward onto a white glossy ceiling to give indirect lighting. This eliminated all shadows in the trough, but not necessarily all light gradient. The lights were controlled from outside the room with an automatic timer or a powerstat (Superior Electric Co. Type 110). The timer took one-half hour to increase to maximum or decrease to minimum light intensity.

Fig. 3 The standard 25-compartment performance channel (entrance and exit of each compartment staggered). The circle in the upper plan view shows the compartment used to release fry.



The tests were conducted at three different temperature regimes 3.5-6.5 C, 9.0-10.5 C, and 16.0-19.0 C. The high temperature was obtained by recycling 10 C Rosewall Creeks water and heating it with a 1000 watt emersion heater (Waage Electric Inc. Model SF100). The medium temperature was maintained by combining river and ground water, while the low temperature was maintained by recycling ground water cooled by a Blissfield refrigerant unit Model BHL-909-B.

D. Method for Behavioral Tests

Testing began shortly after "swim up" at the high temperature (614 C T.U.) and ended at 739 C T.U. of development. The medium temperature tests were run from 759 C T.U. to 836 C T.U., and lower temperature tests from 870 C T.U. to 968 C T.U. of development. Fry tested ranged between 20 and 30 mm fork length and all had started to feed.

In each test, a maximum of 20 fry were used, depending upon the number of survivors per tank. Fry were placed in the central compartment of the test channel and held there by placing aluminum strips over the exit holes. A test was first performed under daylight conditions. Then, the same fish were placed back into the troughs and tests run again in darkness. For the daylight tests the experiments were begun in complete darkness and the lights were slowly increased to maximum intensity over a one-half hour period. Fifteen minutes after the test was begun (when the light was at half its maximum intensity) the screens on the exit holes were removed and the fish were free to move upstream or downstream. After 2½ hours from the start, the

positions of the fish were noted with the lights dimmed to 55 volts and after all the fish in the twelve troughs had been counted the fish were removed and placed back in the centre compartment. For the response of the fry in darkness, the lighting was the reverse. Upon completion of this test, the fry were placed back in the rearing tanks.

For tests in which the water temperature in the experimental troughs was higher than the water in the rearing tanks, the fry were collected approximately 45-60 minutes in advance and held in plastic freezer cartons until the temperature warmed to within 2C of that in the experimental trough. Flow rates were measured both before and after each test. Three to four sets of experiments were done per day for a total of 36 to 48 individual tests.

III

GENETICAL ANALYSES AND STATISTICAL METHODS

There have been several experimental designs devised for estimating the genetic and/or environmental variation in plant and animal populations (Kearsey 1965). The design of this experiment was similar to the North Carolina Design 2, where all the mn progeny families that were obtained by crossing m males with n females were raised, ie a full diallel cross. Wearden (1964) defines a full diallel as "... p^2 possible matings among a set of parental lines including $1/2 p(p-1)$ pairs of reciprocal crosses."

Two methods of analysing the diallel cross are the Hayman analysis and the factorial analysis (Wearden, 1964). These are models for maternal and for reciprocal effects.

Hayman's analysis of the model for reciprocal effects gives the most powerful test for the genetic contribution (σ_g^2) but the factorial analysis for maternal effects gives the best test for maternal factors (σ_m^2) because it tells whether the variance is due to the maternal or to the paternal effects of the parent (Wearden, 1964).

For Hayman's analysis of variance (Hayman, 1954a) there are six basic assumptions: (1) Diploid segregation; (2) No difference between reciprocal crosses; (3) Independent action of non-allelic genes, and in the diallel cross; (4) No multiple allelism; (5) Homozygous parents; (6) Genes independently distributed between parents.

His analysis yields seven statistics: "A" -- genetic variation amongst parents (additive variation), "B" -- variation in reciprocal sums not ascribed to A or non-additivity (dominance), "C" -- average maternal effects of each parental line, "D" -- variation in reciprocal differences not ascribed to C. On the assumption that the genes are independently distributed between parents, the "B" term is divided into three separate statistics: " b_1 " -- testing the mean deviation of F1's from their mid-parental values and is significant only if the dominance deviations are directional, " b_2 " -- testing whether the mean dominance deviation of the F1 from the mid-parental values within each array differs over arrays (i.e. gene assymetry or dominance at some of the loci), and " b_3 " -- testing that part of the dominance deviation unique to each F1. However, because of the assumption underlying these latter three statistics, they have been omitted from the main discussion of the results. For those who wish to carry the analysis to its full extent the b_1 , b_2 , b_3 terms have been left in the anova table in Appendix 3.

Hayman's analysis calls for the mean squares for each main effect to be tested for significance against its own interaction over blocks (environmental component). However, to increase the degrees of freedom the error variances, where homogeneous, may be pooled to give a block interaction mean square as a common error variance (Hayman 1954a). The computer program used was written by J.R. Calaprice of the Pacific Biological Station, Nanaimo, B.C.

Hayman (1954a) gives a graphical analysis of the diallel cross which can show either the additive or dominant effects by the use of a variance-covariance graph. However, it too depends upon the same assumption of independent assortment of genes, so has been omitted from this analysis. A further point is that the experiment is regarded as a fixed effects analysis because the streams containing the adults were specific streams picked from the Loon Lake and from the Pennask Lake areas and was not a sample of streams from a large population.

The factorial analysis of the data is based on the model for maternal effects (Wearden 1964), but is modified to include also the environmental effects of temperature. The interaction terms not only show the genetic contribution of the parent stocks but also show how the animals vary in response to a specific environment, or in other words a genotype by environmental interaction.

The expected mean squares were calculated by the rules set down by Sokal and Rohlf (1969) for a multi-factorial fixed effects analysis of variance. The statistical analysis was carried out at the Pacific Biological Station in Nanaimo, B.C. using the program for factorial anova from Sokal and Rohlf, number C A 3.5.

IV

SCORING SYSTEM

In this analysis, no test for scaling (ie mathematical transformations) was made. However, the analysis was performed on the raw data using several different scoring procedures. These all produced similar F-ratios. The first procedure - the "chance score" - was based on the probability of fish movement being random. For example, the probability of a fish moving from compartment 13 (middle compartment) to the next was one half. Then the probability of it moving to the next was one quarter and so on to the end of the trough. A score was thus calculated for each fish, summed and then divided by the total number of fish, to give a mean. The formula was thus:

$$\frac{\sum_{i=1}^{12} n_i (1-\frac{1}{2}^i)}{N_T}$$

where n_i = number of fish per compartment

$$N_T = \text{total number of fish moving in one direction} = \sum_{i=1}^{12} n_i$$

Two scores were obtained from each experiment, a downstream score and an upstream score.

The second score system tried was one in which a "rank number" was assigned to each compartment with the middle compartment being zero and the upstream, or downstream side, being numbered one to twelve consecutively with the end compartment being number twelve. The number of fish per compartment was multiplied by its corresponding rank number, summed, and then divided by the total number of fish that had moved in one direction. To prevent negative scores, a constant of twelve was added to each score.

The formula was:

$$\frac{\sum_{i=1}^{12} (n_i \times r) + 12}{N_T}$$

where n_i = number of fish per compartment

r = rank number of corresponding compartments

N_T = total number of fish moving in one direction

A third score was also obtained by subtracting the downstream score from the upstream score and again adding a constant of 12 to prevent negative numbers.

The third score tried was very similar to the second except that N_T equalled the total number of fish used in that experimental test. That is, it gave the mean proportion of fish moving in any one direction.

In a comparison of Hayman's analysis on the "chance score" and the first "ranked scores", it was found that the same statistics were significant in both cases, but higher levels of significance were found in the "rank scores". This is probably due to the fact that with the "rank score" system a higher value is given to the fish that move the farthest. In a comparison of the two "rank scores", exactly the same F-ratios were obtained in both cases for the net scores. The mean proportional rank system appeared to be the best to distinguish between a test with little directional movement and a test with a large directional movement. Because all scoring systems were arbitrary and because there was basically little difference between the systems, the proportional ranking system was used. Appendix 1 shows several hypothetical examples of scores for the upstream-downstream movement of fry. This scoring system would not distinguish between the situation where all fry remained in the middle compartment (the score would be 12) and that where half of the fry moved into the downstream trap and the other half moved into the upstream trap (the score again would be 12). However, in no instance did all the fish in any one test remain in the centre release compartment.

V

RESULTS

A. Behavioural Responses of Fry in the Experimental Channels

The directional movement of the fry was largely dependent upon the

light as will be shown later. When the light regime was changing from light to dark, the fry would start to move upstream upon first being released. However, when the light was decreased to a very low level, upstream movement would cease and after two hours of total darkness movement would be predominately downstream. No observations were made of the fish in the dark but in daylight if fry moved passively downstream, they would go from one compartment to the next. This was probably because of the staggered position of the compartment openings and the "swirling" action of the current which hindered any further passive downstream movement.

When moving upstream, the fry had to put forth extra effort to swim through the opening to the next compartment. However, they could hold position in areas of each compartment where the current was minimal. Most movement took place within the first hour of the test. The fish normally showed very little "back-and-forth" movement through the troughs and if they did, it usually involved only two or three compartments.

It was further observed during daylight that when the fry reached the end compartments they would still try to move farther upstream by bumping against the screen covering the inlet.

B. Survival

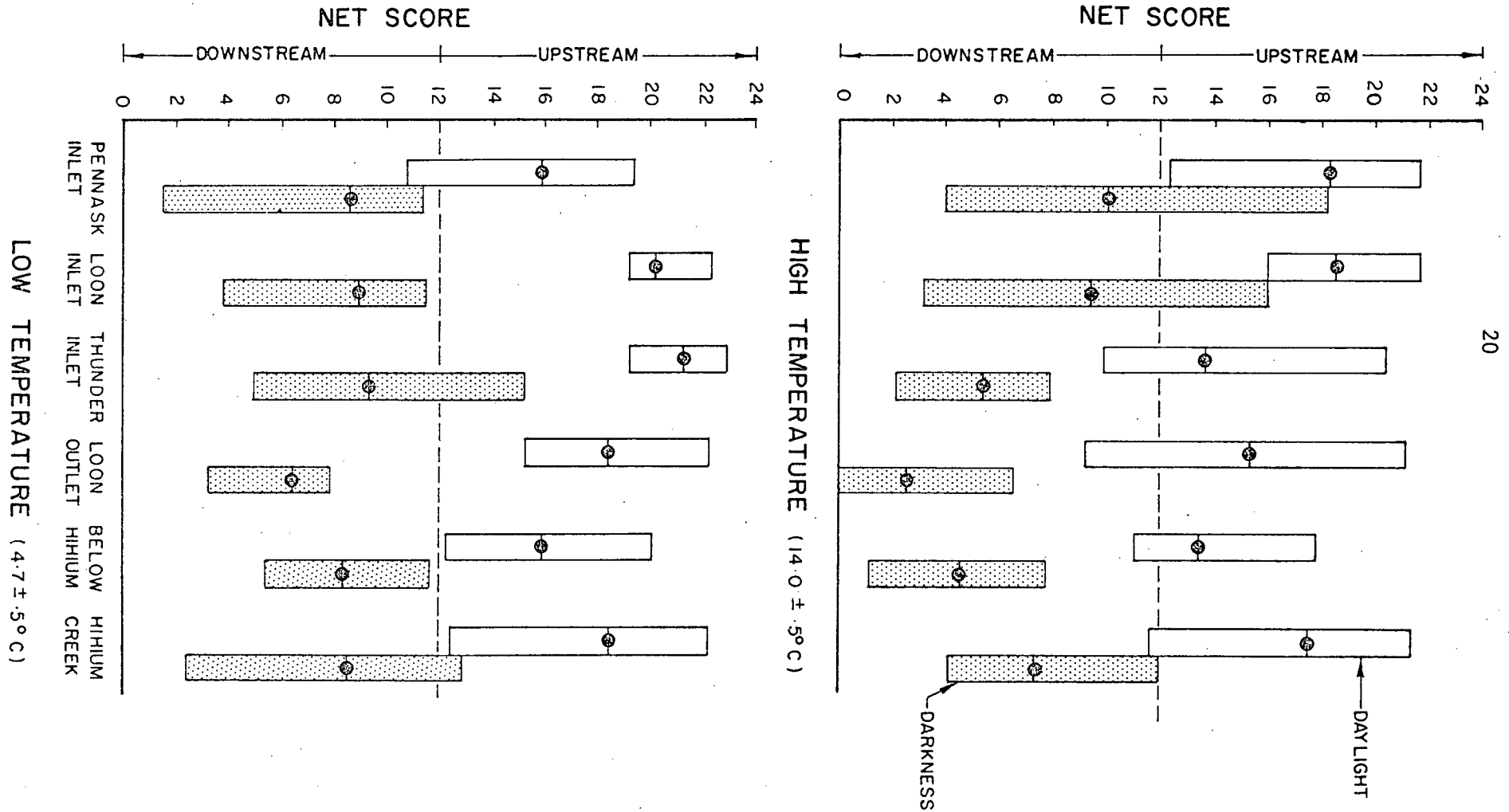
The mortality associated with all crosses will be dealt with in greater detail in a separate paper. However, the number of intra-population crosses were reduced to six stocks as the Pennask Outlet eggs suffered 100% mortality. For the diallel crosses there was an unexplained high mortality in the second cross. This was mainly in the second replica and was caused by the males (rows) of Pennask Inlet, Thunder Creek, and Below Hihium stocks as well as the females (columns) of Below Hihium.

C. Performance Tests

a) Pure stocks

The tests for the six pure stocks (Fig. 4) were run during darkness and daylight at low ($4.7 \pm .5$ C) and high temperature ($14.0 \pm .5$ C). Analysis of variance shows that there was a highly significant difference between each of the three main effects ($P < .001$)

Fig. 4 Net scores for the behavioural tests for the current response of the pure stocks. Solid circles and bars represent means and ranges, respectively of 12 replicas.



of stock, light, and temperature (Table III).

Some of the first order interaction terms were also significant when tested by the graphical method of Sokal and Rohlf (1969). The stocks by light (Fig. 5a), i.e. genotype-environmental interaction, was significant ($P < .01$). Here, the fry show a definite upstream response during daylight and a definite downstream response during darkness. Further, the stocks also change their intensity of movement in relation to each other between day and night tests. For example, Loon Outlet stock was fifth in order of upstream preference during the daylight, but was first in order of downstream preference at night. Pennask Inlet fish also show an interaction between stocks and light.

The genotype-environmental interaction of the stocks at different temperatures is also highly significant ($P < .001$). In the experiment inlet fry had a greater upstream response during the day than the outlet stocks. During darkness, outlet fry showed a greater downstream response than inlet fry. This is directly opposite to field observations where inlet fry move downstream during the night and the outlet fry move upstream during the day.

In other tests conducted on Loon Lake fry (Kelso MS, 1970) in the summer of 1969, there was a definite current response (Fig. 6). The chi-square test for independence for daylight tests was highly significant ($P < .005$). Both the Outlet stock and the Hihium stock showed a strong upstream preference while the Inlet stocks showed a downstream response in the daylight runs. However, here there was very little difference between the day and night tests of the Outlet stock, whereas the Inlet stock showed greater downstream preference during the day than at night (Fig. 6.).

However, the most important result was that a change in temperature did not cause the fry to reverse their direction but only caused a change in the intensity of their movement. The light by temperature interaction was not significant (Fig. 5) nor was the second order interaction of stock by light by temperature.

The highest variance component was that of light - 74.5% of the total variance. Variance of the stocks was 3.2% while the stocks by light was 1.6% and the stocks by temperature was 5.1% (Table IV).

Table III. Analysis of variance on the net scores of the behavioural tests on the pure stocks using the three factors where A = stocks, B = light and C = temperature.

Source	d.f.	M.S.	F
Main effects			
A = stocks	5	105.65	13.40 ***
B = light	1	6881.32	872.85 ***
C = temperature	1	281.34	35.69 ***
A × B	5	31.75	4.03 **
A × C	5	85.78	10.88 ***
B × C	1	4.83	<0 n.s.
A × B × C	5	11.28	1.43 n.s.
Error	264	7.88	
Total	287		

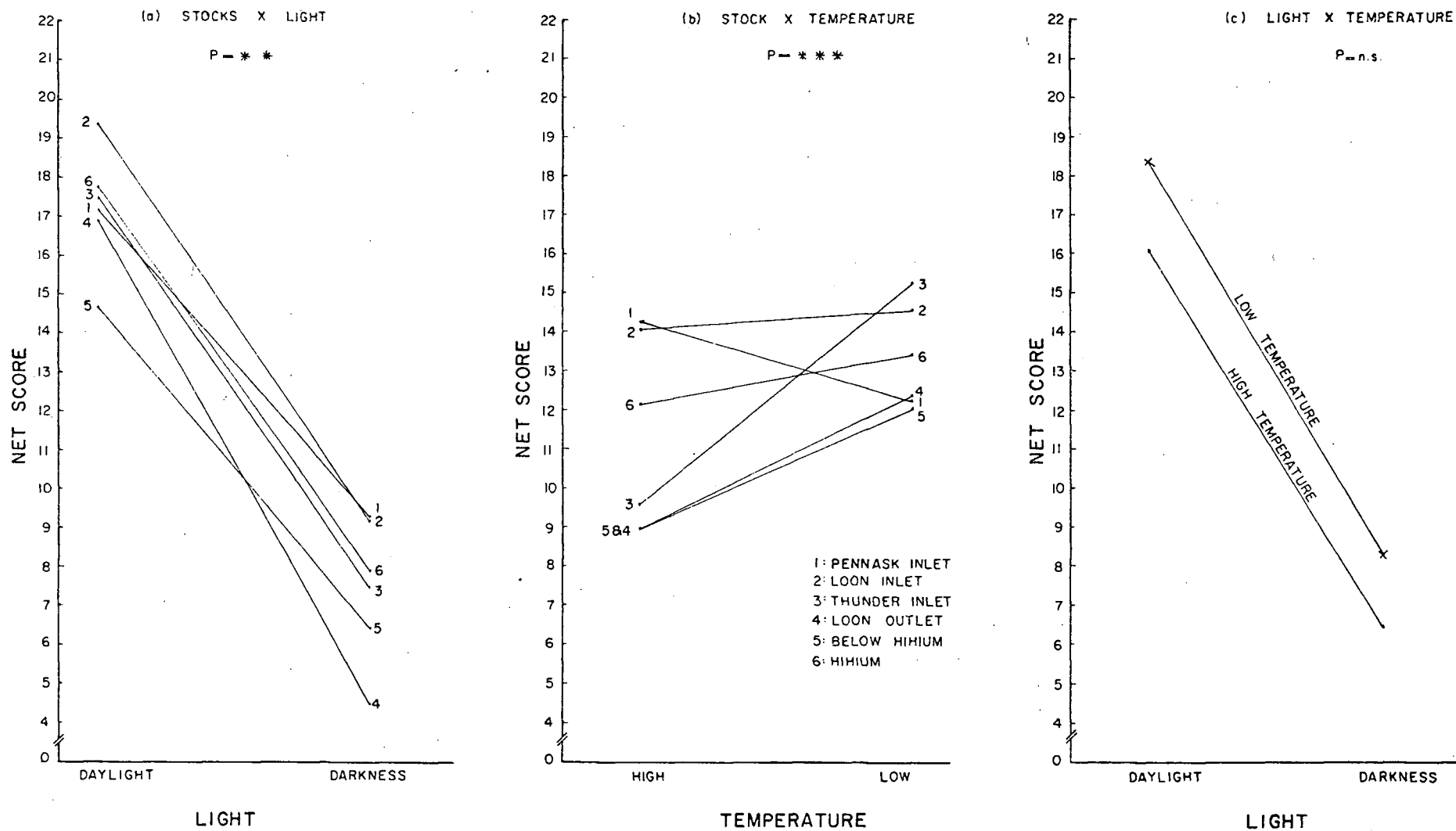


Fig. 5 First order interactions of the analysis of variance (Table III) on the pure stocks; significance indicated by the degree of crossing between lines (see Sokal and Rohlf, 1969 p. 355).

Fig. 6 Upstream-downstream preference tests on three stocks of Loon Lake fry conducted in "Brannon-type" (Brannon, 1967) performance troughs during the summer of 1969. Water temperatures = 9.4C, velocity = 6.7 cm/sec., water source = Loon Creek Hatchery spring water.

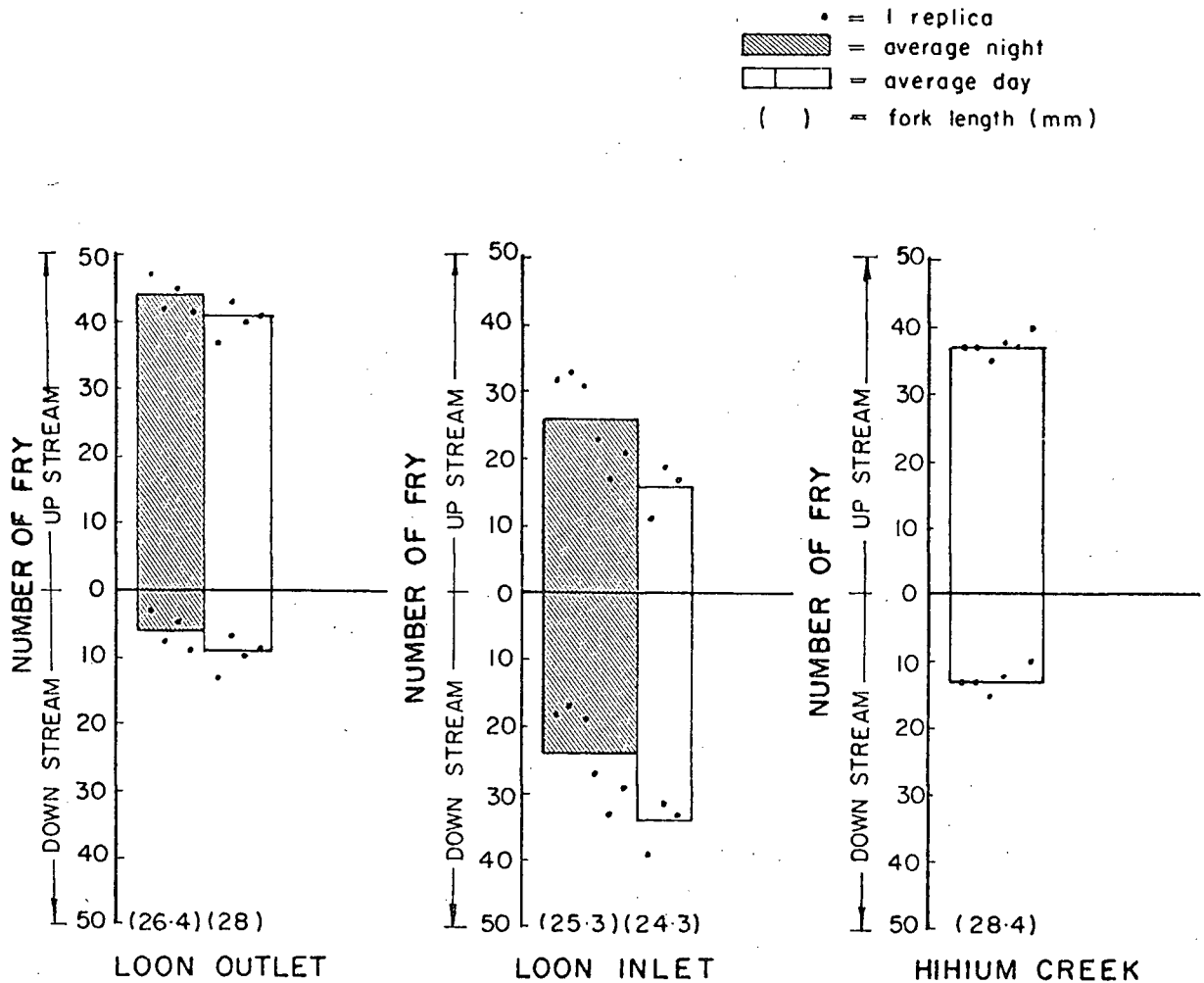


Table IV. The expected mean squares and the variance components for the three-factor analysis of variance on the pure stocks. $n = 12$, $a = 6$, $b = 2$, $c = 2$.

Source	Expected mean square	Variance component	Percent variance
A = stocks	$\sigma^2 + nb\tilde{\sigma}_A^2$	2.0369	3.2
B = light	$\sigma^2 + na\tilde{\sigma}_B^2$	47.7322	74.5
C = temperature	$\sigma^2 + nab\tilde{\sigma}_C^2$	1.8990	3.0
A \times B	$\sigma^2 + nc\tilde{\sigma}_{AB}^2$	0.9943	1.6
A \times C	$\sigma^2 + nb\tilde{\sigma}_{AC}^2$	3.2456	5.1
B \times C	$\sigma^2 + na\tilde{\sigma}_{BC}^2$	0.0	0.0
A \times B \times C	$\sigma^2 + n\tilde{\sigma}_{ABC}^2$	0.2830	0.4
Error	σ^2	7.8837	12.3

b) Diallel crosses

The scores for Blocks 1 and 2, together with their means, are given in Appendix 2 for Crosses I and II at the three temperatures.

(i) Hayman's analysis of the diallel tables

As mentioned earlier, Hayman's analysis of the diallel cross computes four statistics plus a breakdown of his (B) or dominance term into three further statistics (b_1 , b_2 , b_3). The analysis of variance tables for all the tests are shown in Appendix 3 including the b_1 , b_2 , and b_3 terms. The breakdown of the "B" term will not be considered herein. Expected mean squares for the "model for reciprocal effects" are given in Table V, which best shows the genetic effects of a diallel cross.

The score calculations for Hayman's analysis of the diallel cross were first divided into upstream and downstream movement and analysed separately. Then, these scores were combined by subtracting the downstream from the upstream to obtain a net score. An analysis was done on all three sets of scores. The results of each (not shown) were the same whether split or net scores were used with one exception at the medium temperature where dominance was shown ($P < .05$) for Cross II daylight and Cross I darkness, for split scores but not net scores. The net scores of the two replicas were also summed and an analysis done on these scores. The results of the two analyses on the net scores are summarized in Table VI.

The additive effect (A), ie genetic variation among stocks was evident at all temperatures, but not necessarily in both crosses or at both light conditions tested. Only in cross I for daylight tests was there additive genetic variance at high temperature ($P < .05$) while at medium temperature the genetic variation was only significant during the darkness tests. However, when the crosses were summed there was additive genetic variance at both medium and low temperature tests.

The only evidence of dominance (B term) was at low temperature during darkness ($P < .05$) but this was not evident when the crosses were summed.

Table V. Model for expectations of mean squares of a n^2 diallel cross, when the parental lines are fixed, for Hayman's analysis of variance.

Source	Expected mean square
A = parental lines	$\sigma^2 + 2n\sigma_{\epsilon}^2$
B = genetic interaction	$\sigma^2 + \frac{2n}{n-1}\sigma_s^2$
C = average maternal effects	$\sigma^2 + 2\sigma_r^2$
D = reciprocal effects	$\sigma^2 + 2\sigma_r^2$
Error	σ^2

Table VI. Levels of significance for Hayman's analysis of variance of the diallel crosses for the net scores. "P" means the item was tested against the pooled interaction mean square.

(a) Net Movement

		Daylight		Darkness	
		Cross I	Cross II	Cross I	Cross II
High	A	*	n.s.	n.s.	n.s.
	B	n.s.	n.s.	n.s.	n.s.
	C	* P	** P	n.s.	** P
	D	n.s.	n.s.	n.s.	n.s.
Med.	A	n.s.	n.s.	** P	*** P
	B	n.s.	n.s.	n.s.	n.s.
	C	n.s.	n.s.	n.s.	n.s.
	D	n.s.	n.s.	n.s.	*
Low	A	n.s.	** P	* P	*** P
	B	n.s.	n.s.	*	* P
	C	n.s.	n.s.	** P	*** P
	D	n.s.	** P	* P	**

(cont'd)

Table VI (cont'd)

(b) Crosses Summed for Net Movement

Temperature		Daylight	Darkness
High	A	n.s.	n.s.
	B	n.s.	n.s.
	C	** p	** p
	D	n.s.	* p
Med.	A	* P	*** p
	B	n.s.	n.s.
	C	n.s.	n.s.
	D	n.s.	n.s.
Low	A	*** p	*** p
	B	n.s.	n.s.
	C	n.s.	*** p
	D	* p	* p

Maternal effects (C) were quite significant at high temperature for daylight and darkness and at low temperature for darkness, but not at the intermediate temperature. At the medium temperature only in Cross II at darkness was there any reciprocal difference and this was in the "D" term, ie. those effects not ascribed to "C". At the low temperature during daylight there was no significance for maternal effects but the "D" term was significant for Cross II ($P < .01$).

The intrapopulation variability was further outlined in the percentage of the variance components of the diallel tables (Table VII). The additive component of variance ranged from 0 to 20 percent between the different tests, but also varied between crosses. The percent variance of the dominant effect was mostly zero except at low temperature. The error variance, ie the unexplained variance, was high which suggests that rearing and/or testing procedure could be an important factor.

The additive and dominant effects of the genes were shown schematically with the use of a graph. When the score for a hybrid of an inlet and an outlet cross fell exactly half way between the scores of the two parent stocks, then the genes were considered to be completely additive and no dominance existed. If the hybrid score favoured one of the adult stocks then there was evidence of some dominance. However, if the score fell somewhere outside the two parental stocks then this was considered "overdominance" (Falconer, 1960).

The mean scores at high temperature for the leading diagonal (Fig. 2) of the diallel cross and some of the hybrids were plotted on a daylight-darkness graph to show the additive variation of the genes (Fig. 7). There was overdominance in all cases except the Loon Inlet by Loon Outlet cross where the Inlet stock was dominant over the Outlet stock. However, with hybrids of the Pennask Inlet/Pennask Outlet cross; the fry acted more like inlet fry with very little movement during the daylight and a very strong downstream movement in the darkness. The same pattern also followed for the Thunder Inlet by Loon Outlet cross.

Table VII. Percent of the variance components for Hayman's analysis of the diallel tables of the net scores. Pooled mean square used as error variance.

(a) Net Movement

Temperature		Daylight		Darkness	
		Cross I	Cross II	Cross I	Cross II
High	A	4.37	0.47	0	0.79
	B	0	0	0	0
	C	39.66	50.87	11.55	58.77
	D	6.10	11.88	0	0.79
	E	49.86	36.78	88.45	39.65
Med.	A	5.49	2.63	11.02	19.57
	B	0	20.20	17.27	4.22
	C	0	0	0	0
	D	0.47	0	12.27	23.14
	E	94.05	77.17	59.44	53.07
Low	A	3.09	6.58	3.85	6.81
	B	0	9.98	4.33	9.15
	C	0	20.55	38.88	46.03
	D	5.92	27.89	19.97	12.22
	E	90.99	35.00	32.97	25.79

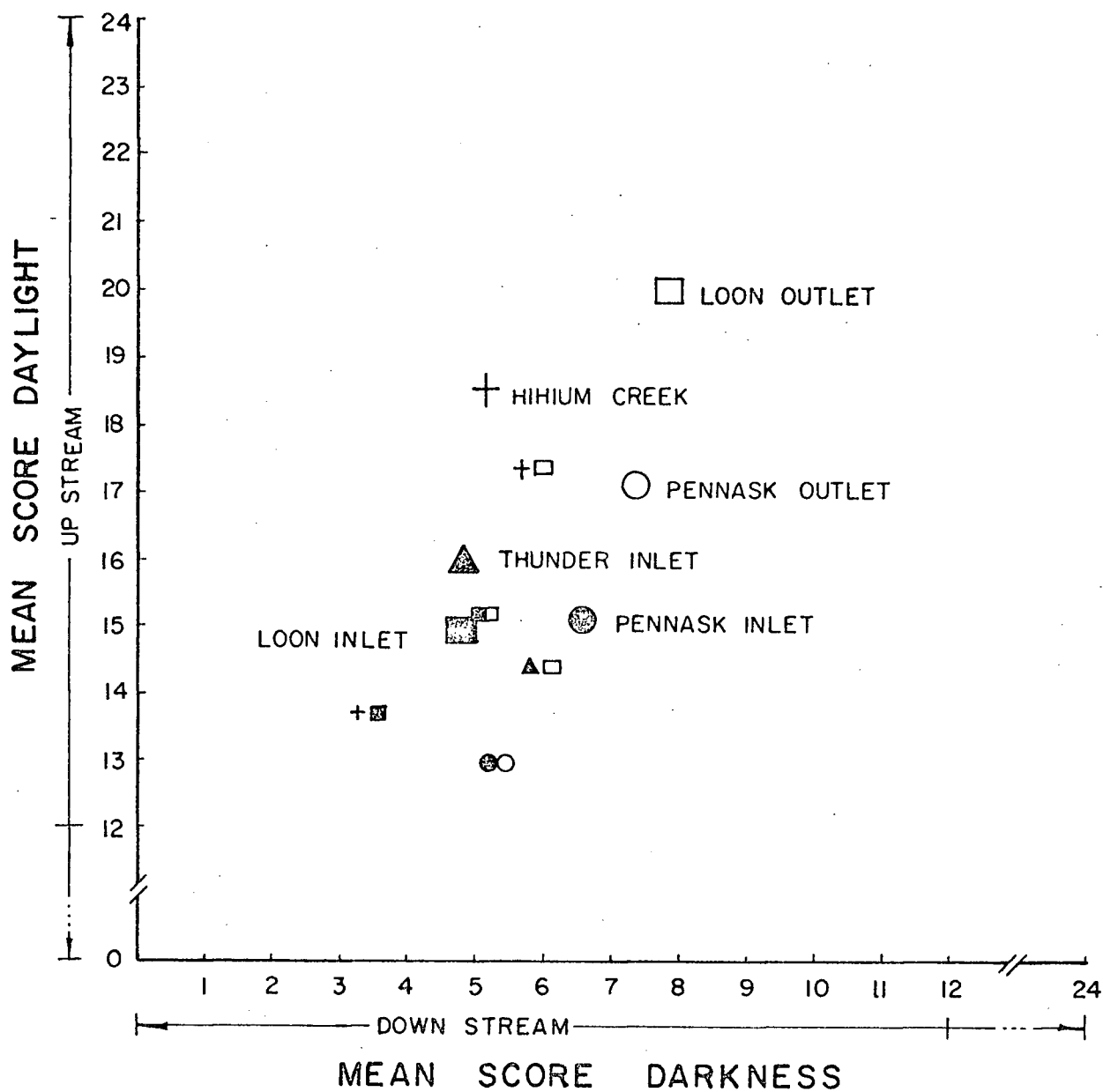
(cont'd)

Table VII(cont'd)

(b) Crosses Summed for Net Movement

Temperature		Daylight	Darkness
High	A	1.49	1.37
	B	0	0
	C	54.38	51.63
	D	10.21	14.38
	E	33.91	32.62
Med.	A	9.50	22.15
	B	5.35	4.59
	C	0	0
	D	0	16.65
	E	85.15	56.61
Low	A	13.38	6.24
	B	6.09	5.79
	C	12.86	54.59
	D	26.08	12.97
	E	41.58	20.42

Fig. 7 The mean scores of both crosses for the "leading diagonal" (see Fig. 2) and some of the hybrids for the daylight and darkness tests conducted at the high temperature (17.5 ± 1.50). Single symbols represent the self-crosses, paired symbols represent the hybrids.



where the influence of the Inlet parent seemed to be the most dominant. This was also evident in hybrids from crosses of Hihium Creek stock with Loon Inlet and Loon Outlet stocks. The Loon Inlet/Hihium Creek hybrids showed little movement during daylight and a strong downstream movement during darkness. Hybrids from the Loon Outlet/Hihium Creek cross showed a greater upstream movement in daylight and less downstream movement during darkness than the inlet fry.

Fig. 7 shows a positive correlation with regards to upstream-downstream movement. The three outlet stocks, Pennask Outlet, Loon Outlet and Hihium Creek, exhibited the greatest upstream movement during the daylight and the least downstream movement during the darkness. The inlet stocks showed the reverse.

At the medium temperature there was overdominance in all cases (Fig. 8). However, here the Pennask Outlet self-crosses show greater downstream movement than the Pennask Inlet self-crosses. Also, Thunder Inlet shows a very high score during the day.

(ii) The factorial analysis of variance

From the results of Hayman's analysis of variance on the experimental current responses of the fry, it would appear that the fish are reacting not only to genetic and maternal components, but also to environmental components as well. Wearden (1964) gives a model to test for maternal effects by using an analysis of variance between the rows and columns of the diallel crosses. One can further extend this analysis into a multi-factorial design and test for the environmental components of light and temperature as well.

The results of such an analysis of variance on all the data is summarized in Table VIII. Of the three main effects (males, females, and temperature), temperature is the most significant for all tests ($P < .001$). The male component is only significant during darkness ($P < .05$) while the female component is significant in all cases except cross I Darkness.

The graphical interpretation of the male by female interaction shows that the female components of the diallel cross had a slightly

Fig. 8 The mean scores of both crosses for the "leading diagonal" and some of the hybrids for the daylight and darkness tests conducted at the medium temperature ($9.7 \pm .5^\circ\text{C}$). Single symbols represent the self-crosses, paired symbols represent the hybrids.

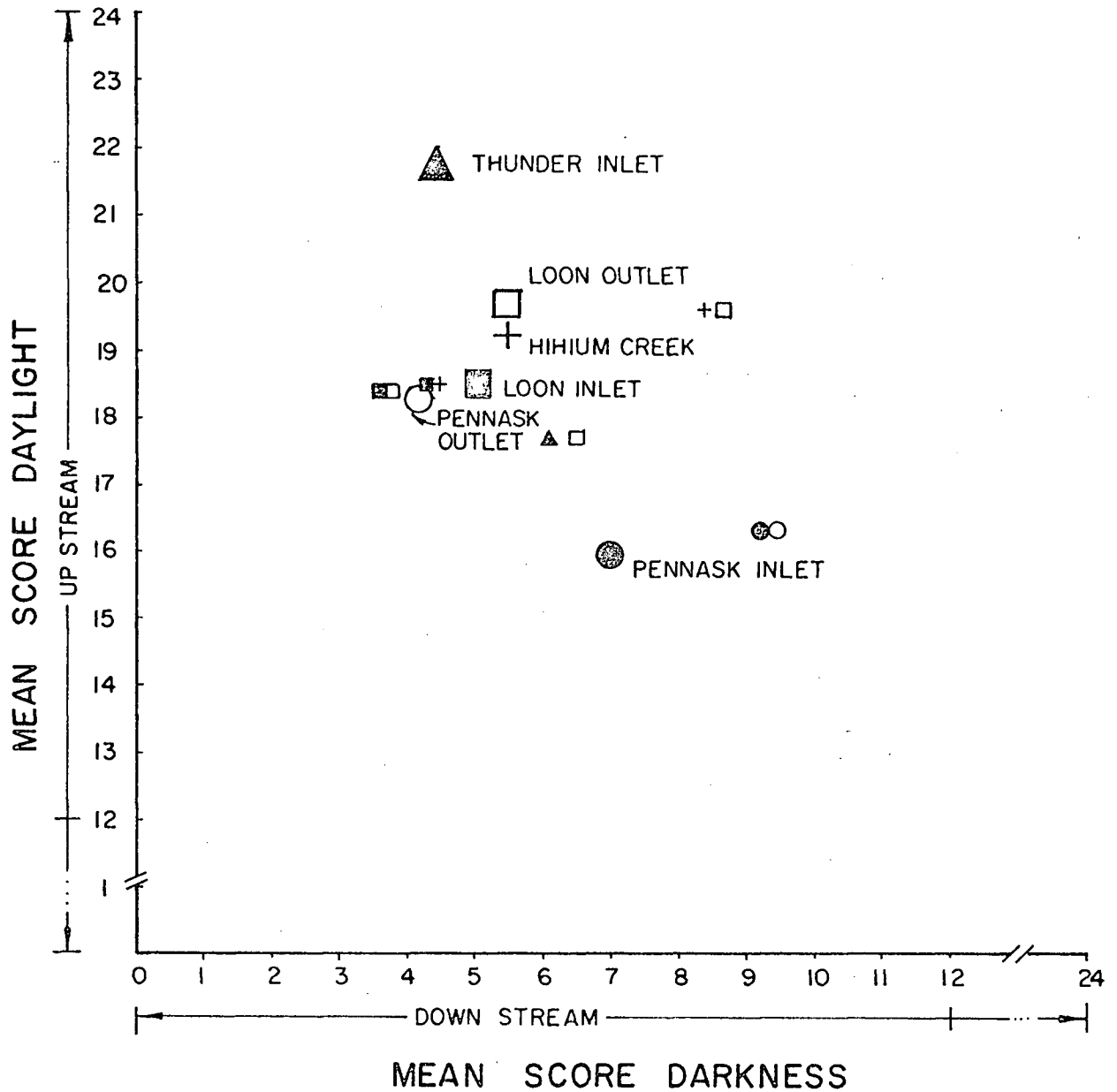


Table VIII Three -factor analysis of variance table on the net scores where A = males, B = females, C = temperature for the daylight and darkness runs for both Cross I and Cross II.

Source	d.f.	Cross I Daylight		Cross II Daylight		Cross I Darkness		Cross II Darkness		
		M.S.	F	M.S.	F	M.S.	F	M.S.	F	
Main effects										
A = males	6	20.9539	1.81 n.s.	37.5122	2.04 n.s.	20.4406	2.39 *	45.3519	2.80 *	
B = females	6	28.9789	2.50 *	39.6986	2.16 *	15.7645	1.84 n.s.	94.1453	5.81 ***	
C = temperature	2	695.0153	59.98 ***	162.5654	8.84 ***	189.2228	22.12 ***	256.5955	15.84 ***	
Interactions										
A × B	36	19.4585	1.68 **	30.6723	1.67 *	8.6791	1.01 n.s.	35.4247	2.19 ***	
A × C	12	12.6842	1.09 n.s.	43.6188	2.37 **	13.2756	1.55 n.s.	59.9597	3.70 ***	
B × C	12	16.4793	1.42 n.s.	31.5760	1.72 n.s.	9.2704	1.08 n.s.	45.1004	2.78 **	
A × B × C	72	6.8715	0.59 n.s.	23.7895	1.29 n.s.	10.4396	1.22 n.s.	15.6011	0.96 n.s.	
Error	147	11.5866		18.3953		8.5547		16.2000		
Total	293									

higher score than the male components, (Fig. 9), although there were some exceptions. These were Loon Inlet and Pennask Outlet, and it was these stocks that showed the greatest degree of interaction.

The two genotype by environmental interactions, i.e. male by temperature and female by temperature, are shown in Figures 10 and 11. Although temperature was highly significant ($P < .001$) in the main effects it did not appear so important in the interactions. For the male by temperature, it was significant for Cross II. This was due mainly to the Thunder Inlet stock at the medium temperature during daylight and the Pennask Outlet and Below Hihium stocks during darkness. For the female by temperature interaction, only cross II Darkness was significant ($P < .01$). Again, this was due to Pennask Outlet and Below Hihium stocks. This shows intrapopulation variability as well as interpopulation variability.

The variance components of the analysis again show that the environmental components of temperature in the interactions was quite low (Table IX). Only for the interaction of males by temperature for cross II darkness does the variance reach 10% of the total variance. Most of the variance in the analyses was due to the error term, which again indicates that rearing and/or testing procedure, ie environmental effects, could be important.

As with Hayman's analysis, the analysis of variance further points out that there are genetic differences among the parents. However, two very important features are shown in the graphical interpretations of the first order interactions. First, the only environmental component that causes an actual change in direction is light. In the daylight, the movement is predominately upstream while in the darkness it is predominately downstream. The second important feature is that the three different temperatures do not cause a change of direction in the fish movement but only a change in the intensity of the movement either upstream or downstream.

The results of the leading diagonal was plotted on a high temperature by low temperature graph (Fig. 12). For the daylight tests the order of the upstream migrants and the downstream migrants are as one would expect with the Inlet fry showing the

Fig. 9 First order interactions of male (A) by female (B) in the factorial analysis of males by females by temperature.

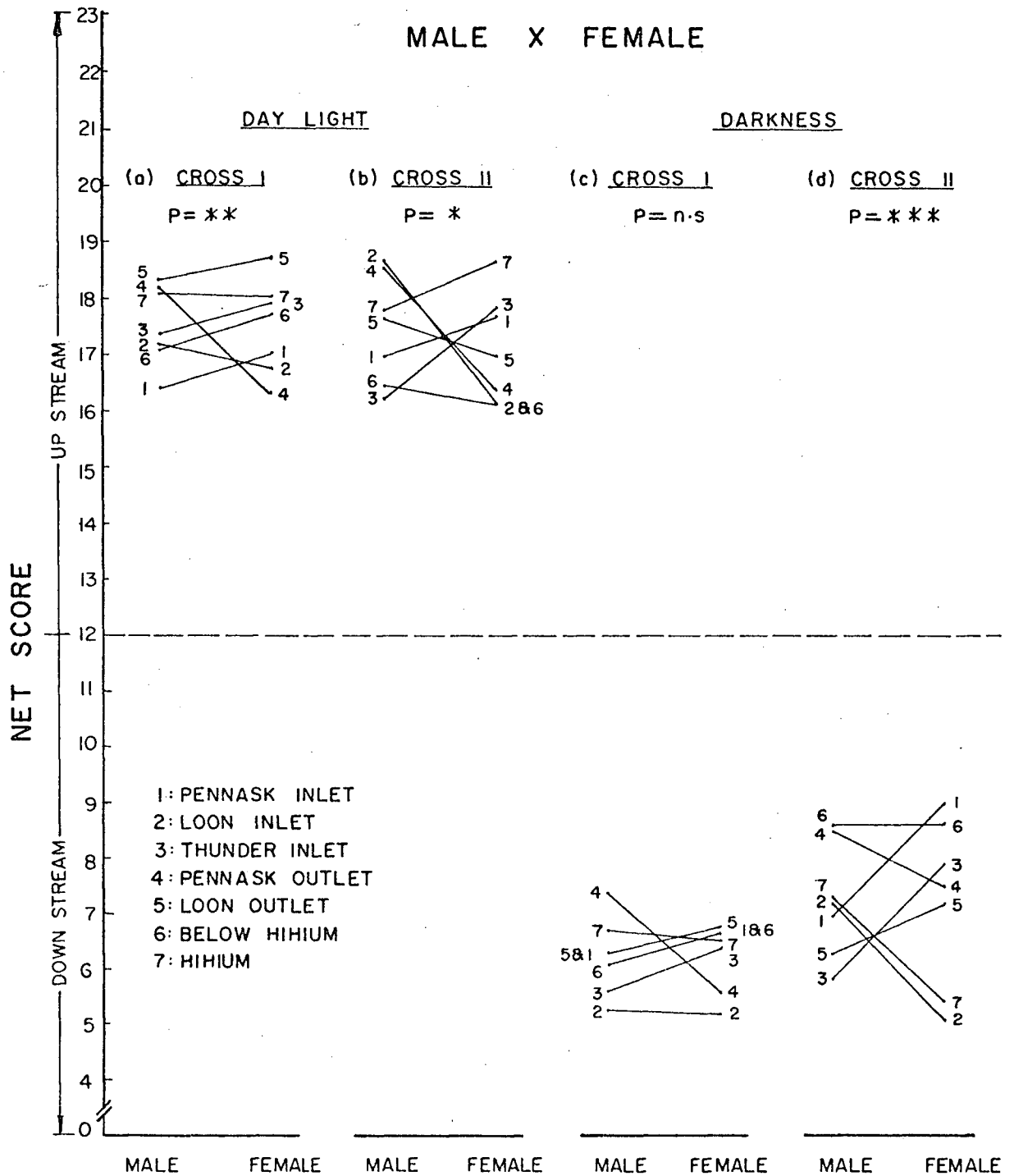


Fig. 10 First order interactions of male (A) by temperature (C) in the factorial analyses of males by females by temperature.

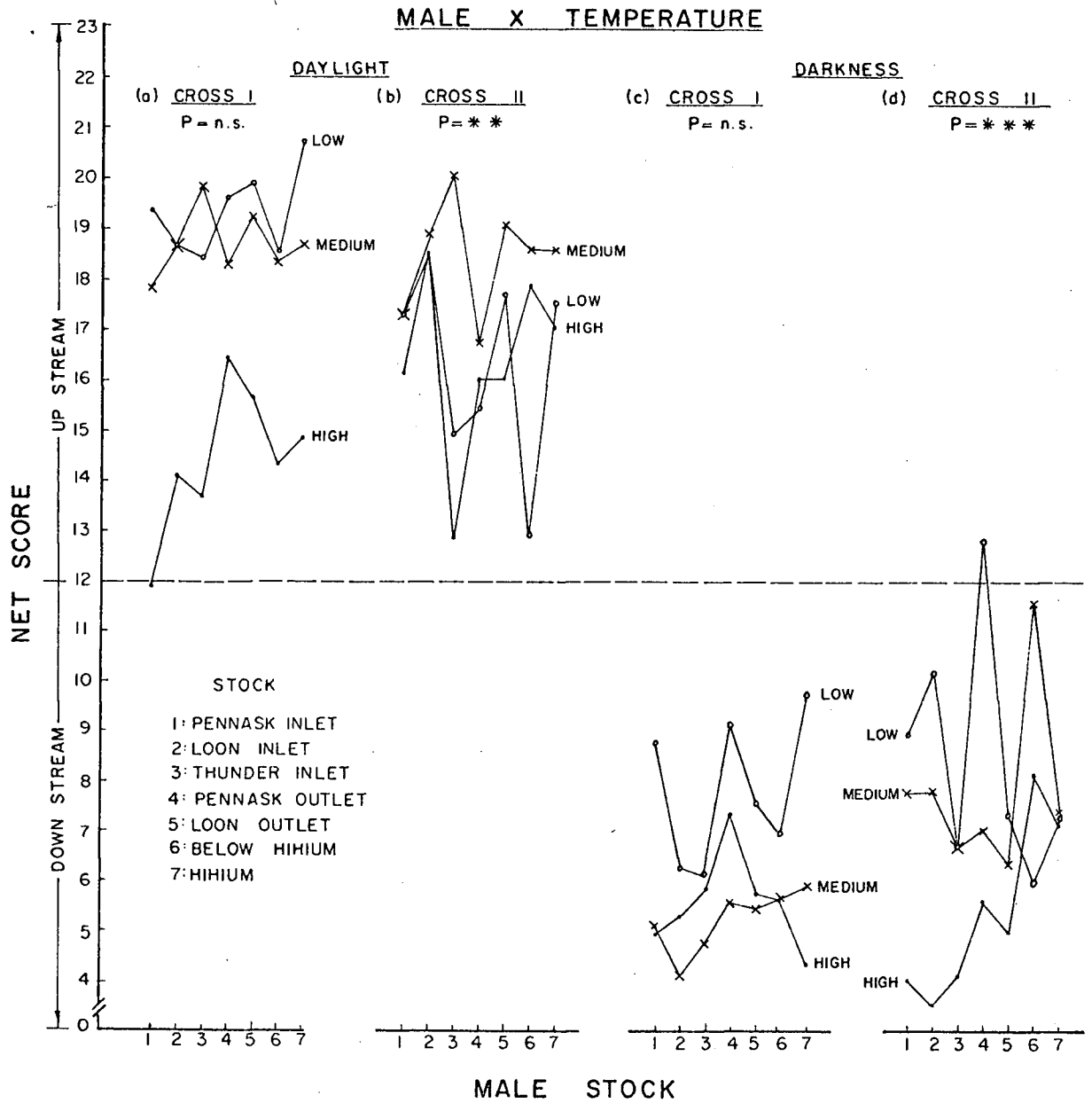


Fig. 11 First order interactions of female (B) by temperature (C) in the factorial analyses of males by females by temperature.

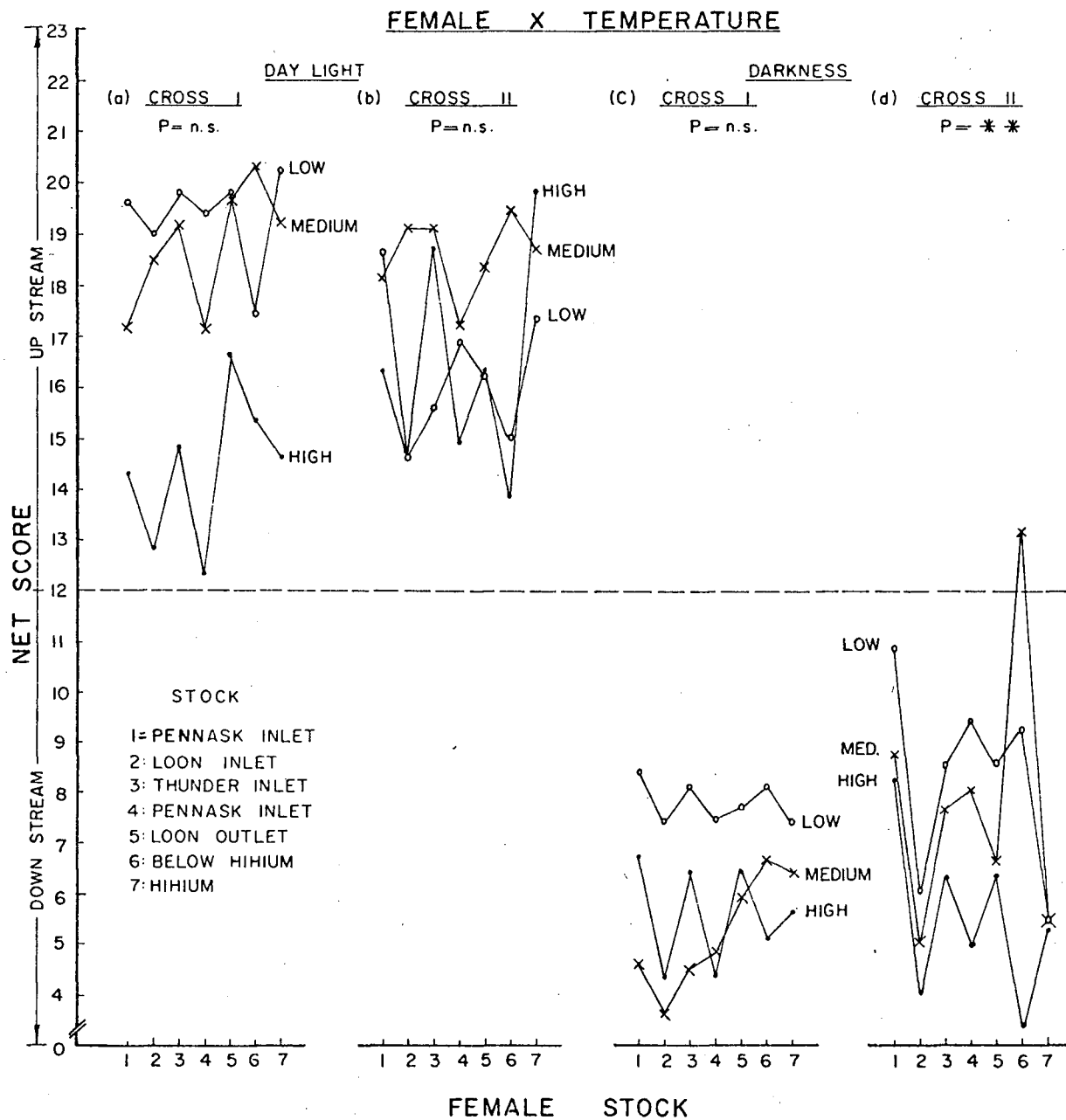
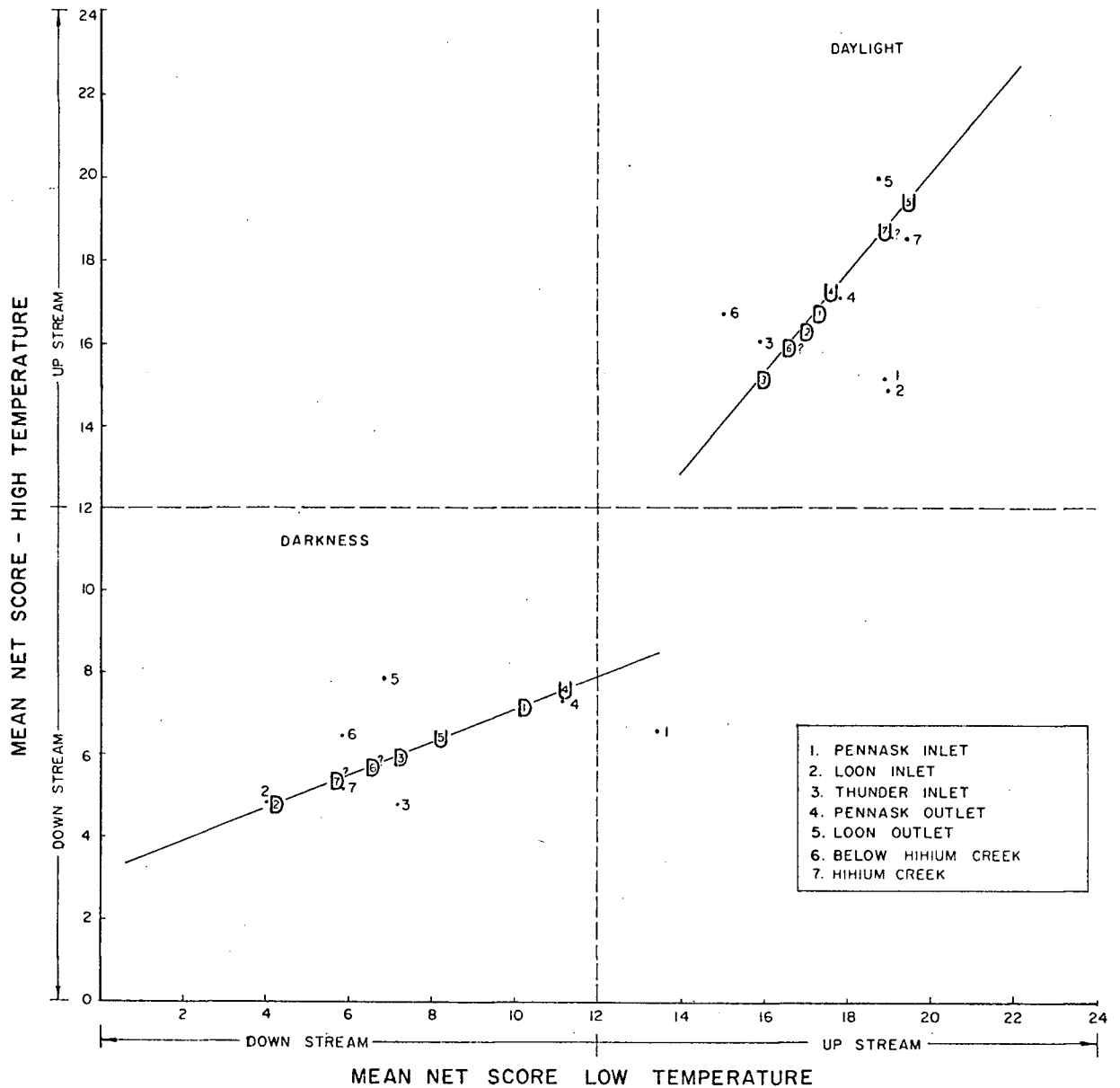


Table IX. Expected mean squares for three-factor analysis of variance, fixed model, and the variance components for the four analyses of Cross I and II for daylight and darkness. $n = 2$, $a = 7$, $b = 7$, $c = 3$.

Source	Expected mean square	Variance component				Percent variance			
		Cross I Daylight	Cross II Daylight	Cross I Darkness	Cross II Darkness	Cross I Daylight	Cross II Daylight	Cross I Darkness	Cross II Darkness
A = males	$\sigma^2 + nbc\tilde{\sigma}_A^2$	0.2230	0.4552	0.2830	0.6941	1.07	1.61	2.32	2.35
B = females	$\sigma^2 + nac\tilde{\sigma}_B^2$	0.4141	0.5072	0.1717	1.8558	1.98	1.79	1.41	6.27
C = temperature	$\sigma^2 + nab\tilde{\sigma}_C^2$	6.9738	1.4711	1.8436	2.4530	33.31	5.20	15.11	8.29
A × B	$\sigma^2 + nc\tilde{\sigma}_{AB}^2$	1.3120	2.0462	0.0207	3.2041	6.27	7.23	0.17	10.83
A × C	$\sigma^2 + nb\tilde{\sigma}_{AC}^2$	0.0784	1.8017	0.3372	3.1257	.37	6.36	2.76	10.56
B × C	$\sigma^2 + na\tilde{\sigma}_{BC}^2$	0.3495	0.9415	0.0511	2.0643	1.67	3.32	0.42	6.97
A × B × C	$\sigma^2 + n\tilde{\sigma}_{ABC}^2$	<0	2.6971	0.9425	<0	0	9.53	7.72	0
Error	σ^2	11.5866	18.3953	8.5547	16.2000	55.34	64.97	70.10	54.74

Fig. 12 The mean scores of both crosses for the "leading diagonal" in a linear regression of high temperature and low temperature for daylight and for darkness. Question mark means it is uncertain if the stock should be called an upstream or a downstream stock.



least movement and the Outlet fry showing the greatest upstream movement. The same applies for the darkness tests except for the Pennask Inlet tests at the low temperature where there was very little movement. Question marks appear beside the Hihium (7) and Below Hihium (6) stocks because one can only postulate as to their behaviour. It is expected the Hihium fry would move upstream during the daylight and downstream during darkness. As for the Below Hihium fry, it is believed that there are some resident stock spawning in this area and it is difficult to speculate as to how their progeny would behave in the experimental channels.

VI

DISCUSSION

Conditions of the inlet and outlet streams of Loon Lake were studied in detail by Northcote (1962) and observed by myself in the summer of 1969. Northcote found that downstream movement of rainbow trout fry occurred in both Inlet and Hihium Creeks where the water temperature rarely exceeded 13 C. This migration took place almost entirely at night when illumination fell below 0.01 foot-candles. He reported some occasional downstream movement in the Outlet Creek when the water temperature was >14 C for several days. Further, he found that the Outlet fry maintained position at night, but only when water temperature was >14 C. The upstream movement of the fry occurred only in the Outlet Creek where the summer water temperature was >15 C. It is also known that the Inlet fry move downstream into the lake shortly after emergence from the gravel, while the Outlet fry remain in the creek for one to two months or even up to one or two years before migrating to the lake.

The diel movement of sockeye salmon is also similar to rainbow trout, where the upstream movement is almost entirely in the daylight and the downstream movement is predominately at night (Brannon 1967; McCart 1967). McDonald (1960) also reports that pink, coho and chum fry movement downstream was nocturnal and rather precisely regulated by light and its changes in intensity.

From more recent studies on sockeye salmon and other stream systems con-

taining trout, it would appear that there are innate as well as environmental controls governing the migratory behaviour of rainbow trout. The results obtained in this study further support this hypothesis. However, the experimental results here suggest that the marked differences of water temperatures between the two types of stream systems are not one of the major controls affecting the lakeward migration of the young trout.

A. Genetic Effects

The Mendelian method of studying genetic traits involves crossing known genotypes differing in phenotype and arriving at the first (F_1) and second (F_2) filial generations of offspring and then backcrossing these to the parent strains. However, Broadhurst (1967) points out that prior to 1956 there were only four cases where this type of analysis was applied to behavioural characteristics. Further, this type of analysis is not very feasible for long lived species. Therefore, the diallel cross is believed to be the best way to determine whether there are genetic differences between families when one can only deal with one generation of progeny (Broadhurst, 1967). Both statistical methods used in the analyses of the data herein show that there is a genetic difference between the behavioural current responses of the seven stocks chosen.

One should expect from field observations that if there are genetic differences between the Inlet and Outlet fry that when they are tested together under the same light conditions, the Inlet fry should hold during daylight while the Outlet fry should move upstream. The reverse should happen during darkness where the Outlet fry should hold and the Inlet fry move downward.

However, in Loon Outlet Creek no fry move up to the lake immediately after hatching. Some remain in the creek for two to three months while others remain in the creek for one to two years. Thus, at the age at which the fry from the diallel cross were tested they should remain in the test apparatus for both daylight and darkness tests. However, Slaney (MS 1972 and personal communication) found that at very low food levels a large number of fry moved out of his test channel, while at high food levels most of the fry remained. Of the inlet fry that moved out of the testing channel at the low food level, 70% moved downstream.

At the high food level 70% moved upstream, but this was a lesser number of fish than at the low food level. For the outlet fry, of those moving out of the test channel 50% went upstream and 50% went downstream at both food levels. This suggests that the absence of food also plays a key role in the start of migration of the young fry. No food was present in the current response channels and this might explain why the Outlet stocks moved upstream during the day. If this explanation is true, then Figure 12 shows that the fry did behave as expected in the experimental troughs with some exceptions.

Variations between replicas can partially be explained by Lindsey et al. (1959). In their study of adult rainbow trout they found that some mixing of the two populations did occur. Thus, there is always the possibility that the adults collected from the Outlet could have included some fish originating from the inlet or vice versa. Thus, at no time would an Inlet or an Outlet stock show 100% movement in the required direction. Lindsey et al. (1959) reported that homing was 94% accurate for both streams. However, the Inlet usually contains two to three times more fish than the Outlet so the chance of picking up an Outlet adult in the Inlet is two to three times less than in the Outlet.

The fact that the Outlet fry do not usually move upstream until they are older than those used in the tests, might also cause some variability in these tests. It is very difficult to say whether there is a greater intrapopulation variability within the outlet stock than within the inlet stock. However, the graphical interpretation of the male by female interactions did show that the Pennask Outlet male-female crosses had the greatest amount of interaction for both replications. Also, the Loon Inlet male-female crosses of the second replica showed a large interaction.

Further, one would expect that there would not always be a 100% movement in one direction and that there should always be some variation in the population. Thus, if a disaster occurred in one stream there would always be a small proportion of the population left to carry on the population.

However, the most important behavioural aspect of these tests is that the fry were behaving in the appropriate way to light and to dark

tests as to what had been observed in the field. Because there was more upstream movement by the Outlet stocks in daylight tests and more downstream movement by the Inlet stocks in darkness tests, the evidence for genetic differences between the stocks is further strengthened.

Evidence for genetic differences between stocks is also seen when one examines the crosses of the inlet stocks with the outlet stocks. Figures 7 and 8 show overdominance as well as additive genetic effects. In most cases the inlet stocks appear to be slightly dominant over the outlet stocks especially for Loon Inlet at the high temperature. The crosses of the Hihium stock with the Inlet and Outlet stocks also show that the Inlet parent was partially dominant with respect to current response. In a similar study by Calaprice (1972b) it was found that there was additive genetic variation between sockeye stocks of the Babine Lake system. The statistical analyses on the tests performed with pure stocks also show that there is a difference between the stocks. However, these differences do not seem consistent with those apparent in the field. That is, while there is a marked difference between daylight and darkness tests, the Inlet stocks showed more upstream movement in the day and the least movement at night. Precautions were taken to ensure that there was no mixing of the stocks, but the absolute possibility of this happening cannot be ruled out.

One possible explanation of the current response of the pure stocks and the intrapopulation variability of the diallel cross could arise from three phenotypic forms of liver lactate dehydrogenase (L.D.H.) that exist in the fish of the two lakes systems. Northcote, et al. (1970) found that there were three phenotypic forms of L.D.H. in stream populations of rainbow trout from above and below a waterfall. These consisted of two homozygous strains (CC and C'C') and a heterozygous strain (CC'). It

has since been shown that the CC strain has the ability to rid itself of lactic acid in the muscle tissues four to five times faster than the other homozygous strain (H. Tsuyuki, personal communication). This allows the above falls population, CC, to remain in the faster flowing water for a much longer time period.

In preliminary studies (unpublished data, H. Tsuyuki) it was found that the three strains existed in the Loon and Pennask Lake systems. Tests were conducted on Loon Inlet fry with the two homozygous strains (CC and C'C') of L.D.H. (Fig. 13). The CC strain showed more upstream movement during the day while the C'C' showed greater downstream movement at night ($P < .001$). Thus, the phenotypic form of the adults could have greatly influenced the current response of the offspring used to test the pure stocks. Unfortunately the L.D.H. types of the parents used in the pure stocks are not known but an analyses was done on the adults of the diallel cross (Table X) and from this it is possible that there could have been a very high percentage of CC strains in the Inlet stocks. The interpopulation variability is not significant ($P > .05$), but there is a large intrapopulation variability ($P < .01$). The Pennask system for both the Inlet and Outlet have a high percentage of the CC strain. However, the stocks taken from the Outlet system of Loon Lake have a larger percentage of the C'C' than the CC. Furthermore, because there is a large variability in the number of L.D.H. strains between replicas of the diallel cross, this could also partially explain the intrapopulation variability in the current response of the diallel cross.

Fig. 13 Net score of the current response of Loon Inlet fry which contain two separate phenotypic forms of liver lactate dehydrogenase. Tests conducted in 25-compartment performance channels. Circle and bar represent mean and ranges, respectively of 12 replicas.. Data provided by T.G. Northcote; to be published elsewhere.

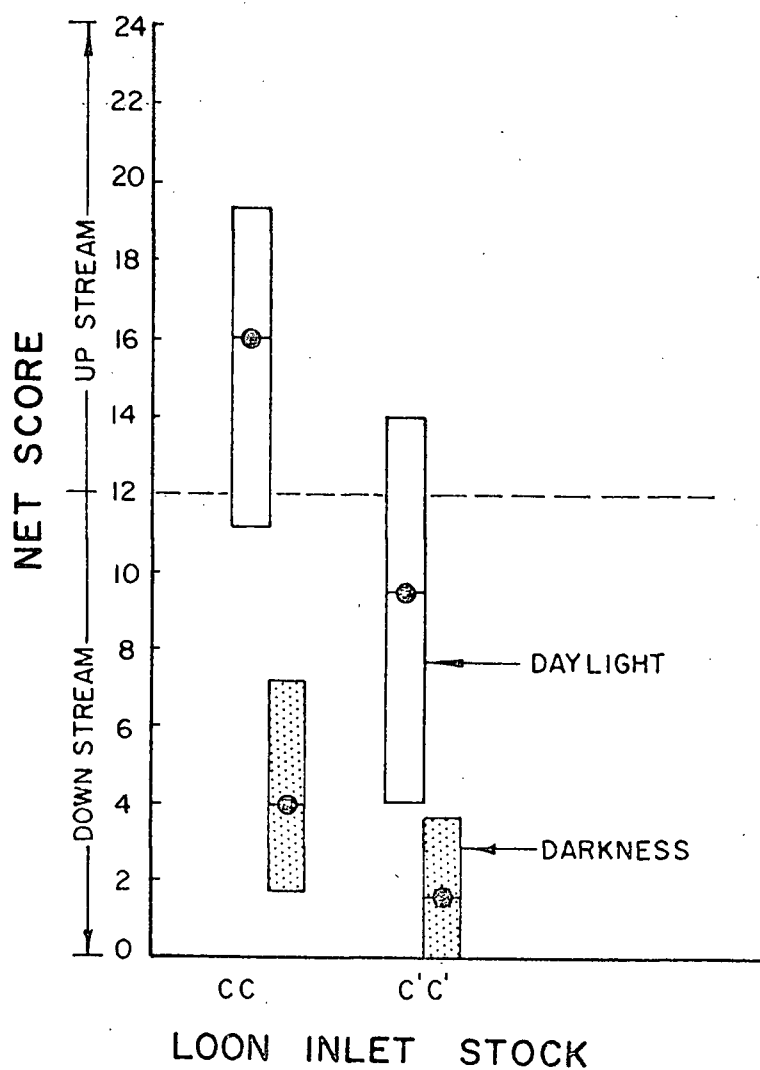


Table X. The percent of the adults used in the diallel cross which contain the three types of liver lactate dehydrogenase (L.D.H.). See text for explanation of the three types of L.D.H.

Stock	Cross	Number	% CC	% CC'	% C'C'
Pennask Inlet	I	16	56	44	-
	II	11	55	36	9
	mean		<u>55.5</u>	<u>40</u>	<u>4.5</u>
Loon Inlet	I	14	7	64	29
	II	15	33	67	-
	mean		<u>20</u>	<u>65.5</u>	<u>14.5</u>
Thunder Creek	I	12	50	17	33
	II	13	<u>15</u>	<u>39</u>	<u>46</u>
	mean		<u>32.5</u>	<u>28</u>	<u>39.5</u>
Pennask Outlet	I	13	46	46	8
	II	14	<u>64</u>	<u>29</u>	<u>7</u>
	mean		<u>55</u>	<u>37.5</u>	<u>7.5</u>
Loon Outlet	I	13	15	39	46
	II	13	<u>31</u>	<u>46</u>	<u>23</u>
	mean		<u>23</u>	<u>42.5</u>	<u>34.5</u>
Below Hihium	I	10	-	50	50
	II	20	<u>10</u>	<u>35</u>	<u>55</u>
	mean		<u>5</u>	<u>42.5</u>	<u>52.5</u>
Hihium Creek	I	12	-	58	42
	II	21	<u>14</u>	<u>62</u>	<u>24</u>
	mean		<u>7</u>	<u>60</u>	<u>33</u>

B. Maternal Effects

Very little can be said about maternal effects other than that the analysis shows they do exist. One possible explanation could be that an extra amount of cytoplasm was contributed by some females to their eggs, and thus, the alevins would have a larger yolk sac. This might allow some fry to be stronger than others in their swimming abilities. Calaprice (1972b) found in his study of sockeye salmon that there was no maternal influence when he tested the fry for current response. However, he did find (Calaprice, 1972a) that maternal effects were directly related to the survival of the young fry. One reason was the presence of parasitic nematodes in the females. In this study of rainbow trout, there was no evidence of internal parasites in the Loon Lake females, but both stocks of females from Pennask Lake carried a very large number in the body cavity. Calaprice also states that maternal effects could be a possible mechanism for decreasing the reproductive potential of a population and thereby affecting regulation.

Eisen (1967) points out that maternal effects can mask genetic effects. However, one would need several generations of trout in order to test for this.

C. Temperature Effects

The most obvious environmental differences between the inlet and outlet streams of both Pennask Lake and Loon Lake are the differences in water temperature. The experimental results here, however, suggest that different water temperatures are not the main influence which causes the appropriate current response of the two types of stocks. Further, Brannon (1967) in his study of sockeye salmon, reports in his field observations that there is only a 2C difference between the water temperatures of the Chilko River (upstream races) and the Stellako

River (downstream races). In his experimental tests he found that temperature had no effect on directional preference.

In these experiments the different water temperatures only caused an intensity change in directional movement. Furthermore, this intensity change was the opposite to what one would expect from field observations. That is, there was even less upstream movement at the high temperature than at the low temperature for both daylight and darkness tests. This was also evident for the tests conducted on the pure stocks. However, when one examines the results as shown in Fig. 10 and 11, at high temperature it is the inlet stocks that moved the least amount during daylight. At the low temperature during the darkness tests the inlet stock moved the farthest, with the exception of the Pennask Inlet stock. Here there could have been some mixing of the two Pennask spawning stocks as there are some differences between the male by female interaction as shown in Fig. 9d. Thus, the different water temperatures may have some influence on the migration behaviour of the fry.

If water temperature was playing a key role in the migration of inlet and outlet fry, one would expect they would have a high upstream score of twenty-four during the high temperature and a very high downstream score of near zero at the low temperature. This is on the assumption that the early outlet fry move upstream because of very low food levels. This would give a slope of zero on a high temperature score by low temperature score graph with the line running parallel to the Y-axis. If temperature was only causing an intensity change, then during the daylight, the Outlet stock would have a score of near twenty-four while the Inlet stock would have a value of twelve or slightly higher due to the mixing of stocks and the slight temperature effect. This would then give a positive correlation on the temperature graph as is shown in

Fig. 12.

It is important to mention that this influence of temperature is not the same as Northcote's further conclusion that sharp rises in water temperature are associated with upstream movement. This has been observed in several other lakes as well (Northcote 1969).

Raleigh and Chapman (1971) found that experimental tests at different temperatures with cutthroat trout fry altered the ratio of outlet fry moving upstream and downstream, but did not alter the direction of movement of inlet fry. Raleigh (1971) found in a study with sockeye salmon, that temperature changes had a greater effect on outlet stocks than inlet stocks, but again, this was only an intensity change and not a directional change. He further points out that temperatures, such as cold fluctuations in the outlet streams, only delay upstream migration and do not prevent the fry from eventually reaching the lake.

D. Other Possible Effects

a) Water sources

In Northcote's (1962) study on Loon Lake an experiment was conducted using Loon Outlet and Hihium Creek water to test for the fry's behavioural response to temperature differences. He found that there was considerably more downstream movement in the cooler Hihium water than the warmer Outlet water where the movement was mainly upstream. Brannon (1967) in a similar test with sockeye fry at Cultus Lake, although his results were similar to Northcote's findings, concluded that the cue eliciting the upstream response was in the lake water and was not due to temperature differences. Raleigh (1971) states that sockeye salmon fry can distinguish between sources of water and obtain directional cues, but that this mechanism

is poorly understood.

b) Other environmental effects

As stated earlier, the environmental component of variance consists of maternal effects as well as any effect that the animal's surroundings may have. In a diallel cross the rearing facilities as well as the testing procedure are thus included as being part of the environmental component. In both Hayman's analysis and the factorial analysis of variance the percent error variance was very high (Table VII and IX). This suggests that rearing and/or testing procedure could also be important, although there could also be unknown factors that caused the error variance to be so high.

VII

CONCLUSIONS

(1) Experimental results of the progeny of a diallel cross between seven inlet and outlet spawning stocks of Loon Lake and Pennask Lake, British Columbia, indicate that there are genetic differences in behavioural responses to current between the stocks.

(2) These differences are such that basically the inlet stocks hold during daylight tests and move downstream during darkness tests while the outlet stocks move upstream during daylight tests and hold during darkness tests.

(3) The most obvious differences between the inlet and outlet streams is the water temperature difference with cool inlet water and warm outlet water. The experiments conducted in this study show that different water temperatures do not cause a direction change in fry's current response, but

only an intensity change in its movement. However, at the temperature of 18 C the outlet fish did show the greatest upstream movement during the daylight and the least downstream movement during the darkness.

(4) Although there is a large intrapopulation variability, genetic differences between populations and light intensity appear to be the most important mechanism controlling migration while temperature differences between streams only play a minor role.

(5) Other mechanisms that may operate in the control of migration are water quality and source, the absence or presence of food, and the heritable trait of liver lactate dehydrogenase and its ability to dissipate lactic acid in the muscle tissue.

BIBLIOGRAPHY

- Brannon, E.L. 1967. Genetic control of migrating behaviour of newly emerged sockeye salmon fry. Int. Pac. Salmon Fish. Comm. Progr. Rep. 16: 31 p.
- Broadhurst, P.L. 1967. An introduction to the diallel cross, p. 287-304. In Behaviour genetic analysis. Series in Psychology. McGraw-Hill Book Co., New York, N.Y.
- Calaprice, J.R. 1972a. Heritable variation in five populations of sockeye salmon, Oncorhynchus nerka. I. Early migration. (Submitted for publication.)
- Calaprice, J.R. 1972b. Heritable variation among populations of sockeye salmon, Oncorhynchus nerka. III. Differences in the migratory behaviour of fry in a current. (Submitted for publication.)
- Eisen, E.J. 1967. Mating designs for estimating direct and maternal genetic variances and direct maternal genetic covariances. Can. J. Genet. Cytol. 9: 13-22.
- Falconer, D.S. 1960. Introduction to quantitative genetics. The Ronald Press Co. N.Y. 365 p.
- Harden-Jones, F.R. 1968. Fish migration. Edward Arnold (Publishers) Ltd., London, W.I. 325 p.
- Hayman, B.I. 1954a. The theory and analysis of the diallel crosses. Genetics 39: 789-809.
- Hayman, B.I. 1954b. The analysis of variance of diallel tables. Biometrics 10: 235-244.
- Hoar, W.S. 1953. Control and timing of fish migration. Biol. Rev. 28: 437-452.
- Kearsey, M.J. 1965. Biometrical analysis of a random mating population: A comparison of five experimental designs. Heredity 20: 205-235.
- Kelso, B.W. 1970. The upstream-downstream migration of rainbow trout in inlet and outlet streams of Loon Lake, British Columbia. Directed studies under T.G. Northcote, Dept. of Animal Resource Ecology, University of British Columbia.
- Lindsey, C.C., T.G. Northcote, and G.F. Hartman. 1959. Homing of rainbow trout of inlet and outlet spawning streams at Loon Lake, British Columbia. J. Fish. Res. Bd. Canada 16: 695-719.

- McCart, P. 1967. Behaviour and ecology of sockeye salmon fry in the Babine River. J. Fish. Res. Bd. Canada 24: 375-428.
- McDonald, J. 1960. The behaviour of Pacific salmon fry during their downstream migration to freshwater and saltwater nursery area. J. Fish. Res. Bd. Canada 17: 655-676.
- Mather, K. 1971. On biometrical genetics. Heredity 26: 349-364.
- Mather, K., and J.L. Jinks. 1971. Biometrical genetics. 2nd ed. Chapman and Hall Ltd., London. 382 p.
- Northcote, T.G. 1962. Migratory behaviour of juvenile rainbow trout, Salmo gairdneri, in outlet and inlet streams of Loon Lake, British Columbia. J. Fish. Res. Bd. Canada 19: 201-270.
- Northcote, T.G. 1969. Patterns and mechanisms in the lakeward migratory behaviour of juvenile trout, p. 181-204. In T.G. Northcote (ed.) Salmon and trout in streams. H.R. McMillan Lectures in Fisheries, University of British Columbia, Vancouver, B.C.
- Northcote, T.G., S.N. Willisroft, and H. Tsuyuki. 1970. Meristic and lactate dehydrogenase genotype differences in stream populations of rainbow trout below and above waterfall. J. Fish. Res. Bd. Canada 27: 1987-1995.
- Raleigh, R.F. 1967. Genetic control in the lakeward migrations of sockeye salmon (*Oncorhynchus nerka*) fry. J. Fish. Res. Bd. Canada 24: 2613-2622.
- Raleigh, R.F. 1971. Innate control of migrations of salmon and trout fry from natal gravels to rearing areas. Ecology, Vol. 52, No. 2: 291-297.
- Raleigh, R.F., and D.W. Chapman. 1971. Genetic control in lakeward migrations of cutthroat trout fry. Trans. Amer. Fish. Soc. No. 1: 33-40.
- Slaney, P.A. 1972. Effects of prey abundance on distribution, density, and territorial behaviour of young rainbow trout in streams. M. Sc. thesis, Univ. of British Columbia. 74 p.
- Sokal, R.R., and F.J. Rohlf. 1969. Biometry. W.H. Freeman and Company, San Francisco. 776 p.
- Wearden, S. 1964. Alternative analysis of the diallel cross. Heredity 19: 669-680.

Appendix 1. Worked examples showing maximum, minimum downstream-upstream movement of fish in twenty-two hypothetical tests using the formula:
 $\Sigma(n+r)/N_T + 12$ where n = number of fish per compartment, r = rank number, N_T = total number of fish used in the test.

Rank #	12	11	10	9	8	7	6	5	4	3	2	1	0	1	2	3	4	5	6	7	8	9	10	11	12	Upstream score	Downstream score	Net score
Compartment #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25			
Test 1	0																							20	12.000	24.000	0.000	
2	1																							19	12.600	23.400	1.200	
3	2																							18	13.200	22.800	2.400	
4	3																							17	13.800	22.200	3.600	
5	4																							16	14.400	21.600	4.800	
6	5																							15	15.000	21.000	6.000	
7	6																							14	15.600	20.400	7.200	
8	7																							13	16.200	19.800	8.400	
9	8																							12	16.800	19.200	9.600	
10	9																							11	17.400	18.600	10.800	
11	10																							10	18.000	18.000	12.000	
12	11																							9	18.600	17.400	13.200	
13	12																							8	19.200	16.800	14.400	
14	13																							7	19.800	16.200	15.600	
15	14																							6	20.400	15.600	16.800	
16	15																							5	21.000	15.000	18.000	
17	16																							4	21.600	14.400	19.200	
18	17																							3	22.200	13.800	20.400	
19	18																							2	22.800	13.200	21.600	
20	19																							1	23.400	12.600	22.800	
21	20																							0	24.000	12.000	24.000	
22	0					2				1			1		4			3			2		1	4	12.833	17.944	6.889	

Appendix 2. The behavioral scores for the diallel cross (for Blocks 1 and 2, Cross I and II) together with their means at the three temperatures ($17.5 \pm 1.5^{\circ}\text{C}$; $9.7 \pm 0.5^{\circ}\text{C}$; $5.0 \pm 1.5^{\circ}\text{C}$).

NET DIALLEL SCORES CROSS I DAYLIGHT, HIGH TEMPERATURE

F E M A L E

	1	2	3	4	5	6	7
1	13.800	16.500	13.399	4.450	15.250	14.350	14.750
	17.850	7.849	8.761	7.700	13.800	5.099	13.050
	15.825	12.175	11.080	6.075	14.525	9.725	13.900
2	12.050	12.650	11.750	12.350	16.500	13.000	11.350
	14.450	11.473	13.449	10.350	13.949	19.150	18.400
	13.250	15.561	12.600	11.350	15.225	16.075	14.875
3	14.300	7.699	14.650	9.400	14.750	14.650	17.300
	11.900	13.950	11.950	14.450	15.700	16.000	16.050
	13.100	10.825	13.300	11.925	15.225	15.325	16.675
4	17.800	14.350	18.450	17.900	18.350	12.764	14.600
	20.700	16.400	15.250	16.150	20.100	13.650	14.400
	19.250	15.375	16.850	17.025	19.225	13.207	14.500
5	6.950	11.199	8.399	9.550	20.100	16.950	20.800
	22.700	12.449	19.750	16.500	20.000	19.700	14.649
	14.825	11.824	14.075	13.025	20.050	18.325	17.725
6	12.200	11.800	22.350	17.300	14.450	15.900	10.800
	13.100	15.550	17.350	8.700	16.650	16.200	8.500
	12.650	13.675	19.850	13.000	15.550	16.050	9.650
7	7.750	8.526	15.850	14.100	17.250	15.705	13.100
	15.200	12.900	17.200	14.684	16.550	22.000	17.200
	11.475	10.713	16.525	14.392	16.900	18.852	15.150

	1	2	3	4	5	6	7
1	16.700 12.400 ----- 14.550 -----	12.750 18.000 ----- 15.375 -----	16.950 18.350 ----- 17.650 -----	16.400 13.300 ----- 14.850 -----	11.850 18.950 ----- 15.400 -----	14.000 16.250 ----- 15.125 -----	19.450 21.050 ----- 20.250 -----
2	18.550 19.950 ----- 19.250 -----	12.200 16.350 ----- 14.275 -----	20.200 16.050 ----- 18.125 -----	14.750 18.150 ----- 16.450 -----	18.150 23.350 ----- 20.750 -----	24.000 24.000 ----- 24.000 -----	11.700 22.350 ----- 17.025 -----
3	22.000 20.000 ----- 21.000 -----	9.399 18.250 ----- 13.825 -----	13.700 24.000 ----- 18.850 -----	12.200 12.833 ----- 12.516 -----	13.700 0.000 ----- 6.850 -----	0.000 0.000 ----- 0.000 -----	16.850 17.333 ----- 17.091 -----
M							
A							
L	4	17.050 6.000 ----- 11.525 -----	18.950 19.350 ----- 19.150 -----	12.150 18.900 ----- 15.525 -----	13.550 21.050 ----- 17.300 -----	13.900 18.500 ----- 16.200 -----	19.200 1.800 ----- 10.500 -----
E							
	5	14.700 14.850 ----- 14.775 -----	13.800 11.149 ----- 12.475 -----	19.250 23.400 ----- 21.325 -----	14.950 11.666 ----- 13.308 -----	22.100 17.800 ----- 19.950 -----	2.599 23.500 ----- 13.050 -----
	6	15.444 13.000 ----- 14.222 -----	20.375 9.600 ----- 14.987 -----	20.500 24.000 ----- 22.250 -----	18.062 12.375 ----- 15.218 -----	15.550 21.000 ----- 18.275 -----	23.500 11.500 ----- 17.500 -----
	7	20.250 18.050 ----- 19.150 -----	9.000 16.050 ----- 12.525 -----	18.700 15.850 ----- 17.275 -----	11.050 19.000 ----- 15.025 -----	15.750 18.050 ----- 16.900 -----	13.875 19.571 ----- 16.723 -----

Appendix 2 (Cont'd.)

NET DIALLEL SCORES CROSS I DARKNESS, HIGH TEMPERATURE

F E M A L E

	1	2	3	4	5	6	7
1	3.700	1.849	3.299	5.150	7.349	0.600	4.750
	11.100	6.000	8.000	4.950	7.000	2.666	2.949
	7.400	3.925	5.650	5.050	7.175	1.633	3.850
2	1.000	8.899	3.900	4.150	7.899	1.599	9.600
	15.600	4.800	7.200	1.200	7.250	1.250	0.000
	8.300	6.849	5.550	2.675	7.575	1.425	4.800
3	10.050	2.399	3.350	4.800	5.800	1.200	20.500
	5.000	0.000	9.399	2.399	4.550	8.949	3.500
	7.525	1.200	6.375	3.599	5.175	5.075	12.000
4	8.300	9.850	13.200	9.550	10.000	6.294	11.300
	8.149	3.500	2.950	6.850	3.399	4.099	5.700
	8.225	6.675	8.075	8.200	6.700	5.197	8.500
5	1.899	1.200	2.399	3.950	10.350	6.149	4.750
	12.900	4.550	5.649	5.250	10.000	7.300	4.650
	7.400	2.875	4.024	4.600	10.175	6.725	4.700
6	3.850	8.749	6.800	4.900	2.100	7.750	2.799
	6.300	8.150	9.350	2.399	4.950	7.599	2.950
	5.075	8.450	8.075	3.650	3.525	7.675	2.875
7	3.350	0.300	4.950	2.700	6.000	3.588	2.000
	3.600	1.149	9.600	3.650	3.699	12.538	3.650
	3.475	0.725	7.275	3.175	4.850	8.063	2.825

F E M A L E

[illegible]

Appendix 2 (Cont'd.)

NET DIALLEL SCORES CROSS I DAYLIGHT, MEDIUM TEMPERATURE

F E M A L E

	1	2	3	4	5	6	7	
1	14.600 22.000 ----- 18.300 -----	20.150 17.700 ----- 18.925 -----	17.700 11.900 ----- 14.800 -----	7.450 18.900 ----- 13.175 -----	21.650 22.500 ----- 22.075 -----	22.312 23.875 ----- 23.093 -----	10.850 18.350 ----- 14.600 -----	
2	14.550 19.400 ----- 16.975 -----	15.250 20.600 ----- 17.925 -----	16.450 21.600 ----- 19.025 -----	19.210 13.899 ----- 16.555 -----	16.850 17.700 ----- 17.275 -----	20.333 23.111 ----- 21.722 -----	20.050 22.800 ----- 21.425 -----	
3	21.600 16.200 ----- 18.900 -----	17.650 22.800 ----- 20.225 -----	22.400 22.700 ----- 22.550 -----	19.150 16.500 ----- 17.825 -----	16.850 20.500 ----- 18.675 -----	20.400 19.600 ----- 20.000 -----	19.000 22.550 ----- 20.775 -----	
M A L E	4	13.600 21.950 ----- 17.775 -----	18.400 16.550 ----- 17.475 -----	20.000 15.400 ----- 17.700 -----	15.950 23.800 ----- 19.875 -----	23.350 21.600 ----- 22.475 -----	20.538 10.000 ----- 15.269 -----	16.750 18.650 ----- 17.700 -----
5	17.650 16.150 ----- 16.900 -----	19.500 16.050 ----- 17.775 -----	17.450 19.600 ----- 18.525 -----	24.000 17.800 ----- 20.900 -----	20.050 16.750 ----- 18.400 -----	21.222 20.450 ----- 20.836 -----	22.800 20.300 ----- 21.550 -----	
6	16.950 10.050 ----- 13.500 -----	17.150 21.000 ----- 19.075 -----	22.050 20.400 ----- 21.225 -----	14.400 14.050 ----- 14.225 -----	18.350 20.750 ----- 19.550 -----	21.631 21.600 ----- 21.615 -----	21.350 17.600 ----- 19.475 -----	
7	17.800 17.750 ----- 17.775 -----	15.200 21.050 ----- 18.125 -----	18.600 22.300 ----- 20.450 -----	15.000 19.550 ----- 17.275 -----	20.850 16.950 ----- 18.900 -----	17.647 21.846 ----- 19.746 -----	18.650 18.894 ----- 18.772 -----	

F E M A L E

[illegible]

F E M A L E

	1	2	3	4	5	6	7
1	2.250	0.000	5.850	8.437	4.250	9.687	5.750
	3.350	2.650	4.800	8.550	3.849	8.375	3.750
	2.800	1.325	5.325	8.493	4.050	9.031	4.750
2	2.450	5.000	3.750	2.450	1.349	9.600	5.849
	2.349	6.100	3.500	4.800	3.299	5.222	2.399
	2.400	5.550	3.625	3.625	2.324	7.411	4.124
3	4.300	2.899	2.399	4.250	6.149	3.950	3.149
	2.649	2.399	6.999	6.600	8.249	3.700	9.100
	3.474	2.649	4.699	5.425	7.199	3.825	6.125
4	6.750	2.650	9.350	0.050	4.550	5.384	8.899
	9.950	6.550	2.250	1.200	3.650	11.470	5.200
	8.350	4.600	5.800	0.625	4.100	8.427	7.050
5	5.200	1.700	3.900	9.450	5.550	5.000	5.700
	4.050	5.399	2.899	3.850	7.550	5.750	10.789
	4.625	3.550	3.400	6.650	6.550	5.375	8.244
6	4.099	1.500	0.400	0.350	8.550	6.578	11.100
	3.450	5.250	5.100	7.150	7.700	10.050	8.100
	3.775	3.375	2.750	3.750	8.125	8.314	9.600
7	3.250	3.399	3.299	1.899	9.550	1.235	4.400
	10.750	4.950	8.699	8.950	9.300	7.692	6.099
	7.000	4.175	5.999	5.425	9.425	4.463	5.250

F E M A L E

	1	2	3	4	5	6	7	
1	9.300 12.750 ----- 11.025 -----	4.800 3.375 ----- 4.087 -----	5.949 6.600 ----- 6.275 -----	1.500 16.928 ----- 9.214 -----	1.150 9.350 ----- 5.250 -----	10.000 12.000 ----- 11.000 -----	5.500 9.857 ----- 7.678 -----	
2	6.500 6.157 ----- 6.328 -----	0.050 8.999 ----- 4.524 -----	6.250 2.450 ----- 4.350 -----	3.450 3.700 ----- 3.575 -----	6.550 5.950 ----- 6.250 -----	24.000 24.000 ----- 24.000 -----	8.200 2.950 ----- 5.575 -----	
3	16.200 8.250 ----- 12.225 -----	6.000 0.000 ----- 3.000 -----	6.550 2.000 ----- 4.275 -----	5.099 3.000 ----- 4.050 -----	7.461 10.500 ----- 8.980 -----	12.000 12.000 ----- 12.000 -----	1.700 2.799 ----- 2.250 -----	
M								
A								
L	4	14.800 7.800 ----- 11.300 -----	5.800 13.650 ----- 9.725 -----	7.250 2.450 ----- 4.850 -----	5.000 10.200 ----- 7.600 -----	2.549 7.349 ----- 4.949 -----	0.333 12.000 ----- 6.166 -----	2.150 7.200 ----- 4.675 -----
E								
5	1.900 10.249 ----- 6.074 -----	3.399 3.578 ----- 3.489 -----	4.050 7.149 ----- 5.600 -----	7.750 7.599 ----- 7.675 -----	3.950 4.899 ----- 4.425 -----	13.500 6.250 ----- 9.875 -----	2.099 12.850 ----- 7.474 -----	
6	12.625 0.000 ----- 6.312 -----	15.200 0.000 ----- 7.600 -----	23.500 20.000 ----- 21.750 -----	17.142 15.000 ----- 16.071 -----	10.500 4.399 ----- 7.450 -----	10.500 24.000 ----- 17.250 -----	8.950 0.000 ----- 4.475 -----	
7	8.350 8.050 ----- 8.200 -----	2.599 3.050 ----- 2.825 -----	5.349 7.500 ----- 6.425 -----	5.650 10.100 ----- 7.875 -----	7.349 10.650 ----- 9.000 -----	14.166 9.142 ----- 11.654 -----	4.000 7.599 ----- 5.800 -----	

Appendix 2 (Cont'd.)

NET DIALLEL SCORES CROSS I DAYLIGHT, LOW TEMPERATURE

F E M A L E

[illegible]

Appendix 2 (Cont'd.)

NET DIALLEL SCORES CROSS II DAYLIGHT, LOW TEMPERATURE

F E M A L E

	1	2	3	4	5	6	7
1	12.500 17.900 ----- 15.200 -----	15.157 17.533 ----- 16.345 -----	15.450 17.722 ----- 16.586 -----	13.200 15.666 ----- 14.433 -----	19.200 16.800 ----- 18.000 -----	20.000 24.000 ----- 22.000 -----	18.150 19.200 ----- 18.675 -----
- 2	21.800 24.000 ----- 22.900 -----	20.900 15.000 ----- 17.950 -----	20.350 17.150 ----- 18.750 -----	21.600 19.850 ----- 20.725 -----	18.050 16.750 ----- 17.400 -----	15.500 15.500 ----- 15.500 -----	18.600 13.899 ----- 16.250 -----
3	21.250 18.000 ----- 19.625 -----	13.000 16.000 ----- 14.500 -----	18.950 12.000 ----- 15.475 -----	19.500 12.000 ----- 15.750 -----	19.200 0.000 ----- 9.600 -----	11.500 11.500 ----- 11.500 -----	16.800 20.000 ----- 18.400 -----
M A L E	4 18.750 16.400 ----- 17.575 -----	15.850 9.900 ----- 12.875 -----	17.800 19.250 ----- 18.525 -----	12.750 17.950 ----- 15.350 -----	20.400 17.800 ----- 19.100 -----	0.000 11.666 ----- 5.833 -----	18.250 20.000 ----- 19.125 -----
5	18.400 23.900 ----- 21.150 -----	15.350 15.200 ----- 15.275 -----	19.700 21.850 ----- 20.775 -----	15.500 16.400 ----- 15.950 -----	15.800 18.850 ----- 17.325 -----	17.000 12.000 ----- 14.500 -----	20.500 17.450 ----- 18.975 -----
6	17.500 15.000 ----- 16.250 -----	19.200 3.000 ----- 11.100 -----	0.000 0.000 ----- 0.000 -----	14.846 21.000 ----- 17.923 -----	19.450 15.300 ----- 17.375 -----	16.000 16.000 ----- 16.000 -----	13.210 10.400 ----- 11.805 -----
7	19.950 15.400 ----- 17.675 -----	10.750 17.650 ----- 14.200 -----	21.950 16.450 ----- 19.200 -----	20.300 16.750 ----- 18.525 -----	16.200 13.800 ----- 15.000 -----	20.000 20.571 ----- 20.285 -----	17.900 18.600 ----- 18.250 -----

F E M A L E

	1	2	3	4	5	6	7
1	10.450	6.000	10.100	4.800	7.149	10.764	5.736
	11.100	8.200	5.849	14.400	9.050	11.988	7.450
	10.775	7.100	7.974	9.600	8.100	11.326	6.593
2	9.149	4.050	6.500	8.750	9.050	3.199	9.050
	7.300	4.050	5.099	7.700	3.900	3.466	6.800
	8.225	4.050	5.800	8.225	6.475	3.333	7.925
3	5.050	4.999	5.750	3.799	6.300	10.450	6.099
	5.199	6.050	6.199	3.949	7.500	7.350	7.300
	5.125	5.525	5.975	3.874	6.900	8.900	6.699
4	13.350	6.750	7.949	6.200	9.149	4.800	11.550
	8.750	13.050	13.750	9.449	11.000	3.214	9.800
	11.050	9.900	10.850	7.825	10.075	4.007	10.675
5	10.149	8.500	8.399	6.599	8.500	7.000	6.800
	3.900	11.250	10.150	4.300	6.300	6.000	7.950
	7.024	9.875	9.274	5.449	7.400	6.500	7.375
6	6.100	7.000	9.600	7.399	4.900	10.894	5.950
	4.500	6.650	9.150	8.200	7.000	4.700	5.300
	5.300	6.825	9.375	7.800	5.950	7.797	5.625
7	9.950	10.000	8.450	7.400	7.300	14.266	3.150
	12.850	7.349	6.900	11.500	10.950	15.461	11.050
	11.400	8.675	7.675	9.450	9.125	14.864	7.100

Appendix 2 (Cont'd.)

NET DIALLEL SCORES CROSS II DARKNESS, LOW TEMPERATURE

F E M A L E

[illegible]

Appendix 3. Analysis of variance tables for the diallel analysis using Hayman's model for reciprocal effects.

(A) Tests run at the high temperature ($17.5 \pm 1.5^\circ\text{C}$) for the net scores.

Source	d.f.	Cross I Daylight		Cross II Daylight		Cross I Darkness		Cross II Darkness	
		M.S.	F	M.S.	F+	M.S.	F+	M.S.	F+
a	6	27.6620	5.94 *	27.2949	1.18 n.s.	7.9520	0.5514 n.s.	16.8775	1.28 n.s.
b1	1	46.1886	3.72 ⁺ n.s.	31.5161	1.36 n.s.	35.1737	2.4394 n.s.	0.4710	0.04 n.s.
b2	6	8.5557	0.69 ⁺ n.s.	17.8158	0.77 n.s.	9.2285	0.6400 n.s.	3.0008	0.23 n.s.
b3	14	8.1307	0.65 ⁺ n.s.	21.7497	0.94 n.s.	10.5877	0.7342 n.s.	7.7807	0.59 n.s.
b	21	10.0644	0.81 ⁺ n.s.	21.0908	0.91 n.s.	11.3701	0.7885 n.s.	6.0670	0.46 n.s.
c	6	32.1790	2.59 ⁺ *	87.0735	3.77 **	18.1852	1.2612 n.s.	52.2473	3.96 **
d	15	15.4602	1.24 ⁺ n.s.	38.0432	1.65 n.s.	12.2430	0.8490 n.s.	13.7047	1.03 n.s.
Blocks	1	18.3208	1.48 n.s.	16.4132	0.71 n.s.	0.5622	0.04 n.s.	40.8078	3.10 n.s.
B × a	6	4.6601		16.9729		28.8467		18.9670	
B × b1	1	8.8917		0.8962		3.7850		2.7880	
B × b2	6	10.3562		27.7934		10.1448		17.4030	
B × b3	14	15.5299		34.5087		9.6880		10.6208	
B × b	21	13.7356		30.9895		9.5374		12.1855	
B × c	6	26.2056		10.9344		29.0964		9.9279	
B × d	15	8.1692		19.4308		9.6107		13.5587	
Blocks pooled	48	12.4204		23.1184		14.4189		13.1801	

*Each item tested against its own block interaction.

⁺All items tested against the pooled interaction mean square.

(cont'd)

Appendix 3 (cont'd)

(B) Test run at the medium temperature ($9.7 \pm 0.5^\circ\text{C}$) for the net scores.

Source	d.f.	Cross I Daylight		Cross II Daylight		Cross I Darkness		Cross II Darkness	
		M.S.	F+	M.S.	F+	M.S.	F+	M.S.	F
a	6	19.1258	1.82 n.s.	25.6927	1.48 n.s.	20.5566	3.60 **	125.7276	6.16 ⁺ ***
b1	1	13.3988	1.27 n.s.	34.4385	1.98 n.s.	2.8547	0.50 n.s.	0.0224	0.00 ⁺ n.s.
b2	6	9.1491	0.87 n.s.	56.7009	3.26 **	17.3922	3.04 *	5.5384	0.27 ⁺ n.s.
b3	14	10.6299	1.01 n.s.	15.2849	0.88 n.s.	6.7310	1.18 n.s.	33.9109	1.66 ⁺ n.s.
b	21	10.3386	0.98 n.s.	28.0301	1.61 n.s.	9.5924	1.68 n.s.	24.1907	1.19 ⁺ n.s.
c	6	7.6317	0.72 n.s.	5.7868	0.33 n.s.	3.0485	0.53 n.s.	19.7808	0.97 ⁺ n.s.
d	15	10.6309	1.01 n.s.	11.6144	0.67 n.s.	8.0756	1.41 n.s.	38.1929	3.10 [*] *
Blocks	1	10.8987	1.04 n.s.	46.0210	2.64 n.s.	35.4910	6.21 *	7.8356	0.38 n.s.
B × a	6	12.0426		24.9864		2.5714		24.7583	
B × b1	1	14.3980		33.3538		3.7362		61.3862	
B × b2	6	7.0447		16.2524		1.5818		35.1984	
B × b3	14	18.8288		20.2152		7.0573		14.0008	
B × b	21	15.2509		19.7086		5.3347		22.3137	
B × c	6	3.3569		16.0333		8.7578		29.5518	
B × d	15	6.1749		11.6868		6.2915		12.3286	
Blocks pooled	48	10.5269		17.4021		5.7162		20.4037	

*Each item tested against its own block interaction.

⁺All items tested against the pooled interaction mean square.

(cont'd)

Appendix 3 (cont'd)

(C) Test run at the low temperature ($5.0 \pm 1.5^\circ\text{C}$) for the net scores.

Source	d.f.	Cross I Daylight		Cross II Daylight		Cross I Darkness		Cross II Darkness	
		M.S.	F+	M.S.	F+	M.S.	F	M.S.	F
a	6	17.5911	1.47 n.s.	51.1315	3.63 **	13.8716	2.63 ⁺ *	68.4220	4.70 ⁺ ***
b1	1	0.2229	0.02 n.s.	0.3687	0.03 n.s.	4.7018	19.15 ⁺ n.s.	0.5583	0.04 ⁺ n.s.
b2	6	12.7076	1.07 n.s.	19.4571	1.38 n.s.	4.7119	0.99 n.s.	22.3197	8.94 **
b3	14	6.9123	0.58 n.s.	26.8248	1.90 *	7.9638	2.75 *	30.3344	2.08 ⁺ *
b	21	8.2496	0.69 n.s.	23.4599	1.67 n.s.	6.8793	2.09 ⁺ *	26.6266	1.83 ⁺ *
c	6	4.0703	0.34 n.s.	30.6211	2.17 n.s.	17.6833	3.36 ⁺ **	66.5623	4.57 ⁺ ***
d	15	13.4789	1.13 n.s.	36.5324	2.59 **	11.6424	2.21 ⁺ *	28.3688	3.94 **
Blocks	1	0.0097	0.00 n.s.	20.6213	1.46 n.s.	2.2863	0.43 n.s.	21.6437	1.49 n.s.
B × a	6	14.3370		15.7473		7.2967		18.1368	
B × b1	1	36.2248		5.2299		0.2455		0.3785	
B × b2	6	13.6220		5.7675		4.7460		2.4976	
B × b3	14	10.4737		14.7840		2.8931		24.4343	
B × b	21	12.5994		11.7529		3.2964		17.0212	
B × c	6	5.7127		16.1852		7.0127		20.8001	
B × d	15	12.5088		15.8438		6.5089		7.2007	
Blocks pooled	48	11.9275		14.0846		5.2649		14.5641	

*Each item tested against its own block interaction.

⁺All items tested against the pooled interaction mean square.

(cont'd)

Appendix 3 (cont'd)

(D) Crosses I and II summed for the net scores - high temperature
($17.5 \pm 1.5^\circ\text{C}$).

Source	d.f.	Daylight		Darkness	
		M.S.	F+	M.S.	F+
a	6	56.3204	1.62 n.s.	30.5441	1.59 n.s.
b1	1	154.0260	4.42 *	32.0418	1.67 n.s.
b2	6	10.9949	0.32 n.s.	12.1986	0.63 n.s.
b3	14	28.8923	0.83 n.s.	15.7424	0.82 n.s.
b	21	29.7375	0.85 n.s.	15.5060	0.81 n.s.
c	6	146.6446	4.21 **	80.1238	4.17 **
d	15	55.8417	1.60 n.s.	36.1977	1.88 *
Blocks	1	69.4127	1.99 n.s.	37.4285	1.94 n.s.
B \times a	6	29.1281		28.6805	
B \times b1	1	15.4347		0.4788	
B \times b2	6	28.8990		15.0303	
B \times b3	14	53.8079		17.7032	
B \times b	21	44.8638		16.1193	
B \times c	6	25.3117		35.8121	
B \times d	15	26.9524		13.1860	
Blocks pooled	48	34.8555		19.2344	

*Each item tested against its own block interaction.

+All items tested against the pooled interaction mean square.

(cont'd)

Appendix 3 (cont'd)

(E) Crosses I and II summed for the net scores - medium temperature
(9.7 \pm 0.5°C).

Source	d.f.	Daylight		Darkness	
		M.S.	F+	M.S.	F+
a	6	82.6032	2.56 *	187.1608	6.48 ***
b1	1	4.9665	0.15 n.s.	2.3457	0.08 n.s.
b2	6	50.3476	1.56 n.s.	13.8296	0.48 n.s.
b3	14	33.5124	1.03 n.s.	45.4321	1.57 n.s.
b	21	36.9631	1.15 n.s.	34.3511	1.19 n.s.
c	6	16.6310	0.52 n.s.	24.7878	0.86 n.s.
d	15	27.7803	0.86 n.s.	45.8894	1.59 n.s.
Blocks	1	12.1264	0.38	76.6764	2.65 n.s.
B \times a	6	46.3912		23.1447	
B \times b1	1	3.8107		95.3973	
B \times b2	6	19.9306		34.6888	
B \times b3	14	48.1729		35.2081	
B \times b	21	37.9912		37.9259	
B \times c	6	30.8300		28.4132	
B \times d	15	19.0824		18.7283	
Blocks pooled	48	32.2370		28.8899	

*Each item tested against its own block interaction.

+All items tested against the pooled interaction mean square.

(cont'd)

Appendix 3 (cont'd)

(F) Crosses I and II summed for the net scores - low temperature
($5.0 \pm 1.5^{\circ}\text{C}$).

Source	d.f.	Daylight		Darkness	
		M.S.	F+	M.S.	F+
a	6	129.9008	5.50 ***	99.5462	5.28 ***
b1	1	0.3468	0.01 n.s.	8.2620	0.44 n.s.
b2	6	11.3712	0.48 n.s.	36.3138	1.92 n.s.
b3	14	42.6111	1.81 n.s.	30.8613	1.64 n.s.
b	21	31.6728	1.34 n.s.	31.3430	1.66 n.s.
c	6	38.2083	1.62 n.s.	119.7470	6.35 ***
d	15	53.2132	2.25 *	42.8404	2.27 *
Blocks	1	12.4879	0.53 n.s.	10.5000	0.56 n.s.
B \times a	6	20.4531		27.5536	
B \times b1	1	16.8327		0.0063	
B \times b2	6	30.8994		5.6309	
B \times b3	14	13.1881		23.5509	
B \times b	21	18.4220		17.3097	
B \times c	6	14.9680		23.5292	
B \times d	15	35.5740		15.7111	
Blocks pooled	48	23.6041		18.8681	

Each item tested against its own block interaction.

⁺All items tested against the pooled interaction mean square.