MERISTIC VARIATION IN THE MEDAKA (ORYZIAS LATIPES) PRODUCED BY TEMPERATURE AND BY CHEMICALS AFFECTING METABOLISM

by

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PARALLELS BETWEEN MERISTIC VARIATION IN THE MEDAKA (ORYZIAS LATIPES) PRODUCED BY TEMPERATURE AND BY CHEMICALS AFFECTING METABOLISM

ABSTRACT

Meristic characters in offspring from 27 pairs of medaka were investigated with respect to some factors known to alter metabolism. Temperature, light, thyroxine, thiourea, dinitrophenol, urethan and salinity were tested. Egg size, egg density, nature of rearing containers, quality of successive day's egg batches from the same parent, mechanical shock of developing eggs and pricking the chorion of eggs were also studied as possible factors producing meristic variations.

Mean vertebral counts showed a V-shaped relation to temperature in 9 out of 15 replications, inverse relation in 2, and no consistent relation in 4. Pectoral fin ray counts were inversely related to temperature. Degree and direction of change of other fin rays with temperature varied between genotypes.

Vertebral counts were not affected by variations in light intensities or duration; fin ray counts were altered but their reaction lacked uniformity.

Mean total vertebral counts of 8 out of 11 replications were altered in thyroxine solution, but magnitude and direction of change differed between genotypes. When eggs were hatched in thyroxine solution, pectoral ray counts were lowered. Exposure of larvae to thyroxine produced significant decrease in pectoral, anal, dorsal, and caudal fin ray counts.

Rearing eggs to hatching in thiourea produced significant increase in mean total vertebral counts in only 2 of 11 replications. Pectoral and anal fin ray counts increased, but total caudal rays decreased, in samples from treated eggs as well as from larvae treated in thiourea after hatching.

Rearing of eggs to hatching in dinitrophenol, urethan, or sea water resulted in an increase in mean vertebral counts. Pectoral rays increased in lower concentrations of dinitrophenol, or in urethan, but were unaffected in sea water. Anal, dorsal, and total caudal rays were not altered in sea water, but variable effects resulted from dinitrophenol or urethan.

No correlation was found between meristic counts and egg size. Vertebral and pectoral ray counts seemed to follow those of the father; paternal influence was very pronounced in inheritance of pectoral rays.

Vertebral, pectoral and dorsal ray counts were not affected by other extraneous factors tested. Effect of egg density of anal and total caudal rays was variable.

Final fixation of total vertebrae occurred at the embryonic stage when eye pigmentation commenced and pectoral buds had appeared. Other characters remained sensitive to environmental influence even after hatching

The relation of metabolism to meristic characters, and evident parallels between effects of the several factors used are discussed.

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INTRODUCTION

It is well known that the meristic characters of cold blooded vertebrates, particularly fish, vary within a single species. Earlier workers (Heincke, 1898; Schnackenbeck, 1931) attributed such variations to genetic differences among the populations. But subsequent findings have demonstrated that the characters, though their limits of variability are genetically controlled, may be modified by environmental influences operating at the time of their fixation. Inf;uences of different environmental factors - e.g. temperature (Schmidt 1917, 1919, 1921; Mottley 1934; Gabriel 1944; Dannevig 1950; Lindsey 1954, 1962, Blaxter, 1957, Seymour 1959; Itazawa 1959), temperature and/or salinity (Schmidt, 1920). temperature, oxygen and carbon dioxide (Taning, 1944, 1946 and 1950), salinity and temperature (Heuts 1947 and 1949), and light (McHugh 1954; Lindsey 1958; Canagaratnam 1959 and Lyubitskaya 1956 and 1961) have been studied.

Although the influence of the environmental factors in determining the final expression of different meristic series is established, it is not clear how these factors operate. Marckmann (1954) suggested that temperature alters meristic counts by altering the metabolism of the developing embryo. According to this hypothesis, the lowest vertebral counts obtained in the intermediate temperature in sea trout, Salmo trutta was the result of a most economic metabolism of the individuals at this temperature. Canagaratnam (1959) suggested that there is probably a relationship between the activity of the pituitary-thyroid complex and the final fixation of many meristic series.

The present work was undertaken to study the relationship of thyroid activity, metabolism and the fixation of different meristic series in the medaka, Oryzias latipes (Temminck and Schlegel), a Japanese cyprinodont, by rearing eggs and larvae in thyroxine and thiourea solutions. In addition experiments were conducted to observe the effects of dinitrophenol, urethan, temperature, light and some extraneous sources of variation.

Several different meristic series were examined which yielded information on the genotypic and phenotypic control of the individual series. Possible role of metabolism and thyroid activity in relation to fixation of meristic characters have been considered in the discussion. Size hierarchies and selective mortality have also been considered in relation to meristic variation.

MATERIALS AND METHODS

Outline of experiments.

The number and purpose of each experiment are listed below.

Experiment 1: effect of malachite green treatment on developing eggs.

Experiment 11: effect of the nature of egg rearing containers (bottles, baskets) and variation in aeration.

Experiment 111: effect of the quality of eggs obtained on successive days from the same parent.

Experiment 1V: effect of mechanical shock given to developing eggs.

Experiment V: effect of pricking the chorion of fertilized eggs.

Experiment V1: effect of the density of egg and young in the rearing containers.

Experiment V11: effect of yolk diameter (egg size).

Experiment V111: determination of the sensitive period for different meristic characters during development by transfer of eggs from high to low and low to high temperatures.

Experiment 1X: effect of sustained temperature.

Experiment X: effect of increased light (intensity and duration).

Experiment X1: effect of thyroxine and thiourea.

Experiment X11: effect of 2, 4-Dinitrophenol.

Experiment X111: effect of urethan.

Experiment X1V: effect of salinity.

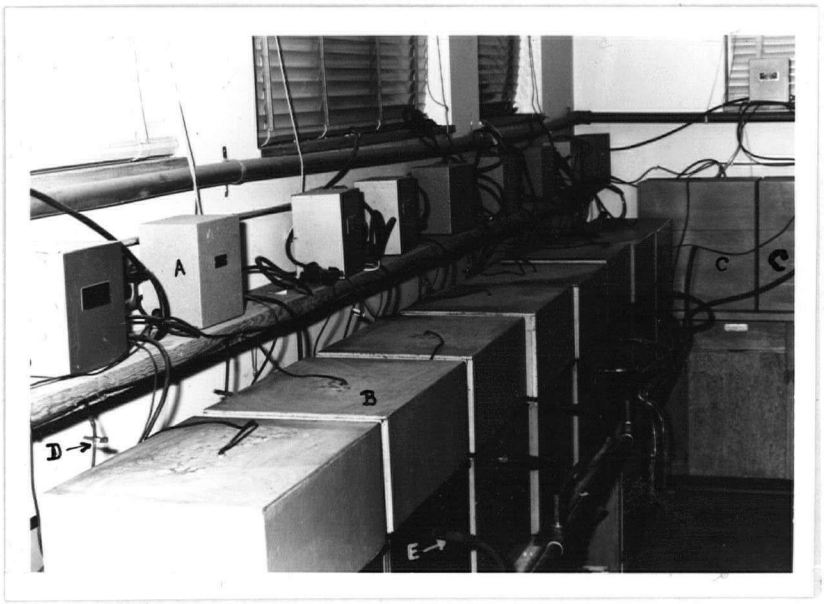

Figure 1. Arrangements of controlled environment units. A-Aminco relay; B-Light hood; C-Tank; D-Water inlet; E-Outlet pipe.

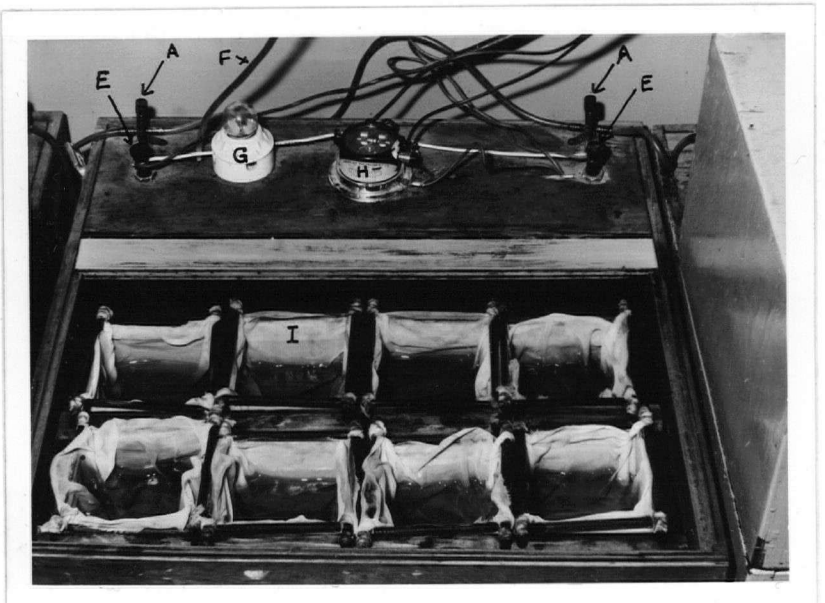

Figure 2. Instrument board and arrangement of small cloth baskets. A-Air valve; E-Heater terminals; F-Water inlet; G-Indicator light; H-Thermoregulator; I-Small cloth basket.

All experiments except II, III, IV, VI and VIII were repeated more than once with eggs from different genotypes in order to obtain a better picture of the complex relationshup between environment and genotype.

Experimental fish

A domestic stock of medaka, Oryzias latipes (Temminck and Schlègel), a small Japanese cyprinodont was used for all the experiments. Briggs and Egami (1959) outline some of the features which make it useful as an experimental animal. Medaka are quite hardy and can withstand a wide range of temperature. The temperature range of the species, according to Rugh (1962), is from 7° to 39°C with the optimum between 20° and 25°C. Time of breeding and egg production can be regulated by controlling the illumimation.. Number of eggs produced at a single spawning vary widely (1-80 eggs according to Rugh, 1962).

Depending upon food, temperature and space, the time required to attain maturity varies from 4 to 6 months. Upon maturity, the female breeds readily on successive days for 3 or 4 months.

Laboratory installations

Controlled environment apparatus was set up in the Biological Sciences Building, University of British Columbia in the winter of 1959 and the experiments were conducted from March, 1960 through May, 1962.

Each unit of the controlled apparatus consisted of a tank (54x54x38cm) made of 3/4" plywood and lined with non-toxic neopreme paint (figure 1). Each tank held approximately 70 liters of water.

Freshwater from the University mains was dechlorinated and filtered before being taken into the laboratory pipes. Water was guided into every tank with 'tygon' tubing and glass T-tubes from a single faucet. The majority of the experiments were conducted in dechlorinated and filtered water but a few were done in straight tap water owing to the removal of the dechlorinating unit. This difference in water, however, did not affect either survival and growth of fish or the results. Little or no chlorine can be detected in the raw water.

Water flow into each tank was maintained at 3 liters per hour, which replaced the entire volume in a tank every 20 hours.

Compressed air was provided in each tank by two air diffuser stones (figure 2), which provided both aeration and mixing. Both water and air flow into each tank were checked twice daily.

Each tank was provided with a 500 watt stainless immersion heater (Aminco) with 80cm effective heating surface and a "Quickset" bimetal thermo regulator (Aminci product). Heating was controlled by a supersensitive relay (Aminco). Both the thermoregulator and the heating element were suspended into water from a plywood instrument board, which also contained an indicator light to signal the working of the heater. Temperature in every tank was recorded twice daily with a thermometer calibrated to 0.1^{o}c. from three points at depths where eggs remained suspended. The temperature was maintained within $\pm 0.1^{o}$c. in every tank throughout the experiments. In a few cases where the temperature of a tank had departed widely from the set temperature, the experimental lots were discarded.

Each tank was provided with a light hood (54x35x22.5cm) made from plywood and painted white inside. Light hoods fitted the top of each

tank except the area occupied by the instrument board. The edges of the hood were lined with 1 cm thick foam rubber strips to insure light proofing. The inside centre of the hood was provided with an electric lamp holder into which was fitted a 7.5 watt, frosted glass filament bulb (G.E.). This bulb, lying at a distance of approximately 17 cm. from the water surface, provided 9 ft.-c. of light on the water surface in the centre of the tank. For all experiments, except the increased light intensity and duration, the above-mentioned light intensity was maintained by a time switch for a day-length of 16 hours.

Containers for rearing eggs and young

For experiments I-X eggs were reared in cloth baskets (10x10x15cm) made from nylon "horsehair" crinoline (10 meshes/cm) with nylon chiffon liners (30 meshes/cm). Eight baskets were suspended in water in a tank in two rows (figure 2) with 10 cm of the height (out of 15 cm) under water. Baskets in the first row (nearer to instrument panel) were always used for rearing the eggs until hatching. Eggs rested on the bottom of the baskets. After hatching, the young were reared in the baskets in the second row (near the outlet) until they were ready for preservation. In some replications of experiment, young were reared in slightly larger baskets (12x12x15.5cm). Except for size, these baskets were similar to the smaller ones. Approximately 12 cm out of 15.5 cm of the height of the larger basket remained under water when suspended in tanks. In a single experiment, the control and the treatments were reared in the same size of baskets to avoid any effects of "space factor" on development (Brown 1946; Comfort 1956).

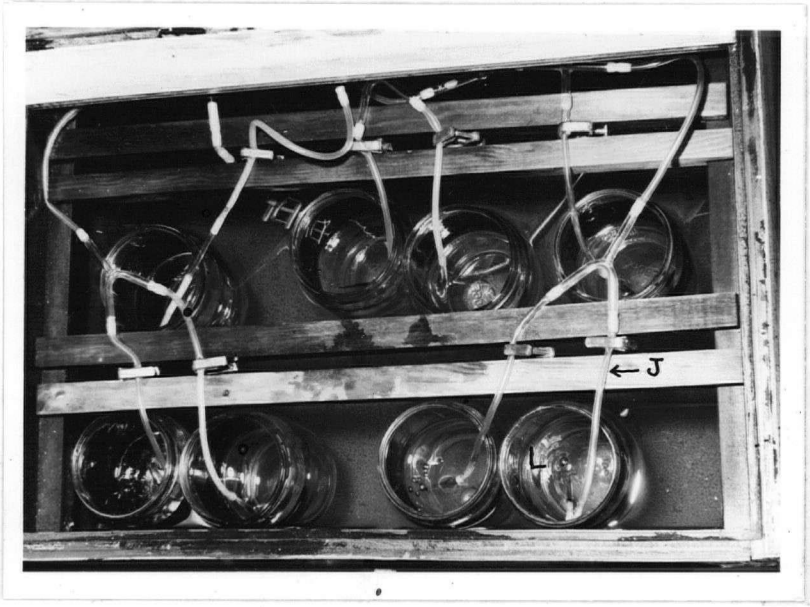

Figure 3. Arrangement of bottles in a tank for experiments XI - XIV. J - Air jet; L - Bottle.

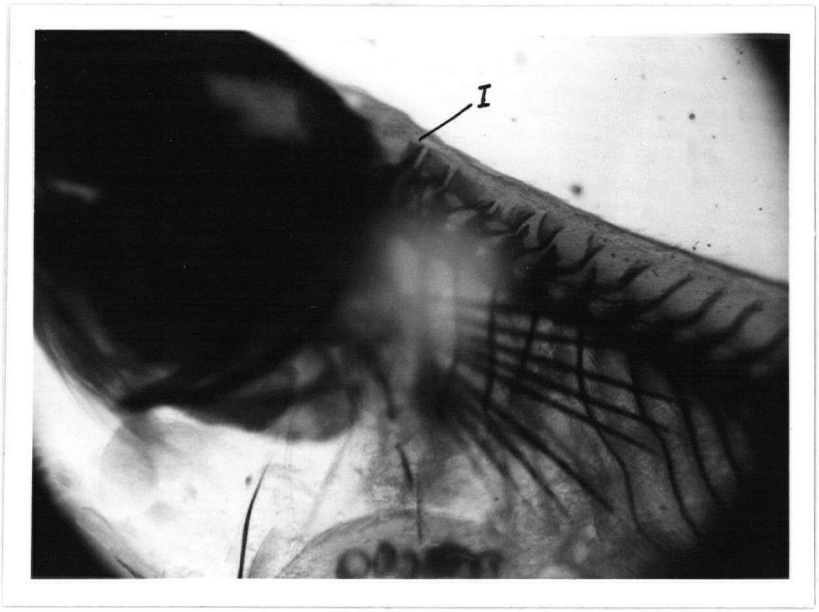

Figure 4. Vertebral column showing the first vertebra (I) in a cleared juvenile medaka.

For experiments XI-XIV, eggs were reared in 710 ml wide-mouthed bottles. Each bottle containing 300 ml of liquid (solution or water) was floated in a tank and provided with an air jet (figure 3). After hatching the young were reared in baskets except in experiment XI(a) where the young were reared in the appropriate solutions in bottles. Altogether 8 bottles were kept in one tank.

Breeding and hatching

One of the fourteen tanks was used for holding the brook stock at 24°C. Eggs from 27 pairs of parents were used for the experiment. Experiment 1 (effect of malachite green) was repeated twice with eggs of parent W and Y, while for experiment II eggs of parent Y and A were utilised. Experiment III was performed with eggs of parent U. Only a single replication of experiment IV was conducted with eggs of parent T but experiment V was repeated twice with eggs of parent R and V. In experiment VI, eggs of parent Y were used. Parent F and J and reciprocal crosses thereof giving small and large eggs respectively were used for the first replication of experiment VII.. For the second, eggs of parent N and S and reciprocal crosses thereof were used. Effects of sustained temperature (experiment IX) were tested on eggs of parents A, B, C, D, E, G, H, I, K, Q, R, V, W, X and Y. Eggs of parent W, Y and A were reared in different intensities and durations of light in experiment X. In thyroxine and thiourea treatments, eggs of parents G, H, O, P, Q, S, V, Y, a and b were utilized. Eggs of parent Y were used for experiment XII (dinitrophenol effect) and XIII (urethan effect). Effect of salinity (experiment XIV) was tested with eggs of parent a.

Breeding and hatching (con'd)

Breeding occurred every morning soon after the light went on. The eggs adhered to the female's abdomen. Some of the parents laid eggs three to four hours after the lights were on. The number of eggs laid, and their frequency, were not uniform. As a result, in some experiments egg numbers were not uniform (see tables on the record of the number and mortality of eggs). As the number of eggs given by a female was small, eggs obtained on several successive days were used for a single treatment in any experiment. Every morning eggs were removed from the female's abdomen within 15 to 30 minutes of spawning. Each egg was then separated, counted and transferred to the desired treatment basket or bottle.

Eggs of different parent were reared in separate containers, and usually allowed to hatch naturally. In a few instances, hatching was induced by giving the eggs a temperature shock (Magnuson 1961) or by putting them in a beaker away from aerated water (Kinne and Kinne 1962) (Details in the tables on eggs numbers and mortality).

Care of eggs

To prevent fungus attack, eggs in baskets were treated twice daily with one eye-dropped full of malachite green solution (1:200,000). As a result of the circulation and mixing of water by aeration, the solution was spread uniformly inside the basket and was replaced by freshwater in fifteen to twenty minutes. Baskets were checked every day for dead eggs which were discarded when discovered.

Malachite green was not used on eggs reared in bottles. The dead or fungus attacked eggs were simply removed with a pipette.

Foods and feeding

In all experiments, living Paramecium sp were fed to the newly hatched medaka four times each day for at least 7 days. Commencing from the third day after hatching, the young were also supplied with live brine shrimp nauplii (Artemia salina) twice every day in conjunction with Paramecium. At the end of the seventh day of hatching the young were fed with brine shrimp nauplii only and this was continued until the fish were preserved. Brine shrimp nauplii was always supplied in excess and the leftover food in the baskets from one day was removed on the morning of the next day. The young were reared for six to eight weeks after hatching and then preserved in 4% neutral formalin. Rearing time was a little longer in lower than in the higher temperature so that samples of approximately the same length frequency were obtained from all treatments.

Clearing and counting of meristic series.

Two to three weeks after preservation, fish were dyed with Alizarin and cleared under an ultraviolet lamp in KOH solutions. In clearing and staining, the procedure outlined by Hollister (1934) was followed with minor modifications as to the concentration of KOH solutions and deviation of exposure to ultraviolet ray.

After clearing, specimens were first measured and then counted in glycerine under a binocular microscope fitted with crosshair and vernier stage.

The following meristic series wer counted: (a) Vertebrae;

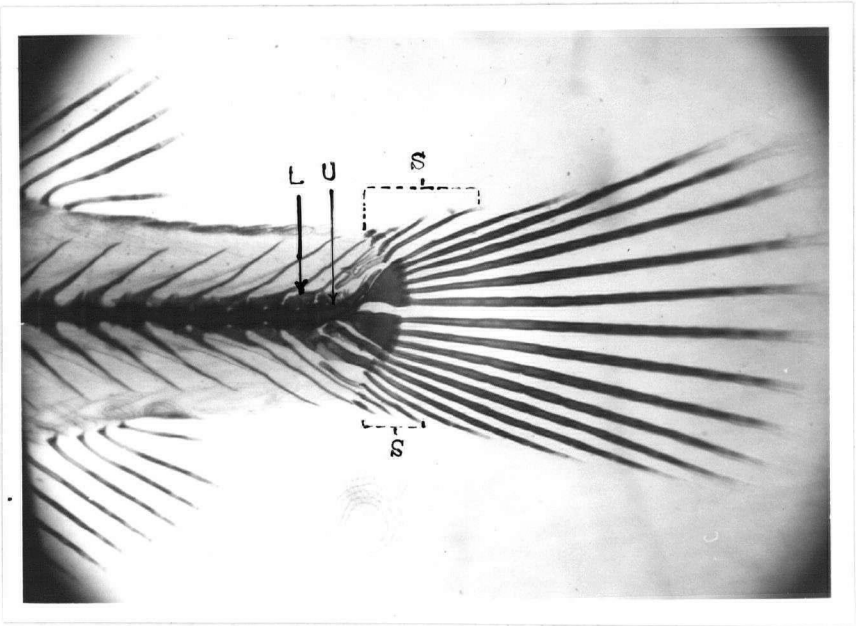

Figure 5. Vertebral column and caudal fin rays in a cleared juvenile medaka. L - Last vertebra; U - Urostyle; S - Secondary caudal fin rays.

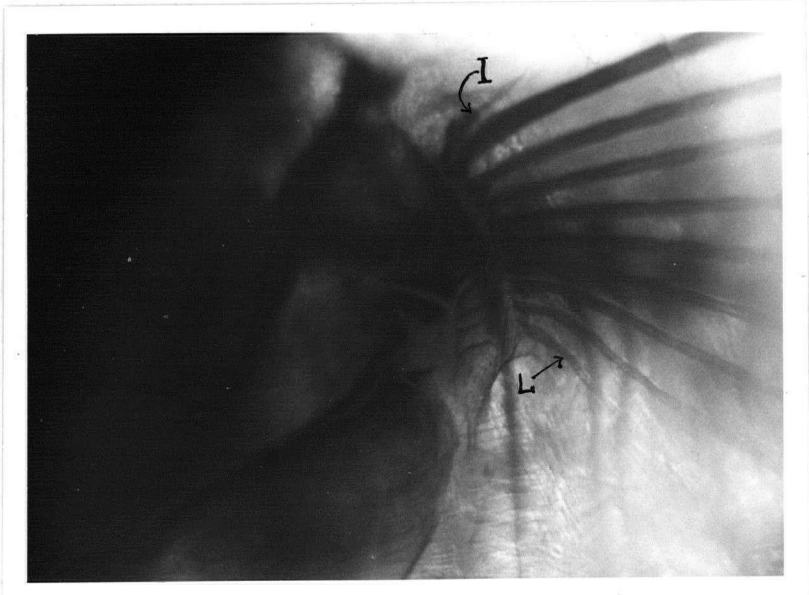

Figure 6. Pectoral fin rays in a cleared juvenile medaka. I - First ray; L - Last ray.

Figure 7. Anal fin rays in a cleared juvenile medaka showing first and last fin ray counted. I - First ray; L - Last ray.

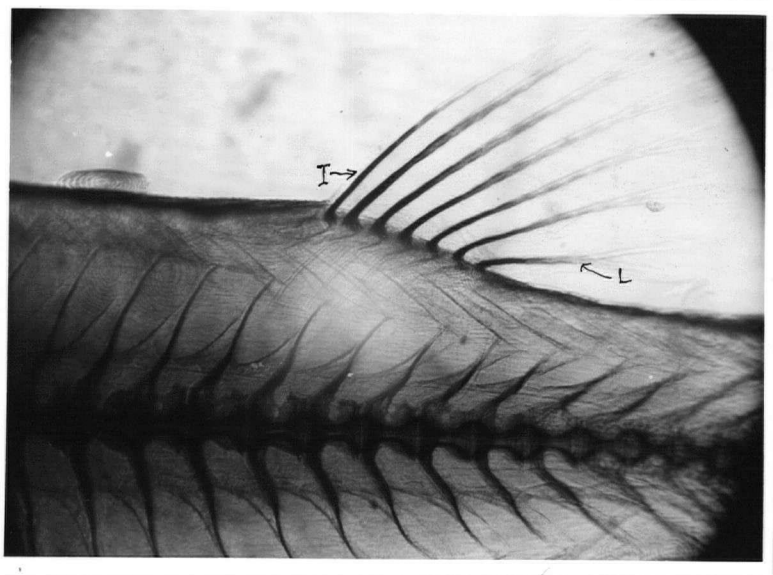

Figure 8. Dorsal fin rays in a cleared juvenile medaka showing the first and last ray counted. I - First ray; L - Last ray.

(b) Pectoral rays; (c) Anal rays; (d) Dorsal rays and (e) Caudal rays.

(a) Vertebrae ... All vertebrae from the basi-occipital to the urostyle were counted. Since the centrum of the first vertebra was not clearly visible, the first neural process behind the occipital region was considered to represent the first vertebra (figure 4). Thereafter each element having a clear neural process and ribs or haemal process was counted as a single vertebra. The urostyle was counted as one vertebra. The vertebral count was subdivided into abdominal and caudal elements; the first centrum showing a haemal process was treated as the first caudal vertebrae. In some cases where two centra were fused or closely apposed, these were counted as two only if there were two distinct neural and haemal processes.

(b) Pectoral fin rays ... The pectoral rays stained well and were clearly visible. When staining was poor, the rays were not counted. Rays from both fins were counted in the majority of the experimental lots but in a few cases only pectoral rays of the left fin were counted. The rays at the periphery on either side of a pectoral fin were very small but they were included in the count (figure 6).

(c) Anal and dorsal fin rays ... These were distinct and all were counted. The last two were counted as two independent rays instead of one as counted by others (Tåning, 1944; Seymour, 1956). (figures 7 and 8).

(d) Caudal fin rays ... Here two types of rays - i.e. primary and secondary rays were distinguished; rays attached to the lower and upper halves of the hypural were treated as the primary rays. These rays tended to branch at their tip. Unbranched and smaller rays on the outer side of both the upper and lower primary rays were considered as secondary rays (figure 5).

The data were analysed mainly by comparing the means by 't' test using the method outlined by Dixon and Massey (1957). Two means were considered different only when the calculated value of 't' was greater than the tabled value of 't' at P=.01.

Calculation of hatching time

A wide variation in the time of hatching of eggs from the same spawning was observed in medaka. This was similar to the results found for sea-trout (Marckmann, 1954) and other fishes. Although most eggs hatch within a short period of time, some required much longer time. In order to avoid the bias introduced by the late hatching eggs in the calculation of hatching time, the time required for 50% hatching was used in comparisons of the time required for different treatments. This calculation was further complicated by the fact that the eggs for the samples were accumulated over three or four days. The mean time to 50% hatching was therefore calculated as shown in the following example:

Time to 50% hatching of the eggs of genotype X in 30°C

Date Eggs Laid	Time	Total Eggs
21 January 62	0945	7
22 " "	0945	29
23 " "	0950	39
		75

Total hours for all eggs

7 x 0	=	0
29 x 24	=	696
39 x 72.08	=	2811
		3507

Average time = 3507 ÷ 75 = 47.0 hours

Total hatched = 72

Time required for 36 eggs to hatch
= February 1, ,962 .... 1700 hours

Total time from 0945 hours January 21, 1962

to 1700 hours February 1, 1962 = 271 hours

Time to 50% hatching = 271.00-47.0 = 224 hours

EFFECT OF MALACHITE GREEN (EXPERIMENT I)

Introduction

In all experiments where eggs were reared in cloth baskets, eggs were treated with malachite green solution (1:200,000) twice daily to control fungus. This experiment was performed to test the effect of malachite green treatment on the meristic series.

Description of experiment

Eggs of parent W and Y were reared in small cloth baskets until hatching. In all except one lot (control of genotype W) eggs hatched naturally. The control lot of genotype W had not done so long after the hatching was due. These eggs were, therefore, induced to hatch by simply removing them into a beaker from the basket. The beaker was floated in the same temperature (i.e., 30°C) bath but aeration was discontinued. Eggs hatched rapidly and the larvae were then reared in large cloth baskets in both replications.

Results

As may be expected, survival of eggs up to hatching was lower in both genotypes reared without malachite green treatment (Table I). In genotype W, survival of the young until preservation was less than 50% in the lot without malachite green treatment. Differences between the mean vertebral (Table II), pectoral ray (Table III), anal ray (Table IV) dorsal ray (Table V) and total caudal rays (Table VI) of the samples from treated and untreated eggs were not significant ($P>.05$) in either genotypes.

Thus it is concluded that malachite green as used in the present series of experiments does not alter the meristic counts of the medaka.

Table I. Egg numbers and mortality in experiment I: Effect of malachite green.

Treatment	No. of fertd. eggs	No. hatched	As % of fertd. egg	Time to 50% hatching (hrs.)	No. survived to preservation	As % of fertd. eggs	Remarks
(a) Parent: W							
Control	100	79	79	460	42	42	Hatching induced by removing eggs into beaker without aeration
Eggs treated with m. green	100	96	96	226	92	92	
(b) Parent: Y							
Control	100	85	85	270	80	80	
Eggs treated with m. green	100	98	98	226	95	95	

Table VII: Egg number and mortality in experiment II. Effect of egg rearing containers and variability of aeration.

Nature of container							
(c) Parent: a							
In bottle with aeration	50	43	86	328	32	64	In bottle up to hatching
In bottle without aeration	50	38	76	156	35	70	In bottle up to hatching
In cloth basket with aeration	50	42	84	387	39	78	

Table II. Frequency distribution of total vertebrae in experiment I: Effect of malachite green.

Treatment	Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
(a) Parent: W								
Control	30°	1	21	20		42	30.45	
Eggs treated with m. green	30°		33	17		50	30.34	
(b) Parent: Y								
Control	30°		13	65	2	80	30.86	
Eggs treated with m. green	30°		13	79	3	95	30.89	

Table VIII. Frequency distribution of total vertebrae in experiment II: Effect of egg rearing containers and variability of aeration.

(a) Parent: a								
In bottle with aeration	30°		21	12		33	30.36	
In bottle without aeration	30°		18	14	1	33	30.51	
In cloth basket with aeration	30°		27	12		39	30.31	
(b) Parent: Y								
In bottle with aeration	26°		12	54	4	70	30.89	
In cloth basket with aeration	26°		8	71	8	87	31.00	

Table III. Frequency distribution of pectoral rays in experiment I: Effect of malachite green.

Treatment	Temp (°C)	Pectoral rays 10	11	12	13	14	Number	Mean	Remarks
(a) Parent: W									
Control	30°	19	59	6			84	10.84	
Eggs treated with m. green	30°	22	77	1			100	10.79	
(b) Parent: Y									
Control	30°		7	128	25		160	12.11	
Eggs treated with m. green	30°		3	140	46	1	190	12.24	

Table IX. Frequency distribution of pectoral rays in experiment II: Effect of egg rearing containers and variability of aeration.

(a) Parent: a									
In bottle with aeration	30°	2	45	19			66	11.26	
In bottle without aeration	30°	2	47	17			66	11.23	
In cloth basket with aeration	30°	3	57	18			78	11.19	
(b) Parent: Y									
In bottle with aeration	26°			63	77		140	12.55	
In basket with aeration	26°			63	106	3	172	12.65	

Table IV. Frequency distribution of anal rays in experiment I: Effect of malachite green.

Treatment	Temp (°C)	Anal rays 16	17	18	19	20	21	Number	Mean	Remarks
(a) Parent: W										
Control	30°	1	3	21	17			42	18.29	
Eggs treated with m. green	30°		7	29	14			50	18.14	
(b) Parent: Y										
Control	30°		4	37	37	2			18.46	
Eggs treated with m. green	30°			39	52	4		95	18.63	

Table X. Frequency distribution of anal rays in experiment II. Effect of egg rearing containers and variability of aeration.

(a) Parent: a										
In bottle with aeration	30°	4	16	11	2			33	17.36	Not different from basket sample (P>.05)
In bottle without aeration	30°	3	13	14	3			33	17.51	
In basket with aeration	30°	4	14	15	6			39	17.59	
(b) Parent: Y										
In bottle with aeration	26°		2	18	37	12	1	70	18.89	
In basket with aeration	26°		2	28	37	12	1	87	18.79	

Table V. Frequency distribution of dorsal rays in experiment I: Effect of malachite green.

Treatment	Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean	Remarks
(a) Parent: W								
Control	30°		34	8		42	6.19	
Eggs treated with m. green	30°		33	16	1	50	6.36	
(b) Parent: Y								
Control	30°		69	10	1	80	6.15	
Eggs treated with m. green	30°		76	18	1	95	6.21	

Table XI. Frequency distribution of dorsal rays in experiment II: Effect of egg rearing containers and variability of aeration.

(a) Parent: a								
In bottle with aeration	30°	1	32			33	5.97	
In bottle without aeration	30°		32	1		33	6.03	
In cloth basket with aeration	30°		38	1		39	6.03	
(b) Parent: Y								
In bottle with aeration	26°		50	20		70	6.29	
In cloth basket with aeration	26°	1	64	22		87	6.24	

Table VI. Frequency distribution of total caudal rays in experiment I: Effect of malachite green.

Treatment	Temp (°C)	Total caudal rays 19	20	21	22	23	24	25	26	27	28	Number	Mean	Remarks
(a) Parent: W														
Control	30°		2	10	13	13	3	1				42	22.19	
Eggs treated with m. green	30°			9	10	23	6	1		1		50	22.64	Not different from control (P>.05)
(b) Parent: Y														
Control	30°			7	18	35	15	4	1			80	22.96	
Eggs treated with m. green	30°			9	17	42	19	6				93	22.96	

Table XII. Frequency distribution of total caudal rays in experiment II. Effect of egg rearing containers and variability of aeration.

(a) Parent a														
In bottle with aeration	30°				3	15	10	4	1			33	23.54	
In bottle without aeration	30°			1	2	10	12	6	2			33	23.81	
In cloth basket with aeration	30°				7	17	8	4	2		1	39	23.51	
(b) Parent: Y														Tend to be lower than basket sample (P<.05; >.02)
In bottle with aeration	26°	1	2	23	23	16	5					70	21.94	
In cloth basket with aeration	26°	5	5	32	29	13	3					87	21.56	

EFFECT OF EGG REARING CONTAINERS AND VARIABILITY OF AERATION (EXPERIMENT II).

Introduction

This experiment was conducted to test the possible effects of differences between containers used for rearing eggs in the present series of experiments on meristic characters.

Description of Experiment.

Eggs of genotype a were reared to hatching in three sets of conditions; in cloth basket in the tank where water was being replaced every 20 hours; in 300 ml of water in a bottle with continuous aeration; and in a bottle in 300 ml of stagnant water without any air supply. Upon hatching, larvae from all containers were transferred to small cloth baskets and reared therein until preservation. All three lots were reared in the same tank in 30°C. temperature bath throughout the experiment. Data obtained from genotype Y reared in basket and in bottle with aeration in connection with experiments IX and XI are also included here for comparison.

Results

Survival of eggs up to hatching in all three lots of genotype a was high. Survival in the lots in aerated bottle and cloth basket was almost identical but in the lot in stagnant and non-aerated water, this was somewhat lower (Table VII). Variation in the method of rearing eggs did not affect the mean vertebral counts in any of the genotypes (Table VIII). Differences between the pectoral (Table IX), anal (Table X), and dorsal rays (Table X) was also not significant ($P > .05$). In genotype a

mean total caudal ray counts of the three samples (Table XII) did not differ from each other significantly but the mean count of the lot of genotype Y in bottle tended to be higher than the mean of the lot reared in basket all through (P = .02 - .05).

Typeof containers used therefore, introduced no bias in meristic series with the possible exception/ofcaudal ray count.

Table XIII. Egg numbers and mortality in experiment III: Effect of quality of eggs on successive days from same parent.

Date eggs obtained	Parent	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs.)	No. survived to preservation	As % of fertd. eggs	Remarks
March 23, 1961	U	47	45	96	265	36	77	
April 8, 1961	U	55	54	98	262	37	67	

Table XIV. Frequency distribution of total vertebrae in experiment III. Effect of quality of eggs on successive days from same parent.

Date eggs obtained	Temp. (°C)	Total vertebrae 30	31	32	Number	Mean	Remarks
March 23, 1961	24°	2	27	1	30	30.97	
April 18, 1961	24°	3	30	3	36	31.00	

Table XV. Frequency distribution of pectoral rays in experiment III. Effect of quality of eggs on successive days from same parent.

Date eggs obtained	Temp (°C)	Pectoral rays 11	12	13	Number	Mean	Remarks
March 23, 1961	24°	14	42	4	60	11.83	
April 8, 1961	24°	13	49	10	72	11.96	

Table XVI. Frequency distribution of anal rays in experiment III. Effect of quality of eggs on successive days from same parent.

Date eggs obtained	Temp (°C)	Anal rays 19	20	21	Number	Mean	Remarks
Parent U							
March 23, 1961	24°	5	16	9	30	20.13	
April 8, 1961	24°	11	12	3	36	19.78	Tends to be lower. ($P<.05; >.02$)

Table XVII. Frequency distribution of dorsal rays in experiment III.

Date eggs obtained	Temp (°C)	Dorsal rays 6	7	Number	Mean	Remarks
March 23, 1961	24°	29	1	30	6.03	
April 8, 1961	24°	34	2	36	6.06	

Table XVIII. Frequency distribution of total caudal rays in experiment III.

Date eggs obtained	Temp (°C)	Total caudal rays 21	22	23	24	25	26	Number	Mean	Remarks
March 23, 1961			2	11	14	3		30	23.60	
April 8, 1961		1	11	12	9	2	1	36	23.08	Tends to be lower. ($P<.05; >.02$)

EFFECT OF SUCCESSIVE DAYS EGGS (EXPERIMENT III)

Introduction

The number of eggs laid by a female on successive days was always small. This necessitated the use of several days eggs for any one treatment and for different treatments. It was therefore necessary to test the effect of successive lots of eggs of different days on the meristic series considered.

Description of Experiment.

Two batches of eggs from genotype U, obtained on two different dates with an interval of 15 days in between, were reared in 24°C under identical conditions (with the exception that the number of fertilized eggs put in was different).

Results

Survival to hatching was more than 95% in both lots. There was also no difference between the two lots in terms of survival at the time these were preserved (Table XIII). Mean vertebral (Table XIV), pectoral (Table XV) and dorsal ray (Table XVII) counts of the two lots showed no significant difference. Mean anal ray count of the sample from eggs of later date tended to be lower than that of the other ($P<.05$; Table XVI. Total caudal ray count of the lot from later date eggs also tended to be lower ($P<.05$) than the other.

Despite the tendency of difference displayed by anal and caudal counts, it is concluded that successive days eggs has no effect

on most meristic characters. Anal caudal counts remain sensitive even after hatching (as will be shown later) and variation may be due to some unknown factors not dependent on the time elapsed between the two egg batches tested.

EFFECT OF MECHANICAL SHOCK (EXPERIMENT IV)

Introduction

Because different egg lots were probably subjected to slightly different handling, this experiment was conducted to test the effect of mechanical shocks during care of eggs on the formation of meristic characters.

Description of experiment

Effects of disturbance was tested by shaking the developing eggs vigorously. Eggs were picked up from the rearing basket and placed in an 8-ounce bottle partly filled with water (approximately 3 ounces) taken from the temperature bath. The bottle was then shaken vigorously with its lid tightly screwed. After four minutes of continuous shaking, eggs were put back in the appropriate baskets. Three lots of eggs of parent T were used for this experiment. The first batch was used as control and allowed to hatch without any disturbance. The second lot of eggs were subjected to shaking immediately after fertilization and thereafter shaking was repeated daily for four minutes until hatching. Eggs of the third lot were allowed to develop undisturbed for the first four days following fertilization. After this period, these eggs were also subjected to four minutes of continuous shaking daily until hatching. All three lots were reared in the same temperature bath.

Results

Shaking eggs from fertilization to hatching did not affect survival to hatching. Compared to the control survival of the lot

subjected to shaking (from the date of fertilization) was slightly higher (Table XIX). In the third lot shaken after 4 days of undisturbed development, survival at the corresponding stage was lower.

Mean total vertebral count (Table XX), pectoral ray (Table XXI), dorsal ray (Table XXIII) and total caudal ray counts (Table XXIV) of the three lots did not differ from each other significantly. Mean anal ray count of the sample from eggs shaken daily from fertilization was not significantly different from the control mean but the mean of the sample from eggs shaken after four days undisturbed development tended to be higher ($P<.05$) than the mean of the control (Table XXII).

The above results for all characters except anal rays show that shaking of eggs does not alter the meristic counts. Anal rays are finally fixed after hatching and as such, observed variation may not be attributable to shaking of eggs.

Table XIX. Egg numbers and mortality in experiment IV. Effect of mechanical shock.

Treatment	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching hrs.	No. survived to preservation	As % of fertd. eggs
Parent T						
Control	40	30	75	755	23	57
Shaken 4 minutes daily from fertilization	55	42	76	287	40	73
First 4 days undisturbed; thereafter shaken 4 minutes daily	39	26	67	287	21	54

Table XXV. Egg numbers and mortality in experiment V: Effect of pricking the chorion.

(a) Parent R						
Control	100	84	84	477	74	74
Chorion pricked	57	17	30	275	12	21
(b) Parent V						
Control	75	71	95	317	70	93
Chorion pricked	75	29	39	321	29	39

Table XX. Frequency distribution of total vertebrae in experiment IV: Effect of mechanical shock.

Treatment	Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
Parent T								
Control	24°		17	5		22	30.23	
Shaken 4 minutes daily from fertilization	24°		27	13		40	30.32	Not different from control (P>.05)
First 4 days undisturbed; shaken 4 minutes daily	24°	1	15	5		21	30.19	Do (P>.05)

Table XXVI. Frequency distribution of total vertebrae in experiment V: Effect of pricking the chorion.

(a) Parent R							
Control	26°		19	54	1	74	30.76	
Chorion pricked	26°		3	7	2	12	30.92	
(b) Parent V							
Control	26°		2	63	5	70	31.04	
Chorion pricked	26°			25	4	29	31.14	Not different from control (P>.05)

Table XXI. Frequency distribution of pectoral rays in experiment IV: Effect of mechanical shock.

Treatment	Temp (°C)	Pectoral rays 10	11	12	Number	Mean	Remarks
Parent T							
Control	24°		9	11	20	11.55	
Shaken 4 minutes daily from fertilization	24°		27	13	40	11.33	Not different from control (P>.05)
First 4 days undisturbed; thereafter shaken 4 minutes daily.	24°		14	7	21	11.33	Do (P>.05)

Table XXVII. Frequency distribution of pectoral rays in experiment V: Effect of pricking the chorion.

(a) Parent R							
Control	26°	3	88	57	148	11.37	
Chorion pricked	26°		15	9	24	11.37	
(b) Parent V							
Control	26°	8	113	19	140	11.08	
Chorion pricked	26°	2	42	14	58	11.21	Not different from control (P>.05)

Table XXII. Frequency distribution of anal rays in experiment IV: Effect of mechanical shocks.

Treatment	Temp (°C)	Anal rays 17	18	19	20	21	22	Number	Mean	Remarks
Parent T										
Control	24°	1	11	7	3			22	18.53	
shaken 4 minutes daily from fertilization	24°		14	20	6			40	18.80	Not different from control (P>.05)
First 4 days undisturbed; thereafter shaken 4 minutes daily.	24°		5	10	5	1		21	19.09	Tends to be higher than control (P<.05;>.02)

Table XXVIII. Frequency distribution of anal rays in experiment V: Effect of pricking the chorion.

(a) Parent R										
Control	26°		4	14	48	7	1	74	19.82	
Chorion pricked	26°		3	7	2			12	18.92	Lower than control (P<.01)
(b) Parent V										
Control	26°		8	34	26	2		70	19.31	
Chorion pricked	26°	4	13	5	4	3		29	18.62	Do (P<.01)

Table XXIII. Frequency distribution of dorsal rays in experiment IV: Effect of mechanical shock.

Treatment	Temp (°C)	Dorsal rays 6	7	8	Number	Mean	Remarks
Parent T							
Control	24°	13	9		22	6.42	
Shaken 4 minutes daily from fertilization.	24°	26	14		40	6.35	
First 4 days undisturbed; thereafter shaken 4 minutes daily	24°	12	9		21	6.43	

Table XXIX. Frequency distribution of dorsal rays in experiment V: Effect of pricking the chorion.

(a) Parent R							
Control	26°	30	43	1	74	6.60	
Chorion pricked	26°	11	1		12	6.08	Lower than control. ($P<.01$)
(b) Parent V							
Control	26°	53	13		*70	6.13	*5 fish with 5 dorsal rays.
Chorion pricked	26°	16	7		**29	6.03	**6 fish with 5 dorsal rays.

Table XXIV. Frequency distribution of total caudal rays in experiment IV. Effect of mechanical shock.

Treatment	Temp (°C)	Total caudal rays 20	21	22	23	24	25	Number	Mean	Remarks
Parent T										
Control	24°		4	4	9	4	1	22	22.73	
Shaken 4 minutes daily from fertilization	24°		2	5	k7	13	3	40	23.25	Not different from control (P>.05)
First 4 days undisturbed; thereafter shaken 4 minutes daily	24°		1	5	9	3	3	21	23.09	Not different from control. (P>.05)

Table XXX. Frequency distribution of total caudal rays in experiment V. Effect of pricking the chorion.

(a) Parent R										
Control	26°	1	22	15	28	7	1	74	22.28	
Chorion pricked	26°	1	3	5	2	1		12	21.92	Not different from control (P>.05)
(b) Parent V										
Control	26°		5	8	41	13	2	69	23.50	
Chorion pricked	26°		5	10	12	2		29	23.38	

EFFECT OF PRICKING THE CHORION (EXPERIMENT V)

Introduction

In one experiment with thyroxine and thiourea, chorion pricked eggs were used. It was therefore considered necessary to test if pricking of chorion as such alters any of the meristic characters.

Description of experiment

This experiment was replicated twice with eggs of genotypes R and V. The chorion of each egg was pricked under a binocular microscope with a very sharp dissecting needle shortly after fertilization. Both control and the chorion pricked eggs of genotype R were reared in small baskets in 26°C in the same tank from fertilization to preservation. In the case of genotype V, control and chorion pricked egg lots were reared in 300 ml of aerated water in bottle in 26°C bath. After hatching the young were reared in small cloth baskets until they were finally preserved.

Results

In both replications, mortality before hatching was greater in the chorion pricked egg lots (Table XXV). Mean vertebral (Table XXVI), pectoral ray (Table XXVII) and total caudal ray (Table XXX) counts of the samples from chorion pricked eggs were not significantly different from the control means in either of the replications. Mean anal ray counts of the sample from chorion pricked eggs in both replicates were lower ($P<.01$) than the respective control mean (Table XXVIII). Mean dorsal ray count of the sample from pricked eggs of genotype R was lower than the control mean ($P<.01$) but no difference was found in the other replication (Table XXIX).

From the above, it appears that most meristic counts are not affected by pricking of the chorion. As in other experiments, the anal ray counts produced ambiguous results. Because of their late fixation (demonstrated later), they may have been influenced by other variables than that under investigation.

Table XXXI. Egg numbers and mortality in experiment VI. Effect of egg density.

Parent	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs).	No. survived to preservation	As % of fertd. eggs	Remarks
Y	25	25	100	297	25	100	
Y	50	50	100	273	50	100	
Y	100	96	96	291	90	90	
Y	200	193	97	283	178	89	

Table XXXII. Frequency distribution of total vertebrae in experiment VI.

No. of eggs	(°C) Temp	Total vertebrae 30	31	32	Number	Mean	Remarks
25	24°	1	22	2	25	31.04	
50	24°	3	42	5	50	31.04	
100	24°	5	79	6	90	31.01	
200	24°	12	155	11	178	30.99	

Table XXXIII. Frequency distribution of pectoral rays in experiment VI.

No. of eggs	Temp (°C)	Pectoral rays 11	12	13	14	Number	Mean	Remarks
25	24°		10	38	2	50	12.84	
50	24°	1	20	76	2	99	12.80	
100	24°		41	136	3	180	12.79	
200	24°	3	120	215	14	352	12.68	Not different from 25 egg sample. (P>.05)

Table XXXIV.. Frequency distribution of anal rays in experiment VI. Effect of egg density.

No. of eggs	Temp (°C)	Anal rays 17	18	19	20	21	Number	Mean	Remarks
Parent Y									
25	24°		1	13	8	3	25	19.52	
50	24°	1	4	30	15		50	19.18	Not different from 25 eggs sample (P>.05)
100	24°		21	50	18	1	90	18.99	(1) Lower than 25 eggs sample (P<.01) (2) Not different from 50 & 200 eggs sample (P>.05)
200	24°	2	33	101	39	1	176	19.02	(1) Lower than 25 egg sample (P<.01) (2) Not different from 50 eggs sample (P>.05)

Table XXXV.. Frequency distribution of dorsal rays in experiment VI.

No. of eggs	(°C) Temp	Dorsal rays 6	7	8	Number	Mean	Remarks
Parent Y							
25	24°	15	10		25	6.40	
50	24°	33	16	1	50	6.36	
100	24°	68	22		90	6.24	Not different from any other sample (P>.05)
200	24°	122	54		176	6.31	

Table XXXVI. Frequency distribution of total caudal rays in experiment VI.

No. of eggs	Temp (°C)	Total caudal rays 17	18	19	20	21	22	23	24	25	Number	Mean
Parent Y												
25	24°				1	3	6	10	5		25	22.60
50	24°					17	12	14	5	2	50	22.26 [1]
100	24°			2	5	35	33	11	3	1	90	21.67 [2]
200	24°	1	1	3	7	55	64	33	9	1	174	21.81 [3]

(1) Not different from 25 egg sample ($P>.05$); higher than 100 egg sample ($P<.01$).

(2) Lower than 25 eggs and 50 eggs samples ($P<.01$); not different from 200 egg samples.

(3) Lower than 25 eggs sample ($P<.01$); tends to be lower than 50 eggs sample ($P<.02; >.01$).

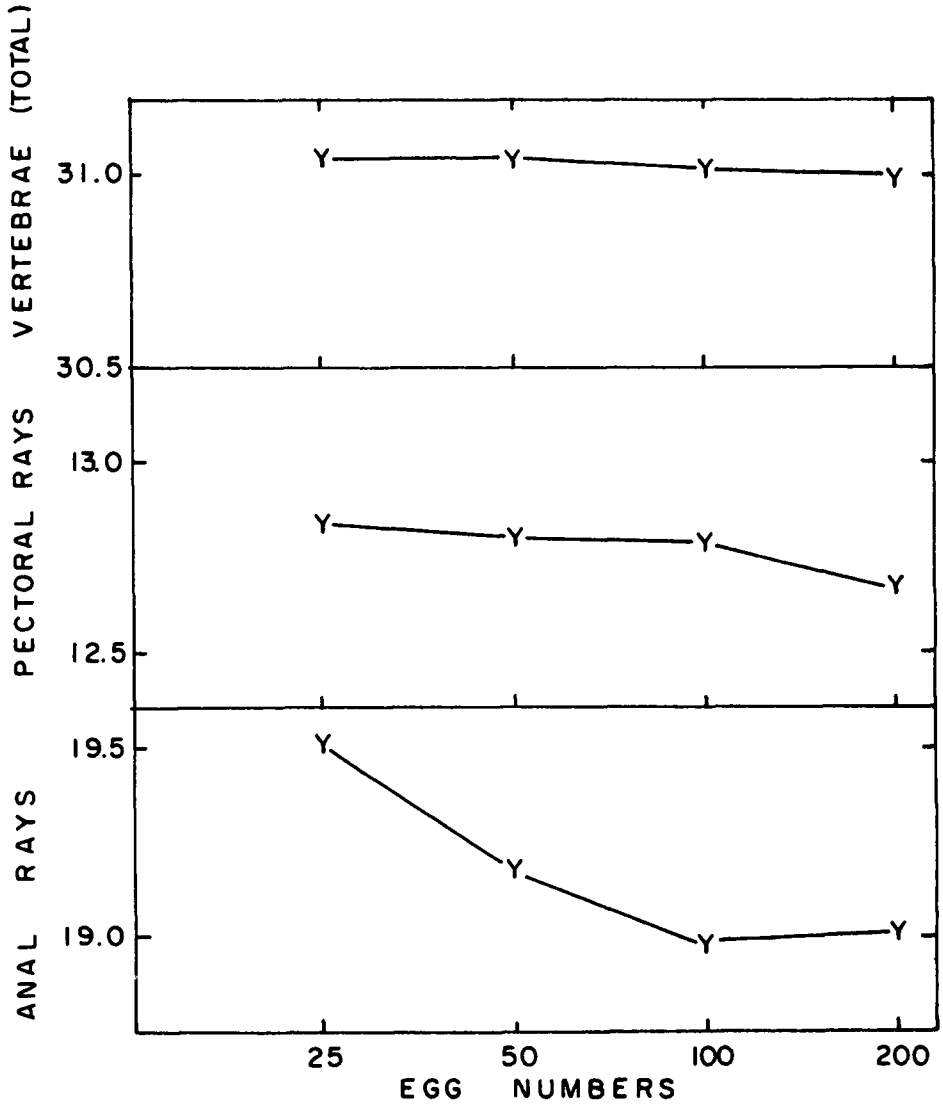

Figure 9. Effect of egg density on mean total vertebrae and pectoral and anal fin rays of genotype Y (Experiment VI)

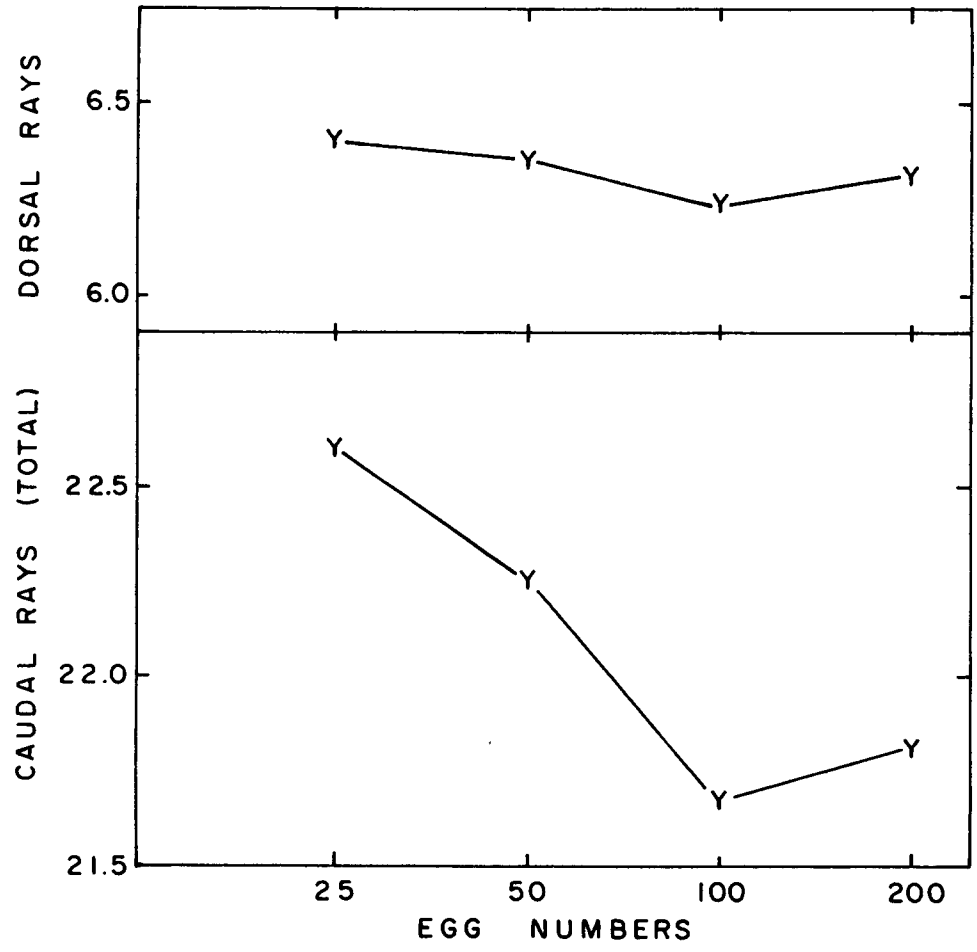

Figure 10. Effect of egg density on mean dorsal and total caudal fin rays of genotype Y (Experiment VI)

EFFECT OF EGG DENSITY (EXPERIMENT VI)

Introduction

The purpose of experiment VI was to explore the extent and direction of the influence of the different density of eggs and young upon different meristic series, since egg numbers were not identical in other experiments designed to test other variables.

Description of experiment

Eggs of genotype Y were used. Eggs were reared in lots of 200, 100, 50 and 25. For the first three lots, eggs obtained in consecutive spawning days were used. The lot in the last sample was from a single day's spawning. All the lots were reared in small cloth baskets in 24°C bath in the same tank, both before and after hatching.

Results

Survival up to hatching in different lots ranged from 96 to 100 percent (Table XXXI). From 25 and 50 eggs, 25 and 50 young survived up to preservation. Survival up to hatching in the lots of 100 and 200 eggs was slightly lower (96%). Mean total vertebral (Table XXXII: Figure 9), pectoral ray (Table XXXIII: Figure 9) and dorsal ray (Table XXXV: Figure 10) were not affected by egg density. A four or eight fold increase in density appeared to influence the expression of final anal ray counts (Table XXXIV: Figure 9). Mean count of the lot from 25 eggs was significantly higher than the means from lots of

100 and 200 eggs. Statistical tests failed to reveal any difference between the means of the samples from 25 and 50 eggs but the latter showed a trend towards decrease. Mean counts of samples from 50, 100 and 200 egg lots were compared with each other, but no significant difference was observed. Results with respect to total caudal rays are presented in Table XXXVI and Figure 10. Mean total caudal rays of the sample from 25 egg-lot was not different from that of the 50 egg lot sample but was significantly higher than the mean counts of samples from 100 and 200 egg lots ($P<.01$). There was significant difference between the mean caudal rays of the samples from 50 and 100 eggs-lot where the mean of the latter was lower ($P<.01$). The mean counts of the samples from 50-eggs lot and 200-eggs lot revealed a strong tendency of being different ($P<.02$). The means of 100 and 200-eggs lot samples showed no difference ($P>.05$).

Density of eggs or of the young does not therefore influence the number of vertebrae and pectoral or dorsal fin rays. Effects of density alone on the number of anal and total caudal rays are not conclusive. The effect becomes apparent perhaps only when the density has reached a certain critical level, not reached in most other experiments.

Table XXXVII. Egg numbers and mortality in experiment VII. Effect of egg size.

Parent	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching	No. survived to preservation	As % of fertd. eggs	Mean egg diameter (mm)
Replication I							
F	98	48	49	263	38	39	1.03
F♀ J♂	116	58	50	280	54	47	1.06
J♀ F♂	164	116	71	273	96	59	1.13
J	105	63	60	302	36	34	1.14
Replication II							
N	100	72	72	360	40	40	1.09
N♀ S♂	100	78	72	360	40	40	1.09
S♀ N♂	100	67	67	524	47	47	1.16
S	100	53	53	316	41	41	1.14

Table XXXVIII. Frequency distribution of total vertebrae in experiment VII.

Parent	Egg size	Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
Replication I									
F	small	24°		4	30	4	38	31.00	Lower than J(P<.01)
F♀ J♂	small	24°	1	23	12		36	30.31	(1) Lower than F (P<.01) (2) Not different from J(P>.01)
J♀ F♂	large	24°	1	19	15	1	36	30.44	(1) Lower than F (P<.01) (2) Higher than J(P<.01)
J	large	24°	3	28	5		36	30.05	Lower than F(P<.01)

Table XXXVIII continued. Frequency distribution of total vertebrae in experimetn VII.

Parent	Egg size	Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
Replication II									
N	small	24°		18	22		40	30.53	Lower than S(P<.01).
N♀ S♂	small	24°		6	47	1	54	30.91	(1) Higher than N(P<.01) (2) Lower than S(P<.01) (3) Lower than S♀N♂ (P<.01)
S♀ N♂	large	24°		1	27	14	42	31.31	Tends to be lower than S (P<.02;>.01)
S	large	24°			17	23	40	31.56	

Table XXXIX. Frequency distribution of pectoral rays in experiment VII: Effect of egg size.

Parent	Egg size	Temp (°C)	Pectoral rays 10	11	12	13	Number	Mean
Replication I								
F	small	24°	3	31	4		38	11.03
F♀ J♂	small	24°		19	17		36	11.47 [1]
J♀ F♂	large	24°		29	7		36	11.19 [2]
J	large	24°		23	12	1	36	11.39 [3]
Replication II								
N	small	24°		17	57	5	79	11.85 [4]
N♀ S♂	small	24°		70	34		104	11.33 [5]
S♀ N♂	large	24°		7	64	11	82	12.05
S	large	24°		29	39	2	70	11.61 [6]

Note:
1. Higher than F(P<.01); not different from J.
2. Not different from J(P>.05); tend to be lower than F♀J♂(P<.02;>.01)
3. Higher than F(P<.01)
4. Higher than S(P<.01) and N♀S♂(P<.01)
5. Lower than S♀N♂and S(P<.01)
6. Lower than S♀N♂(P<.01)

Table XL. Frequency distribution of anal rays in experiment VII.

Parent	Egg size	Temp (°C)	Anal rays 16	17	18	19	20	21	22	Number	Mean
Replication I											
F	small	24°	1	20	14	3				38	17.50
F♀ J♂	small	24°		9	24	3				36	17.83 [1]
J♀ F♂	large	24°			10	20	6			36	18.89
J	large	24°			6	23	6	1		36	19.06 [2]
Replication II											
N	small	24°			8	16	10	6		40	19.35 [3]
N♀ S♂	small	24°			2	17	24	11		54	19.81 [4]
S♀ N♂	large	24°				6	26	9	1	42	20.12 [5]
S	large	24°			2	8	13	15	1	39	20.13

Note:
1. Lower than J(P<.01); tend to be higher than F(P<.05;>.02); lower than J♀F♂(P<.01)
2. Higher than F(P<.01)
3. Lower than S(P<.01)
4. Tends to be higher than N(P<.02;>.01); not different from S♀N♂(P>.05); tend to be lower than S(P<.05;>.02)
5. Not different from S(P>.05)

Table XLI. Frequency distribution of dorsal rays in experiment VII: Effect of egg size.

Parent	Egg size	Temp (°C)	Dorsal rays 5	6	7	Number	Mean
Replication I							
F	small	24°	5	33		38	5.86
F♀ J♂	small	24°		34	2	36	6.06[1]
J♀ F♂	large	24°		28	8	36	6.22[2]
J	large	24°	1	34	1	36	6.00
Replication II							
N	small	24°		20	20	40	6.50[3]
N♀ S♂	small	24°		50	4	54	6.35[4]
S♀ N♂	large	24°		22	20	42	6.47[5]
S	large	24°	5	27	7	39	6.05

Note:
1. Not different from F
2. Not different from J
3. Higher than S ($P<.01$)
4. Not different from N ($P>.05$); higher than S ($P<.01$)
5. Higher than S ($P<.01$); Not different from N and N♀ S♂ ($P>.05$)

Table XLII. Frequency distribution of total caudal rays in experiment VII: Effect of egg size.

Parent	Egg size	Temp (°C)	Total caudal rays 17	18	19	20	21	22	23	24	25	26	Number	Mean	
Replication I															
F	small	24°			1		8	13	12	4			38	22.24	
F♀ J♂	small	24°						4	15	14	3		36	23.44	1
J♀ F♂	large	24°					4	11	13	7	1		36	22.72	2
J	large	24°					2	8	13	10	3		36	23.11	
Replication II															
N	small	24°					1	12	10	15	1	1	40	23.15	3
N♀ S♂	small	24°			1	1	2	9	14	19	6	2	54	23.31	4
S♀ N♂	large	24°			2	2	8	18	6	5			41	21.96	5
S	large	24°	2	1	4		1	16	10	5			39	21.82	

Note:
1. Higher than F($P<.01$)
2. Not different from J($P>.05$)
3. Higher than S($P<.01$)
4. Not different from N($P>.05$)
5. Not different from S($P>.05$); lower than N and N♀S♂ ($P<.01$)

EFFECT OF EGG SIZE (EXPERIMENT VII)

Introduction

This experiment was designed to study the possible effects of yolk size, expressed as egg size, on different meristic series. Öaning (1952) found no relationship between yolk size and the vertebral number, but Garside and Fry (1959) hypothesized that yolk size of the egg influences the number of myomeres.

Description of experiment

As yolk diameter was the factor under consideration, each individual egg used was measured under a binocular microscope equipped with an ocular micrometer. The ocular micrometer was calibrated to a piece of circular stage micrometer placed inside a Syracuse watch glass under water. Quantity of water in the watchglass was just enough for immersing one egg. In making the calibration, an egg under water in the watch glass was brought into sharpest focus by adjusting the height of the eye pieces. Then the stage micrometer was placed inside water in glass and it was brought into sharpest focus (same as that for the egg) by altering the height of the watchglass and leaving the eye piece height undisturbed. The level of the watchglass with the stage micrometer in it was altered by putting glass slides and cover glasses under it. The same calibration was used for measuring the yolk diameter of all eggs used in both the replications.

The yolk diameter of medaka eggs inside the chorion is clearly visible. The diameter of the yolk was measured twice from

two positions before the completion of first cleavage and the mean of the two was recorded as the diameter of that egg. While measuring the yolk diameter, the animal pole of the egg was avoided in all cases.

This observation was repeated twice with different sets of parents. In each replication two sets of parents were selected on the basis of the yolk diameter of the females. In the first replication, parents F and J were used. The mean yolk diameter of eggs from the female of parent F was significantly smaller ($P<.01$) than that of the eggs from the female of parent J. After obtaining eggs from these sets of parents, a reciprocal cross of the two was made; female of F was crossed with the male of J and vice versa. Mean diameter of eggs obtained from the two sets of parents after crossing was still significantly different ($P<.01$), female of F and J giving small and large eggs respectively. Small and large egg lots from the original parents and their crosses were reared in the same tank in 24°C temperature bath. For rearing the fertilized eggs and the young, small cloth baskets were used.

Parents N and S giving small and large eggs respectively were used for the second replication of the experiment. Here also the female giving smaller egg (N) was crossed with the male of the parent giving large egg and vice versa. Crossing was done after obtaining samples of eggs from the original parent. Mean yolk diameter of the eggs was significantly different ($P<.01$) in the original parent and in the crosses. All four lots in the second replication were reared in 24°C in small cloth baskets in the same tank.

Results

Survival: Percent survival up to hatching was lower than in some of the other experiments and ranged from 49 to 78 (Table XXXVII). After hatching, mortality of the young too was comparatively higher and survival up to the time of preservation ranged from 34 to 58% in the first replicate and 39 ti 48% in the second one.

Total vertebrae: Mean vertebral counts of the different samples are presented in Table XXXVIII. Mean vertebrae of the sample from smaller egg (genotype F) was significantly higher than that from large egg (genotype J). Upon crossing, this difference in the mean vertebral counts between the sample from small and large eggs was removed ($P>.05$). Crossing of the female of parent J (large egg) with the male of parent F (small egg) resulted in a significant rise in the mean vertebral count. On the other hand, crossing the female of parent F (small egg) with the male of J (large egg parent) reduced the vertebral count ($P<.01$). In both cases influence of the father in the final determination of the vertebrae of the offspring was apparent and followed a pattern of blending inheritance.

In the second replication, mean vertebrae of the sample from eggs with large yolk diameter (parent S) was higher ($P<.01$) than that from eggs with smaller yolk diameter (parent N). As in the first replication, reciprocal crossing of the females resulted in a decrease in the higher mean count (from large egg: parent S♀ and N♂) and an increase in the mean of the sample from smaller egg (parent: N♀ S♂). This increase was statistically significant ($P<.01$) but the decrease brought about in the mean of the sample from large egg (large egg of

parent S vs the large egg from S ♀ and N ♂) only tended to be significant (P = .01-.02).

Although the crossing of the genotypes in the second replication resulted in intermediate mean counts, the difference between these two means (small egg from N ♀ S ♂ and large egg from S ♀ N ♂) was still significant (P<.01). This was different from the result of crossing in the replication I where the initial difference between the mean counts disappeared completely after reciprocal crossing of the parents.

Pectoral rays: Results are presented in Table XXXIX. Mean pectoral rays of the sample from eggs with large yolk diameter (parent J) in the first replicate was higher (P<.01) than the mean of the sample from eggs with smaller yolk diameter (parent F). Upon crossing the female of parent F (giving small eggs) with the male of parent J (giving large eggs), mean counts of the sample from small eggs increased significantly (P<.01). Similarly in the other cross (large egg yielding female of parent J with the male of the small egg yielding parent F) the mean pectoral count showed a strong tendency of decrease (P = .01-.02) as compared to the mean of the sample from original parent J. In both cases the male appeared to decide the final expression of the pectoral rays. The original difference between the means of the sample from eggs with small and large yolk (parent F vs J) tended to become slightly reduced after the reciprocal cross (F ♀ J ♂ vs J ♀ F ♂ : P = .01-.02).

In the second replication with parents N (eggs with smaller yolk diameter) and S (eggs with large yolk diameter), mean pectoral

ray counts of the former was significantly higher than the latter ($P<.01$). Upon crossing the female of N with the male of S, this higher pectoral ray was reduced significantly ($P<.01$) although the eggs still remained small. The influence of the male in this case was very strong and the resultant mean pectoral ray of the cross was even lower ($P<.01$) than the mean of the parent S (large yolk diameter). The other cross (i.e. crossing the female of parent S giving eggs with large yolk diameter with the male of the parent giving small egg i.e.N) increased the mean count ($P<.01$) of large egg sample ($P<.01$; parent S vs parent S♀ N♂). Here also, the mean count was higher ($P<.01$) than the mean pectoral ray of the offspring of parent (parent N) whose father contributed to this increase. In both crosses in this replication, the influence of male's genetic constitution was strong and pronounced in inheritance of pectoral rays.

Anal rays: Mean anal ray counts of different samples are presented in Table XL. Mean anal ray count of the sample from eggs with small yolk diameter was lower than the mean of the sample from large yolked egg ($P<.01$) in the first replication (parents F and J). Crossing the female giving smaller egg with the male of the parent giving larger egg (F♀ with J♂) did not change the mean ray count from the eggs with smaller yolk significantly although a strong trend for increase ($P = .02-.05$) was observed as compared with the original sample from small eggs. Crossing the female giving large egg with the male from the small egg parent (J♀ with F♂) did not produce any difference in the mean anal ray counts of the samples from eggs with large yolk diameter.

The mean anal ray counts of the samples from eggs with large yolk diameter and from eggs with small yolk diameter were still different ($P<.01$) after crossing of the males.

Results obtained in the second replication were almost identical with those of the first. Here also, mean count of the sample from smaller egg (after crossing) showed a tendency to become higher than the mean of the sample from smaller egg from the original parent (N and N♀ S♂: $P = .01-.02$). The mean count of the sample from smaller eggs after crossing became higher to the extent that its difference with the mean of the sample from the large egg (original) and large egg (crossing) was respectively reduced and obliterated. In the other cross (large egg female with small egg male), the anal count was not altered significantly. Results in the case of the samples from eggs with larger yolk diameter in both the replications suggest that anal rays are dependent on the mother. Insofar as the samples from eggs with smaller yolk are concerned, crossing the female with the male from the large egg parent tended to increase the mean counts in both the replicates but the increase was not significant, (not at $P<.01$). Mean anal ray counts of the samples from smaller eggs in both replicates were lower than the means of samples from large egg and this difference remained the same even after crossing in the first replicate while in the second replicate the difference disappeared.

Dorsal rays: In the first replication there was no difference in the mean dorsal ray of the samples from eggs with small and large yolk diameters. The crossing of the parents also did not alter the counts in any significant manner (Table XLI).

In the second replication with parents N and S giving eggs with small and large yolk diameter respectively, the mean count of the offspring of N was significantly higher. No significant alteration in dorsal ray counts occurred in the sample from eggs with small yolk diameter when the female of N was crossed with the male of S. The mean dorsal ray count showed an increase ($P<.01$) in the case of the samples, from eggs with large yolk diameter after crossing as compared to the mean of the offspring from original large egg sample. Results from the second replication indicate some influence of the father's genetic constitution on dorsal rays.

Total caudal rays: Data on mean total caudal rays are presented in Table XLII. Compared to the sample from eggs with large yolk diameter (parent J), the mean of the sample from eggs with small yolk diameter (parent F) in the first replication was significantly lower ($P<.01$). But after crossing, (F♀ J♂) the mean count from the sample from eggs with small yolk diameter increased appreciably (by 1.20 rays). The increase in this mean was even slightly higher than the mean of the sample from large egg (parent J). Mean caudal rays of the sample from eggs with large yolk diameter showed a significant decrease ($P<.01$) as a result of crossing the female with the male from the other genotype. Results from this replication indicate a marked paternal influence on the determination of the total caudal rays of the offspring. But the results of the second replication showed a different trend in that the mean counts of the sample from eggs with smaller yolk diameters were higher ($P<.01$). Reciprocal crossing did not produce any appreciable alteration in the mean counts of samples from eggs with large and small yolk diameters.

A comparison of the results of both replications showed no relationship between yolk size and caudal rays. These results also failed to indicate any consistency in the relationship between the caudal ray and the influence of the genetic make-up of the parents.

Conclusion

There is no direct casual relation between yolk size of the egg and different meristic characters of medaka.

Table XLIII. Egg numbers and mortality in experiment VIII:
(a) Transfer from 20°C to 30°C: Parent U.

Period of dev. in 20°C (hrs)	No. of eggs in 20°C	No. of eggs to 30°C	No. hatched	*As % of fertd. eggs	Time to 50% hatching in 30°C (hrs)	No. survived to preservation	*As % of fertd. eggs
24	43	36	36	95	161	32	84
48	49	44	41	93	144	41	93
72	41	35	32	89	143	24	67
96	46	41	39	95	123	34	83
120	40	35	33	94	128	33	94
144	34	28	28	97	118	23	79
168	39	25	24	71	96	22	65
192	39	34	34	100	98	31	91
216	48	41	41	95	95	36	84
240	40	34	33	94	83	30	86
264	51	46	46	100	60	39	85
288	32	27	27	100	46	22	81

*Assuming 5 eggs preserved at time of transfer as alive.

Table XLIII. Egg numbers and mortality in experiment VIII: (cont'd) (b) Transfer from 30°C to 20°C.

Period of dev. in 30°C	No. of eggs in 30°C	No. of eggs to 20°C	No. hatched	*As % of fertd. eggs	Time to 50% hatching (hrs)	No. survived to preservation	*As % of fertd. eggs
24	62	53	40	70	441	18	32
48	49	38	25	57	388	15	34
72	46	41	37	90	419	23	56
96	38	27	25	76	263	8	24

*Assuming 5 eggs preserved at time of transfer as alive.

(c) Control samples.

Temp (°C)	No. of eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs)	No. survived to preservation	As % of fertd. eggs
20°	40	40	100	483	36	90
30°	35	34	97	168	30	86

Table XLIV . Frequency distribution of total vertebrae in experiment VIII: (a) Transfer of eggs from 20° to 30°C: Parent U.

Hours (and day degrees) in 20°C before transfer	Developmental stage at transfer	Total vertebrae 30	31	32	33	Number	Mean	Diff. with 30°C control	Diff. with 20°C control
0		7	22	1		30	30.80	30°C control	yes P<.01
24 (20)	Blastula complete.	11	21			32	30.65	None P>.05	Yes P<.01
48 (40)	Embryonic shield	28	13			41	30.32	Yes P<.01	Yes P<.01
72 (60)	Optic cup forming; 8 somites	7	16	1		24	30.75	None P>.05	Yes P<.01
96 (80)	Somites 20 Auditory vesicle formed; tail bud	5	28	1		34	30.88	None P>.05	Yes P<.01
120 (100)	Circulation started; eyes slightly rounded		25	8		33	31.24	Yes P<.01	None P>.05
144 (120)	Elongated embryo; pectoral buds seen; eye pigmentation commenced		9	14		23	31.61	Yes P<.01	Strong trend P<.05; >.01
168 (140)	Post. part free and moving. Rectorals triangular.		10	12		22	31.54	Yes P<.01	None P>.05

Table XLIV. (Cont'd). Frequency distribution of total vertebrae in experiment VIII: Transfer of eggs from 20°C to 30°C: Parent U.

Hours (and day degrees) in 20°C before transfer	Developmental stage at transfer	Total vertebrae 30	31	32	33	Number	Mean	Diff. with 30°C control	Diff. with 20°C control
192 (160)	Eyes darkly pigmented; pectorals distinct	16	13	2		31	31.55	Yes $P<.01$	None $P>.05$
216 (180)	not recorded	16	20			36	31.35	Yes $P<.01$	None $P>.05$
240 (200)	not recorded		15	15		30	31.50	Yes $P<.01$	None $P>.05$
264 (220)	" "		25	14		39	31.36	"	"
288 (240)	" "	1	11	10		22	31.41	"	"
Entire		1	22	13		36	31.33	"	20°C Control

Table XLIV. Frequency distribution of total vertebrae in experiment VIII. (b) Transfer of eggs from 30°C to 20°C: Parent U.

Hours (and day degrees) in 30°C before transfer	Developmental stage at transfer	Total vertebrae 30	31	32	Number	Mean	Diff. with 30°C Control	Diff with 20°C control
0		1	22	13	36	31.33	Yes P<.01	20°C control
24 (30)	Optic cup forming; 5-6 somites		8	10	18	31.55	Yes P<.01	None P>.05
48 (60)	Circulation started; auditory vesicle distinct	1	12	2	15	31.07	None P>.05	Tends P<.05; >.01
72 (90)	Embryo elongated; pectorals triangular; posterior part free and moving	5	18		23	30.78	None P>.05	Yes P<.01
96 (120)	Pectorals moving; melanophore along dorsal line	1	7		8	30.87	None P>.05	Yes P<.01
Entire		1	22	7	30	30.80	30°C con.	Yes P<.01

Table XLV. Frequency distribution of pectoral rays in experiment VIII.
(a) Transfer of eggs from 20°C to 30°C, Parent U

Period of dev. in 20°C before transfer (hrs)	Pectoral rays 10	11	12	13	Number	Mean	Diff. with 30°C control	Diff. with 20°C control
0		23	7		30	11.23	30°C	Yes P<.01
24	4	47	7		58	11.05	None P>.05	"
48	1	72	9		82	11.01	"	"
72	6	33	9		48	11.06	"	"
96	4	37	5		46	11.02	"	"
120	2	27	15		44	11.29	"	"
144	1	14	6		21	11.24	"	"
168		10	10		20	11.50	Tends P=.05	"
192		30	32		62	11.51	Yes P<.01	"
216		15	52	5	72	11.86	"	"
240		7	65	6	78	11.99	"	"
264		7	65	6	78	11.99	"	"
288		13	28	3	44	11.77	"	"
Entire		2	50	20	72	12.25	"	20°C

Table XLVI. Frequency distribution of anal rays in experiment VIII.
(a) Transfer of eggs from 20°C to 30°C: Parent U.

Period of dev. in 20°C before transfer (hrs)	Anal rays 18	19	20	21	22	Number	Mean	Diff. with 30°C control	Diff. with 20°C control
0	1	9	17	3		30	19.73	30°C	None P>.05
24	3	12	15	2		32	19.50	None P>.05	"
48		17	16	8		41	19.78	"	"
72	1	7	13	3		24	19.74	"	"
96	8	16	9	1		34	19.09	Yes P<.01	Yes P<.01
120	4	14	9	6		33	19.21	Tends P<.02	"
144		6	8	9		23	20.13	None P>.05	None P>.05
168	1	4	12	4	1	22	20.00	"	"
192	1	10	12	8		31	19.87	"	"
216		15	17	4		36	19.69	"	"
240	3	13	11	1	2	30	19.53	"	"
264	5	16	15	3		39	19.41	"	Tends P<.05;>.02
288	2	11	8	1		22	19.36	"	"
Entire	2	9	18	5	2	36	19.89	"	20°C control

Table XLVII. Frequency distribution of dorsal rays in experiment VIII: (a) Transfer of eggs from 20°C to 30°C: Parent U.

Period of dev. in 20°C before transfer (hrs)	Dorsal rays 6	7	Number	Mean	Diff. with 30°C control	Diff. with 20°C control
0	25	5	30	6.17	30°C control	None P>.05
24	25	7	32	6.22	None P>.05	"
48	33	8	41	6.19	"	"
72	23	1	24	6.04	"	"
96	31	3	34	6.09	"	"
120	30	3	33	6.09	"	"
144	19	4	23	6.17	"	"
168	20	2	22	6.09	"	"
192	23	8	31	6.23		
216	23	13	36	6.08	"	"
240	18	12	30	6.40	Tends P=.05	Yes P<.01
264	25	14	39	6.36	None P>.05	None P>.05
288	16	6	22	6.27	"	"
Entire	31	4	36*	6.08	"	20°C control

* 1 fish with 5 rays

Table XLVIII. Frequency distribution of total caudal rays in experiment VIII: (a) Transfer of eggs from 20°C to 30°C: Parent U.

Period of dev. in 30°C before transfer (hrs.)	Total caudal rays 21	22	23	24	25	26	Number	Mean	Diff. with 30°C control	Diff with 20°C control
0	1	7	13	8			29	22.96	30°C control	None P>.05
24	3	3	20	6			32	22.91	None P>.05	"
48	3	5	22	10	1		41	23.00	"	"
72		2	12	10			24	23.33	"	"
96		4	15	14	1		34	23.35		
120		3	12	16	2		33	23.51	Yes P<.01	Tends P=.05
144	2	4	8	7	2		23	23.13	None P>.05	None P>.05
168	1	9	3	8	1		22	22.95	"	"
192	2	6	11	11	1		31	23.10	"	"
216	1	8	15	11	1		36	23.08	"	"
240		9	11	8	2		30	23.10	"	"
264	1	10	15	12	1		39	23.05	"	"
288		5	10	7			22	23.09	"	"
Entire	1	7	20	6		2	36	23.08	"	20°C control

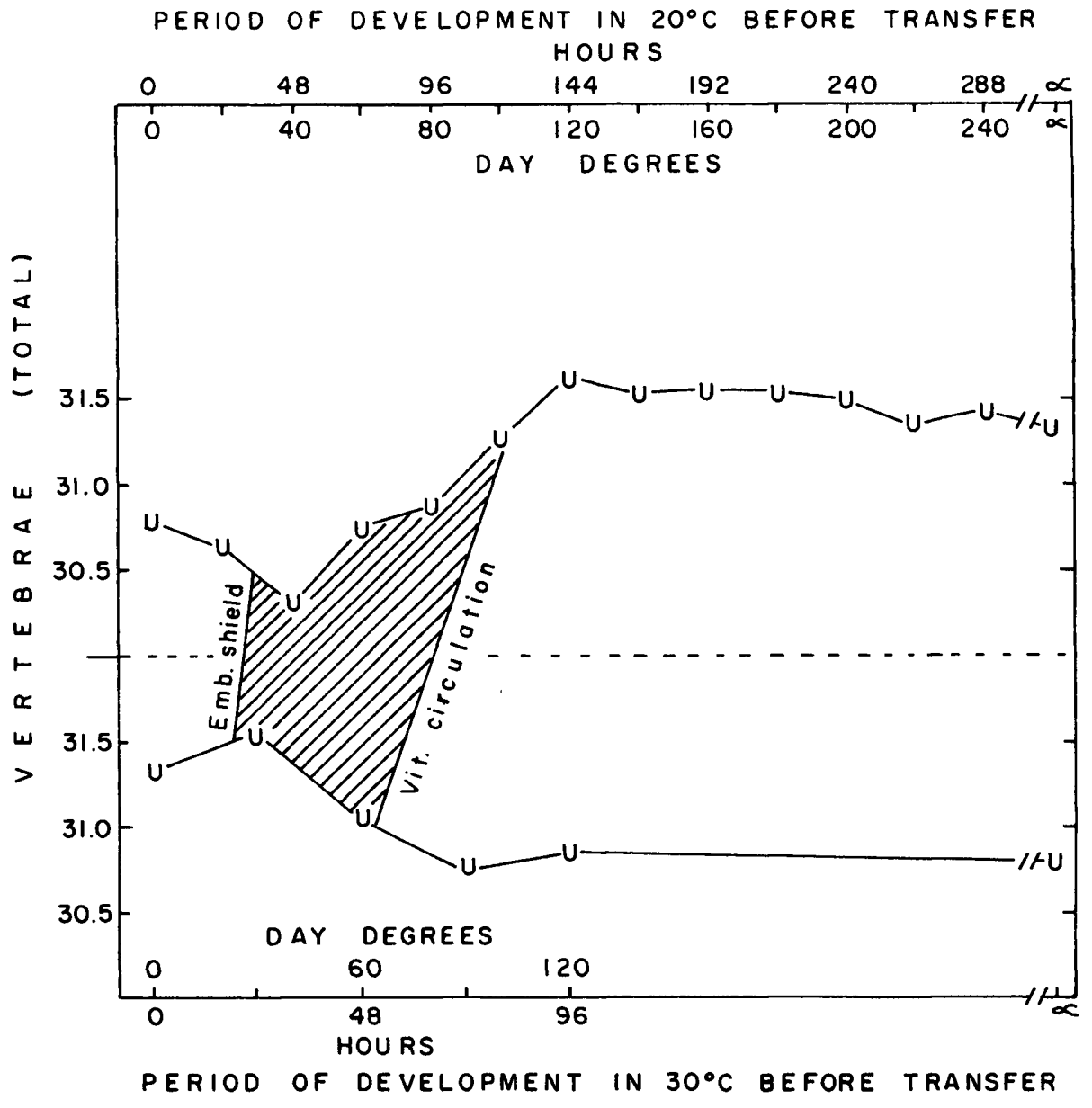

Figure 11. Mean vertebral counts of fish transferred from 20° to 30°C (top) and from 30° to 20°C (bottom). Shaded area indicates period between embryonic shield and vitelline circulation. (Experiment VIII).

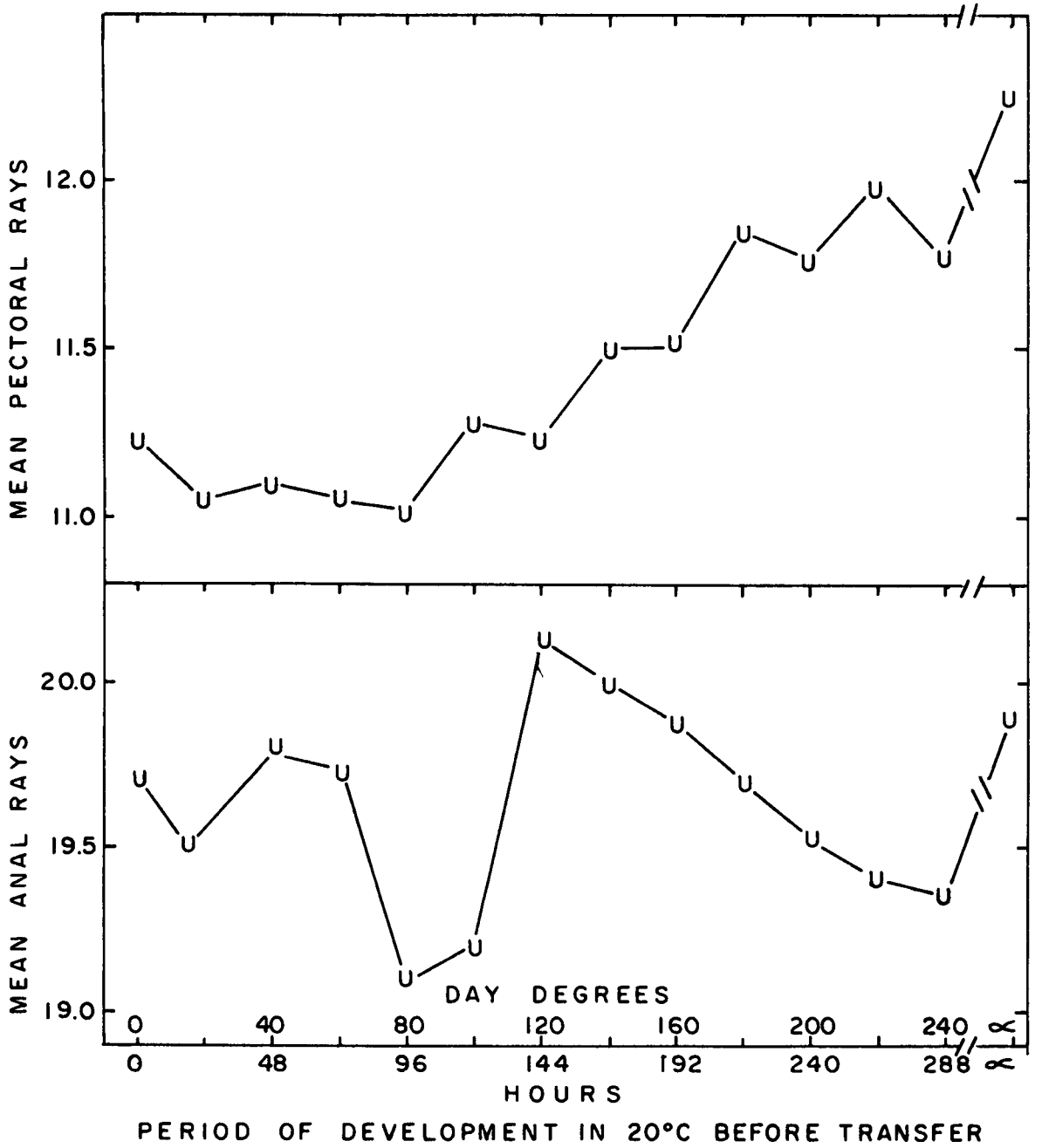

Figure 12. Effect of transfer of developing embryo from 20° to 30°C on mean pectoral and anal fin rays of genotype U. (Experiment VIII).

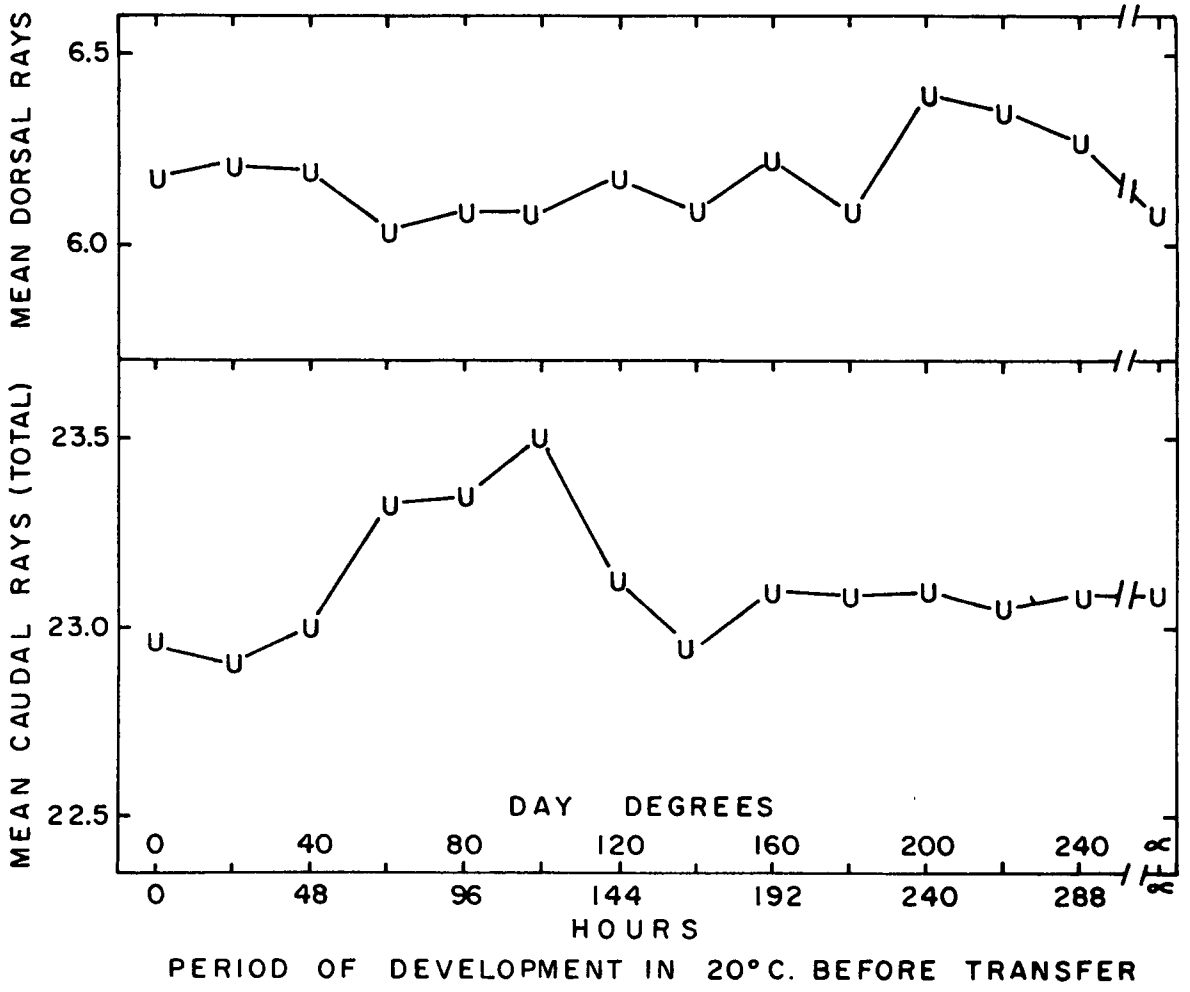

Figure 13. Effect of transfer of developing embryo from 20° to 30°C on mean dorsal and total caudal fin rays of genotype U (Experiment VIII)

STAGE OF FIXATION OF MERISTIC SERIES (EXPERIMENT VIII)

Introduction

This experiment was performed to determine the time of vertebral fixation in medaka. Batches of eggs at different stages of development were transferred from low to high and from high to low temperature. A comparison of the mean vertebral count with the mean count of controls at high and low temperature was expected to reveal if the number of vertebrae had become finally determined at certain stage of development.

Description of experiment

Eggs from parent U were used for all the transfers made from 20°C to 30°C and vice versa. For each transfer and control (in 30° and 20°C) eggs obtained on a single day's spawning were used. As the number of eggs obtained each day varied, the number initially used for each transfer was not the same. The control lot in 30°C was reared entirely in that temperature while the corresponding lot in 20°C were hatched and reared in 20°C (15.5 to 22.5 days after hatching) up to the stage when the anal, caudal and dorsal fins were distinctly visible to the naked eye. Thereafter, the young were reared in 24°C until such time that they could be preserved. Transfers in the two directions were made at the end of periods of development as shown in Tablex XLIII and XLIV. At the time of each transfer, five developing eggs were taken from the basket and preserved. Before preservation, the stage of development of these five eggs were observed under a binocular microscope and recorded (Column 2: Table XLIV. The remaining eggs were transferred to the desired

temperature by removing the baskets directly from the low to high or high to low temperatures without any conditioning. All the samples transferred to high temperature were reared in that temperature until they could be preserved. The lots transferred to lower temperature (20°C) were reared in the low temperature until hatching. Thereafter, these lots were reared in 24°C for further growth and as such, only vertebral counts (which were found to be fixed before hatching) were considered.

Eggs and young in all cases were reared in small cloth baskets.

In calculating percentage survival, the 5 eggs that were preserved at the time of transfer were deducted from the initial number of eggs used for rearing and in the calculations shown in Table XLIII the remaining transferred eggs were treated as the initial number of eggs.

Results

In all the lots transferred from low to high temperature, survival until hatching and up to the time of preservation ranged from 70 to 100% and 66 to 94% respectively (Table XLIII). Transfers in the opposite direction affected the survival rate and were not as satisfactory.

Total Vertebrae: a) Transfer from 20 to 30°C. Mean vertebral counts of samples of different days transfers from low to high temperature are presented in Figure II and Table XLIVa. The sensitive period for the vertebrae appeared to extend from the embryonic shield stage to the stage when eye pigmentation commenced and pectoral buds had appeared.

These stages of development corresponded to 48 hours (40 day degrees) and 144 hours (120 day degrees) of complete development in 20°C. Furthermore, these stages correspond roughly to Fundulus stages 14 to 22 (Oppenheimer 1937).

The mean vertebral count of the lot transferred at the end of 48 hours (beginning of sensitive period) was significantly lower than the mean of the control at 30°C.

The mean count of the lot transferred at the end of 144 hours (120 day degrees), on the other hand, showed a strong tendency to become higher than the control in 20°C ($P<.05; >.01$). Mean counts of all transfers made after 168, 192, 216, 240, 264 and 288 hours of development did not differ from the mean of the control in 20°C ($P>.05$ in all cases).

b) Transfer from 30° to 20°C:

The number of vertebrae seemed almost fixed after 48 hours incubation in 30°C ($P>.05$ as compared to 30°C; $P<.05$ as compared to the 20°C mean count) (Table XLIVb and Figure II). The stage of development reached by the eggs at the end of 48 hours in 30° corresponded more or less to the stage obtained in 20° in 120 hours (100 day degrees). The mean count of the sample transferred at the end of 72 hours (90 day degrees) showed that the vertebrae has become finally fixed by this time in 30°C. The developmental stage at this time closely corresponded to the stage obtained in 168 hours development in 20°C.

From the data the sensitive period for the fixation of the number of vertebrae appears to extend between the stage when the embryonic shield is formed and the time when pectoral buds appear and eye pigmentation starts, after which changes in the temperature fail to alter the number.

Other meristic series: Although this experiment was designed to determine time of fixation of vertenrae, other meristic series were counted only for the lots transferred from low to high temperature.

Pectoral rays: Mean pectoral rays of the samples transferred at the end of 24, 48, 72, 96 and 120 hours were lower ($P<.01$) than the mean of 20°C control but were not significantly different from 30°C control (Table XLV: Figure 12). Mean count of the lot transferred at the end of 168 hours (7 days) development tended to be higher ($P=.05$) than the mean of the lot in 30°C (control). Mean counts of all the lots transferred thereafter were higher than that of 30°C control but lower than the mean of the control in 20°C.

Anal rays: Mean anal ray counts of the sample transferred at the end of 96 hours was lower ($P<.01$) than the mean of the high and low temperature control (Table XLVI: Figure 12). Mean of the sample transferred after 120 hours was lower than the mean of the control in 20°C and showed a marked tendency of being lower ($P=.01-.02$) than the mean of the control in 30°C. Compared to the control in 20°C, the mean counts of the lots transferred at the end of 264 and 288 hours were somewhat lower ($P=.02-.05$) but a comparison with the control in the high temperature showed no difference. Mean counts of the remaining samples exhibited no difference with the mean of the controls at 20° or 30°C.

Dorsal rays: Mean dorsal count of the sample tramsferred at the end of 240 hours was higher than the mean of the low temperature control ($P<.01$) and also showed a tendency to be higher than the mean of the control in 30°C (Table XLVII: Figure 13). Mean counts of the other transfer lots showed no difference with either of the control mean counts.

Total caudal rays: The mean count of the lot transferred at the end of 120 hours was higher ($P<.01$) than the mean of the control in 30°C and furthermore displayed a tendency of being higher ($P=.05$) than the mean count of the control in 20°C (Table XLVIII: Figure 13). Total mean caudal ray counts of the remaining transfer lots did not reveal any significant difference with the mean of either the control in 20° or 30°C.

Conclusion

Transfer of developing embryo revealed that the sensitive period for the vertebral number in medaka extends between the embryonic shield stage and the stage when pectoral buds had appeared. High temperature shock in the beginning of the sensitive period lowers the number of vertebrae but an increase in the number results when similar shock is applied towards the end of the sensitive period. Sensitive period for the fixation of pectoral and other fin rays, although commencing early, extends to periods after hatching.

Table XLIX. Egg numbers and mortality in experiment IX: Effect of temperature.

Temp (°C)	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs.)	No. survived to preservation	As % of fertd. eggs	Remarks
(1) Parent A							
20°	49	28	57		16	33	414 hrs. in 20°C; hatched and reared in 24°C
22°(a)	65	26	40	661	10	15	
22°(b)	88	40	45		26	29	Hatched by temp. shock after 512 hrs.
24°	133	40	30	366	33	30	
26°	33				6	18	
28°	73	42	58	339	28	38	
30°	95	27	28	276	6	6	
32°(a)	95	27	28	188	11	12	
32°(b)	80	19	24	200	10	13	Reared in 26° after hatching.
(2) Parent B							
22°	31	19	61		15	48	
28°	32	26	81		26	81	
32°	35	24	69	274	16	46	

Table XLIX continued. Egg numbers and mortality in experiment IX: Effect of temperature.

Temp (°C)	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs)	No. survived to preservation	As % of fertd. eggs	Remarks
(3) Parent C							
20°(a)	156	94	60		39	25	64 hatched in 20°; 30 hatched in 26°; 13 reared entirely in 20°C
20°(b)	40	21	52		9	23	
22°	33				20	61	
24°(a)	68	56	82		50	74	
24°(b)	37	28	76	311	26	70	
26°(a)	82	66	80		60	73	
26°(b)	30	30	100		26	87	
30°	76	35	46		35	46	
32°	35	28	80		28	80	
(4) Parent D							
22°	20	14	70	724	11	55	
24°(a)	22	12	54	311	11	50	
24°(b)	17	13	76	288	10	59	
24°(c)	13				6	46	
26°	20	20	100		20	100	
32°	16				9	56	

Table XLIX continued. Egg numbers and mortality in experiment IX: Effect of temperature.

Temp (°C)	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs)	No. survived to preservation	As % of fertd. eggs	Remarks
(5) Parent E							
20°	71	56	79		36	51	552 hours in 20°C. Hatched and reared in 24°C.
24°	29	17	59	299	16	55	
28°	23	22	96	198	21	91	
32°	27	20	74	312	10	37	
(6) Parent G							
20°	23	5	22	386	4	17	Reared in 24°C after hatching
22°	20	12	60	601	8	40	
24°	66	55	83	498	46	70	
26°	90	67	74	277	34	51	
28°	40	17	43	266	17	43	
32°	94	67	71	213	33	35	
(7) Parent H							
20°	125	104	83	459	65	52	Reared in 24°C after hatching
22°	119	100	84	360	52	44	
24°	125	144	91		80	64	
26°	75	69	92	357	36	48	
28°	77	58	75	227	40	52	
30°	125	110	88	221	71	57	
32°	150	137	91	185	126	84	

Table XLIX continued. Egg numbers and mortality in experiment IX: Effect of temperature.

Temp (°C)	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs.)	No. survived to preservation	As % of fertd. eggs	Remarks
(8) Parent I							
20°	62	20	32	465	14	23	
22°	103	55	53	433	33	32	
24°	100	82	82	268	61	61	
26°	104	41	39	263	28	27	
28°	114	72	63	265	54	47	
30°	101	84	83	176	75	74	
32°	100	75	75	177	60	60	
34°	150	24	16	204	7	5	Reared in 32°C after hatching
(9) Parent K							
20°	107	42	39		34	32	504 hrs. in 20°C Hatched and reared in 24°C.
22°	104	60	58	432	28	27	
24°	164	116	71	273	96	59	
26°	44	27	61	243	27	61	
28°	41	28	68	292	22	54	
30°	83	46	55	236	46	55	
32°(a)	77	48	62	164	20	26	
32°(b)	103	47	46	168	28	27	
(10) Parent Q							
24°	50	42	84	317	30	60	
28°	93	58	62	228	27	29	
32°	108	35	32	174	26	24	

Table XLIX continued. Egg numbers and mortality in experiment IX: Effect of temperature.

Temp (°C)	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs.)	No. survived to preservation	As % of fertd. eggs	Remarks
(11) Parent R							
20°	100	31	31	1059	14	14	Reared in 24° after hatching
22°	100	61	61	730	36	36	
26°	100	84	84	477	74	74	
28°	100	60	60	490	41	41	
30°	100	48	48	560	29	29	
32°	100	69	69	457	19	19	
34°	100	50	50	290	18	18	Reared in 32°C after hatching.
(12) Parent U							
20°	40	40	100	483	36	90	
24°(a)	47	45	96	265	36	77	
24°(b)	55	54	98	262	46	84	
28°	53	53	100	202	51	96	
30°	35	34	97	168	31	89	
32°	88	81	92	162	71	81	

Table XLIX continued. Egg numbers and mortality in experiment IX. Effect of temperature.

Temp (°C)	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs.)	No. survived to preservation	As % of fertd. eggs	Remarks
(13) Parent W							
22°	100	99	99	439	88	88	
26°	100	100	100	365	93	93	
30°	100	96	96	226	92	92	
32°	100	90	90	383	60	60	
34°	100	20	20	331	7	7	Reared in 32°C after hatching.
(14) Parent X							
22°	75	70	93	478	62	83	
26°	75	59	79	316	51	68	
30°	75	72	96	200	70	93	
32°	75	71	95	221	66	88	
34°	75	61	81	198	41	55	
(15) Parent Y							521 hrs. in 20°; hatched and reared in 26°C.
20°	100	95	95	554	90	90	
24°	100	96	96	289	90	90	
26°	100	93	93	346	87	87	
30°	100	98	98	226	95	95	
32°	100	93	93	226	64	64	
34°(a)	100	a. 31 b. 43	74	279 343	7 9	16	a. From 50 eggs hatched & reared in 34°C. b. From 50 eggs hatched & reared in 30°C.
34°(b)	100	67	67	325	4	4	
34°(c)	100	43	43	255	9	9	Reared in 32°C after hatching.

EFFECT OF SUSTAINED TEMPERATURE (EXPERIMENT IX)

Introduction

The purpose of this experiment was to determine the effect of sustained rearing temperature on the different meristic characters. Information on the effect of temperature was also necessary to determine whether temperature induced changes are comparable to changes induced by thyroxine, thiourea or dinitrophenol. This experiment was repeated with several genotypes to discover differences in their response.

Description of experiment

Temperatures of 20°, 22°, 24°, 26°, 28°, 30°, 32° and 34°C were used. Altogether, fifteen replications of the experiment were made. Eggs of each replicate could not be treated in all the above-noted temperatures. Also, in some of the replicates, the number of eggs put in each temperature was not the same. These departures from uniformity were imposed by the irregularity in the number of eggs obtained daily and loss of breeding activity of different sets of parents. Details of the genotypes used, number of eggs treated in different temperatures etc. are outlined in Table XLIX.

Eggs obtained on several successive days were placed in one particular temperature treatment.

Spawning always occurred at 24°C and the eggs were put into different temperatures directly without any conditioning in the temperatures concerned. As will appear from Table XLIX this technique of egg transfer did not affect the mortality of eggs appreciably.

Eggs were allowed to hatch naturally in the baskets in all temperatures except in some instances in 20°C bath where developed eggs did not hatch out even after a prolonged incubation in that temperature. Hatching in those lots was induced by transferring the eggs to a higher temperature, i.e. 24°C. The temperature shock induced hatching of the eggs within about an hour of transfer. After hatching such lots were reared in the higher temperature until preservation. Particulars of such transfers are recorded in Table XLIX. In 34°C, the highest temperature used, mortality after hatching was very high for most genotypes. In some cases, as shown in the table, the young were transferred to lower temperatures for further growth and fattening. For lots so transferred in the course of their growth and differentiation, only vertebrae were taken into account for the present analysis as the number of vertebrae was found to become fixed very early in development (Experiment VIII).

Young in all replicates were reared in the small baskets (10 x 10 x 15 cm) except samples of replicates number XIII to XV, which were reared in the larger basket (12 x 12 x 15.5 cm) after hatching.

Results

Survival of eggs to hatching and survival of young to the stage when these were preserved were recorded for every genotype (Table XLIX). Survival was greatest in temperatures from 24° to 30°C in almost all genotypes. In extreme high or low temperatures survival was lower; here also, the genotypes responded differently.

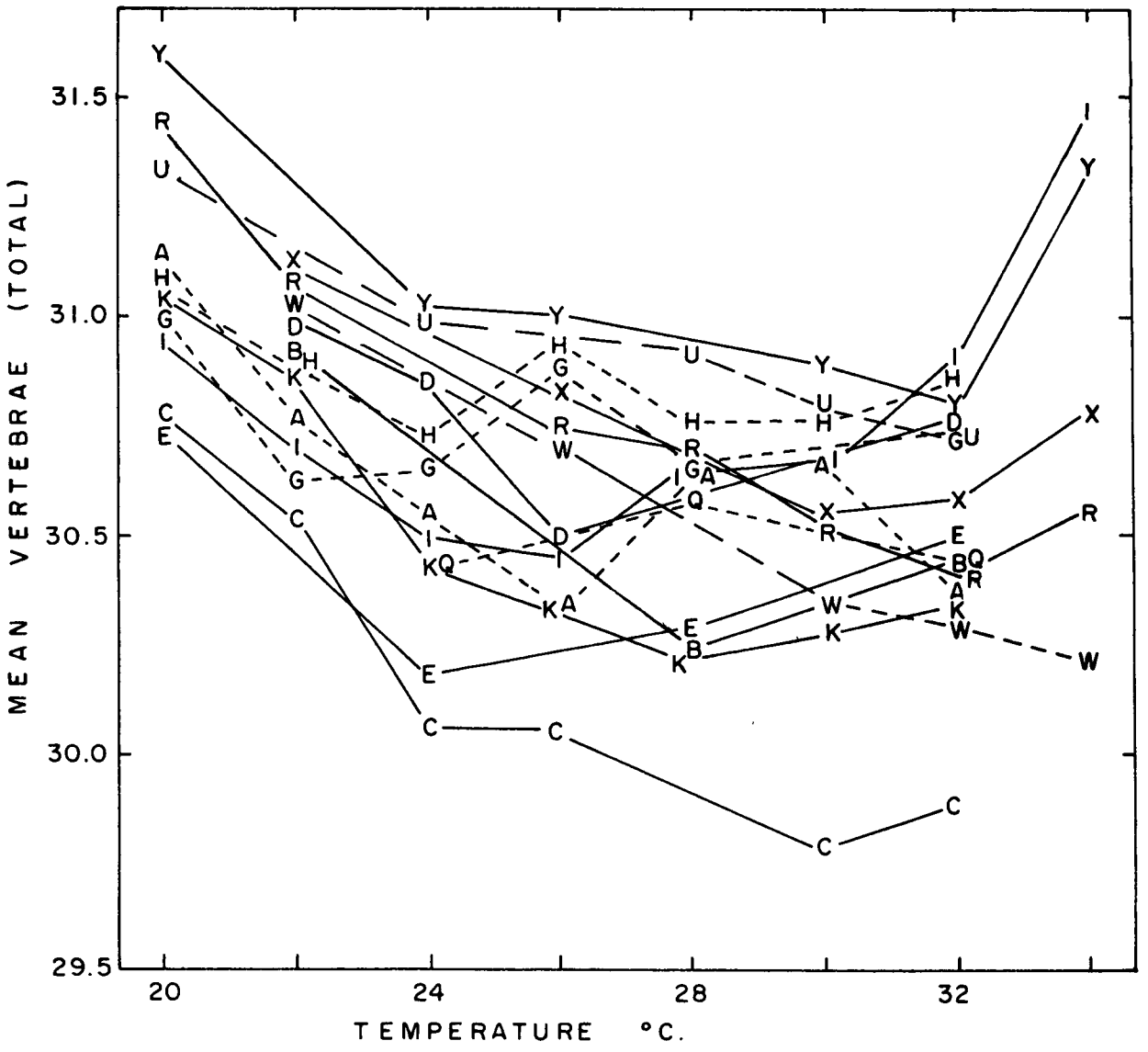

Figure 14. Effect of sustained temperature on mean total vertebrae. Letters indicate genotypes (Experiment IX)

In genotypes U, W, X and Y, percentage survival up to hatching in all temperatures ranged between 67 and 100 (except in 34°C for W). The percentage of fry surviving in these genotypes was also higher and ranged between 55 and 95 (except for genotypes W and Y in 34°C). In spite of this, significant variation was found in the meristic characters of these genotypes.

Total vertebrae: Main vertebral counts of each genotype are plotted against the appropriate temperatures (Figure 14) and are also shown in appendix I. In some cases where there was more than one replication of one genotype in the same temperature, samples from all replications are lumped together if the means of individual samples were not different.

Of the fifteen genotypes, mean counts of nine were high in the lowest and highest temperature with the lowest mean in an intermediate temperature. The mean count in the lowest temperature was significantly higher ($P<.01$) than the lowest mean count in the intermediate temperature in all the nine genotypes but this is not true of the difference between the mean counts in the higest temperature and intermediate temperature. In genotypes I and Y, difference between the means in the highest and intermediate temperature (lowest mean) was significant ($P<.01$) and in genotype W, the difference was indicative of being significant ($P<.05$). Difference between the means in highest and intermediate temperature in the remaining six genotypes were not statistically significant.

The intermediate temperature that produced the lowest mean vertebrae was different for different genotypes and varied

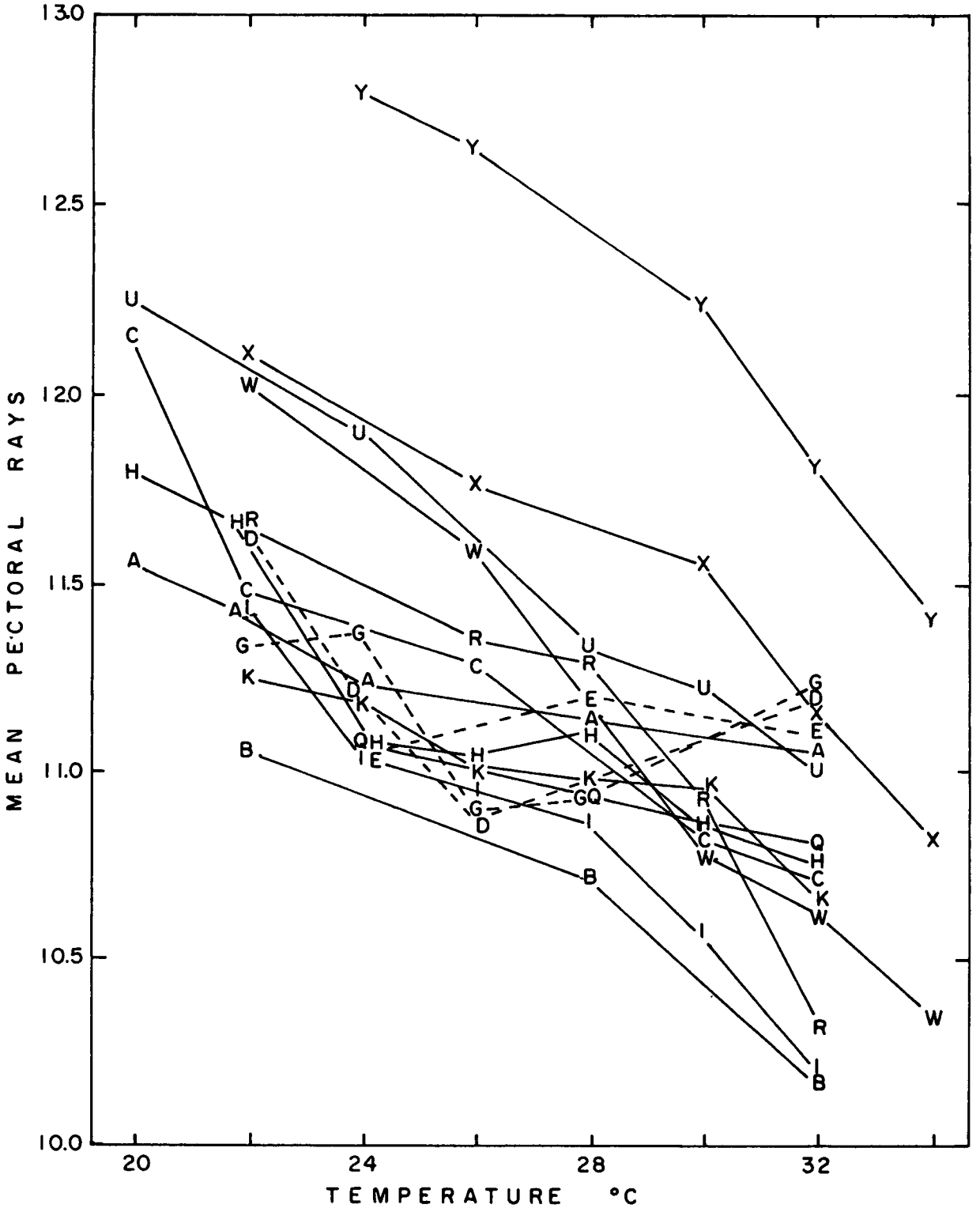

Figure 15. Effect of sustained temperature on mean pectoral fin rays. Letters indicate genotypes (Experiment IX)

between 26° and 32°C except in genotype E where the lowest count was obtained in 24°C; but for this genotype no data are available at 26°C.

Mean counts of three out of fifteen genotypes (G, H and Q) were not affected by temperature treatments.

Mean counts of two out of fifteen genotypes (U and W) showed a progressive decrease with increase in the temperature. The mean counts in the highest temperature (32°C for U and 34°C for W) were the lowest and both the means were significantly lower than those in the lowest temperature ($P<.01$).

In genotype A the mean counts showed a V-shaped relationship to temperature in all treatment except in the two highest temperatures where the mean tended to decrease again. Difference between the mean at the highest temperature and high mean counts in the previous two temperatures was not significant.

A large variation in the mean vertebral count between the genotypes in any single temperature was also found.

Vertebral count of all the replications were also analysed for abdominal and caudal vertebrae separately. Except for genotype H and U, the abdominal vertebrae did not alter significantly in the temperature treatments; variations were due almost entirely to alterations in the caudal vertebrae (data not included).

Pectoral rays: Mean pectoral ray counts of individuals reared in different sustained temperatures are shown in Figure 15 and appendix II. Mean counts of samples from 20° or 34°C where the eggs were hatched or reared in higher or lower temperature respectively are omitted from

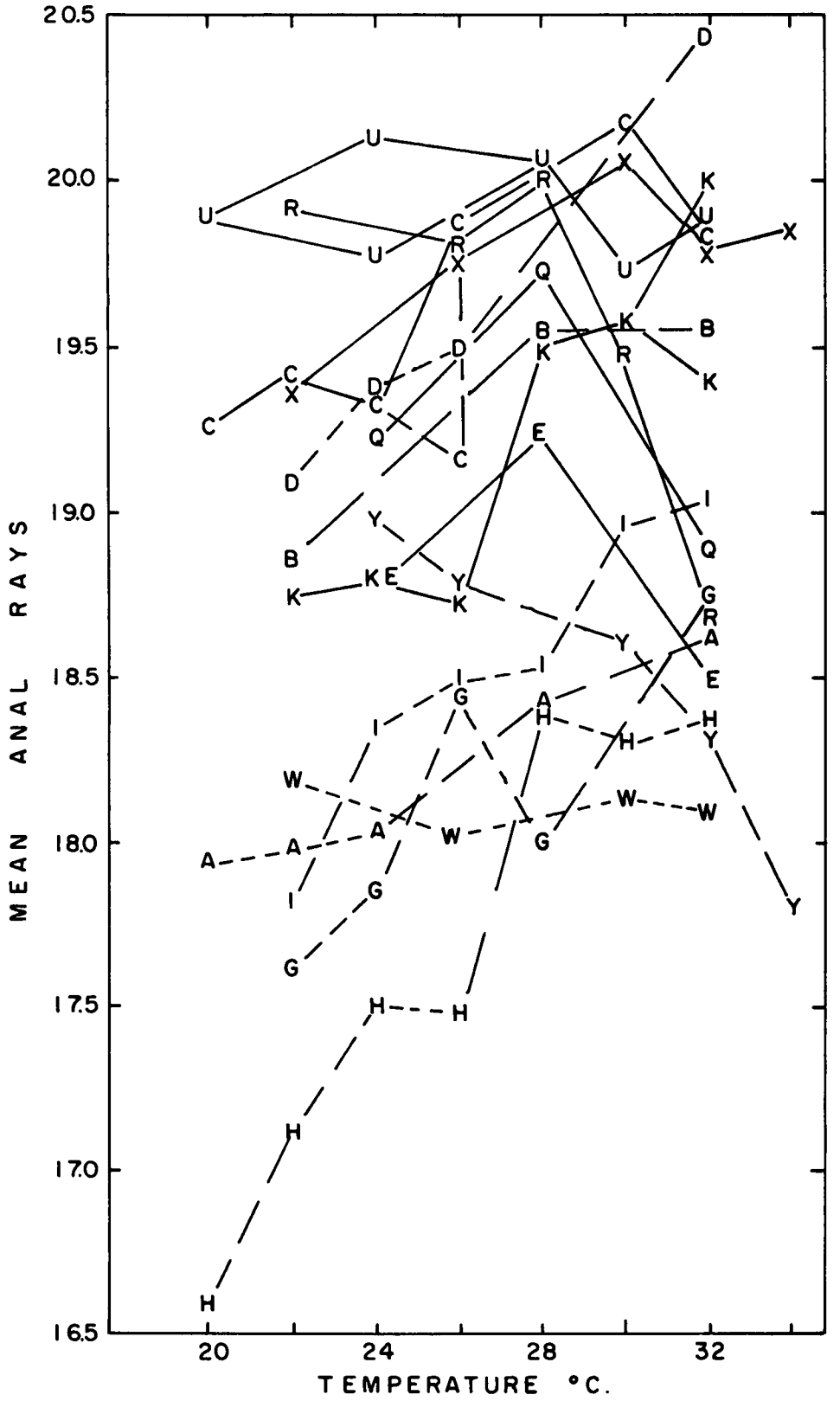

Figure 16. Effect of sustained temperature on mean anal fin rays. Letters indicate genotypes (Experiment IX)

considerations. Unlike the vertebral series, pectoral rays were progressively reduced by the increase in temperature. All genotypes except E and G responded to the treatment in a similar manner, i.e. a progressive decrease with increase in temperature. In E and G, no difference was caused by the treatments. It may be recalled that the vertebral counts of genotype G and H were also not altered by the temperature treatments.

Significant variation in the pectoral ray counts between genotypes were noticeable; most genotypes having a higher vertebral count also gave a higher pectoral ray count.

In all but the two replicates mentioned above, the difference between the mean counts in the lowest and highest temperature was significant ($P<.01$).

Anal rays: Mean anal ray counts in different temperatures are shown in Figure 16 and appendix III. Mean counts of samples reared in two different temperatures during pre- and post- hatching period of development and growth, (i.e. 20°C samples raised in higher temperature after hatching) have not been taken into account.

The genotypic influence with respect to temperature effect was most pronounced in this meristic character. Temperature - effect on anal rays can be summarized into the following categories:

a) Mean anal ray count increased with increase in temperature. The increase was progressive in genotypes D and I. Anal rays increased with increase in temperature up to a point and beyond that, the increase was not proportional to the temperature rise. This type of response

was obtained in genotypes B and H. In A, C, G and K, although there was an increase, the pattern was not consistent. Differences between the low mean count (in lower temperature) and highest mean count in the high temperature was statistically significant.

b) Mean anal counts decreased progressively with increase in temperature. This effect was pronounced in genotype Y. Mean count in 24°C (lowest temperature) was higher ($P<.01$) than the mean in 34°C (highest temperature).

c) Anal ray counts of genotypes E, Q and E showed an inverted V-shaped relationship to temperature. The mean count of genotype E and Q in the intermediate temperature was higher than the mean count in the highest temperature ($P<.01$) and tended to be higher than that in the lowest temperature ($P<.05$; $> .02$).

d) In genotypes U, W and X, anal counts were not appreciably altered by temperature differences.

In addition to the variation in response between the genotypes, variable results in anal count were obtained from samples of the same genotype reared in two separate lots at the same temperature. Of the two samples of genotype C in 26°, the sample with the higher number of fish in basket gave a higher mean anal ray count ($P<.01$). Mean counts of two samples of the same genotype in 24°C exhibited no difference, though the population size of one was double the other. Similarly, no difference was found in the means of three lots of genotype D in 24°C ($P<.05$). The mean counts of two samples of genotype K in 32° tended to be different ($P = .02$-$.05$), mean count of the sample from

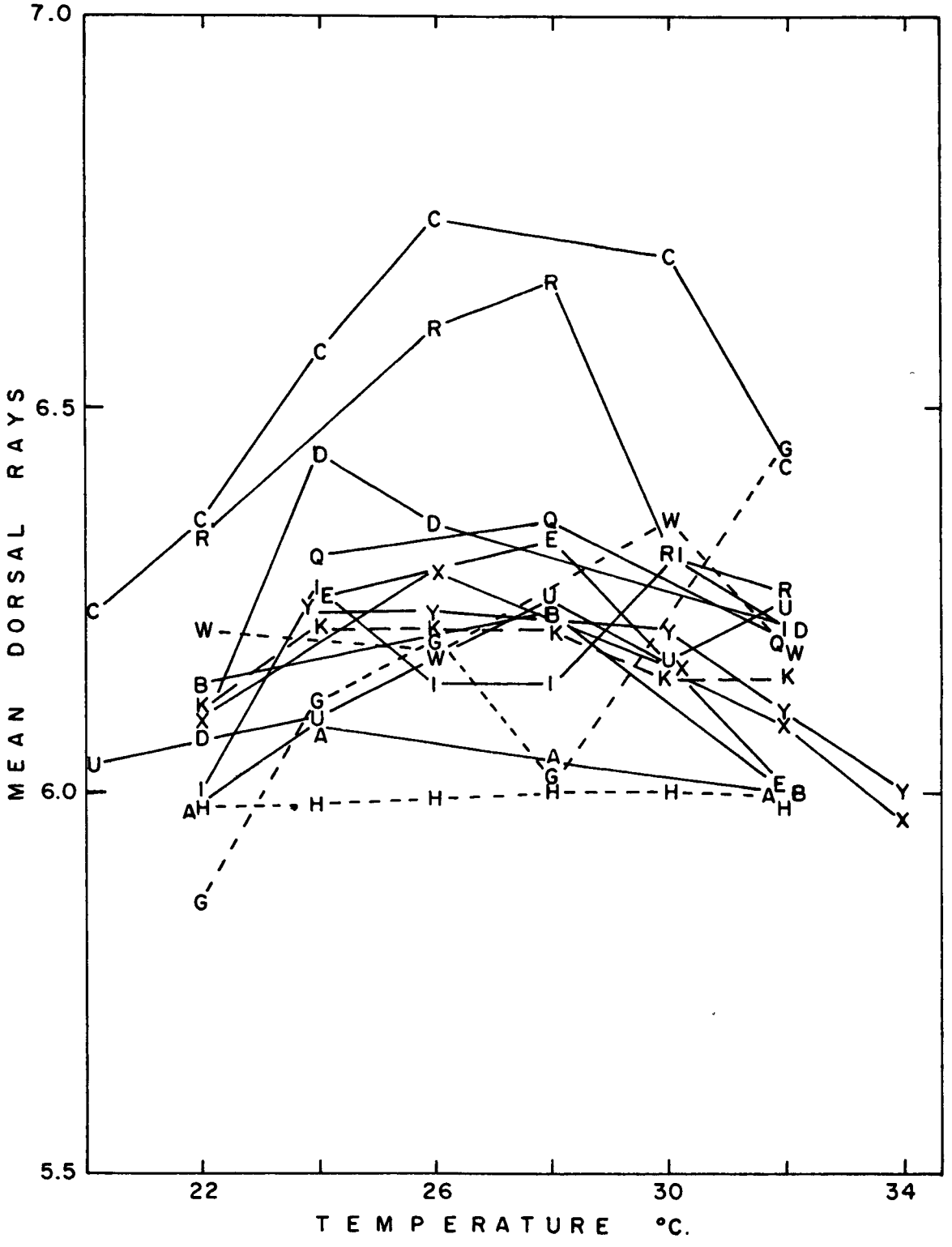

Figure 17. Effect of sustained temperature on mean dorsal fin rays. Letters indicate genotypes (Experiment IX)

denser population being lower in this case. This was identical to the result obtained in experiment VI but opposite to that found in genotype C in 26°C (see above). Mean counts of the two samples of genotype U in 24°C also showed a tendency to be different ($P = .02-.05$) and here the sample with denser population had a lower mean count.

Significant variation was also found between genotypes in respect of mean ray counts in any single temperature. Relationship between anal rays and other meristic series was not very clear. Some genotypes with high vertebral and/or pectoral rays also had high anal rays. In others, the relationship was inverse.

Dorsay rays: In ten out of fifteen replicates, the mean counts formed an inverted V when plotted against temperatures (Figure 17; Appendix IV). In others similar plotting of the counts revealed no pattern. In genotypes C and R, the count in the intermediate temperature was significantly higher than both in the lowest and highest temperature. Most of remaining replications showed the same pattern but differences in differemt temperatures were not statistically significant.

The variation between genotypes in their mean dorsal count was also not significant except for genotypes C and R in which the mean counts were higher than in the rest.

Insofar as the relationship of the dorsal rays to other meristic series is concerned, no definite trend was displayed by the genotypes.

Total caudal rays: As in the anal rays, a large genotypic variability was observed for total caudal rays (Appendix V and Figure 18).

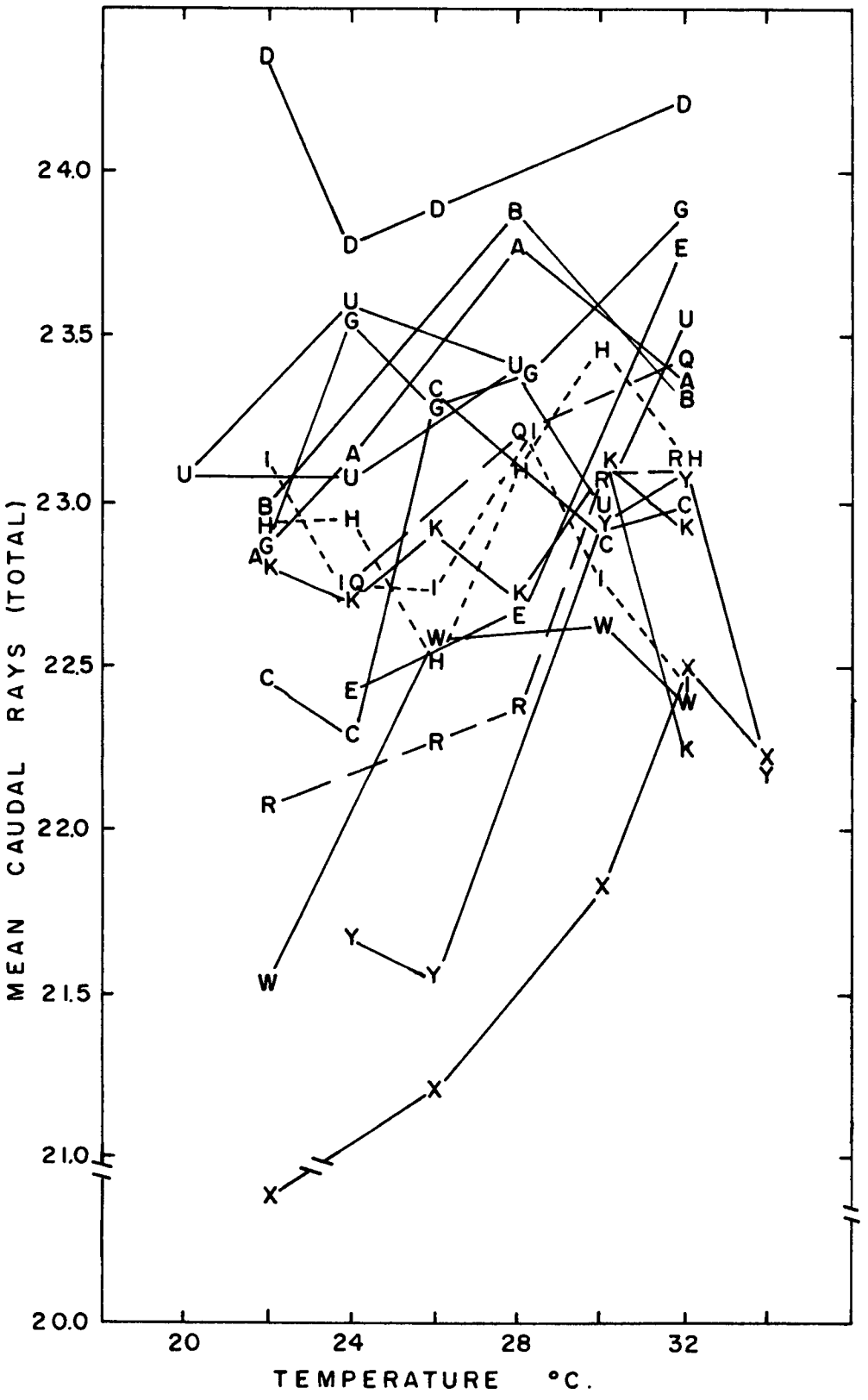

Figure 18. Effect of sustained temperature on mean total caudal fin rays. Letters indicate genotypes (Experiment IX)

Plotting of the mean total caudal rays against temperatures resulted in an inverted V-shaped curve in genotypes A, B, K, W, X and Y. Compared to the means in the intermediate temperature, mean counts in the lowest temperature were lower ($P<.01$) for these genotypes but the same was not true for the means in the highest temperature.

In genotypes E, Q and R, total caudal rays tended to increase with rise in temperature. Difference between the means in lowest and highest temperature was significant in E and R ($P<.01$) while in Q, the means showed a tendency to differ ($P = .02-.05$).

In genotype D, the mean total caudal rays in the highest and lowest temperature were higher than in the intermediate temperatures showing a V-shaped relationship to temperature. Differences between the mean counts, however, were not significant. In the remaining genotypes, i.e. C, G, H, I and U, no definite trend of temperature effect was observed.

In addition to the genotypic variations described above, variable results in total caudal ray counts were obtained by rearing eggs of the same genotype in separate lots at the same temperature. Mean counts of the two samples of genotype K in 32° tended to be different ($P<.05;>.02$), where mean total caudal ray of the sample with denser population tended to be higher. This trend was in contrast to the results obtained in experiment VI. Of the two samples of genotype U in 24°C, mean count of the sample with denser population tended to be higher ($P<.05;>.02$).

Conclusion

Mean vertebral counts in extreme low and high sustained temperature become higher than in the intermediate temperature. The intermediate temperature producing the lowest count varies with genotypes. The V-shaped relationship of vertebral counts to temperature can be obtained in most genotypes if extreme temperatures nearer the upper and lower lethal limits are used. Vertebral counts of some genotypes, however, are not temperature labile. Pectoral fin ray counts in medaka decreases consistently with increase in temperature. Dorsal ray count shows in general an inverted V-shaped relation to temperature, although mean counts in different temperatures are not strikingly different. Effects of sustained temperature on anal and total caudal ray counts do not reveal any clear pattern.

Table L. Egg numbers and mortality in experiment X: effect of increased light (intensity and duration.

Treatment	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs.)	No. survived to preservation	As % of fertd. eggs
(a) Parent: W						
9 ft.-c. for 16 hrs.	100	96	96	226	92	92
9 ft.-c. for 24 hrs.	100	91	91	301	67	67
170 ft.-c. for 16 hrs.	100	82	82	370	53	53
(b) Parent: Y						
9 ft.-c. for 16 hrs.	100	98	98	226	95	95
9 ft.-c. for 24 hrs.	100	100	100	278	97	97
170 ft.-c. for 16 hrs.	100	99	99	223	97	97
(c) Parent: a						
9 ft.-c. for 16 hrs.	50	42	84	387	39	78
9 ft.-c. for 24 hrs.	50	44	88	395	34	68
170 ft.-c. for 16 hrs.	50	49	98	208	44	88

Table LI. Frequency distribution of total vertebrae in experiment X: Effect of increased light (intensity and duration).

Treatment	Temp (°C)	Total vertebrae 30	31	32	Number	Mean	Remarks
(a) Parent: W							
9 ft.-c. for 16 hrs.	30°	33	17		50	30.34	
9 ft.-c. for 24 hrs.	30°	23	27		50	30.54	Not different from control (P>.05)
170 ft.-c. for 16 hrs.	30°	35	15		50	30.30	
(b) Parent: Y							
9 ft.-c. for 16 hrs.	30°	13	79	3	95	30.89	
9 ft.-c. for 24 hrs.	30°	13	36	1	50	30.76	
170 ft.-c. for 16 hrs.	30°	11	38	1	50	30.80	
(c) Parent: a							
9 ft.-c. for 16 hrs.	30°	27	12		39	30.31	
9 ft.-c. for 24 hrs.	30°	20	14		34	30.41	
170 ft.-c. for 16 hrs.	30°	32	11	1	44	30.29	

Table LII. Frequency distribution of pectoral rays in experiment X: Effect of increased light (intensity and duration)

Treatment	Temp (°C)	Pectoral rays 10	11	12	13	14	Number	Mean
(a) Parent: W								
9 ft.-c. for 16 hrs.	30°	22	77	1			100	10.79
9 ft.-c. for 24 hrs.	30°	13	82	5			100	10.92 [1]
170 ft.-c. for 16 hrs.	30°	19	78	3			100	10.84
(b) Parent: Y								
9 ft.-c. for 16 hrs.	30°		3	140	46	1	190	12.24
9 ft.-c. for 24 hrs.	30°		10	72	18		100	12.08 [2]
170 ft.-c. for 16 hrs.	30°	1	20	77	2		100	11.80 [3]
(c) Parent: a								
9 ft.-c. for 16 hrs.	30°	3	57	18			78	11.19
9 ft.-c. for 24 hrs.	30°	2	49	16			67	11.21
170 ft.-c. for 16 hrs.	30°	12	69	7			88	10.94 [4]

Note:
1. Not different from control ($P>.05$) i.e., 16 hrs. 9 ft.-c.
2. Lower than 16 hrs. 9 ft.-c. ($P<.01$).
   Higher than 16 hrs. 170 ft.-c. ($P<.01$).
3. Lower than 16 hrs. 9 ft.-c. ($P<.01$).
4. Lower than 16 hrs. 9 ft.-c. ($P<.01$).

Table LIII. Frequency distribution of anal rays in experiment X: Effect of increased light (intensity and duration).

Treatment	Temp (°C)	Anal rays 16	17	18	19	20	Number	Mean
(a) Parent: W								
9 ft.-c. for 16 hrs.	30°		7	29	14		50	18.14
9 ft.-c. for 24 hrs.	30°	1	12	27	9	1	50	17.94
170 ft.-c. for 16 hrs.	30°	2	11	26	10	1	50	17.94
(b) Parent: Y								
9 ft.-c. for 16 hrs.	30°			39	52	4	95	18.63
9 ft.-c. for 24 hrs.	30°		2	18	25	5	50	18.66
170 ft.-c. for 16 hrs.	30°			9	30	11	50	19.04[1]
(c) Parent: a								
9 ft.-c. for 16 hrs.	30°	4	14	15	6		39	17.59
9 ft.-c. for 24 hrs.	30°	3	18	13			34	17.29
170 ft.-c. for 16 hrs.	30°	1	15	21	7		44	17.77

Note: 1. Higher than 16 hrs. 9 ft.-c. ($P<.01$).

Table LIV. Frequency distribution of dorsal rays in experiment X: Effect of increased light (intensity and duration).

Treatment	Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean
(a) Parent: W							
9 ft.-c. for 16 hrs.	30°		33	16	1	50	6.36
9 ft.-c. for 24 hrs.	30°		43	6	1	50	6.16[1]
170 ft.-c. for 16 hrs.	30°		38	12		50	6.24
(b) Parent: Y							
9 ft.-c. for 16 hrs.	30°		76	18	1	95	6.21
9 ft.-c. for 24 hrs.	30°		36	13	1	50	6.30
170 ft.-c. for 16 hrs.	30°		29	21		50	6.42[2]
(c) Parent: a							
9 ft.-c. for 16 hrs.	30°		38	1		39	6.03
9 ft.-c. for 24 hrs.	30°	1	32	1		34	6.00
170 ft.-c. for 16 hrs.	30°		41	3		44	6.07

Note: 1. Tends to be lower than 16 hrs. 9 ft.-c. ($P<.05$).
2. Higher than 16 hrs. 9 ft.-c. ($P<.01$).

Table LV. Frequency distribution of total caudal rays in experiment X: Effect of increased light (intensity and duration).

Treatment	Temp (°C)	Total caudal rays 20	21	22	23	24	25	26	27	28	Number	Mean
(a) Parent: W												
9 ft.-c. for 16 hrs.	30°		9	10	23	6	1		1		50	22.64
9 ft.-c. for 24 hrs.	30°		4	13	24	8	1				50	22.78
170 ft.-c. for 16 hrs.	30°	2	5	6	31	5	1				50	22.72
(b) Parent: Y												
9 ft.-c. for 16 hrs.	30°		9	17	42	19	6				93	22.96
9 ft.-c. for 24 hrs.	30°		4	2	28	10	6				50	23.24
170 ft.-c. for 16 hrs.	30°		2	7	17	16	8				50	23.44 [1]
(c) Parent: a												
9 ft.-c, for 16 hrs.	30°			7	17	8	4	2		1	39	23.51
9 ft.-c. for 24 hrs.	30°		1	1	15	14	3				34	23.53
170 ft.-c. for 16 hrs.	30°			1	7	20	13	3			44	24.23 [2]

Note: 1. Higher than 16 hrs. 9 ft.-c. ($P<.01$).
2. Higher than 16 hrs. 9 ft.-c. ($P<.01$).

EFFECT OF INCREASED LIGHT (EXPERIMENT X)

Introduction

This experiment was designed to determine if altered light intensity or duration will change any one or all of the meristic series. The experiment was repeated with genotypes W, Y, and a. Treatments in 9 ft.-c. of light for 16 hours a day were used as the controls. In the second treatment, duration of light of the same intensity as the control was increased to 24 hours a day. In the third treatment, intensity of light was increased to 170 ft.-c. by replacing the 7.5 watt lamp with a 60 watt lamp. The increased intensity was maintained for 16 hours daily during the entire course of the experiment. Eggs in all the replications were reared in small baskets (10x10x15 cm.) up to hatching. Except for genotype a, the young were transferred upon hatching to the larger rearing baskets (12x12x15.5cm.) and reared therein until preservation. Young of the genotype a were reared in the small basket. Total number of fertilized eggs used, percentage of hatching and other relevant information are presented in Table L. All the lots were reared in 30°C temperature bath all throughout the experiment.

Results

Survival in all the treatments was satisfactory for all genotypes. Survival up to hatching ranged from 82 to 100 percent but the result was variable with respect to survival up to preservation. Three genotypes showed a different proportion of survival under identical conditions (Table L). Mean vertebral counts were not

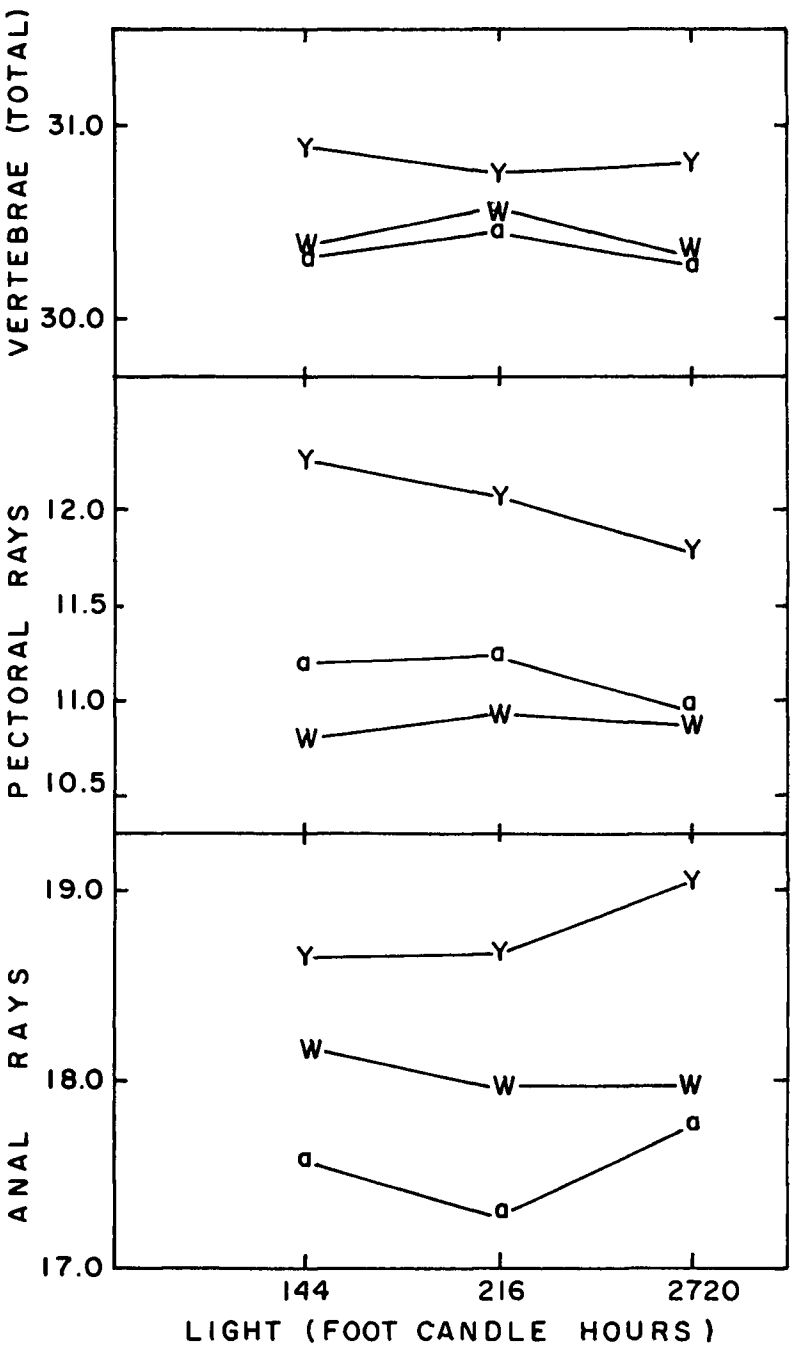

Figure 19. Effect of increased light on mean total vertebrae and pectoral and anal fin rays. Letters indicate genotypes (Experiment X)

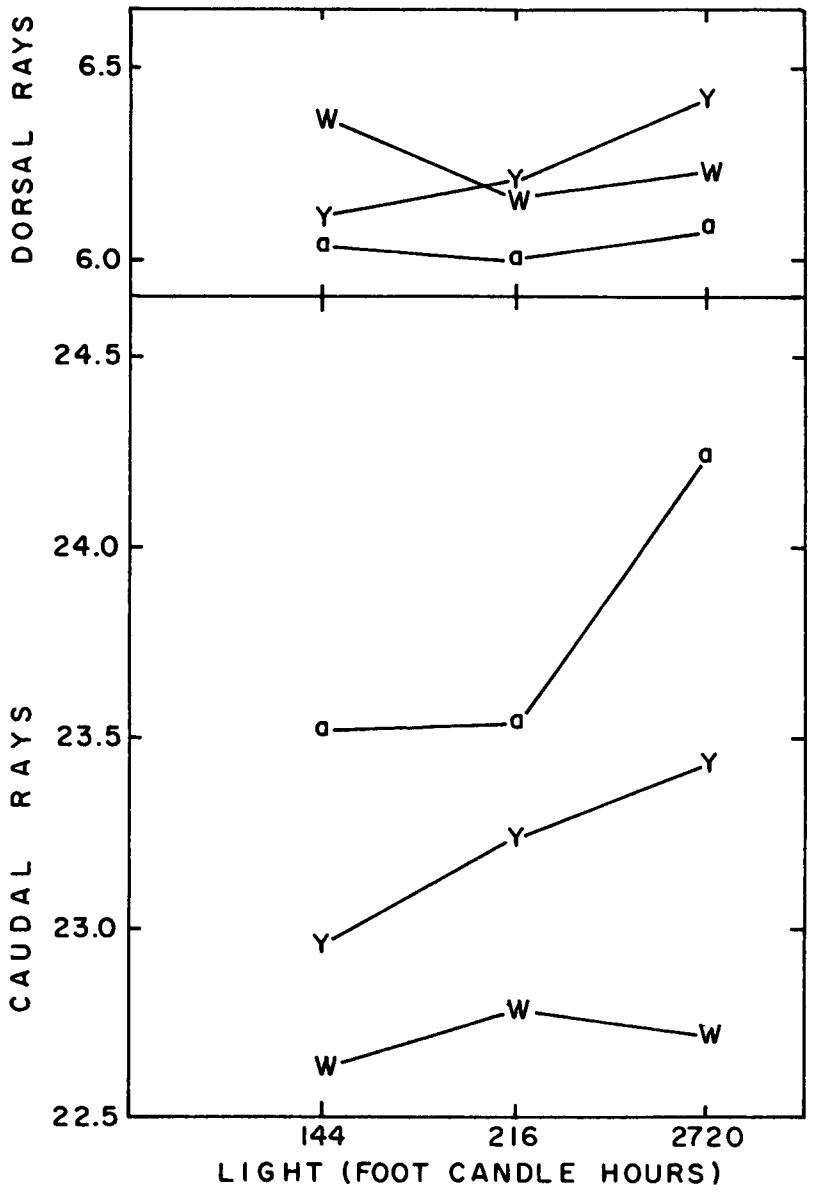

Figure 20. Effect of increased light on mean dorsal and total caudal fin rays. Letters indicate genotypes (Experiment X)

affected by either increased duration or increased intensity of light (Table LI and Figure 19). Effects of the treatments on pectoral ray counts were variable on the genotypes used (Table LII and Figure 19). The mean ray count was not altered by increased light duration or intensity in genotype W, while in genotype Y, under increased intensity and duration, mean counts were reduced ($P < .02$). The effect of increased intensity was most pronounced in this case and greatest reduction of pectoral ray was obtained. Genotype a was affected only by increased light intensity where the mean count decreased ($P < .01$). Mean anal ray counts also varied with genotypes (Table LIII and Figure 19). No effect was apparent in genotype W. The control (in 9 ft.-c. for 16 hours) mean of genotype a was not different from the means of either of the treatment lots. In case of genotype Y, an increase ($P < .01$) in the mean count occurred under increased light intensity. Increased light duration tended to decrease ($P < .05$) the dorsal rays of genotype W (Table LIV and Figure 20). In others no difference was produced. The effect of increased light intensity was apparent only in genotype Y where the dorsal ray count increased ($P < .01$). Effects of treatments on the total caudal rays were variable (Table LIV and Figure 20). Mean counts of genotype W were not affected by increased duration or increased intensity. Increased intensity increased the caudal ray counts in genotype Y and a ($P < .01$) but increased light duration had no effect on either of these genotypes.

Conclusion

Increased light conditions do not affect vertebral counts

in medaka raised in 30°C temperature bath, but effects on pectoral, anal and other fin rays are variable.

Table LVI. Egg numbers and mortality in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs.)	No. survived to preservation	As % of fertd. eggs.
(1) Parent: G						
.0025% thiourea	75	33	44	276	21	28
.005% "	75	43	57	244	34	45
.01% "	42	31	74	314	27	64
.02% "	18	16	89	274	16	89
.04% "	27	17	63	288	12	44
.05% "	26	18	69	290	17	65
(2) Parent: M						
Fresh water control	44	32	75	283	28	64
0.1 PPM thyroxine	100	68	68	289	46	46
0.2 " "	99	37	37	395	16	16
0.4 " "	103	48	46	323	29	28
(3) Parent: O						
Fresh water control	26	17	65	317	9	35
Tapwater control	97	66	68	382	46	47
0.2 PPM thyroxine	71	39	55	333	8	11
0.4 " "	70	29	41	288	20	29
0.8 " "	56	17	30	360	15	27
1.6 " "	60	12	20	352	8	13
.01% thiourea	73	31	42	346	19	26
.02% "	61	26	43	278	21	34
.04% "	52	29	56	307	24	46

Table LVI. Egg numbers and mortality in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs.)	No. survived to preservation	As % of fertd. eggs
(4) Parent: P						
Fresh water control	40	26	65	288	23	58
0.2 PPM thyroxine	94	33	35	348	23	24
0.4 " "	51	29	57	323	20	39
0.8 " "	50	23	46	320	15	30
.01% thiourea	46	33	72	312	30	65
.02% "	50	22	44	269	17	34
.04% "	50	27	54	302	15	30
(5) Parent: Q						
Fresh water control	50	33	66	269	26	52
0.8 PPM thyroxine	61	33	54	260	21	34
.02% thiourea	54	31	57	251	22	41
(6) Parent: S						
Fresh water control	52	51	98	278	33	63
0.2 PPM thyroxine	119	92	77	272	48	40
0.4 PPM "	71	68	96	276	62	87
0.8 " "	66	57	86	312	51	77
1.6 " "	50	26	52	406	25	50
.01% thiourea	74	69	93	283	64	86
.02% "	64	60	94	264	57	89
.04% "	66	57	96	279	55	83

Table LVI continued. Egg number and mortality in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs.)	No. survived to preservation	As % of fertd. eggs
(7) Parent: V						
Fresh water control	75	71	95	317	70	93
0.4 PPM thyroxine	75	55	73	489	52	69
0.8 " "	75	71	95	409	66	88
3.2 " "	75	54	72	497	50	67 [1]
.05% thiourea	75	67	89	371	63	84
(8) Parent: Y						
Fresh water control	100	80	80	223	70	70
0.8 PPM thyroxine	100	91	91	291	88	88
.02% thiourea	100	89	89	252	42	42

Note: 1. 43 hatched in water after 489 hours in the solution.
11 hatched in water by temperature shock after 379 hours in the solution.

Table LVI continued. Egg number and mortality in experiment XI: Effect of thyroxine and thiourea.

(b) Eggs fertilized and reared in the solutions up to h hatching.

Treatment	No. of fertd. eggs	No. hatched.	As % of fertd. eggs	Time to 50% hatching (hrs.)	No. survived to preservation	As % of fertd. eggs
(1) Parent: Y						
Fresh water control	100	80	80	223	70	70
0.8 PPM thyroxine	100	97	97	350	90	90
0.2% thiourea	100	100	100	249	95	95
(2) Parent: a						
Fresh water control	50	43	86	328	32	64
0.8 PPM thyroxine	50	33	66	343	33	66
.04% thiourea	50	43	186	362	32	64
(3) Parent: b						
Fresh water control	50	49	98	270	46	92
0.8 PPM thyroxine	50	27	54	439	27	54
.04% thiourea	50	19	38	328	15	30

(c) Chorion pricked eggs reared in the solutions up to hatching.

Parent: V						
Fresh water control	75	29	39	321	29	39
3.2 PPM thyroxine	75	(1). 7		397	6	/1
		(2). 22			16	/2
		29	39		22	29
.02% thiourea	75	29	39	356	21	28

Note: /1. Hatched in solution.
/2. Hatched in water after 475 hours in solution.

Table LVI continued. Egg numbers and mortality in experiment XI: Effect of thyroxine and thiourea.
(d) Larvae reared in the solutions after hatching.

A. Number of eggs used.

No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching	Remarks
Parent: V				
150	123	82	328	Reared & hatched in tapwater (in bottle)

B. Number of larvae reared.

Treatment	No. of larvae in solution	Mean rearing time in solution (hrs.)	No. survived to preservation	As % of larvae
Parent: V				
Fresh water	41	663	37	90
3.2 PPM thyroxine	41	227	29	71
.05% thiourea	41	650	37	90

EFFECT OF THYROXINE AND THIOUREA (EXPERIMENT XI)

Introduction

The object of this experiment was to determine the effect of thyroxine and thiourea solutions upon different meristic series in medaka. Marckmann (1954 and 1958) implied that meristic variation is related to the rate of metabolism. Canagaratnam (1959) speculated that some of the variations are under the control of thyroid activity. Dales and Hoar (1954) suggested that the thyroid hormone of cold blooded vertebrates is more directly concerned with growth and differentiation than it is with general metabolism. But according to Hoar (1951) the thyroid is evidently involved in the regulation of the metabolism of fish whether or not it is involved in the control of oxidative metabolism. On the basis of these suggestions, it was expected that rearing of eggs and young in thyroxine and thiourea solutions would alter some or all of the meristic series, if these were under the control of the activity of thyroid or metabolism.

Description of experiments

This experiment was performed in four different manners with eggs from several genotypes as detailed below. Thyroxine and thiourea used in the experiments were the products manufactured by the British Drug Houses and the Eastman Organic Chemicals respectively. Solutions of thyroxine and thiourea were made in tapwater in all cases. The following concentrations of thyroxine and thiourea solutions were used although all the concentrations were not used for all the replications (Table LVI).

Thyroxine: 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 parts per million

Thiourea: .0025%, .005%, .01%, .02%, .04% and .05%

a) Rearing of fertilized eggs in the solutions up to hatching.

This was replicated with eggs of genotypes G, M, O, P, Q, S, V and Y. In all cases, eggs were reared in 300 ml of the appropriate solution or tapwater (controls) in a 710 ml bottle. The bottles were floated in the desired temperature bath and were provided with air jets. The control and the treatment lots of a single genotype were kept in the same waterbath. Solutions and water in the bottles were replaced every sixth day with fresh solutions or water until the eggs hatched out. Particulars of the concentrations of solutions used are given in Table LVI.

Temperature baths used for the different replications were not the same. Replications involving genotypes G, M, O, P, Q and S were made in 24°C waterbath while those of genotypes V and Y were reared in 26°C bath.

Except for the egg lot of genotype V in 3.2 thyroxine solutions, eggs were allowed to hatch naturally in the bottles. In case of V in 3.2 parts per million thyroxine, the eggs did not show any sign of hatching even after 490 hours in the solution (over 20 days in 26°C); 53 out of the total of 75 eggs were, therefore, washed thoroughly in water and hatched naturally in water in the bottle in about 8 hours. Remaining 21 eggs of this treatment were also transferred to water earlier (at the end of 378 hours) but the hatching was induced by temperature shock in 30°C.

Upon hatching, the larvae from the treatments and the control of any one genotype were reared until preservation in small cloth baskets in the same tank. Larvae of genotype Y were reared up to preservation in three large cloth baskets in the same waterbath. The amount of time the larvae spent in the bottles after hatching varied from 0 - 8 hours, depending on the time of the day the eggs hatched. Eight hours exposure to the solution occurred only in such cases where the egg had hatched out shortly after the light went off at night. During the 8-hour dark period, hoods were not lifted from the tank. But this comparatively long period of exposure was rare. In most cases, eggs hatched out during the period of daylight (16 hours). In the sample from eggs of genotype V in 3.2 ppm thyroxine solutions, larvae hatched naturally in water were reared separately from the ones hatched by temperature shock. The lot of fish obtained by temperature induced hatching was not used for analysis of any of the meristic series considered.

b) Eggs fertilized and reared in the solutions up to hatching.

Although the chorion of medaka eggs is known to be freely permeable to crystalloids and small molecules (Yamamoto 1936) it was considered useful to fertilize the eggs in the solutions and find out if any variation in the result could be obtained. Three replications of this experiment were made with eggs of genotypes Y, a and b. In the three replications, parents were placed in a bottle containing the appropriate solution where the eggs were laid and fertilized. Eggs were then removed from the female and transferred to the rearing bottle containing the proper solution. The entire procedure of transferring

the eggs was done in the appropriate solution. In all cases, eggs were allowed to hatch naturally in the solutions. On hatching, the young were placed in baskets and reared in water for further growth and fattening. Small baskets were used for rearing the young of genotypes a and b, while the offspring of genotype Y were reared in large baskets. Control and treatment lots of the same genotype were reared in the same tank. Samples of genotype Y and b were raised in 26°C. Egg lots of genotype a were reared in 30°C. Solutions and water in the control lots were replaced with fresh solution and water every sixth day until hatching. Particulars of the number of eggs and mortality are presented in Table LVI(b).

c) Chorion pricked hatching eggs reared in the solutions up to hatching.

Eggs of genotype V were used for this experiment. Chorion of all the eggs used for control and treatments were pricked with a sharp dissecting needle soon after fertilization under a binocular microscope. Immediately after pricking the chorion, the eggs were transferred into 710 ml bottles containing 300 ml of appropriate solution. Eggs were allowed to hatch normally inside the solution, but in 3.2 ppm thyroxine treatment, only 7 eggs hatched naturally. The remaining eggs were allowed to remain in the solution for 475 hours (over a mean period of 19 days) and then washed thoroughly in water and hatched in water in a bottle. Eggs and young of this experiment were reared in 26°C. Upon hatching, the larvae were transferred to small baskets and reared therein until young were large enough to be preserved.

d) Larvae reared in the solutions after hatching.

150 eggs of genotype V were reared and hatched in 710 ml bottles containing 300 ml of freshwater. A total of 123 eggs hatched (Table LVId.) and the larvae were equally distributed into three 710 ml bottles containing 275 ml of freshwater, 275 ml of 3.2 ppm thyroxine and 275 ml of .05% thiourea. The liquid in each bottle was brought to 300 ml by adding 25 ml of water containing Paramecium culture in order to feed the larvae. Water containing Paramecium was added to each bottle once daily for seven days. Feeding with brine shrimp nauplii was also started after two days of hatching of larvae in each case. Brine shrimp was given twice daily. The solution containing Paramecium and left over brine shrimp was replaced with fresh solutions every night approximately two hours before the lights went off. Thus the young were exposed to pure solutions of thyroxine and thiourea for at least 10 hours daily for the entire period they were reared in the solutions.

Young reared in thyroxine became emaciated and unhealthy. Mortality of young in this solution was higher. Consequently, the young fish of this bottle were transferred to water in small cloth baskets after they were exposed to thyroxine for a mean period of 227 hours. The larvae in water and in thiourea lots did not show any sign of distress or ill health and they were reared in the bottles for 663 and 650 hours respectively. Thereafter, these two samples were transferred to baskets in tank and were reared there until time of preservation.

Results

As shown in Table LVI the initial egg numbers reared in different treatments and the control in a single replication were not uniform in several replications of experiment XIa. In order to find out if initial egg density has in any way influenced the number of vertebrae and pectoral rays, the data for all the replications (1-VI) with variable number of eggs were tested for an association. Chi-square test for vertebral count and pectoral ray count showed no relationship between the initial egg density and either of these two characters.

In replications numbers VI, VII, and VIII in experiment XIa, survival ranged from 52 to 92 percent. Except for two instances (genotype S in 0.2 ppm thyroxine and genotype Y in .02% thiourea) survival up to preservation in these replications ranged from 50 to 93%. In other replications of this experiment, rate of survival was not as satisfactory. In all the replications except no.1 (where a control lot is lacking) the control lots showed a tendency to survive better up to hatching than the treatment lots. This pattern was lost when survival rates up to preservation were compared.

In the second set of replications, where the eggs were fertilized in the solution and reared therein until hatching, percent survival was greater than 50% in different treatment in all the three replicates except for genotype b in .04% thiourea (Table LVIb.).

Compared to the egg lot of genotype V in experiment XIa, survival of chorion pricked eggs of genotype V in experiment XIc was appreciably lower in the control as well as in the treated lots. There was no difference in survival between the control and treated lots.

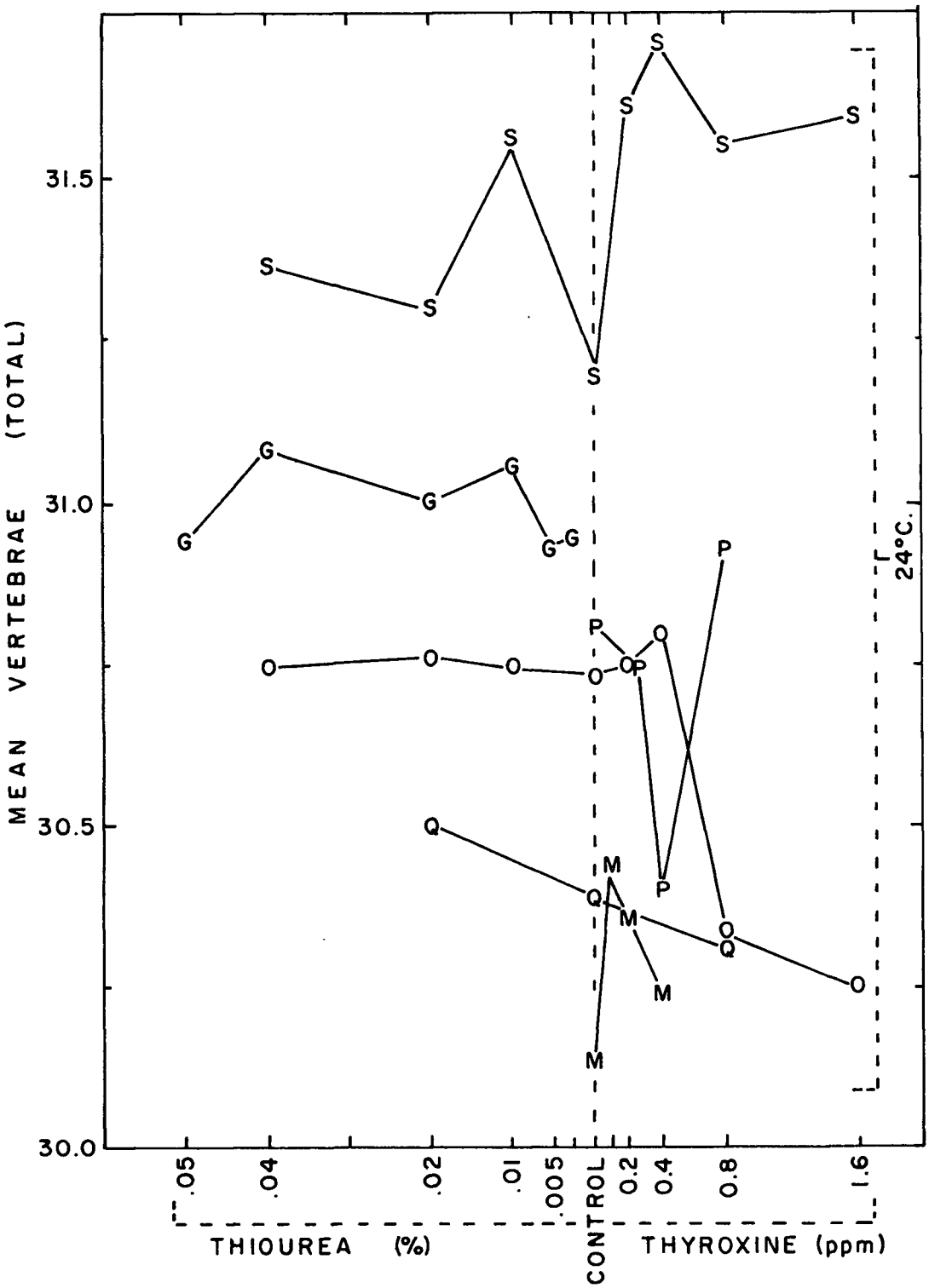

Figure 21a. Effect of thyroxine and thiourea to hatching on mean total vertebrae of genotypes reared in 24°C. Letters indicate genotypes (Experiment XI)

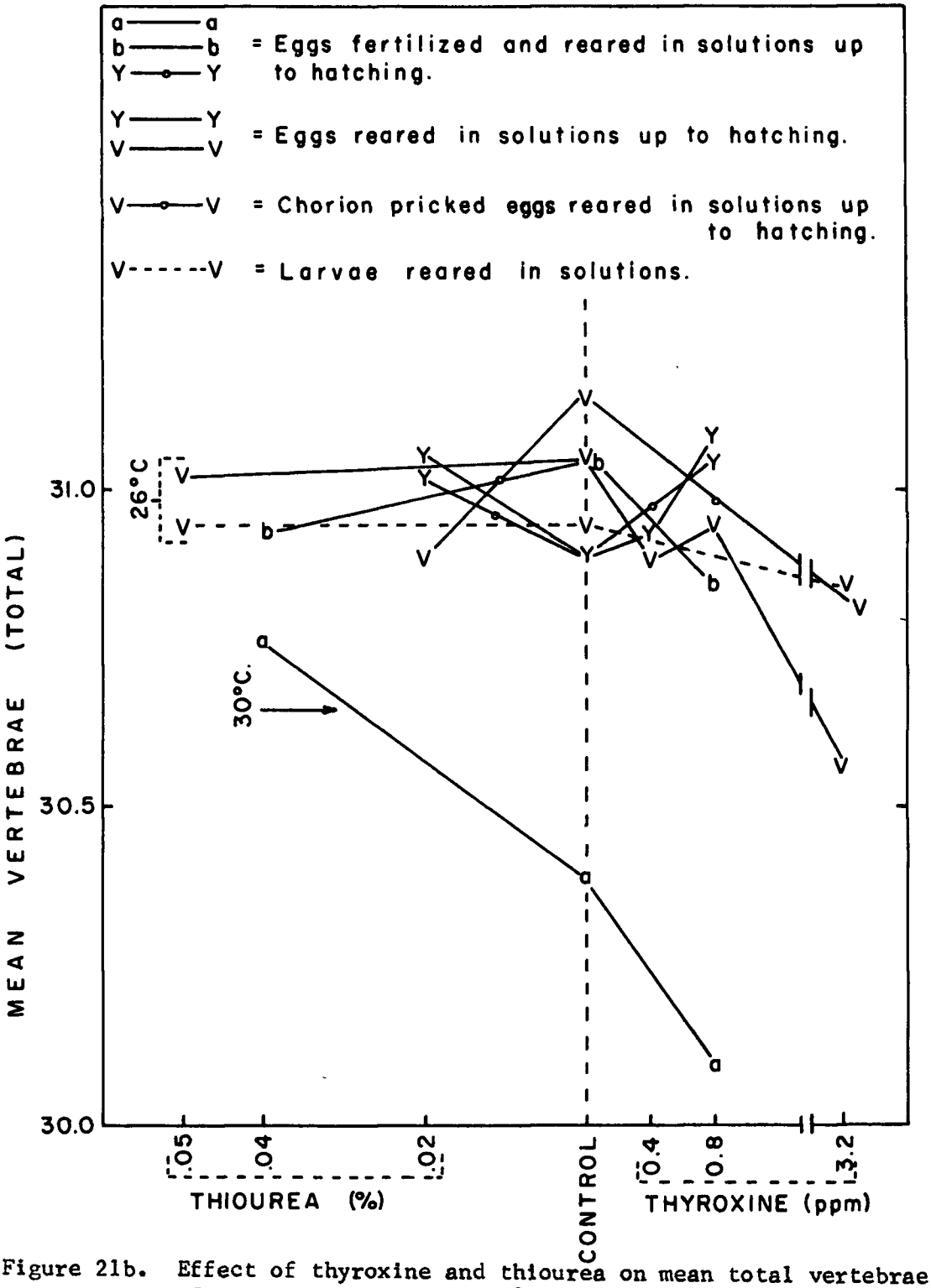

Figure 21b. Effect of thyroxine and thiourea on mean total vertebrae of genotypes reared in 26° and 30°C. Letters indicate genotypes (Experiment XI)

Lower survival in these lots was apparently caused by the shock involved in pricking the chorion of eggs. Compared to the control, survival up to preservation was lower in both thyroxine and thiourea treated lots.

Rearing of the larvae and young in .05% thiourea solution did not affect the survival rate. But in 3.2 ppm thyroxine solution, survival rate was affected and only 71% survived as against 90% in thiourea and in water (control).

Total vertebrae

a) Fertilized eggs reared in the solutions up to hatching.

Response of the genotypes to thyroxine treatment was variable (Appendix VI and Figure 21a and b). The effect was apparent in lots treated in high concentrations of the solution. Mean vertebral counts of the samples of genotype O in 1.6 and 0.8 ppm solution were lower ($P<.01$) than the control. Mean count of the sample of genotype P in 0.8 ppm solution was not altered, but the mean of the lot of this genotype in 0.4 ppm (next lower concentration) was lower ($P<.01$) than the control. In concentrations of 0.1 and 0.2 ppm solutions, vertebral counts of both O and P remained unaffected. Mean count of the sample of genotype V in 3.2 ppm solution was significantly lower ($P<.01$) than the control, even though the eggs were washed and hatched in water after being in the solution for a period of 489 hours. Mean count of the sample of V in 0.8 ppm solution was not altered but the same of the sample in 0.4 ppm solution showed a strong tendency of being lower ($P = .02-.05$) than the control.

Alteration in the vertebral count in thyroxine treated samples was not always in the same direction. In genotype S, mean vertebral counts of samples reared in all the different concentrations of thyroxine solution were higher ($P<.01$) than the mean of the control lot. Although mean count in each concentration was higher than the control, there was no difference between the mean counts of the samples in different concentrations of the solution. In two other genotypes, vertebral counts of thyroxine treated samples tended to increase. In genotype Y, the mean of the sample in 0.8 ppm solution tended to be higher ($P = .02-.05$) than the control. Mean count of the lot of genotype M in 0.1 ppm solution also tended to increase ($P=.05$).

Contrary to thyroxine, vertebral counts in thiourea treated lots were not affected except in genotype S in 01% solution, where the mean was higher ($P<.01$) than the control. Mean of the sample of genotype Y in 02% solution showed a tendency to be higher ($P=.05$) than the control. In others, no variation in the means between control and thiourea treated lot and between lots treated in different concentrations of thiourea was observed.

b) Eggs fertilized and reared in the solutions up to hatching.

Results are presented in appendix VI and figure 21b. Although there was a decrease in the mean vertebrae in the thyroxine (0.8 ppm) treated samples of genotypes a and b, the decrease was not significant ($P>.05$). Mean count of the lot of genotype Y in 0.8 ppm solution, on the other hand, tended to be higher ($P = .02-.05$) than the control. This was similar to the result obtained for this genotype in experiment XIa. where eggs were reared in thyroxine after fertilization.

Mean vertebral counts of genotypes Y and b in .04% thiourea solution in 26°C temperature-bath were not affected ($P>.05$) (Figure 21b). But the mean of the sample of genotype a in .04% and reared in 30°C bath was significantly higher ($P<.01$) than the control (Figure 21b).

c) Chorion pricked fertilized eggs reared in the solutions to hatching.

Mean vertebral count of the sample reared in 3.2 ppm thyroxine solution showed a strong tendency of being lower ($P=.02$) than the control (Figure 21b and Appendix VIc). Comparison of this result with that obtained by simply rearing the fertilized eggs in 3.2 ppm solution indicated that pricking the chorion of egg did not alter the effect of thyroxine solution. Mean count of the present sample, though slightly higher, was not significantly different from the mean of sample from eggs with intact chorion. Mean count of the control sample from chorion pricked eggs was similarly slightly higher (but not significant) than the mean of the control lot from intact eggs.

In contrast to the other results in thiourea solutions already described, the mean vertebral count of the sample in this case in .02% solution was lower ($P=.02$) than the control mean (Figure 21b and Appendix VIc). Difference between the means of this sample from chorion pricked egg and the sample from eggs reared in thiourea with chorion intact was not significant ($P>.05$)

d) Larvae reared in the solutions after hatching

Mean vertebral counts of the samples reared in thyroxine and thiourea solutions after hatching did not differ from the mean of the control lot (Appendix VId and Figure 21b). This result was expected

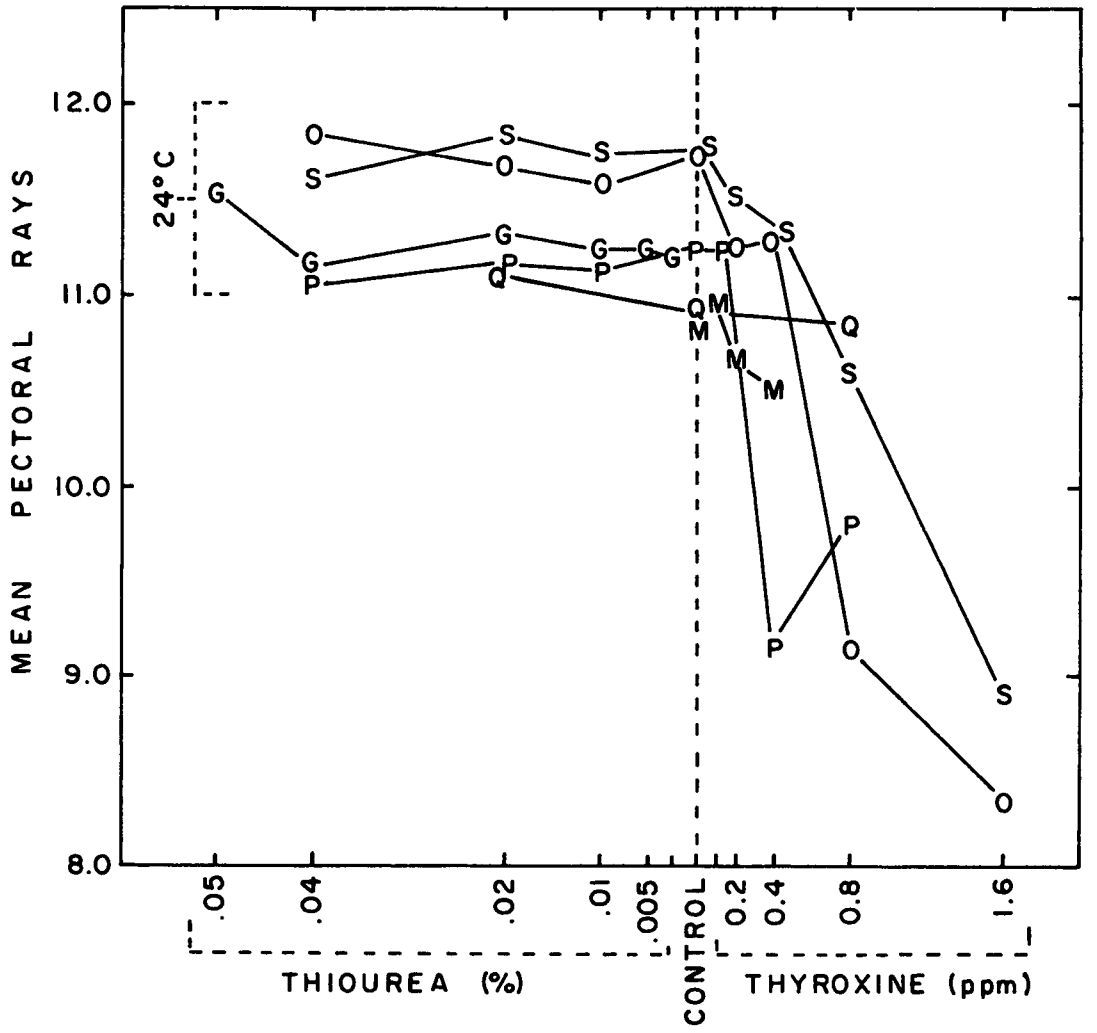

Figure 22a. Effect of thyroxine and thiourea to hatching on mean pectoral fin rays of genotypes reared in 24°C. Letters indicate genotypes (Experiment XI)

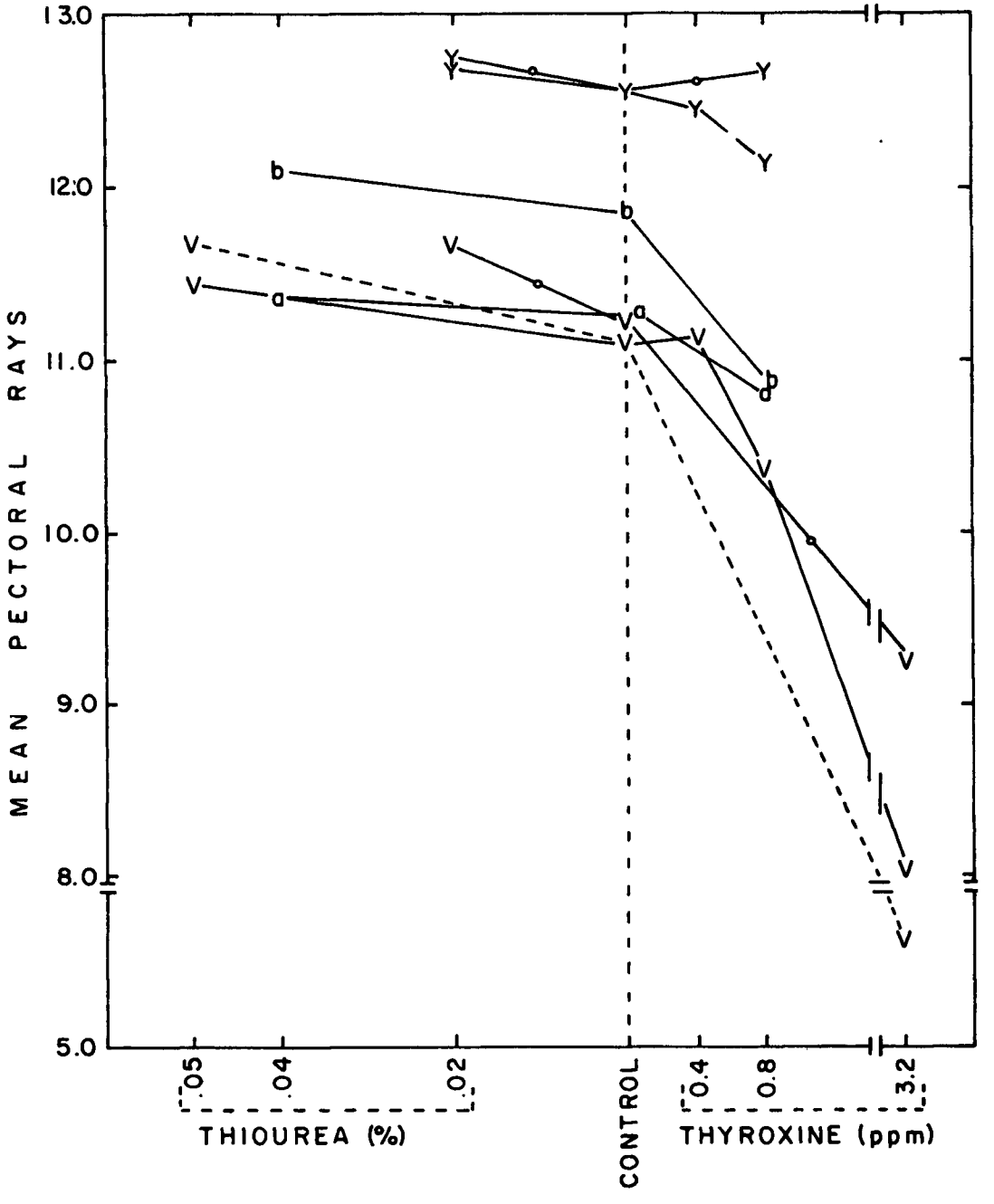

Figure 22b. Effect of thyroxine and thiourea on mean pectoral fin rays of genotypes reared in 26° and 30°C (only a). Legends are same as in Figure 21b. (Experiment XI)

in the perspective of the findings in experiment VIII.

Pectoral rays.

a) Fertilized eggs reared in the solutions to hatching.

Results are presented in appendix VIIa and figure 22a and b. In 1.6 and 3.2 ppm thyroxine solutions, decrease in the mean pectoral rays was very pronounced in all genotypes. In lower concentrations, the effect was variable and the reduction in the mean counts was not as pronounced.

Fertilized eggs of only one genotype - (i.e. genotype V) were reared in 3.2 ppm thyroxine solution. Although the eggs were removed from the solution after 489 hours of rearing and then washed and hatched in water, the pectoral ray count of the sample was reduced drastically. The mean pectoral ray of this sample was lower than the control by 3.04 rays.

The next lower concentration of the solution was 1.6 ppm and eggs of genotype O and S were reared therein. In both cases, the effect on the pectoral rays was very pronounced. Mean pectoral counts in both were reduced by about 3 rays.

A solution of the strength of 0.8 ppm was used for genotypes O, P, Q, S, V and Y. Effects of this concentration upon the pectoral rays were variable. The decrease in the mean pectoral ray count in all genotypes reared in this solution was significant ($P<.01$) except, in the case of genotype Q where pectoral rays remained unaffected ($P>.05$).

Eggs of genotypes M, O, P, S, V and Y were also reared in 0.4 ppm thyroxine solution. Effect of the concentration was variable.

In three genotypes - i.e. O, P and S, pectoral rays were decreased and in P the decrease was even greater than that obtained in the lot reared in 0.8 ppm solution. In the remaining three genotypes, pectoral counts were not affected in this concentration except in M where a tendency of decrease was noticeable (P = .02-05).

Thyroxine solutions in concentrations lower than 0.4 ppm had no effect on the fixation of pectoral ray in genotypes M, P and S. In genotype O, a significant decrease in the mean count resulted in the sample reared in 0.2 ppm solution.

Treatment in thiourea solutions of different concentrations as shown in the table produced no effect on the mean pectoral ray count of most genotypes. Of the eight genotypes used, mean pectoral ray count of genotype V in .05% thiourea solution was significantly higher than the control ($P<.01$). Mean counts of the sample of genotype Y in .02% thiourea solution tended to be higher than the control (P = .02-.05). In genotype G, the mean total pectoral ray in .05% solution tended to be higher (P = .02-.05) than that in .0025% solution.

b) Eggs fertilized and reared in the solutions up to hatching.

Results are summarized in appendix VIIb and figure 22b. In all replicates, eggs were reared only in 0.8 ppm thyroxine solution. Mean pectoral ray counts in genotypes a and b were significantly lower than the respective controls ($P<.01$). In genotype Y, there was no difference between the mean rays of the control and thyroxine treated sample, although the mean count of the sample of genotype in identical concentration of thyroxine solution in experiment XIa was lower than the control.

The mean pectoral ray count of the sample of genotype Y in thiourea solution (.02%)was higher than the control (P<.01). In the other replication of this genotype in identical solution in experiment XIa mean pectoral ray of the sample in thiourea solution showed merely a strong tendency to become higher than control. In both cases, the direction of response was identical.

Of the remaining replications, the mean pectoral ray count of the thiourea treated sample of genotype b was higher than the control (P = .01-.02). The mean count of thiourea treated sample of genotype a in 30°C bath showed no difference (P>.05) with the mean count of the control.

c) Chorion pricked fertilized eggs reared in the solutions to hatching.

Results are presented in appendix VIIc.and figure 22b. The mean pectoral ray count in 3.2 ppm thyroxine solution was significantly lower than the control in spite of the fact that the eggs were washed and hatched in water after 475 hours in thyroxine. This was similar to the result obtained in experiment XIa. But compared to the mean pectoral ray in 3.2 ppm in experiment XIa this mean count was higher, although the eggs were from the same genotype and the concentrations of thyroxine solution were identical. Eggs of the present sample were, however, exposed to thyroxine solution for a lesser period of time (475 as against 489 hours in XIa).

The mean pectoral ray count of the sample reared in .02% thiourea solution was significantly higher (P<.01) than the control. Compared with the mean of the sample of this genotype in thiourea

solution (.05%) in experiment X1a, mean pectoral ray count of the present sample showed a strong tendency of being higher (P = .02-.05) in spite of the fact that the solution of thiourea here was weaker (.02%).

From the overall comparison of mean pectoral counts of the control, thyroxine and thiourea treated lots, from eggs with and without the chorion pricked, it is observed that pricking the chorion of eggs did not alter the effect of thyroxine or thiourea upon the pectoral rays.

d) Larvae reared in the solutions after hatching.

Results are presented in appendix VIId and figure 22b. Mean pectoral count of the lot reared in 3.2 ppm thyroxine solution was lower than the mean of the control by 5.46 rays. This mean count was also lower than the mean counts of the samples from eggs of this genotype reared in the same solution up to hatching without and with their chorion pricked.

The mean pectoral ray of the sample reared in .05% thiourea solution was higher ($P<.01$) than the control. Compared to the mean count of the sample from eggs reared in an identical solution up to hatching (experiment X1a) the mean count of the lot reared in the solution after hatching was higher ($P<.01$). But there was no difference between this mean and the mean count of the sample from eggs reared with their chorion pricked (experiment X1c).

Anal rays.

a) Fertilized eggs reared in the solution up to hatching.

Results are summarized in appendix VIII and figures 23a and b.

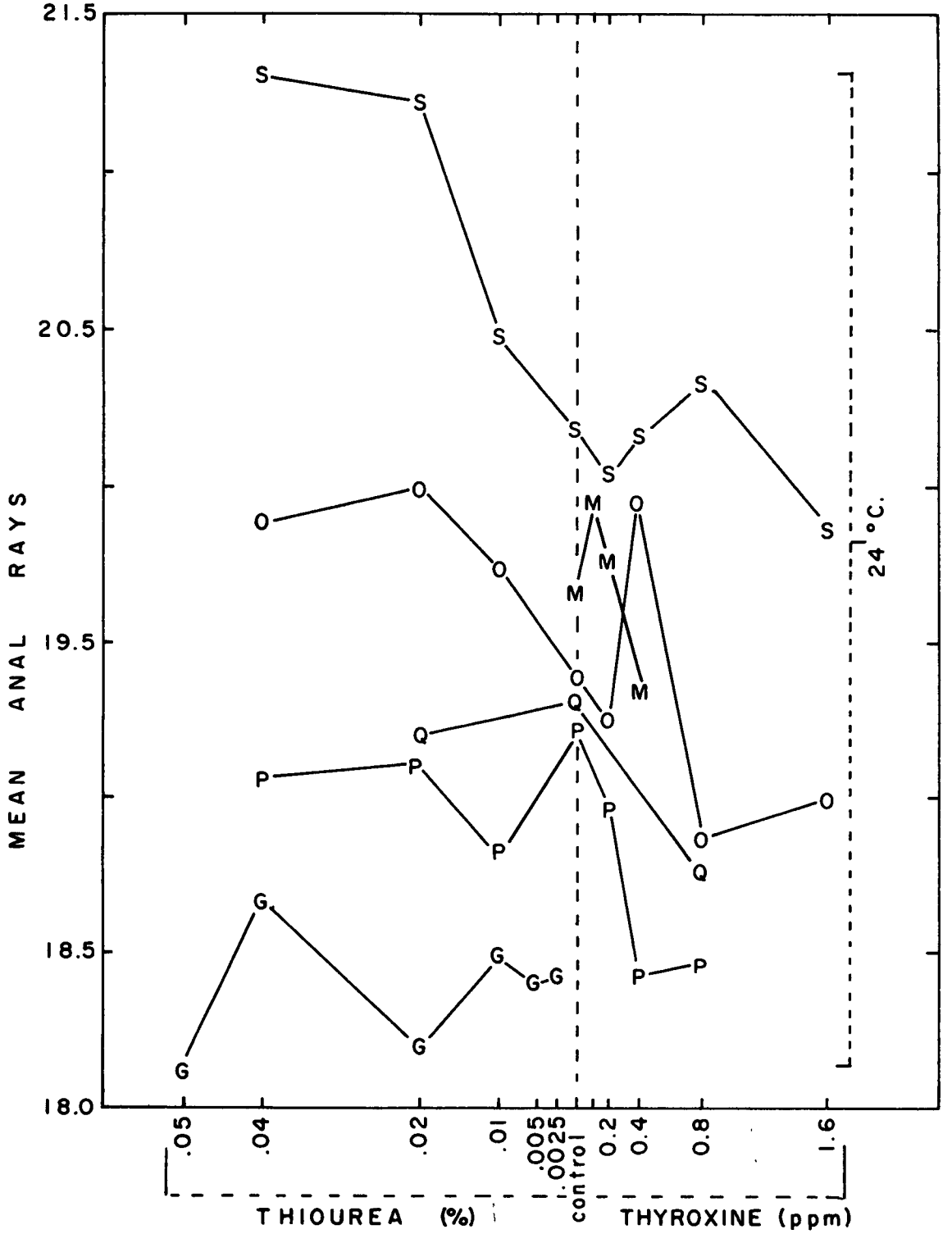

Figure 23a. Effect of thyroxine and thiourea to hatching on mean anal fin rays of genotypes reared in 24°C. Letters indicate genotypes (Experiment XI)

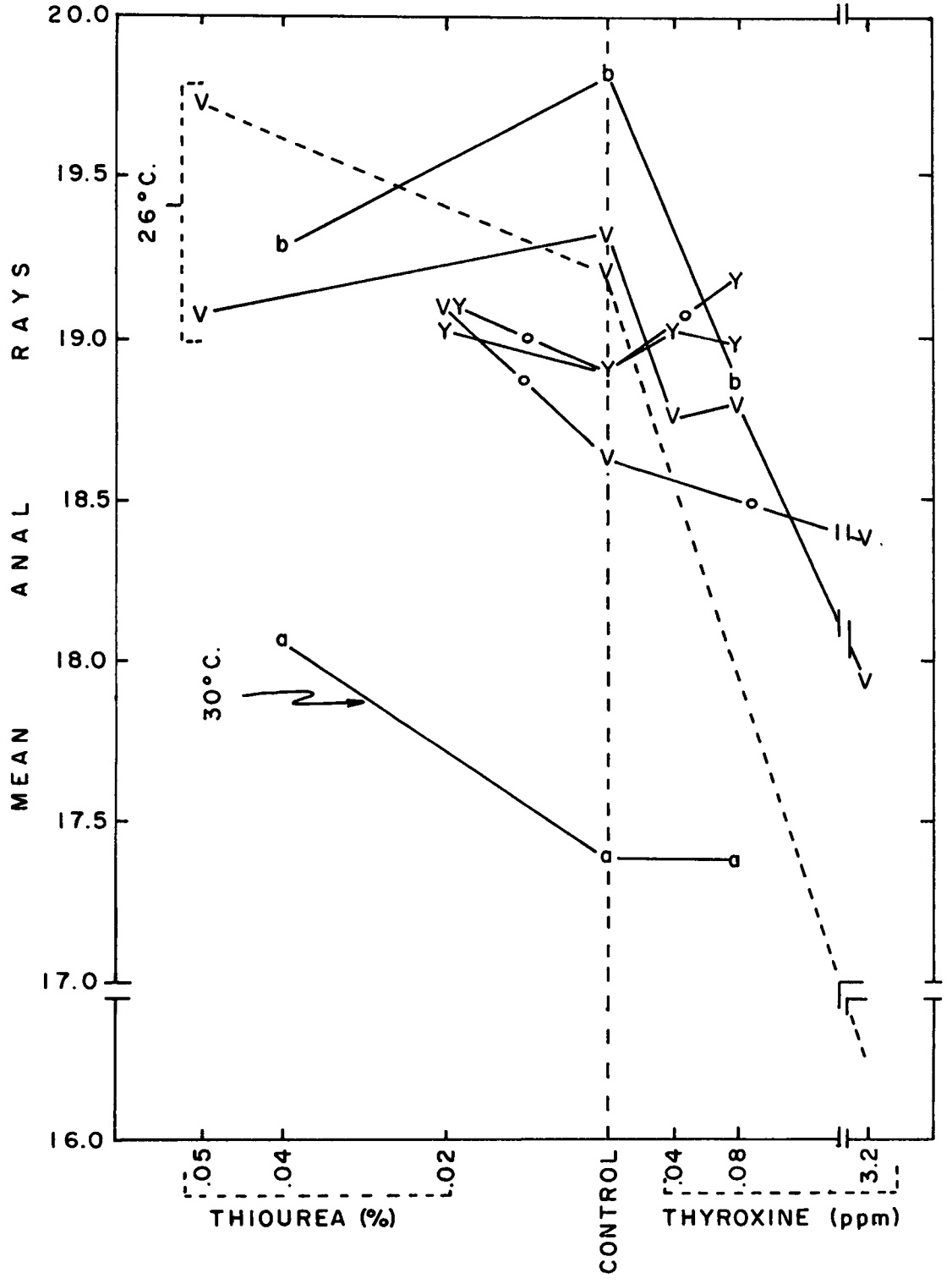

Figure 23b. Effect of thyroxine and thiourea on mean anal fin rays of genotypes reared in 26° and 30° C (only a). Legends are same as in Figure 21b (Experiment XI).

As the larvae were to be removed from the solutions soon after hatching and reared in renewable tap water, no effect of the solutions on anal counts was anticipated. But the results obtained showed that in at least some genotypes, effects were produced on anal rays.

Anal ray counts of genotype M in different concentrations of thyroxine did not show any variation. In case of genotype O the mean count of the sample in 0.8 ppm tended to be lower ($P = .05$) than the control but the mean in the next higher concentration (in 1.6 ppm) showed no difference ($P > .05$); size of this latter sample was however very small (8 fish). In 0,4 ppm thyroxine solution, the anal ray count showed a strong tendency of increase ($P = .01$-$.02$) whereas in the lowest concentration of thyroxine, i.e. in 0.2 ppm, the count remained unaffected. Mean anal counts of the samples of genotype P in 0.8 and 0.4 ppm thyroxine were lower than the control in each case ($P < .01$). The mean of the sample from eggs reared in 0.2 ppm was not affected.

In genotype Q the mean anal ray count of the sample from thyroxine (0.8 ppm) treated eggs tended to be lower than control ($P < .05$).

Mean anal ray of genotype S remained unaffected in all concentrations of the thyroxine solution. Contrary to the above, the mean anal ray count of each sample of genotype V obtained by rearing eggs in 3.2 ppm, 0.8 ppm and 0.4 ppm thyroxine solution was lower than the control ($P < .01$). Compared to the mean count of the control lot, mean of the samples of genotype Y in 0.8 ppm and 0.4 ppm thyroxine solution was not different.

Rearing and hatching the eggs in thiourea solutions produced no effect on the anal ray counts in the majority of the genotypes cited above. In genotype 0 the mean count in the sample in .04% thiourea solution showed a strong tendency to increase ($P = .02-.05$). Mean counts of the samples of genotype S in .04% and .02% thiourea solutions were higher ($P<.01$) than the mean of the control.

b) Eggs fertilized and reared in the solutions up to hatching.

Results are shown in appendix VIII and figure 23b. The mean anal ray count of genotype Y in 0.8 ppm tended to be higher ($P = .02-.05$) than the control. Sample of genotype b in 0.8 ppm thyroxine had a mean anal ray count lower than the control ($P<.01$). In the third replication of this experiment in 30°C bath, the mean anal ray of the sample of genotype a in 0.8 ppm thyroxine solution was the same as that of the control.

The mean count of the thiourea treated sample of genotype a strongly tended to be higher than the control ($P = .01-.02$). In the other two replicates there was no difference between the mean anal ray counts of the control and respective samples from eggs fertilized and hatched in thiourea solutions.

c) Chorion pricked fertilized eggs reared in the solutions to hatching.

Results are summarized in appendix VIIIc and figure 23b. The mean anal ray count of the sample in 3.2 ppm thyroxine solution was not different from the control ($P>.05$). This was in contrast to the result obtained by rearing the fertilized eggs of the genotype in

thyroxine solution of identical concentration where the mean anal ray count was significantly lower than the corresponding control (experiment Xla). In both, eggs were reared in thyroxine solution for a period of time (489 hours in Xla and 475 hours in this case) but hatched in water.

The mean anal ray of the sample from eggs reared and hatched in .02% thiourea showed no difference ($P>.05$) from the mean of the control. This mean again is also not different from the mean of the sample of this genotype reared in .05% thiourea solution in experiment Xla.

d) Larvae reared in the solutions after hatching.

Results are presented in appendix VIII and figure 23b. The effect of thyroxine solution on the anal ray of the young was pronounced. Compared to the mean anal rays of the control, the mean of the sample reared in 3.2 ppm thyroxine was lower by 2.98 rays. This was also lower than the mean count of the sample in identical thyroxine solution in experiments XI a and c. Comparison of the mean anal ray counts of the three respective control lots showed no difference (P .05) between them.

Mean anal ray of the lot reared in .05% thiourea solution strangely tended to be higher than the control (P = .01-.02). This was not seen in the replicates of this genotype V in experiments where eggs were reared and hatched in thiourea solution (experiments Xl a and c).

Dorsal rays

a) Fertilized eggs reared in the solutions up to hatching.

Results are summarized in appendix IX and figure 24. In

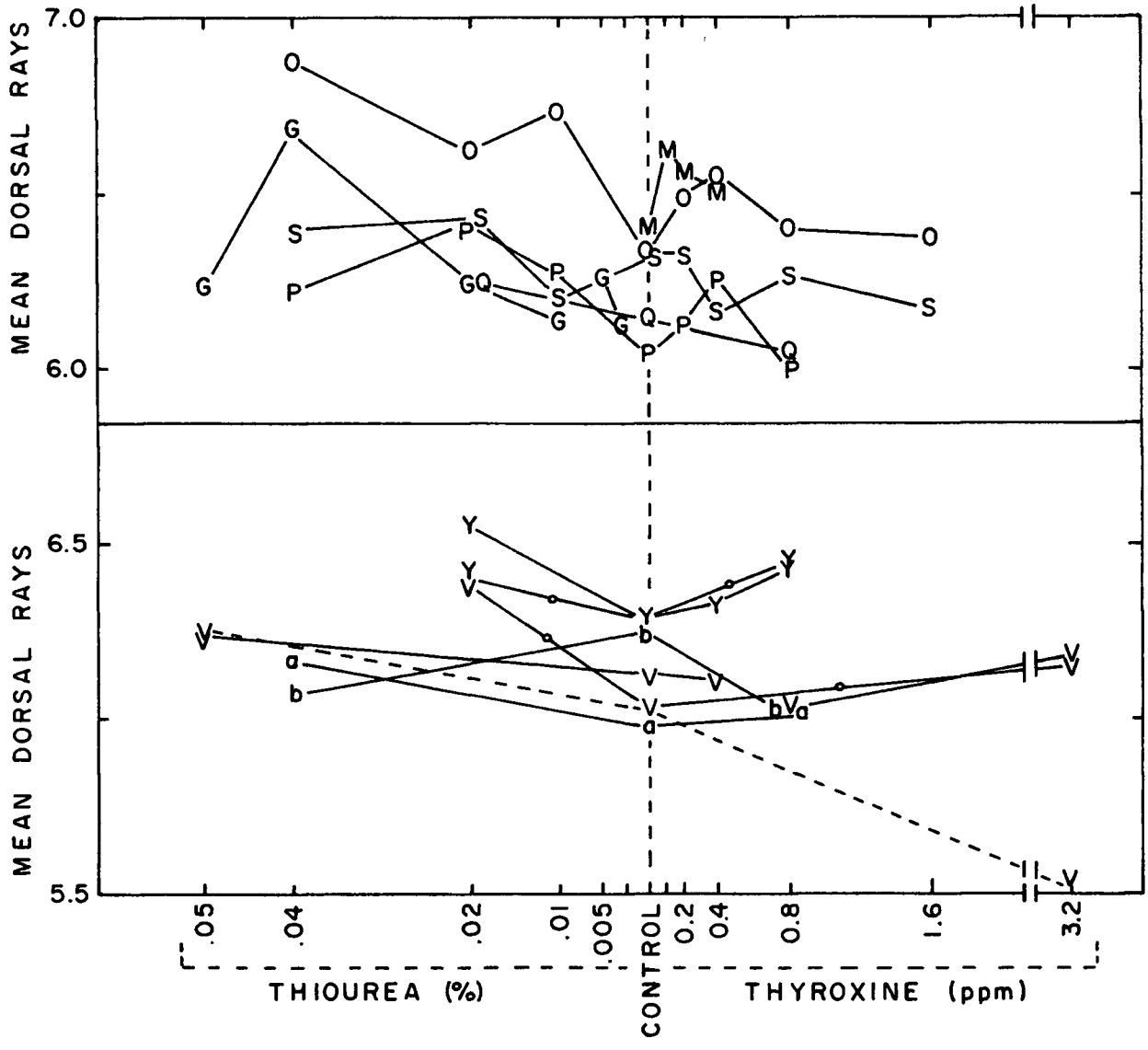

Figure 24. Effect of thyroxine and thiourea to hatching on mean dorsal fin rays of genotypes reared in 24°C (upper) and in 26° and 30°C (lower). For lower figure, legends are same as in Figure 21b. (Experiment XI)

none of the samples treated in thyroxine solution, the mean dorsal ray count differed from the mean of the control lots. But the mean count of the samples in thiourea solution was altered in some of the genotypes and this alteration was in the direction of increase in every such case. The mean count of the sample of genotype G in .04% thiourea was higher ($P<.01$) than the mean of the lot in .0025% thiourea solution. But the mean count of the sample in the highest concentration of thiourea solution (.05%) did not differ from that in .0025% solution. Samples of genotype 0 reared in .04% and .01% thiourea solutions had a mean dorsal ray count higher ($P<.01$ in each case) than the control but the mean of the lot in .02% solution did not differ from the control. Although the mean of the sample of genotype P in the highest (.04%) concentration of thiourea did not differ from the control, the mean of the sample reared in .02% solution was higher ($P<.01$) than the control. A significant increase in the mean dorsal ray count of genotype Y was also obtained in .02% thiourea solution.

b) Eggs fertilized and reared up to hatching in the solutions.

Results are presented in appendix IXb and figure 24. As in experiment Xla, treatment in thyroxine showed no effect on the dorsal ray counts of any of the replicates of this experiment. Rearing in thiourea solutions also had no effect on the dorsal ray counts of all the genotypes including Y, the mean count of the sample of which in identical solution of thiourea in experiment Xla showed an increase. In the present case, however, there was a weak suggestion of increase in the mean count in thiourea solution but this was not significant.

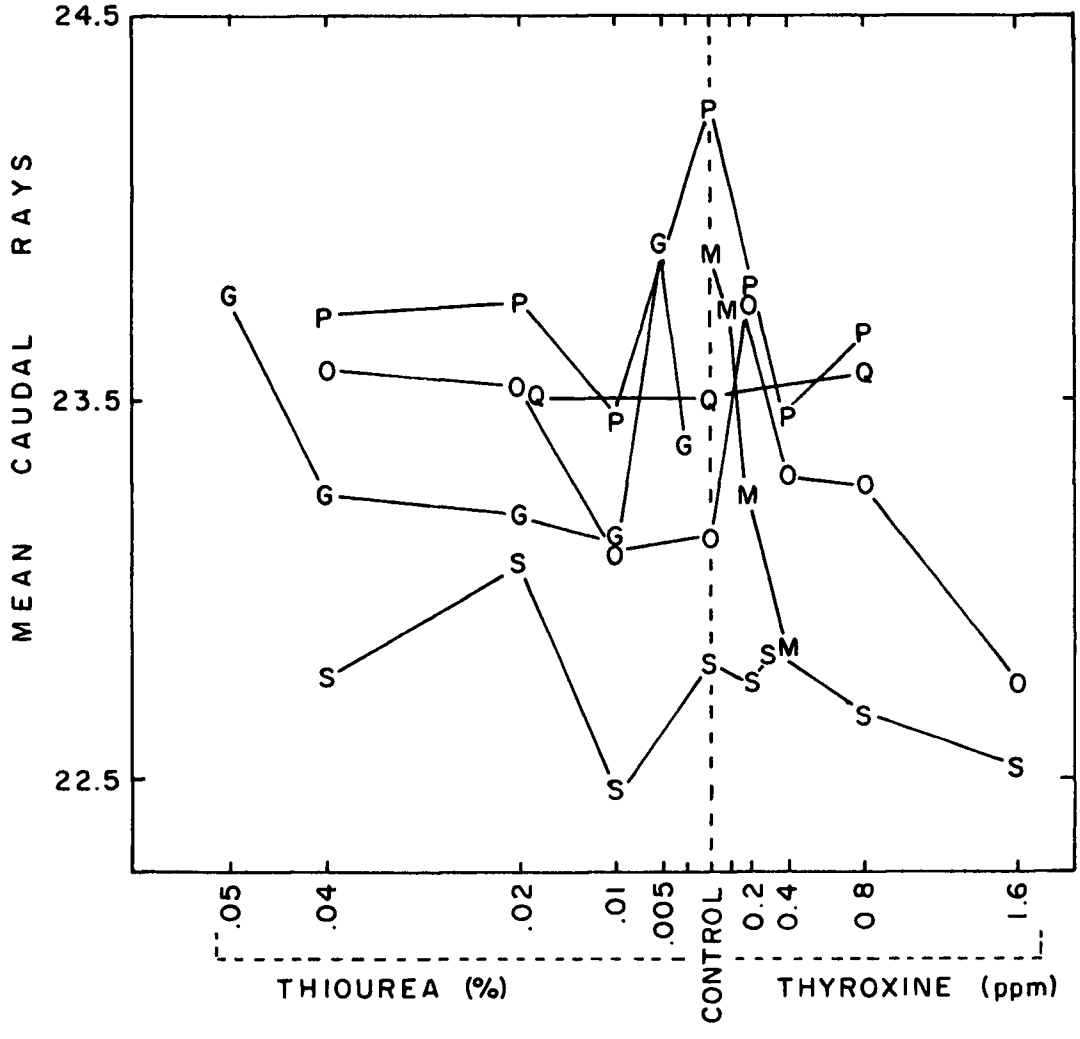

Figure 25a. Effect of thyroxine and thiourea to hatching on mean total caudal fin rays of genotypes reared in 24°C. Letters indicate genotypes (Experiment XI)

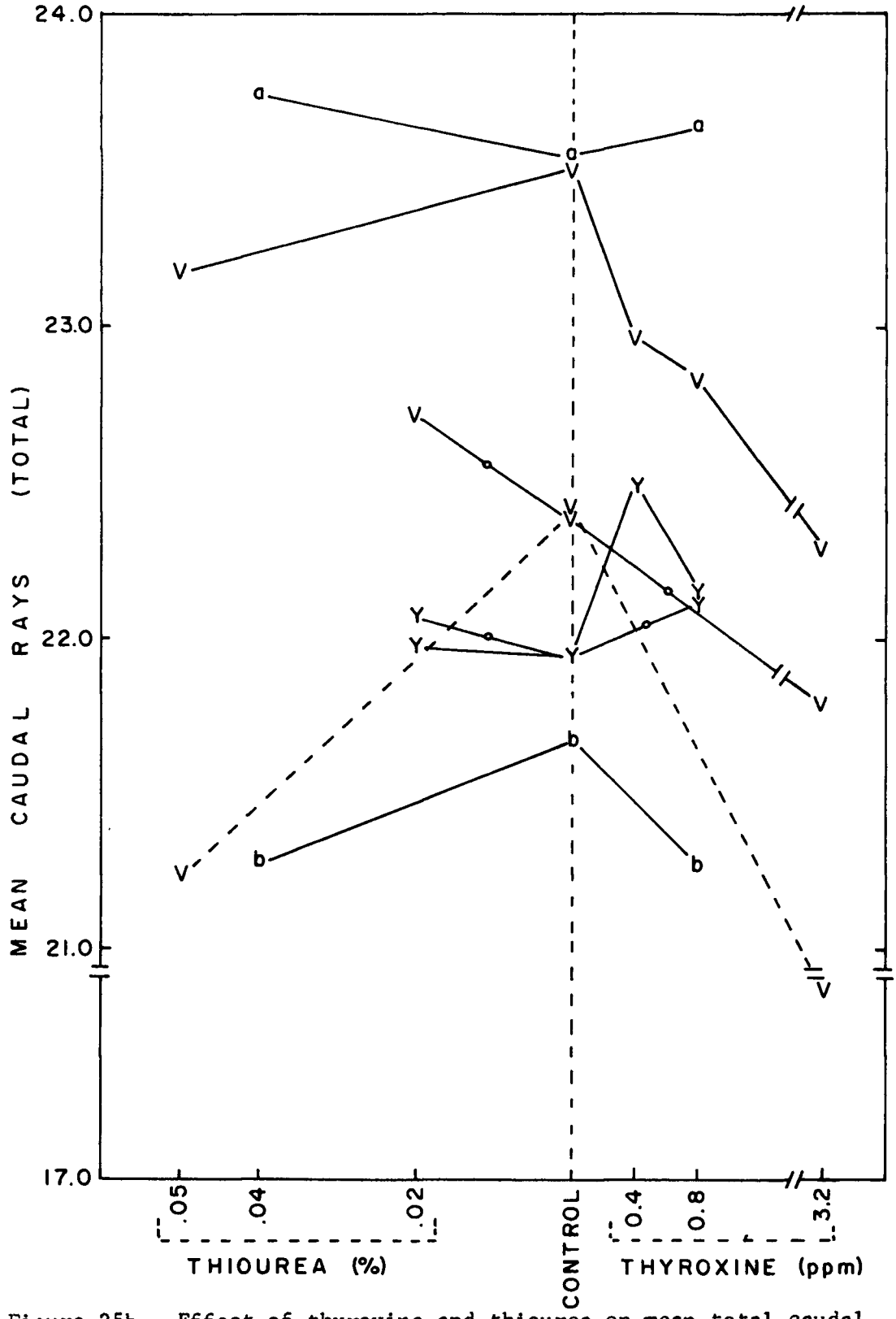

Figure 25b. Effect of thyroxine and thiourea on mean total caudal fin rays of genotypes reared in 26° and 30°C (only a). Legends as in Figure 21b (Experiment XI)

c) Chorion pricked fertilized eggs in the solution to hatching.

Rearing of the chorion pricked eggs in thyroxine and thiourea solution caused no difference in the mean dorsal ray counts of the samples (Appendix IXc and Figure 24). The results were also not different from those where eggs were reared with intact chorion (experiment XIa).

d) Larvae reared in the solutions after hatching.

Mean count of the lot in 3.2 ppm thyroxine solution was significantly lower ($P<.01$) than the control (Appendix IXd and Figure 24). Though the mean of the lot in thiourea solution was higher than the control, the difference was not significant ($P>.05$).

Total caudal rays

a) Fertilized eggs reared in the solutions up to hatching,

Results are presented in appendix Xa and figure 25 a and b. In four of the replicates, the mean caudal ray counts of samples reared in thyroxine solutions differed from the controls or showed some tendency of becoming different. But this difference was not in the same direction in different genotypes. Mean counts of genotype M in 0.4 ppm solution tended to be lower ($P<.05$). Mean total caudal ray of genotype P was lower in only 0.4 ppm solution but the same in the higher 0.8 ppm) and lower (0.2 ppm) concentration merely showed a tendency of being lower ($P<.05$). Mean counts of the samples of genotype V in all concentrations of the solution were lower ($P<.01$) than the control. Mean of the sample of genotype Y was higher ($P<.01$) than control in 0.4 ppm solution but the same in the higher concentration (0.8 ppm) was not significantly different from control.

In all but two genotypes, mean total caudal rays of lots reared in thiourea solution did not differ from the controls. Of the genotypes showing difference, mean count of the sample of genotype P in .01% solution was lower ($P<.01$) than control. Means of the samples of this genotype reared in .02% and .04% solutions showed a tendency to decrease ($P<.05$). In genotype V, mean count of the sample in thiourea solution showed a tendency of being lower than the mean of the control ($P<.05$).

b) Eggs fertilized and reared up to hatching in the solution.

In all three replicates with genotypes Y, a and b, the rearing of the eggs in thyroxine and thiourea solutions did not alter the mean total caudal rays in any significant manner (Appendix Xb and Figure 25b). Results obtained here for genotype Y did not differ from those obtained in experiment Xla in identical concentrations of the solutions.

c) Chorion pricked fertilized eggs reared in the solution up to hatching.

Although the mean total caudal ray of the sample in 3.2 ppm thyroxine was somewhat lower than the control, the difference was not significant ($P>.05$: Appendix Xc and Figure 25b). This result was different from that obtained in experiment Xla with the same genotype, where the thyroxine treated sample had a lower mean than the control.

Mean count of the sample hatched in .02% thiourea solution was slightly higher than the control but the increase was not statistically significant ($P>.05$). This result also differed from the result in Xla where the mean in .05% solution was lower than the control.

d) Larvae reared in the solutions after hatching.

Mean count of the sample in thyroxine solution was lower ($P<.01$) than the control by 4.77 rays (Appendix Xd and Figure 25b. This decrease was also larger than that found in the sample of the genotype from eggs hatched in similar solution in experiment Xla. The mean count of the lot raised in .05% thiourea solution was lower ($P<.01$) than the control. This result is also similar to that obtained with this genotype in the replication in connection with experiment Xla, but the decrease in the mean in the present case was greater.

Conclusions

Both thyroxine and thiourea alter meristic characters of medaka. In high concentrations, thyroxine generally decreases the meristic characters whereas thiourea tends to increase them. This is, however, not always true. The effect of thyroxine on vertebral number was a duplication of high (or low) temperature whereas on pectoral ray counts, the effect appeared to be parallel to that obtained by increase in temperarures. The effect of thiourea on the pectoral ray appeared to be a duplication of the effect produced by low temperatures. In respect of anal, dorsal and total caudal ray counts, the effects of thyroxine and thiourea are not clear and consistent, particularly in replications where eggs were reared only to hatching in the solutions.

Table LVII. Egg number and mortality in experiment XII: Effect of 2, 4-Dinitrophenol.

Treatment	No. of fertd. eggs	No. hatched	As % of fertd. eggs	Time to 50% hatching (hrs.)	No. survived to preservation	As % of fertd. eggs
Parent: Y						
Fresh water control	100	80	80	223	70	70
1: 1,000,000 dinitrophenol	100	24	24	229	18	18
1: 800,000 dinitrophenal	100	70	70	231	51	51

Table LXIII. Egg number and mortality in experiment XIII: Effect of urethan.

Parent: Y						
Fresh water control	100	80	80	223	70	70
0.5% Urethan	100	48	48	255	17	17

Table LXVI. Egg number and mortality in experiment XIV: Effect of salinity.

Parent: a						
Fresh water	50	43	86	431	39	78
Sea water	50	43	86	460	35	70

Table LVIII. Frequency distribution of total vertebrae in experiment XII: Effect of 2,4-Dinitrophenol.

Treatment	Temp (°C)	Total vertebrae 30	31	32	Number	Mean	Remarks
Parent: Y							
Fresh water control	26°	12	54	4	70	30.89	
1: 1,000,000 dinitrophenol	26°	4	14		18	30.78	
1: 800,000 dinitrophenol	26°	2	39	10	51	31.16	Higher than fresh-water ($P<.01$).

Table LXIV. Frequency distribution of total vertebrae in experiment XIII: Effect of urethan.

Parent: Y							
Freshwater control	26°	12	54	4	70	30.89	
0.5% Urethan	26°	2	10	5	17	31.18	Tends to be higher than fresh water ($P<.05;>.02$).

Table LXVII. Frequency distribution of total vertebrae in experiment XIV: Effect of salinity.

Parent: a							
Fresh water control	26°	17	22		39	30.56	
Sea Water	26°	5	29	1	35	30.89	Higher than fresh-water ($P<.01$).

Table LIX. Frequency distribution of pectoral rays in experiment XII: Effect of 2,4-dinitrophenol.

Treatment	Temp (°C)	Pectoral rays 11	12	13	14	15	Number	Mean
Parent: Y								
Fresh water control	26°		63	77			140	12.55
1: 1,000,000 dinitrophenol	26°		8	26	2		36	12.83 [1]
1: 800,000 dinintrophenol	26°		38	63	1		1102	12.64

Table LXV. Frequency distribution of pectoral rays in experiment XIII: Effect of urethan.

Treatment	Temp (°C)	Pectoral rays 11	12	13	14	15	Number	Mean
Parent: Y								
Freshwater control	26°		63	77			140	12.55
0.5% Urethan	26°		7	22	4	1	34	12.97 [2]

Table LX. Frequency distribution of anal rays in experiment XII. Effect of 2.4-Dinitrophenol.

Treatment	Temp (°C)	Anal rays 17	18	19	20	21	Number	Mean
Parent: Y								
Freshwater control	26°	2	18	37	12	1	70	18.89
1: 1,000,000 dinitrophenol	26°		6	8	4		18	18.89
1: 800,000 dinitrophenol	26°	4	24	20	2	1	51	18.45 [3]

Note: 1. Higher than fresh water ($P<.01$).
2. Higher than fresh water ($P<.01$).
3. Lower than fresh water ($P<.01$).

Table LXI. Frequency distribution of dorsal rays in experiment XII: Effect of 2,4-Dinitrophenal.

Treatment	Temp (°C)	Dorsal rays 5	6	7	Number	Mean
Parent: Y						
Fresh water control	26°		50	20	70	6.29
1: 1,000,000 dinitrophenol	26°		13	5	18	6.28
1: 800,000 dinitrophenol	26°		37	14	51	6.27

Table LXII. Frequency distribution of total caudal rays in experiment XII: Effect of 2,4-Dinitrophenol.

Treatment	Temp (°C)	T 19	20	Total caudal rays 21	22	23	24	25	26	Number	Mean
Parent: Y											
Fresh water control	26°	1	2	23	23	16	5			70	21.94
1: 1,000,000 dinitrophenol	26°			3	9	5	1			18	22.22
1: 800,000 dinitrophenol	26°			6	24	13	7	1		51	22.47[1]

Note: 1. Higher than fresh water ($P<.01$).

EFFECT OF 2, 4 - DINITROPHENOL (EXPERIMENT XII)

Introduction

This experiment was performed to study the effect of dinitrophenol on the fixation of different meristic series and to ascertain if the effect of the chemical parallels the effect of temperature in this respect. Dinitrophenol increases oxygen consumption by uncoupling oxydative phosphorylation and probably makes less energy available for chemical work. It was anticipated that the resultant alteration in the metabolic pattern of the eggs would produce changes in the meristic series fixed early in development.

Description of experiment

Eggs of genotype Y were reared in two concentrations (1: 1,000,000 and 1: 800,000) of dinitrophenol. The chemical used is the product manufactured by Eastman Kodak Company, Rochester, New York, and solutions were made in tap water. Fertilized eggs were put into 300 ml of the solution of required concentration and reared therein until hatching. After hatching, the young were transferred to small baskets in tap water for further growth. A control lot was reared in an identical manner in a bottle containing 300 ml of water up to hatching and reared thereafter in a basket. All the lots were reared in 26°C temperature both before and after hatching.

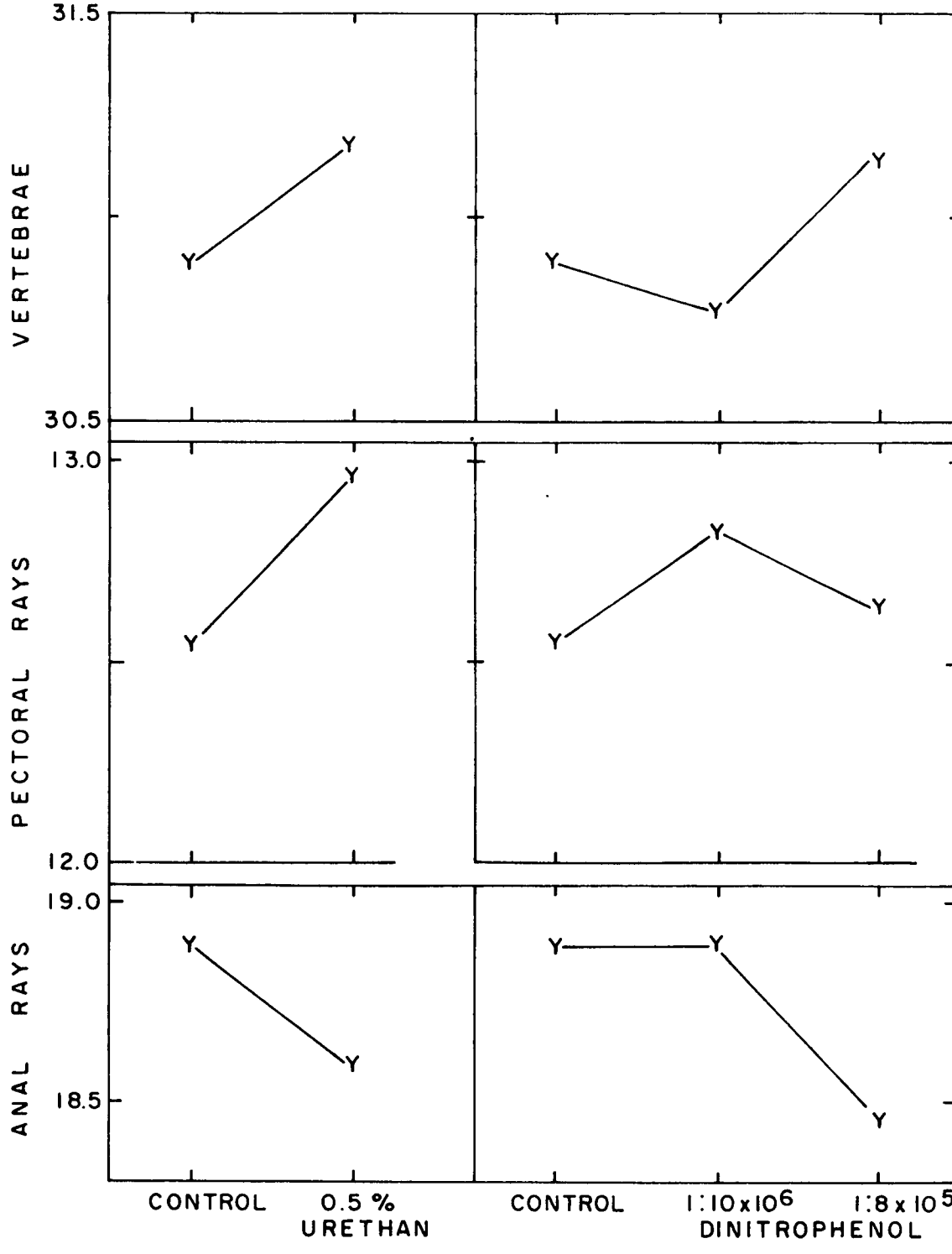

Figure 26. Effect of urethan and dinitrophenol on mean total vertebrae and pectoral and anal fin rays of genotype Y (Experiments XII and XIII)

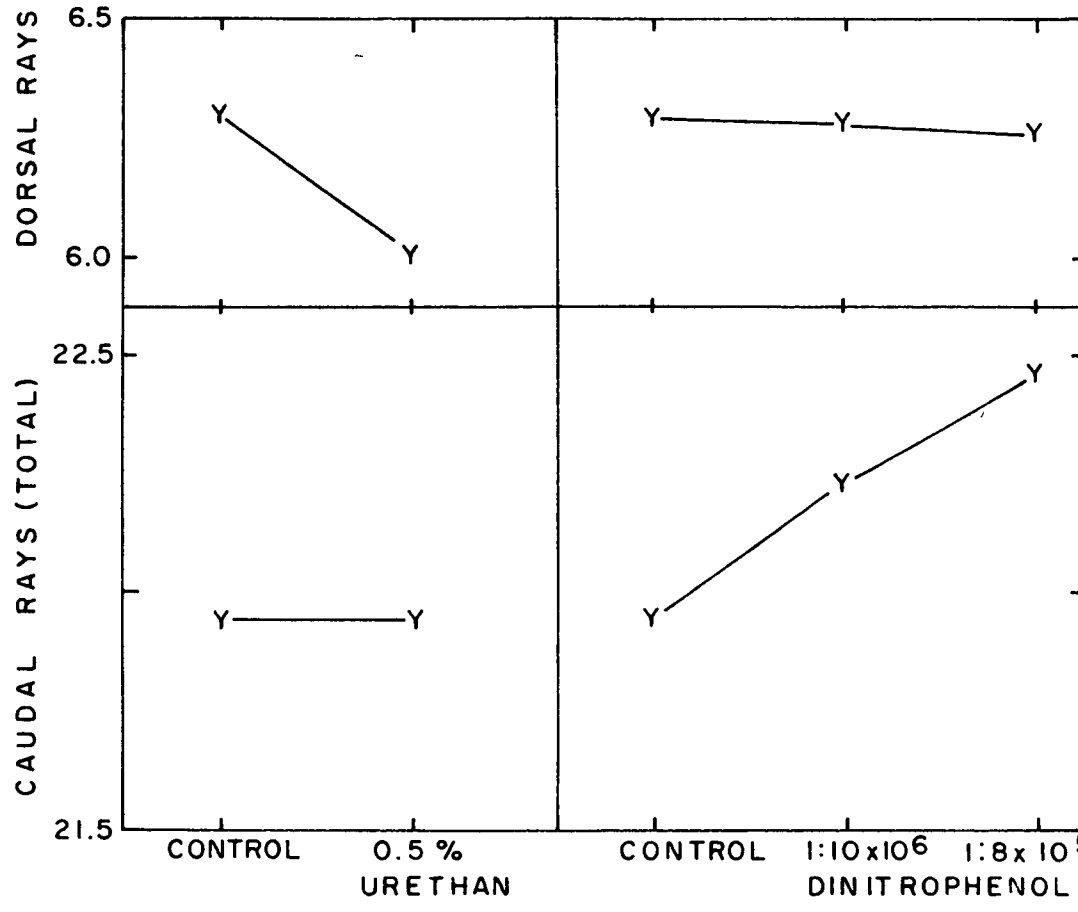

Figure 27. Effect of urethan and dinitrophenol on mean dorsal and total cauday fin rays of genotype Y (Experiments XII and XIII)

Results

70% of the eggs survived to hatching in the higher concentration of the solution while in the lower concentration a large mortality occurred as a result of heavy fungus attack (Table LVII).

Mean vertebral count of the lot hatched in the stronger solution was higher ($P<.01$) than the control (Table LVIII and Figure 26). Mean pectoral ray count of the sample in the weaker solution was higher than the control ($P<.01$) but the stronger solution produced no significant difference (Table LIX and Figure 26). In lower concentration, the mean anal ray count was not altered but in the higher, mean count decreased significantly ($P<.01$. Table LX and Figure 26). While mean dorsal ray counts remained unaffected in dinitrophenol solutions (Table LXI and Figure 27), total caudal ray count of the sample from eggs hatched in stronger solution was higher than the control mean (Table LXII and figure 27).

Conclusion

Dinitrophenol alters the meristic counts by upsetting the availability of energy in the developing embryo. The effect of the chemical on vertebrae appears to be parallel to that obtained in extreme low (or high) temperature whereas the same on pectoral rays resembles the effect of low temperature. In view of the inconsistent results obtained for anal and total caudal rays in temperature, effects on these characters cannot be compared.

EFFECT OF URETHAN (EXPERIMENT XIII)

Introduction

Urethan (Ethyl carbamate) is known as a mitotic poison and its ability to arrest or reduce mitotic activity was demonstrated in case of sea urchin eggs (Lillie 1941; Cornman 1950) and in ciliates (Burt 1945). Urethan exerted a retarding effect on growth and differentiation of the embryonic structures in Zebra fish, Brachydenio rerio (Battle and Hisaoka 1952). The object of the present experiment was to find out how urethan influences the meristic characters, particularly the vertebrae, when eggs are reared in the solution.

Description of experiment

Eggs of genotype Y were used for this experiment. 100 eggs were placed in 300 ml of 0.5% urethan solution in a 710 ml bottle and reared in 26°C temperature bath. The solution was made in tap water with urethan manufactured by Fisher Scientific Company. The solution in the bottle was replaced every six days unt-il the eggs hatched out. Eggs were placed inside the solution immediately after fertilization and reared continuously therein until hatching. Immediately upon hatching, the larvae were transferred to small cloth basket in tap water and reared there until preservation. The lot used as control for thyroxine, thiourea and dinitrophenol was also used as a control for this experiment. Aeration was maintained in the bottles containing the eggs.

Results

48% survived up to hatching but a great mortality occurred after hatching. The number that survived up to preservation was less than 50% of the number hatched (Table LXIII). Mean vertebral count of the urethan treated sample displayed a strong tendency to become higher ($P = .02$-$.05$; Table LXIV and Figure 26). An increase in the mean pectoral ray count also was obtained by the treatment in urethan ($P<.01$: Table LXV and Figure 26) but anal, dorsal and total caudal ray counts remained unaffected (Figures 26 and 27).

Conclusion

Urethan alters the meristic characters of medaka and the effect resembles those obtained in low temperature.

EFFECT OF SALINITY (EXPERIMENT XIV)

Introduction

In an aquatic organism which must regulate both water and salt, there will probably be some effect of salinity on metabolism. This experiment was conducted to test the effect of salinity and resultant altered metabolism on the meristic characters in medaka.

Description of experiment

Eggs of genotype a were used for this experiment. Sea water (25.88‰ salinity) available in the Biological Sciences Building of the University was used for the treatment. 50 eggs were placed in 300 ml of sea water in a 170 ml bottle and floated in a 26°C bath. For comparison, another lot of 50 eggs were reared in 300 ml of fresh water (tap water) in a similar bottle alongside the treatment bottle. Water in both bottles was aerated. On hatching, larvae from both bottles were transferred to small cloth basket in fresh (tap) water and reared therein until preservation.

Results

Survival of eggs up to hatching was 86% in both lots but mortality of fry was slightly greater in the lot hatched in sea water (Table LXVI). Mean vertebral count of the sample from eggs reared in sea water was higher ($P<.01$) than that in fresh water (Table LXVII). Pectoral, anal, dorsal and total caudal ray counts were not altered significantly by sea water.

Salinity induced alteration of metabolism therefore, affects the meristic counts in medaka.

HATCHING TIME AND MERISTIC VARIATION

The data of all experiments were analysed with respect to hatching time. Times to 50% hatching are recorded in Tables I, VII, XIII, XIX, XXV, XXXI, XXXVII, XLIII, XLIX, L, LVI, LVII, LXIII and LXVI. In some of these experiments marked differences occurred in hatching times of lots from different parents or treatments, while in others hatching times were similar. Analysis for association between hatching time and meristic characters were made for different genotypes in the same treatment, and for different egg lots of the same genotype reared separ-ately but under identical conditions. In all the experiments, no correlation was found between time to hatching and number of meristic parts formed.

SIZE HIERARCHY AND MERISTIC VARIATION

As expected,a considerable variation in the relative sizes of individuals occurred within a single lot. This variation was found in lots where young fish were obtained from eggs accumulated on successive days as well as in lots where these were from eggs of a single day's spawning. To test for correlation between relative size and meristic differences of the individuals within the same lot, data of the following lots were analysed: (a) Individuals from 25 egg lot, 100 egg lot and 200 egg lot in experiment VI. (b) Lots from eggs obtained on March 23 and April 8, 1961 in experiment III and all lots of genotype J, F and crosses thereof in experiment VII. Individuals in each lot were divided into small and large fish on the basis of their standard length. Individual count of each

character was then recorded against the length and then a chi-square test (Dixon and Massey 1957) was made for each character separately. If the calculated chi-square value was higher than the tabled value with appropriate degrees of freedom, an association between the character and length was concluded.

These tests revealed a significant positive association between size and the total caudal ray of individuals (within the same lot) in the lot from 200 eggs (egg density experiment) and in the lot from eggs of April 8, 1961 (genotype U). In other lots, there was no correlation between relative size and caudal rays. A significant positive association between anal ray and length was found in the lot obtained from 200 eggs (genotype Y; egg density experiment) and in the lot obtained by the cross of J♀ and F♂ (Egg size experiment replicate #1). No correlation between length and vertebrae, pectoral rays and dorsal rays was found in any of the lots analysed.

Effect of treatments on the mean counts of different meristic series.

Treatments and number of replications (in figure)	Total vertebrae	Pectoral fin rays	Anal fin rays	Dorsal fin rays	Total caudal fin rays
Malachite green treatments of eggs up to hatching: 2	None	None	None	None	None
Nature of egg rearing containers and variations in aeration up to hatching: 2 for first part and 1 for second part	None	None	None	None	Sample from bottle reared eggs tends to have lower mean
Successive days eggs from same parent: 1	None	None	Mean of earlier lot tends to be higher	None	Mean of earlier lot tends to be higher
Mechanical shock to eggs (shaking) up to hatching: 1	None	None	Mean of lot shaken after 4 days undisturbed development tends to be higher	None	None
Pricking chorion of fertilized eggs: 2	None	None	Mean lower in both pricked chorion lots.	Mean lower in pricked chorion lot in one.	None

Summary of results (cont'd)

Treatments and number of replications (in figure)	Total vertebrae	Pectoral fin rays	Anal fin rays	Dorsal fin rays	Total caudal fin rays
Egg density effect: 1	None	None	Erratic. Fourfold increase in density decreased mean in one lot: in others no effect	None	Increased density reduced mean count but effect not progressive
Egg size effect: 2	Independent of egg size.	Independent of egg size.	In one, large egg lot gave higher rays, in the other no clear pattern.	Independent of egg size.	Independent of egg size.
Temperature transfer of developing eggs (a) Low to high temperature	In early part of sensitive period temperature shock reduced vertebrae. Towards end of sensitive period, vertebrae increased.	Not fixed before hat-ching	Not fixed before hatching.	Not fixed before hatching.	Not fixed before hatching.
(b) High to low temperature	Vertebrae fixed by 100-120 day degrees.				
Sustained temperature. 15.	V-shaped curve in 9; no effect in 3, decreased with increase in temperature in 2. Erratic in the last.	Inversely related to temperature in 13. In 2 effect irregular	Altered, but no consistent pattern.	Alteration not statistically significant. But majority gave an inverted V curve against temperature.	Altered but no consistent pattern.

Summary of results (Cont'd)

Treatments and number of replications (in figure)	Total Vertebrae	Pectoral fin rays	Anal fin rays	Dorsal fin rays	Total caudal fin rays
Increased light					
(a)Duration:3	None	Lowered in 1	None	None	None
(b)Intensity: 3	None	Lowered in 2	Increased in 1	Increased in 1	Increased in 2
A. Thyroxine (a) Fertilized eggs hatched in solutions. 7	Decreased in 3; increased in 3; no effect in 1	Decreased	Reduced in 3; no effect in the rest.	None	Reduced in 3; increased in 1; and no effect on the rest.
(b) Fertilized and hatched in solution, 3	No effect on 2. Tended to increase in 1.	Decreased in 2. No effect in 1.	Reduced in 3; no effect in the rest	None	None
(c) chorion pricked eggs hatched in solution. 1.	Tended strongly to decrease.	Decreased	None	None	None
(d) Larvae reared in solution. 1.	No effect	Decreased	Decreased	Decreased	Decreased
B. Thiourea (a) Fertilized eggs hatched in solution 7	Increased in 1; no effect on the rest.	Increased in 3; no effect in the rest.	Increased in 2; no effect in the rest.	Increased in 4; no effect in the rest	Tended to reduce in 2; no effect in the rest.
(b) Fertilized and hatched in solution. 3.	Increased in 1; no effect on the rest.	Increased in 2; no effect in the rest.	Tended to increase in 1; no effect in the rest.	None	None
(c) Chorion pricked eggs hatched in solution. 1.	None	Increased	None	None	None
(d) Larvae reared in solution. 1	None	Increased	Tended to increase	None	Reduced

Summary of results (cont'd)

Treatments and number of replications (in figure)	Total Vertebrae	Pectoral fin rays	Anal fin rays	Dorsal fin rays	Total caudal fin rays
2,4-Dinitrophenol; 1	Increased in higher concentrations.	Increased in lower concentrations.	Decreased in higher concentrations.	No effect	Increased in high concentrations.
Urethan; 1	Tended to increase.	Increased.	No effect.	No effect.	No effect.
Seawater; 1	Increased.	No effect.	No effect.	No effect.	No effect.

DISCUSSION

Metabolism and meristic characters:

A large number of diversified chemical processes are basic to the activities of all living organisms. These processes,which may be collectively called metabolism, are controlled by a number of widely different interlocked enzyme systems. Some metabolic processes are very general in nature and supply the energy whereby the organism merely continues to exist,while others are more specific, providing energy with which the organism can undergo growth or differentiation, or carrying on some special activity such as muscular movement. The former has been termed as the 'standard' or resting metabolism while the latter has been expressed as 'active metabolism' (Fry 1957). The difference between the active and standard rates of metabolism has been termed as 'scope of activity' (Fry 1947). Metabolism, whether 'active' or 'resting', will become altered as a result of the alteration of the activity of the complex interlocking enzyme system that together constitute metabolism. Alteration of the processes would determine the amount of energy available for growth or differentiation. In other words, growth and differentiation in a developing embryo are the resultant effects of the activity of the various enzyme systems. The rate of activity of a single enzymatic reaction or a complex of enzyme reactions is subject to alteration by various environmental conditions of which temperature is the most important.

Temperature acts as a general stimulant for all processes that constitute metabolism. Over a wide range of temperature, the rate of activity first increases with increasing temperature up to a certain optimum, beyond which the rate drops abruptly in spite of further increase

in temperature. In extreme high temperature, a depression in metabolism may be expected and therefore a reverse effect on the process of differantiation or growth.

Unlike temperature, effect of certain other factors on metabolism is not generalized in nature. Photoperiod, for example, has been suggested to influence metabolism by increasing the activity of thyroid. Role of thyroid activity in the regulation of metabolism is not clear. Thyroxine is known to stimulate metabolism but it probably does so by affecting specific points on any chemical cycle or by acting upon some specific aspects of metabolism. Thiourea depresses metabolism probably by interfering with the production of thyroid hormone. Dinitrophenol alters the energetics of a living cell by uncoupling phosphorylation and reducing the availability of ATP (Gorbman and Bern,1962) Urethan, like many anaesthetics, is known to depress rate of respiration at relatively high concentration (Giese 1961) but in low concentration, it acts as a stimulant and increases oxygen consumption (Heilbrunn 1955). Salinity has been found to alter the metabolic activity of certain aquatic organism probably as a result of the altered needs of energy for osmotic or ionic regulation (Hickman, 1959).

Vertebrae:

In the present investigations with sustained temperature, mean vertebral counts in 9 out of 15 replications decreased gradually with increase in temperature and then showed an abrupt rise with further increase in temperature. This pattern of response of vertebral count suggests that increase in temperature up to a certain level increases the metabolism by accelerating the activity of the enzyme systems responsible

for the reduction of the number of vertebrae. An optimum acceleration of these biochemical activities occurred in the intermediate temperatures where lowest mean count was obtained but further increase in temperature perhaps inactivated or depressed the reaction systems whereby an increase in the mean count resulted. Similarity of response of vertebral count in extreme high and low temperature is probably due to the fact that in both the extreme conditions metabolism was uneconomic and greater part of available energy had to be spent in maintaining the basic activity of life. It is not, however, clear why lowest number of vertebrae should result in the intermediate temperature producing economic metabolism.

Similar results are reported by Marckmann (1954). He found that the sea trout larvae having lowest mean vertebral count in the intermediate temperature also had maximum body weight while rearing the larvae at both higher and lower temperature produced higher number of vertebrae and lower body weights. According to him, larvae with maximum body weight in the intermediate temperature had the lowest or most economic metabolism per day degree, possibly conditioned by the most harmonic interaction between single processes taking place during the development of the larvae. But in both higher and lower temperature, the metabolism was greater and uneconomic and this was reflected in higher vertebral number and lower body weight. This hypothesis was corroborated by Marckmann (1958) by actual measurement of oxygen consumption of sea trout larvae in different temperatures. The V-shaped relationship of vertebral counts to temperature as obtained in medaka can also be

explained in the light of the above hypothesis.

Although the V-shaped relationship of vertebral number to temperature was obtained in a large number of similar experiments with different species of fish (Täning, 1944 in sea trout; Seymour, 1956 in chinook salmon, Oncorhynchus tshawytscha; Lindsey, 1952 in paradise fish, Macropodus operculcris; Molander and Molander - Swedmark, 1957, in plaice, Pleuronectes platessa; Hazawa, 1959 in Channa argus and Lindsey, 1962, in three spine stickleback, Gasterosteus aculeatus), this is not the invariable rule. In some investigations inverse relationship between the vertebral number and temperature was observed (Gabriel, 1944 in killi fish, Fundulus heteroclitus; Blaxter (1957) and Hempel and Blaxter (1961) in herring, Clupea harengus). In the present investigation 2 out of 15 replications made with different genotypes showed a similar inverse relationship to temperature. This was probably due to the fact that the highest temperature used for these genotypes (32°C for U and 34° for W) were not intense enough to bring about inactivation of the enzymatic processes involved. Rearing eggs in still higher temperature would probably have resulted in a V-shaped curve for these genotypes as well. This seemed to be the situation when the temperature effect on genotype Y is considered in which case, an inverse relationship would have been the conclusion if eggs were reared only in temperatures up to 32°C.

In the remaining 4 out of 15 genotypes subjected to sustained temperature treatment, mean vertebral counts failed to reflect a consistent pattern of the effect of temperature. The activity systems regulating the vertebral numbers were probably not labile to the temperatures used.

Light, like temperature, also influences chemical reaction systems in a living organism. The processes by which various physiological systems are modified may be, according to Eisler (1961), attributed to thermal and photochemical properties of the experimental light source. In the present study, mean vertebral counts of the samples reared under longer and brighter light conditions was not significantly altered. All the lots for this experiment were reared in a high temperature(30°C)bath. Between light and temperature, the latter perhaps acted as the dominant controlling factor and altered the vertebral numbers to the maximum of the genetic limit. Canagaratnam (1959) obtained similar results with the vertebral counts of sockeye salmon. Light effect was negated or neutralized in high temperature (12°C) although the same altered the vertebral count significantly at a lower temperature (8°C). This suggests that under appropriate conditions, light may modify the activity of the reaction systems responsible for vertebral numbers probably through the pituitary-thyroid axis. This is further evident from the results obtained in other species of fish reared under experimental light conditions (Dannevig, 1932; McHugh,1954; Lindsey, 1958 and Eisler, 1961). The mechanism through which light controls metabolism is not well understood. Canagaratnam (1959). however, indicated that photoperiod or length of period of illumination may alter metabolism by altering the activity of the thyroid.

If this hypothesis were correct, it would be possible to alter the meristic characters by altering the thyroid activity by other known agents. Results of the present investigations with thyroxine and thiourea support this hypothesis.

In 8 out of 12 replications of the experiment where eggs were reared in thyroxine solution, mean vertebral counts of the samples were significantly altered as shown by statistical analysis. The direction of change was however, not the same in all of them (in 4 the count decreased and in the remaining 4, this increased). The alterations in the vertebral counts in thyroxine treated eggs probably resulted from changed metabolic pattern induced by thyroxine through its influence upon the thyroid tissue. Canagaratnam (1959) found the existence of thyroid follicles in sockeye embryos of the stage (with optic vesicle, otic capsule and pectoral buds) when the number of vertebrae was being finally fixed. The sensitive period for vertebrae in medaka approximately corresponded to similar stages in sockeye, and it can be assumed that development of endocrine organs in these embryos also reached the same level. Variation in the direction of change of the vertebral count is, however, difficult to explain.

As opposed to thyroxine, [illegible] thiourea treatment was effective only in two replications (genotype S in .01% solution in 24°C and genotype a in .04% solution in 30°C) where the mean counts were significantly higher. Thiourea is known to depress metabolism in fish. From this point of view, effect of thiourea is perhaps similar to that of low temperature which increased the vertebral count by altering the metabolism to an uneconomic level. As for the instances where no alteration of vertebral count occurred, it may be suggested that thiourea in the low concentrations used in the present investigation was probably not strong enough to induce alterations in the activity complex in these genotypes or the target organ in those perhaps had not reached a definitive stage of development to respond to thiourea.

Although the thyroid is evidently involved in the regulation of the metabolism of fish (Hoar 1951), results of earlier investigations vary as to the specific aspects of metabolism that are under its control (Pickford and Atz, 1957).

In so far as the effect of thyroid hormone on the respiratory metabolism of fish is concerned, conflicting results were obtained in different as well as in the same species. Etkin, Roof and Mofskin (1940) and Hasler and Meyer (1942) found no change in the rate of oxygen consumption in gold fish, Carassius auratus treated in thyroxine or thyroid preparations but Muller (1953; cited in Pickford and Atz, 1957) observed a significant increase in oxygen uptake in the same species after injecting the individuals with thyroxine. Similar conflicting results on the effects of anti-thyroid drugs (thiourea, thiouracil) on the respiratory metabolism in fish have also been reported (no effect in gold fish, Chavin and Rossmoore, 1956; reduction in oxygen consumption in thiouracil treated specimens of Campostoma anomalum, Osborn, 1951).

Though the role of thyroid on respiratory metabolism is not clear, there are evidences that other metabolic functions (nitrogen and protein metabolism, carbohydrate metabolism, fat metabolism etc) are under the control of the thyroid (Pickford and Atz, 1957). One or more of these metabolic functions may be instrumental in the final expression of the number of vertebrae and other meristic characters. Trifonova, Vernidube and Phillipov (1939) suggested that periods of growth and differentiation in fish

embryos were characterized by differences in carbohydrate metabolism. In the developing eggs of medaka, evidence for the early use of fat was found (Yamamoto, 1951; Nakano, 1953) and according to Smith (1957) fat metabolism is of major importance to the development of fish embryo. Alterations in the vertebral counts obtained in thyroxine and thiourea treated samples may therefore be attributable to influence of the drugs upon different aspects of the metabolism of developing medaka embryos.

Increase in the vertebral count of the sample in 2,4-Dinitrophenol was perhaps due to lack of energy for growth or differentiation. Dinitrophenol acts by uncoupling respiratory oxidation from phosphorylation and thus reduces the supply of ATP (Gorbman and Bern, 1962). The net result of this process would be uneconomic metabolism for the embryo as a whole, and growth or differentiation would be slowed down or inhibited. Ishida (1951) also found that the development of medaka eggs was inhibited in dinitrophenol although the oxygen consumption was greatly increased. From the point of metabolism, this effect of dinitrophenol is comparable to that obtained in low (or high) temperature and thiourea. The present result is not, however, in agreement with those of Waterman (1939) who, by rearing eggs of medaka in dinitrophenol solutions (1: 40,000 to 1: 200,000), obtained a reduction in the number of myotomes as well as deformities in the body axis. In the present case, no deformities in the body axis of any of the individuals from the treated sample was found. The differences in the two results may be attributed to the weaker solutions (1: 800,00 and 1: 1,000,000) used in the present case. In higher concentration,

dinitrophenol probably exerts a toxic effect in addition to uncoupling phosphorylation and this may account for deformities and reduction in myotomes.

In urethan solution (0.5%) there was also an increase in the number of vertebrae. Urethan in very low concentrations acts as a stimulating agent (Heilbrunn, 1955) but in relatively high concentrations (0.1 to 0.5 molar) decreases respiration (Giese, 1961). Bodine and Fitzgerald (1948) found that in low concentration, ethyl urethan causes an increase in the oxygen consumption of grasshopper embryos whereas higher concentrations tend to decrease respiration. The solution used in the present investigation was very low (0.055 molar) and the effect produced on vertebrae was perhaps the outcome of metabolic imbalance induced by increased oxygen consumption. The increased oxygen consumption indicates that more energy was perhaps required by the embryos for maintaining basic activities leaving no surplus for growth or differentiation. Thus the action of urethan upon the metabolism of embryos is comparable to those caused by dinitrophenol, low or extreme high temperature, and thiourea, insofar as the differentiation of vertebrae is concerned.

Results of the present investigation differed from the findings of Battle and Hisaoka (1952) with eggs of zebra fish, Brachydenio rerio. By treating eggs in concentrations from 0.25% to 1.00% urethan, they obtained embryos with lesser number of myotomes and shorter body axis. They also observed deformities in the embryos reared in the solution but in the present case, none of the surviving individuals showed any deformity in their body axis.

Vertebral count was also increased by rearing eggs in sea water (25.88%) in the present investigation. In saline medium, osmoregulatory activity of the embryo demands more energy (Hickman, 1959) and this resulted in an uneconomic metabolism which caused an increase in the mean vertebral count. Thus salinity effect on the formation of vertebrae by altering the metabolic pattern may be said to be parallel to those of low or extreme high temperature, thiourea, dinitrophenol (in low concentration) and urethan. Increase in mean vertebral count in higher salinity was also obtained in threespine sticklebacks (Lindsey, 1962).

Pectoral rays:

As far as the fixation of pectoral rays are concerned, effects of increasing temperature, thyroxine and increased light appeared to be identical. All three factors apparently increased the metabolism of the embryo and fry and brought about a decrease in the mean pectoral ray counts of the samples. In other words, the biochemical processes involved in the fixation of pectoral rays are different than those for vertebrae and responded to the alteration of metabolism in an identical manner, except that in thyroxine this was much more pronounced. In contrast to the above, low temperature, low light conditions, thiourea, dinitrophenol, and urethan increased the mean pectoral ray counts by influencing the metabolism in a similar manner. Though the response of pectoral rays was consistent for most genotypes, in some no effect was produced by the treatments. From the data, however, it may be concluded that it is possible to bring about similar alteration in a single meristic character by duplicating the effects of low and high temperature upon metabolism with other environmental agents that alters the energetics of a developing embryo in a similar manner.

Other meristic series:

The above conclusions cannot, however, be applied wholly to the dorsal, anal and total caudal fin ray counts obtained in the majority of the experiments. In sustained temperature, dorsal ray counts of different genotype followed a somewhat similar pattern, but anal and caudal ray counts differed widely. This makes it difficult to compare the effect of temperature on these characters with those obtained in thyroxine, thiourea, dinitrophenol and urethan. Results obtained by rearing larvae in thyroxine and thiourea solution indicate that by stimulating the metabolism with thyroxine, dorsal, anal and caudal fin ray counts can be reduced whereas inhibition induced by thiourea increases anal rays and tends to increase dorsal rays. Decrease of total caudal rays in both thyroxine and thiourea solutions is comparable to the results obtained in some genotype where caudal ray counts showed an inverted V-shaped relationship to temperature with lowest mean counts at both highest and lowest temperatures. Decrease or increase of metabolism by both low or high temperature and thiourea or thyroxine perhaps affect the enzyme systems in an identical manner thereby producing similar or closely similar effects on the ultimate expression of the number of caudal fin rays. The effect of dinitrophenol on anal and caudal fin rays was similar to the effect of high temperature on that genotype (genotype Y). Dawson (1938) suggested similarity between the effects produced by dinitrophenol, high temperature and thyroxine on the embryo of frog, Rana pipiens. As pointed out earlier, dinintrophenol resembled the action of low (or high) temperature and thiourea in its

effect on vertebrae, but resembled the action of low temperature and thiourea in its effect on pectoral rays. Although it is difficult to visualize how dinitrophenol can simulate effects of both thyroxine and thiourea at the same time, it may be pointed out that differentiation of the different meristic series is a very complex process and manifestation of each series is probably controlled by different enzyme systems which become activated at different stages of development. Dinitrophenol alters the energetics of the growing embryo and the alteration may have completely different effect on these different reaction systems.

Conclusion:

The expression of meristic characters are apparently dependent upon the metabolic activity and therefore on the energetics of the growing embryo. At an intermediate temperature metabolism is high, and low vertebral counts are produced. At higher temperatures metabolism becomes uneconomic and the control breaks down as reflected by a reverse effect on the number of vertebrae. Similarly, in low temperature also uneconomic metabolism occurs which produces the same effect on vertebrae as high but unfavourable temperature. Pectoral rays, on the other hand, are reduced progressively with increase in temperature. Temperature affects all the metabolic processes and acts as a general controlling factor. Thyroxine and thiourea, on the other hand, probably alters some aspects of metabolism only. Thyroxine probably increases metabolism in some of its aspects and also lowers meristic series in general, whereas thiourea lowers metabolism and also tends to increase the meristic characters. Dinitrophenol reduces supply of energy necessary for growth or differentiation by uncoupling phosphorylation thereby increasing the number of vertebrae. Urethan acts also in a similar manner.

Salinity upsets the energetics of the developing embryo by forcing the expenditure of energy for osmotic or ionic regulation. Comparable meristic increases resulting from unfavourable conditions (low oxygen concentration or high carbon dioxide concentration) were shown in sea trout by Tåning (1952). Thyroxine duplicates the effect of high temperature and photoperiod in its effect on certain characters whereas thiourea produces the effect of low temperature. Dinitrophenol, urethan and salinity parallels the effect of low or high (unfavourable) temperature in regard to vertrebrae whereas/the effects of dinitrophenol on pectoral rays, and urethan are identical to the effects of thiourea and low temperature. Since metabolism is the sum of total of all enyzme activities, it would be worth while to isolate the enzyme system or systems for each meristic series and study the effects of the environment upon each enzyme system separately.

Certain factors which do not bear directly on the theme of metabolism had to be tested in the course of these experiments and these are discussed briefly below.

Effect of yolk diameter:

Tåning (1952) mentioned that in sea trout, no relationship was found between the egg size and the number of vertebrae. No relationship between yolk diameter and different meristic series in steelhead trout Salmo gairdneri was also found (Lindsey, 1962). The data in medaka also failed to show any influence of the yolk diameter on the number of vertebrae and the fin rays. With respect to vertebral count, this result does not agree with Garside and Fry (1959), who suggested that below a critical size, yolk diameters become a

limiting factor and prevents the formation of the normal myomere complement. But no such relationship was evident in the present case, although the eggs of medaka are small and would be lower than the critical size of Garside and Fry. On the contrary, the resultant counts obtained in reciprocal crossings indicated that the characters tended to follow the genetic makeup of the parents regardless of yolk size. Possibility of an association between length of individual fish within the sample and meristic series was looked for in one of the replicates with eggs of genotype F and J and crosses thereof. There was no correlation between size and any of the characters studied i.e. vertebrae, pectoral, anal, dorsal and caudal rays, except anal rays of the sample from the cross between J♀ and F♂ . In other words meristic differences between the individuals were not the result of differences in egg size.

Fixation of meristic characters:

With a series of transfer experiments, Tåning (1946) established that the determination of vertebrae in the sea trout begins during the gastrulation period and a sensitive period for this character prevails during 145-165 day degrees of development. Transfers during this sensitive period may bring about wide variations in the vertebral count as a result of the temperature shock. (Tåning, 1950). Lindsey (1954) demonstrated in the paradise fish that sensitive period for the abdominal and caudal vertebrae are different and the abdominal vertebrae are fixed earlier in development. He also pointed out that emergence from the egg is not a criterion for cessation of environmental influence on the fixation of meristic characters. Using light as the environmental factor, Canagaratnam (1959) found that the sensitive period for the vertebrae in sockeye salmon extended from 142 to 300 day degrees which corresponded

to the stage of development when optic cup and otic capsule have appeared to the stage when the notochord was bent up at the tail and eyes became distinct.

As the correspondence between 0°C and the lowest temperature at which development takes place is not known, the use of day degrees to reflect developmental process is somewhat arbitrary and questionable. This may account in part for the differences in stages of development at comparable day degrees in two sets of transfers shown in Figure 11. Day degrees (calculated assuming biological zero as equal to 0°C) have been used here only to represent the results of transfers made from both directions of temperature on the same scale.

Results of the present experiment demonstrate that the temperature-sensitive period for vertebrae in medaka extends from approximately 40 to 120 day degrees (corresponding to the embryonic shield stage to the stage when pectoral buds have appeared: 48 to 144 hours in 20°C).

This result is more or less similar to the findings of Tåning (1946) in terms of the stages of embryonic development. Results on the effect of temperature shock are, however, not in agreement. It has been hypothesized that consistently fewer elements form when the embryos in their sensitive period for a particular meristic series are transferred from low to high temperature (Barlow, 1961). But the mean vertebral counts of the transfer lots in this experiment suggest that this is not always true. Similar exception to the generalization of Barlow (1961) was also demonstrated by Orska (1957: in vertebral count) and Lindsey (1954: in anal rays).

Although the number of pectoral rays are not yet finally fixed even after hatching (as demonstrated in experiment XId: rearing of larvae in thyroxine and thiourea after hatching), the sensitive period for this character seemed to commence quite early in the embryonic life, i.e. shortly after the pectoral fin bud has appeared. During the early part of the sensitive period, the number of rays increased in spite of the temperature shocks. This is another instance where Barlow's generalization (consistent lower number of element in low to high temperature transfer shocks) is not applicable.

Experiment XId also demonstrated that the final number of anal, dorsal and total caudal rays are still alterable after hatching. But the results of the transfers from low to high temperature suggest that formation of anal rays commences quite early and in the earliest part of the sensitive period, temperature shock results in a significant decrease in the mean anal ray count (see the result of samples transferred at the end of 96 and 120 hours of development in the lower temperature). Lindsey (1954) found that mean anal count in paradise fish showed a significant increase when transferred from low to high temperature after 22 days development in the former. Though no transfer corresponding to the transfer for paradish fish was made for medaka, it may be postulated that the anal rays are decreased or increased by temperature shock during the sensitive period depending on the stage of differentiation (biochemical and biophysical) reached at the time shock is applied.

From the data on dorsal and caudal rays, it can only be suggested that the sensitive period probably commences in the former at a quite later stage in development (approximately 220 day degrees) while in the latter, this period coincides with the stage of development reached at 100 day degrees.

Unfortunately, the mean counts of anal, dorsal and caudal rays of low and high temperature controls were not different and as such, a satisfactory interpretation of the variations obtained in the transferred lots is difficult.

The mechanism through which temperature transfer affects the meristic character is probably the alteration of metabolic pattern. In his work with sea trout, Marckmann (1958) found that the rate of respiratory metabolism of the eggs transferred permanently to high temperature changes and becomes adapted to the rate of the eggs reared continuously in the higher temperature. The metabolic rate of eggs transferred from high to low temperature, on the other hand, continued to be somewhat lower than that of the lot reared continuously in the lower temperature. In other words, development of the lot transferred to lower temperature was delayed compared to the control lot in that temperature. Information on hatching time in the present experiment, however, does not reveal any clear pattern in regard to alteration of metabolism.

Effect of extraneous factors:

(Battle 1944) found that developing eggs of threespined sticklebacks, killifish and fourbearded rockling, Enchelyopus cimbrius were sensitive to mechanical shocks. This sensitivity was most acute in the early cleavage and blastula stages and decreased gradually with progress in gastrulation and epiboly. Similar results were also described for cod eggs by Rollefsen (1930, 1932, cited in Battle, 1944). Application of mechanical shock by dropping the eggs resulted in abnormality in the notochord. In the present experiments, eggs of medaka were shaken daily beginning from fertilization.

No abnormality in the vertebral column was found in treated individuals nor any significant alteration in vertebral counts or other meristic characters except anal rays. Anal rays appear late in the ontogeny and the variation (decrease in the shaken lots) found in the treated lots was probably not the result of shaking. Similar variation in the anal counts but in no other series was observed when two or three lots of eggs from the same parent were reared separately under identical conditions. The source of this variation was not clear.

Retarding effects of low levels of dissolved oxygen upon developing embryos have been described for many species of fish, (Seymour 1956, 1959: Garside 1959, 1960). This effect increases at higher temperature (Garside, 1959). Further, the effect tends to be stronger in stagnant water, as gaseous exchange on the egg surface is less efficient than in water with movement (Kinne and Kinne 1962). But the hatching time of the eggs of medaka reared in bottles without any aeration at a high temperature does not indicate any lowering of developmental rate. In terms of incubation period and hatching time, the lot in non-aerated bottle required less than half of the time required for the control lot (in aerated bottle) to complete 50% hatching. This accelerated hatching may be due to lowered oxygen availability in water of the non-aerated bottle. Condition of asphyxia was found to induce hatching of salmon eggs 5 to 7 days in advance of the due period (Trifonova, 1937). Milkman (1954) reported that hatching of the eggs of Fundulus can be delayed indefinitely by putting them in sea water with high oxygen tension. Hatching time does not really reflect the rate

of development. Mean vertebral count of the lot in basket (water aerated and gradually replaced in the surrounding bath) did not differ from lots in aerated water or in nonaerated water in bottles. Variation of mean vertebral count of medaka cannot, therefore, be attributed to a variation in the oxygen level in water.

Wide variation in the initial number of eggs used in any particular treatment or the quality of eggs obtained from the same parent on different days did not produce any change in the mean vertebrae, pectoral and dorsal ray counts. Anal and caudal ray counts, however, responded differently. The mean anal and caudal ray counts of the lot from smallest number of eggs (25 eggs) was greater as compared to the mean counts of samples with higher density. The difference in anal ray became significant when the egg density was increased by four or eight times. The caudal ray counts were altered in some instances where the density was doubled only. Contrary to the above, in successive days eggs experiment, the anal and caudal ray counts were higher in lots with lesser density. This is not attributable to the difference in the time of rearing of the two lots as the lot with higher mean anal and caudal rays was reared for lesser time than the lots having lower mean counts. Other possible factors contributing to this variation could be social interaction or "space factor" (Vladykov 1934) involving the accumulation of hormones, waste products and other solutes in confined quarters. In the remaining experiment on extraneous factors, no difference in anal and caudal ray counts of the control and treatment lots of each genotype occurred although the density of the corresponding populations was somewhat different. From this, it appears that density has to be beyond a certain level before any effect becomes apparent on the anal and caudal rays.

Other extraneous factors like altering the medium with malachite green or pricking the chorion also had no influence on meristic characters. The decrease in the anal count in the samples from pricked eggs was caused by some unknown factor. In summary, extraneous factors (malachite green treatment of eggs, quality of eggs of different dates from same parent, nature of rearing containers and slight alteration in aeration, egg density, shaking of eggs, pricking the chorion of eggs) do not affect the number of vertebrae, pectoral rays and dorsal rays, whereas the fixation of anal and caudal rays is complicated by some factors that could not be isolated by these experiments

Selective mortality:

Some experimental studies with different environmental factors (Tåning 1952, Lindsey 1962 and Molander and Molander-Swedmark 1957 with temperature; Canagaratnam 1959 with light) showed that the influence of selective mortality on meristic variability could not be excluded, except in Tåning's (1944) experiment on sea trout. Heuts (1947) demonstrated that in sticklebacks selective mortality with respect to number of lateral plates occurred before hatching.

In the present series of experiment, mortality before hatching and up to preservation was lower in general, and almost insignificant in many cases (e.g. genotypes U, W, X and Y in experiment IX; genotypes S, N and reciprocal cross thereof in experiment VII; genotypes S, V and Y in experiment XI.a). Significant variation in one or more meristic series in different treatment as demonstrated in the genotypes cited above cannot

therefore be attributed to the effect of selective mortality. Results obtained from genotypes showing higher mortality in some experiments were identical to those from genotypes with low mortality,(e.g. vertebral count in higher concentration of thyroxine of genotype V and P). From these and other results presented, it may be concluded that meristic variations are not a by-product of selective mortality amongst fish with different genetically controlled meristic counts, but are the direct outcome of phenotypic alteration by the treatments.

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Appendix I. Frequency distribution fo total vertebrae in experiment IX: Effect of sustained temperature.

Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
(1) Parent: A							
20°			14	2	16	31.13	Higher than 26°C (P<.01).
22°(a)		2	8		10	30.80	
22°(b)		7	18		25	30.72	
24°		16	16	1	33	30.54	
26°		4	2		6	30.33	Not different from 28° and 32°C (P>.05).
28°		10	18		28	30.64	
30°		2	4		6	30.66	
32°(a)		6	5		11	30.45	
32°(b)		7	3		10	30.30	
(2) Parent: B							
22°		2	12	1	15	30.93	
28°		19	6		25	30.24	Lower than 22°C (P<.01).
32°		9	7		16	30.44	Not different from 28°C (P>.05).

Appendix I. contd. Frequency distribution of total vertebrae in experiment IX: Effect of sustained temperature.

Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
(3) Parent: C							
20°(a)		3	10		13	30.77	Reared entirely in 20°C.
20°(b)		9	16	1	26	30.69	Hatched and reared in 26°C after 15-19 days in 20°C.
20°(c)		2	6	1	9	30.89	Reared in 26°C after hatching.
22°	1	7	8	1	17	30.53	
24°(a)	4	35	10		49	30.12	
24°(b)	3	20	3		26	30.00	
26°(a)	5	38	12		55	30.12	
26°(b)	2	21	3		26	30.04	
30°	6	23			29	29.79	
32°	4	23	1		28	29.89	
(4) Parent: D							
22°			11		11	31.00	
24°(a)		1	10		11	30.91	
24°(b)		1	9		10	30.90	
24°(c)		2	4		6	30.67	Not different from 24°(a) of (b).
26°		10	10		20	30.50	
32°		2	7		9	30.78	

Appendix I continued. Frequency distribution of total vertebrae in experiment IX: Effect of sustained temperature.

Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
(5) Parent: E							
20°	9	27			36	30.75	
24°	13	3			16	30.19	Lower than 20°C (P<.01).
28°	15	6			21	30.29	
32°	5	5			10	30.50	
(6) Parent: G							
20°		4			4	31.00	
22°	3	5			8	30.62	
24°	10	19			29	30.65	
26°	4	30			34	30.88	
28°	5	10			15	30.67	
32°	8	25			33	30.76	
(7) Parent: H							
20°	3	54	8		65	31.09	Reared in 24°C after hatching.
22°	6	45	1		52	30.90	
24°	17	46			63	30.73	
26°	3	32	1		36	30.94	
28°	6	20			26	30.77	
30°	15	55	1		71	30.77	
32°	21	103	2		126	30.85	

Appendix I continued. Frequency distribution of total vertebrae in experiment IX: Effect of sustained temperature.

Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
(8) Parent: I							
20°		1	13		14	30.93	Reared in 24°C after hatching.
22°	1)	10	23		33	30.70	
24°		31	30		61	30.49	
26°		15	13		28	30.46	Lower than 20°C (P<.01).
28°		20	34		54	30.63	
30°	1	23	51		75	30.67	
32°		5	55		60	30.91	Higher than 26°C (P<.01).
34°			3	4	7	31.57	Reared in 32°C after hatching.
(9) Parent: K							
20°		1	30	3	34	31.06	Higher than 28°C (P<.01).
22°		5	22	1	28	30.86	
24°	1	25	19	1	46	30.43	
26°		18	9		27	30.33	
28°		17	5		22	30.22	
30°		33	13		46	30.28	
32°(a)	1	11	8		20	30.35	Not different from 28°C (P>.05).
32°(b)	1	16	11		28	30.36	Not different from 28°C (P>.05).

Appendix I continued. Frequency distribution of total vertebrae in experiment IX: Effect of temperature.

Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
(10) Parent: Q							
24°		17	13		30	30.43	
28°		11	16		27	30.59	Not different from 24° and 32°C.
32°	1	7	8		16	30.44	
(11) Parent: R							
20°			8	6	14	31.43	
22°		1	31	4	36	31.08	
26°		19	54	1	74	30.76	
28°		13	28		41	30.68	
30°	1	13	14	1	29	30.51	
32°	1	9	9		19	30.42	
34°		9	8	1	18	30.56	Reared in 32°C after hatching.
(12) Parent: U							
20°		1	22	13	36	31.33	
24°(a)		2	27	1	30	30.97	
24°(b)		3	30	3	36	31.00	
28°		7	41	3	51	30.92	
30°		7	22	1	30	30.80	
32°		20	50	1	71	30.73	Lower than 20°C ($P<.01$).

Appendix I continued. Frequency distribution of total vertebrae in experiment IX: Effect of sustained temperature.

Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
(13) Parent: W							
22°		4	41	5	50	31.02	
26°		15	35		50	30.70	Lower than 22°C (P<.01).
30°		33	17		50	30.34	Lower than 26°C (P<.01).
32°		35	15		50	30.30	
34°		7	2		9	30.22	Lower than 22° (P<.01).
(14) Parent: X							
22°		6	46	14	66	31.12	
26°		13	36	4	53	30.83	
30°		30	39		69	30.56	Lower than 22°C (P<.01).
32°		27	39		66	30.59	
34°		11	28	2	41	30.78	Tends to be higher than 30°C (P<.05) Lower than 22°C (P<.01).
(15) Parent: Y							
20°			37	54	91	31.59	Higher than 24°, 26°, 30°, & 32°C (P<.01).
24°		5	79	6	90	31.01	
26°		8	71	8	87	31.00	
30°		13	79	3	95	30.89	
32°		17	42	5	64	30.81	
34°(a)			12	4	16	31.25	
34°(b)			1	3	4	31.75	a+b+c=Mean= 31.34 is higher than 32°(P<.01) and lower than 20°(P<.01).
34°(c)			6	3	9	31.33	and lower than 20°(P .01).

Appendix II. Frequency distribution of pectoral rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Pectoral rays 9	10	11	12	13	Number	Mean	Remarks
(1) Parent: A								
20°			11	14		25	11.56	
22°			33	22	1	56	11.43	
24°		3	19	10		32	11.22	
28°			24	4		28	11.14	
32°			13	1		14	11.07	
(2) Parent: B								
22°		1	12	2		15	11.07	
28°		7	18			25	10.72	
32°		13	3			16	10.19	Lower than 20°C (P<.01).
(3) Parent: C								
20°				11	2	13	12.15	Reared entirely in 20°C.
22°		1	7	9		17	11.47	
24°(a)		1	16	9		26	11.31	
24°(b)		1	27	20		48	11.39	
26°		2	35	18		55	11.29	
30°		4	25			29	10.86	
32°	2	5	19	2		28	10.75	Lower than 20°C and 22°C (P<.01).

Appendix II. continued. Frequency distribution fo pectoral rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Pectoral rays 9	10	11	12	13	Number	Mean	Remarks
(4) Parent: D								
22°			4	7		11	11.64	
24°		1	18	6		25	11.20	
26°		4	13	2		19	10.89	Not different from 32°C (P>.05).
32°		1	5	3		9	11.22	
(5) Parent: E								
20°		3	17	15		35	11.34	Hatched by temp. shock and reared in 24°C after hatching.
24°			14	1		15	11.07	
28°		1	15	5		21	11.19	
32°			8	1		9	11.11	
(6) Parent: G								
20°				4		4	12.00	Hatched and reared in 24° after hatching.
22°			4	2		6	11.33	
24°			18	11		29	11.38	
26°		5	24	2		31	10.90	
28°		1	13			14	10.93	
32°		1	23	9		33	11.24	

Appendix II continued. Frequency distribution of pectoral rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Pectoral rays 9	10	11	12	13	Number	Mean	Remarks
(7) Parent: H								
20°			20	32	2	54	11.67	Hatched by temp. shock and reared in 24°C after hatching.
20°			3	6	1	10	11.80	Reared entirely in 20°C.
22°			19	31	2	52	11.67	
24°		4	28	7		39	11.08	
26°		2	28	3		33	11.03	
28°		2	19	5		26	11.11	
30°		9	43	2		54	10.87	
32°		31	85	4		120	10.77	
(8) Parent: I								
20°			17	11		28	11.39	Reared in 24° after hatching.
22°		1	36	25	2	64	11.44	
24°		3	86	8		97	11.05	
26°		6	46	4		56	10.96	
28°		14	93	1		108	10.88	
30°	2	55	85			142	10.58	
32°		47	12			59	10.20	

Appendix II continued. Frequency distribution of pectoral rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Pectoral rays 9	10	11	12	13	Number	Mean	Remarks
(9) Parent: K								
20°			40	22	4	66	11.45	
22°	1	2	34	19		56	11.27	
24°			29	7		36	11.19	
26°		2	47	3		52	11.02	
28°		5	35	4		44	10.98	
30°		9	75	7		*92	10.95	* 1 fish with 8 rays.
32°	1	35	57	3		96	10.64	
(10) Parent: Q								
24°		6	41	11		58	11.08	
28°		8	38	5		51	10.94	
32°		7	24	1		32	10.81	
(11) Parent: R								
20°			1	23	4	28	12.11	Reared in 24°C after hatching.
22°		1	22	49		72	11.67	
26°		3	88	57		148	11.37	
28°		3	23	14		40	11.30	
30°		9	43	5		57	10.93	
32°	1	24	13			38	10.32	Lower than 22°C ($P<.01$).

Appendix II continued. Frequency distribution of pectoral rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Pectoral rays 9	10	11	12	13	14	Number	Mean	Remarks
(12) Parent: U									
20°			2	50	20		72	12.25	
24°			27	91	14		132	11.90	Two samples combined; lower than 20°C (P<.01).
28°		2	65	33	2		102	11.34	
30°			23	7			30	11.23	
32°		10	121	11			142	11.00	
(13) Parent: W									
22°			5	87	8		100	12.03	
26°			42	56	2		100	11.60	Lower than 22°C (P<.01).
30°		22	77	1			100	10.79	Lower than 26°C (P<.01).
32°		39	59	2			100	10.63	
(14) Parent: X									
22°			8	101	23		132	12.11	
26°			32	67	7		106	11.76	Lower than 22°C (P<.01).
30°		1	59	77	1		138	11.56	
32°		4	100	26			130	11.17	Lower than 30°C (P<.01).
34°		16	64	2			82	10.83	Lower than 32°C (P<.01).
(15) Parent: Y									
24°				41	136	3	180	12.79	
26°				63	106	3	172	12.65	
30°			3	140	46	1	190	12.24	Lower than 26°C (P<.01).
32°			28	96	4		128	11.81	Lower than 30°C (P<.01).
34°			13	9			22	11.40	Lower than 32°C (P<.01); Fin rays of fish reared entirely in 34°C.

Appendix III. Frequency distribution of anal rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Anal rays 17	18	19	20	21	22	Number	Mean	Remarks
(1) Parent: A									
20°	4	9	3				16	17.94	Reared in 24°C after hatching.
22°	9	17	6	1			33	17.97	Tends to be lower than 28°C (P<.02).
24°	6	21	5	1			33	18.03	
26°	1	2	3				6	18.33	
28°	2	14	11		1		28	18.43	
30°	2	3	1				6	17.83	
32°	1	4	4	2			11	18.64	Tends to be higher than 22°C (P<.02).
(2) Parent: B									
22°		3	11	1			15	18.87	
28°		1	12	9	3		25	19.56	Higher than 22°C (P<.01).
32°		3	3	8	2		16	19.56	Higher than 22°C (P<.01).
(3) Parent: C									
20°		1	8	4			13	19.25	Reared entirely in 20°C.
22°			11	5	1		17	19.41	Lower than 30°C (P<.01).
24°(a)		5	25	15	3		48	19.33	
24°(b)		2	13	11			26	19.35	
26°(a)		2	14	28	11		55	19.87	
26°(b)		3	16	7			26	19.15	
30°			3	18	8		29	20.17	Higher than 20°C (P<.01).
32°		1	7	16	4		28	19.82	Tends to be higher than 20°C (P<.02).

Appendix III. Frequency distribution of anal rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Anal rays 17	18	19	20	21	22	Number	Mean	Remarks
(4) Parent: D									
22°		1	8	2			11	19.09	Lower than 32°C (P<.01).
24°(a)		1	5	4	1		11	19.45	
24°(b)			7	1	2		10	19.50	
24°(c)		1	3	2			6	19.17	Not different from 24°a or c.
26°			11	8	1		20	19.50	
32°			1	4	3	1	9	20.44	Higher than both 24°a and c.
(5) Parent: E									
20°		7	26	2			35	18.86	Reared in 24°C after hatching.
24°		4	11	1			16	18.81	
28°		2	12	7			21	19.24	Tends to be higher than 24°C (P<.05;>.02).
32°		5	5				10	18.50	Tends to be lower than 24°C (P<.05;>.02). Lower than 28°C (P<.01).

Temp (°C)	Anal rays 15	16	17	18	19	20	21	Number	Mean	Remarks
(6) Parent: G										
22°		1	1	6				8	17.62	
24°			6	21	2			29	17.86	
26°		1	2	11	18	1		33	18.48	Higher than 22°C (P<.01).
28°			3	9	3			15	18.00	Not different from 26°C.
32°				12	17	4		33	18.76	Higher than 22°C (P<.01).

Appendix III continued. Frequency distribution of anal rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Anal rays 15	16	17	18	19	20	21	Number	Mean	Remarks
(7) Parent: H										
20°	1	3	5	1				10	16.60	Reared entirely in 20°C.
22°		5	36	11				52	17.12	Higher than 20°C (P<.01).
24°		1	28	26	2			57	17.51	
26°		2	14	15	2			33	17.48	
28°				16	10			26	18.38	Higher than 24° & 22°C (P<.01)
30°			5	40	23	1		69	18.30	
32°			12	63	45	6		126	18.36	
(8) Parent: I										
22°			11	17	5			33	17.82	
24°			4	31	25			60	18.35	Higher than 22°C (P<.01).
26°			4	8	14	2		28	18.50	
28°			3	21	28	2		54	18.54	
30°				18	42	13	1	74	18.92	Higher than 24°C (P<.01).
32°				3	19	4		26	19.04	Higher than 24°C (P<.01).
(9) Parent: K										
22°			1	10	12	5		28	18.75	
24°			1	15	22	8		46	18.80	
26°				10	14	3		27	18.74	
28°				1	10	10	1	22	19.50	Higher than 22°C (P<.01).
30°				2	20	20	4	46	19.57	
32°(a)				1	5	7	7	20	20.00	Higher than 22°C (P<.01).
32°(b)				5	10	10	3	28	19.39	Lower than 32°(a) (P<.05). Higher than 22°C (P<.01).

Appendix III continued. Frequency distribution of anal rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Anal rays 15	16	17	18	19	20	21	Number	Mean	Remarks
(10) Parent: Q										
24°			1	3	15	10	1	30	19.23	
28°					11	11	4	26	19.73	Tends to be higher than 24°C (P<.05).
32°				5	8	3		16	18.88	Lower than 28°C (P<.01). Not different from 24°C.

Temp (°C)	Anal rays 17	18	19	20	21	22	Number	Mean	Remarks
(11) Parent: R									
22°		2	9	17	6	2	36	19.92	Lower than 32°C (P<.01).
26°		4	14	48	7	1	74	19.82	
28°			11	19	11		41	20.00	
30°		2	9	14	3		*29	19.48	*1 fish with 15 rays. Not different from 22°C (P>.05)
32°	2	6	8	2	1		19	18.68	Tends to be lower than 30°C (P<.02).
(12) Parent: U									
20°		2	9	18	5	2	36	19.89	
24°(a)			5	16	9		30	20.13	Tends to be higher than 24°C (b).
24°(b)			11	22	3		36	19.78	
28°			14	22	13	2	51	20.06	
30°		1	9	17	3		30	19.73	
32°		3	19	35	11	3	71	19.89	

Appendix III continued. Frequency distribution of anal rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Anal rays 17	18	19	20	21	22	Number	Mean	Remarks
(13) Parent: W									
22°	7	29	12	1	1		50	18.20	
26°	9	31	10				50	18.02	
30°	7	29	14				50	18.14	
32°	11	24	14	1			50	18.10	
(14) Parent: X									
22°		9	30	23	3	1	66	19.35	
26°		22	16	28	6	1	53	19.77	Higher than 22°C (P<.01).
30°		1	14	38	12	4	69	20.06	
32°		4	17	34	10	1	66	19.80	
34°		2	12	17	10		41	19.85	Higher than 22°C (P<.01).
(15) Parent: Y									
24°		21	50	18	1		90	18.99	
26°	2	28	44	12	1		87	18.79	
30°		39	52	4			95	18.63	
32°	5	36	19	4			64	18.34	
34°	4	5	2				11	17.81	Lower than 24°C (P<.01). Tends to be lower than 32°C (P<.05).

Appendix IV. Frequency distribution of dorsal rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean	Remarks
(1) Parent: A							
22°	2	30	1		33	5.97	
24°		30	3		33	6.09	
28°		27	1		28	6.04	
32°		14			14	6.00	
(2) Parent: B							
22°		12	2		14	6.14	
28°		19	6		25	6.24	
32°		16			16	6.00	
(3) Parent: C							
20°		10	3		13	6.23	Reared entirely in 20°C.
22°		11	6		17	6.35	
24°(a)		19	28		47	6.60	
24°(b)		12	14		26	6.54	
26°		15	39	1	55	6.74	
30°		9	20		29	6.69	
32°		16	12		28	6.43	
(4) Parent: D							
22°		10	1		11	6.09	
24°		15	12		27	6.44	
26°		13	7		20	6.35	
32°		7	2		9	6.22	

Appendix IV. continued. Frequency distribution of dorsal rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean	Remarks
(5) Parent: E							
20°		19	17		36	6.47	Reared in 24°C after hatching.
24°		12	4		16	6.25	
28°		14	7		21	6.33	
32°		9			9	6.00	
(6) Parent: G							
22°	1	6			7	5.86	
24°		26	3		29	6.10	
26°		27	6		33	6.18	
28°		15			15	6.00	
32°		20	11	2	33	6.45	
(7) Parent: H							
22°	1	51			52	5.98	
24°	5	50	4		59	5.98	
26°	1	32			33	5.99	
28°		26			26	6.00	
30°		65			65	6.00	
32°	1	124			125	5.99	

Appendix IV continued. Frequency distribution of dorsal rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean	Remarks
(8) Parent: I							
22°		32			32	6.00	
24°		42	15		57	6.26	
26°		24	4		28	6.14	
28°		46	8		54	6.14	
30°		52	22		74	6.32	
32°		41	7		48	6.15	
(9) Parent: K							
22°		25	3		28	6.10	
24°		28	8		36	6.22	
26°		21	6		27	6.22	
28°		17	5		22	6.23	
30°		39	7		46	6.15	
32°		41	7		48	6.15	
(10) Parent: Q							
24°		20	9		29	6.31	
28°		17	9		26	6.35	
32°		13	3		16	6.19	

Appendix IV continued. Frequency distribution of dorsal rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean	Remarks
(11) Parent: R							
22°	2	20	14		36	6.33	
26°		30	43	1	74	6.60	
28°		14	27		41	6.66	
30°		20	9		29	6.31	
32°		14	5		19	6.26	
(12) Parent: U							
20°	1	31	4		36	6.08	
24°		59	7		66	6.10	
28°		38	13		51	6.25	
30°		25	5		30	6.17	
32°		53	18		71	6.25	
(13) Parent: W							
22°		39	11		50	6.22	
26°		41	9		50	6.18	
30°		33	16	1	50	6.36	
32°		41	9		50	6.18	

Appendix IV continued. Frequency distribution of dorsal rays in experiment IX: Effect of sustained temperature.

Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean	Remarks
(14) Parent: X							
22°		59	7		66	6,10	
26°		38	15		53	6.28	
30°		58	11		69	6.16	
32°		60	6		66	6.09	
34°		40	1		41	6.02	
(15) Parent: Y							
24°		68	22		90	6.24	
26°	1	64	22		87	6.24	
30°		76	18	1	95	6.21	
32°	2	57	5		54	6.05	
34°		7			7	6.00	

Appendix V. Frequency distribution of total caudal rays in experiment IX. Effect of temperature.

Temp (°C)	Total caudal rays 19	20	21	22	23	24	25	26	Number	Mean	Remarks
(1) Parent: A											
22°	1	1	3	4	12	4	4		29	22.83	Lower than 28°C (P<.01).
24°				5	18	8	1		32	23.15	
28°				1	9	13	5		28	23.79	Not different from 32°C (P>.05).
32°				1	5	5			11	23.36	Not different from 22°C (P>.05).
(2) Parent: B											
22°			1	3	6	5			15	23.00	Lower than 28°C (P<.01).
28°				2	6	10	7		25	23.88	
32°			2	2	7	1	2	2	16	23.31	Not different from 28° or 22°C (P>.05).
(3) Parent: C											
22°			2	8	4	3			17	22.47	
24°(a)			14	13	18	3			48	22.21	
24°(b)	1		5	6	11	3			26	22.35	
26°(a)				8	22	18	5	2	55	23.47	
26°(b)			1	6	8	10	1		26	23.15	Not different from 26°(a) (P>.05).
30°			1	7	14	7			29	22.93	Not different from 22°C (P>.05).
32°			2	7	9	9	1		28	23.00	
(4) Parent: D											
22°					4	1	4	2	11	24.36	
24°				4	7	8	7	1	27	23.78	Not different from 22°C (P>.05).
26°				2	3	12	2		*20	23.90	*1 fish with 27 rays.
32°				1		5	2	1	9	24.22	

Appendix V continued. Frequency distribution of total caudal rays in experiment IX: Effect of temperature.

Temp (°C)	Total caudal rays 19	20	21	22	23	24	25	26	Number	Mean	Remarks
(5) Parent: E											
24°			4	4	5	3			16	22.44	
28°			2	4	8	6			*21	22.67	* 1 with 18 rays.
32°				1	2	4	2		9	23.78	Higher than 24° and 28°C (P<.01).
(6) Parent: G											
22°			1	1	3	2			7	22.86	Not different from 24°C (P>.05).
24°				2	13	10	3	1	29	23.58	
26°		1	2	2	12	14	1	1	33	23.30	
28°			1	2	4	6	2		15	23.40	Not different from 32°C (P>.05).
32°			1	2	5	17	8		33	23.88	Tends to be higher than 26°C (P<.05).
(7) Parent: H											
22°		3	3	6	26	10	3	1	52	22.96	
24°			7	11	29	3			50	22.96	
26°			7	3	21	2			33	22.54	Not different from 22°C (P>.05).
28°			4	3	9	6	4		26	23,11	
30°		1	4	13	20	18	3	2	61	23.48	Tends to be higher than 22°C (P<.05).
32°		1	14	21	26	44	11	1	118	23.14	Tends to be lower than 30°C (P<.05).
(8) Parent: I											

Appendix V continued. Frequency distribution of total caudal rays in experiment IX: Effect of temperature.

Temp (°C)	Total caudal rays 19	20	21	22	23	24	25	26	Number	Mean	Remarks
(8) Parent: I											
22°			1	8	13	6	2	1	31	23.13	
24°			6	10	30	9			55	22.76	
26°			1	2	11	7	1		28	22.75	
28°			5	7	17	22	3		54	23.20	
30°		1	11	13	25	17	3		70	22.78	
32°			6	10	7	6			29	22.45	
(9) Parent: K											
22°			3	5	15	4	1		28	22.82	
24°			4	11	13	7	1		36	22.72	
26°			2	4	14	7			27	22.93	
28°			4	4	8	6			22	22.73	
30°		1	3	8	11	19	4		46	23.21	
32°(a)			5	7	6	2			20	22.25	
32°(b)			3	5	11	9			28	22.93	Tends to be higher than 32°(a).
(10) Parent: Q											
24°		1		11	10	7			29	22.76	
28°			3	5	5	11	3		27	23.22	Not different from 24°C ($P>.05$).
32°		1		2	3	8	2		16	23.44	Tends to be higher than 24°C ($P<.05$).

Appendix V continued. Frequency distribution of total caudal rays in experiment IX: Effect of temperature.

Temp (°C)	Total caudal rays 19	20	21	22	23	24	25	26	Number	Mean	Remarks
(11) Parent: R											
22°			12	12	9	3			36	22.08	
26°		1	22	15	28	7	1		74	22.28	
28°		1	10	9	16	4	1		41	22.37	
30°			3	3	14	6	3		29	23.10	Higher than 28°C (P<.01).
32°			2	2	10	2	3		19	23.10	Tends to be higher than 28° (P<.05).
(12) Parent: U											
20°			1	7	20	6		2	36	23.08	
24°(a)				2	11	14	3		30	23.60	
24°(b)			1	11	12	9	2	1	36	23.08	Tends to be lower than 24°(a) (P<.05).
28°			1	4	23	21	1		*51	23.41	*1 with 27 rays.
30°			1	7	13	8			29	22.97	Tends to be lower than 28°C (P<.05).
32°			2	8	34	13	14		71	23.56	Higher than 30°C (P<.01).
(13) Parent: W											
22°	2	1	27	9	11				50	21.54	
26°			8	13	21	7	1		50	22.60	Higher than 22°C (P<.01).
30°			9	10	23	6	1		*50	22.64	*1 with 27 rays.
32°		2	11	9	23	4		1	50	22.40	

Appendix V continued. Frequency distribution of total caudal rays in experiment IX: Effect of temperature.

Temp (°C)	Total caudal rays 19	20	21	22	23	24	25	26	Number	Mean	Remarks
(14) Parent: X											
22°	14	21	22	5	3				*66	20.38	*1 with 18 rays.
26°	1	12	18	15	5	1			*53	21.21	* 1 with 18 rays. Higher than 22°C (P<.01).
30°		6	22	21	17	3			69	21.84	Higher than 26°C (P<.01).
32°	1	1	12	22	25	4	1		66	22.51	Higher than 30°C (P<.01).
34°		3	11	8	14	3	2		41	22.22	Not different from 32°C (P>.05).
(15) Parent: Y											
24°	2	5	35	33	11	3	1		90	21.67	
26°	5	5	32	29	13	3			87	21.56	
30°			9	17	42	19	6		93	22.96	Higher than 26° (P<.01).
32°		1	5	11	24	16	6	1	64	23.10	Higher than 26°C (P<.01). Not different from 30°C.
34°			4	3	3		1		11	22.18	Tends to be lower than 32°C (P<.05).

Appendix VI. Frequency distribution of total vertebrae in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
(1) Parent: G								
.0025% thiourea	24°		2	18	1	21	30.95	
.005% "	24°		4	29	1	34	30.91	
.01% "	24°		1	23	3	27	31.07	
.02% "	24°		1	14	1	16	31.00	
.04% "	24°			11	1	12	31.08	
.05% "	24°		1	16		17	30.94	
(2) Parent: M								
Fresh water control	24°	1	11	3		15	30.13	
0.1 PPM thyroxine	24°		26	20		46	30.43	Tends to be higher than control (P=.05).
0.2 PPM thyroxine	24°		10	6		16	30.37	
0.4 PPM thyroxine	24°		22	7		29	30.24	
(3) Parent: O								
Fresh water control	24°		16	39		55	30.73	
0.2 PPM thyroxine	24°		2	6		8	30.75	
0.4 PPM "	24°		4	16		20	30.80	
0.8 PPM "	24°		10	5		15	30.33	Lower than control (P<.01).
1.6 PPM "	24°		6	2		8	30.25	Lower than control (P<.01).
.01% thiourea	24°		5	14		19	30.74	
.02% "	24°		5	16		21	30.76	
.04% "	24°		6	18		24	30.75	

Appendix VI continued. Frequency distribution of total vertebrae in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
(4) Parent: P								
Fresh water control	24°		4	19		23	30.83	
0.2 PPM thyroxine	24°		6	17		23	30.74	
0.4 PPM "	24°		12	8		20	30.40	Lower than control (P<.01).
0.8 PPM "	24°		2	12	1	15	30.93	Not different from control (P>.05).
.01% thiourea	24°		7	22	1	30	30.80	
.02% "	24°		3	14		17	30.82	
.04% "	24°		2	13		15	30.86	
(5) Parent: Q								
Fresh water control	24°		16	10		26	30.38	
0.8 PPM thyroxine	24°		15	6		21	30.29	
.02% thiourea	24°		10	10		20	30.50	
(6) Parent: S								
Fresh water control	24°			25	6	31	31.19	
0.2 PPM thyroxine	24°			17	27	44	31.61	Higher than control (P<.01).
0.4 PPM "	24°			17	41	58	31.71	Higher than control (P<.01).
0.8 PPM "	24°			14	16	30	31.53	Higher than control (P<.01).
1.6 PPM "	24°			9	13	22	31.59	Higher than control (P<.01).
.01% "	24°			14	16	30	31.53	Higher than control (P<.01).
.02% "	24°			21	9	30	31.30	Not different from control (P>.05).
.04% "	24°			19	11	30	31.37	"

Appendix VI. continued. Frequency distribution of total vertebrae in experiment XI: Effect of thyroxine and thiourea.

(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
(7) Parent: V								
Fresh water control	26°	2	63	5		70	31.04	
0.4 PPM thyroxine	26°	11	36	5		52	30.88	Tends to be lower than control (P<.05; > .02).
0.8 PPM "	26°	8	51	4		63	30.94	Not different from control (P>.05).
3.2 PPM "	26°	16	19	1		36	30.57	489 hrs. in thyroxine & hatched in water; Lower than control (P<.01).
.05% thiourea	26°	5	49	6		60	31.02	Not different from control (P>.05).
(8) Parent: Y								
Fresh water control	26°	12	54	4		70	30.89	
0.4 PPM thyroxine	26°	4	46			50	30.92	
0.8 PPM "	26°	2	42	6		50	31.08	Tends to be higher than control (P<.02).
.02% thiourea	26°	1	38	3		42	31.05	Not different from control (P=.05).

(b) Eggs fertilized and reared in the solutions up to hatching.

(1) Parent: Y								
Fresh water control	26°	12	54	4		70	30.89	
0.8 PPM thyroxine	26°	6	62	9		77	31.04	Tends to be higher than control (P<.05; > .02).
.02% thiourea	26°	2	45	3		50	31.02	Not different from control (P>.05).

Appendix VI continued. Frequency distribution of total vertebrae in experiment XI: Effect of thyroxine and thiourea.

(b) Eggs fertilized and reared in the solutions up to hatching.

Treatment	Temp (°C)	Total vertebrae 29	30	31	32	Number	Mean	Remarks
(2) Parent: a								
Fresh water control	30°		21	12		33	30.36	
0.8 PPM thyroxine	30°	5	21	6	1	33	30.09	Not different from control (P>.05).
.04% thiourea	30°		7	25		32	30.78	Higher than control (P<.01).
(3) Parent: b								
Fresh water control	26°		1	42	3	46	31.04	
0.8 PPM thyroxine	26°		6	19	2	27	30.85	Not different from control (P>.05).
.04% thiourea	26°		1	14		15	30.93	Not different from control (P>.05).

(c) Chorion pricked eggs reared in the solutions up to hatching.

Parent: V								
Fresh water control	26°			25	4	29	31.14	
3.2 PPM thyroxine	26°	1	3	17	1	22	30.81	Tends to be lower than control (P<.02; > .01).
.02% thiourea	26°		4	16	1	21	30.86	Tends to be lower than control (P=.02); Not different from .05% lot in expt. XIa(VII).

(d) Larvae reared in the solutions after hatching.

Parent: V								
Fresh water control	26°		4	29	2	35	30.94	
3.2 PPM thyroxine	26°		6	19	2	27	30.85	
.05% thiourea	26°		4	28	2	34	30.94	

Appendix VII. Frequency distribution of pectoral rays in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	Temp (°C)	Pectoral rays 6	7	8	9	10	11	12	13	Number	Mean	Remarks
(1) Parent: G												
.0025% thiourea	24°						16	4		20	11.20	
.005% "	24°						25	9		34	11.26	
.01% "	24°					1	17	7		25	11.24	
.02% "	24°						10	5		15	11.33	
.04% "	24°						10	2		12	11.17	
.05% "	24°						8	9		17	11.53	*1
(2) Parent: M												
Fresh water control	24°					2	13			15	10.87	
0.1 PPM thyroxine	24°					3	34	1		38	10.95	
0.2 PPM "	24°					5	11			16	10.69	*2
0.4 PPM "	24°				1	12	16			29	10.51	*3

Notes: 1. Tends to be higher than .0025% ($P<.05$).

2. Not different from control ($P>.05$).

3. Tends to be lower than control ($P<.05$).

Appendix VII continued. Frequency distribution of pectoral rays in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	Temp (°C)	Pectoral rays 6	7	8	9	10	11	12	13	Number	Mean	Remarks
(3) Parent: O												
Fresh water control	24°						27	68	2	97	11.74	
0.2 PPM thyroxine	24°						6	2		8	11.25	*1
0.4 PPM "	24°					3	8	9		20	11.30	*2
0.8 PPM "	24°		1	2	6	6				15	9.13	
1.6 PPM "	24°	1	1	2	2	2				8	8.37	
.01% thiourea	24°					1	6	12		19	11.58	*3
.02% "	24°						7	14		21	11.67	
.04% "	24°						5	18	1	24	11.83	
(4) Parent: P												
Fresh water control	24°						18	5		23	11.22	
0.2 PPM thyroxine	24°						16	7		23	11.30	
0.4 PPM thyroxine	24°		1	4	8	5	2			20	9.15	*4
0.8 PPM "	24°				4	10	1			15	9.80	*5
.01% thiourea	24°					1	24	5		30	11.13	
.02% "	24°						14	3		17	11.18	
.04% "	24°					1	12	2		15	11.07	*6

Note:
1. Lower than control ($P<.01$).
2. Lower than control ($P<.01$).
3. Not different from control ($P>.05$).
4. Lower than control ($P<.01$).
5. Lower than control ($P<.01$); Higher than .4PPM ($P<.01$).
6. Not different from control ($P>.05$).

Appendix VII continued. Frequency distribution of Pectoral rays in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	Temp (°C)	Pectoral rays 6	7	8	9	10	11	12	13	Number	Mean	Remarks
(5) Parent: Q												
Fresh water	24°					4	18	2		24	10.91	
0.8 PPM thyroxine	24°					5	14	2		21	10.85	
.02% thiourea	24°					3	11	6		20	11.15	
(6) Parent: S												
Fresh water	24°						6	19		25	11.76	
0.2 PPM thyroxine	24°						22	20	2	44	11.54	
0.4 PPM "	24°						40	18		58	11.31	*1
0.8 PPM "	24°				2	10	16	2		30	10.60	*2
1.6 PPM "	24°			6	13	2	1			22	8.91	
.01% thiourea	24°						10	18	2	30	11.73	
.02% "	24°						6	23	1	30	11.83	
.04% "	24°						12	18		30	11.60	
(7) Parent: V												
Fresh water	26°					8	113	19		140	11.08	
0.4 PPM thyroxine	26°					6	78	20		104	11.13	
0.8 PPM "	26°				4	71	51			126	10.37	*3
3.2 PPM "	26°	1	22	27	19	3				72	8.04	*4
.05% thiourea	26°					3	65	49	3	120	11.43	*5

Note: 1. Lower than control ($P<.01$).
2. Lower than control ($P<.01$).
3. Lower than control ($P<.01$).
4. 489 hrs. in thyroxine and hatched in water.
5. Higher than control ($P<.01$).

Appendix VII continued. Frequency distribution of Pectoral rays in experiment XI: Effect of thyroxine and thiourea.

(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	Temp (°C)	Pectoral rays 6	7	8	9	10	11	12	13	14	Number	Mean	Remarks
(8) Parent: Y													
Fresh water control	26°							63	77		140	12.55	
0.4 PPM thyroxine	26°							54	46		100	12.46	
0.8 PPM "	26°						1	84	15		100	12.14	*1
.02% thiourea	26°							28	53	2	83	12.69	*2

(b) Eggs fertilized and reared in the solutions up to hatching.

(1) Parent: Y													
Fresh water control	26°							63	77		140	12.55	
0.8 PPM thyroxine	26°						1	53	99	1	154	12.65	*3
.02% thiourea	26°							28	71	1	100	12.73	*4
(2) Parent: a													
Fresh water control	30°					2	45	19			66	11.26	
0.8 PPM thyroxine	30°					17	45	4			66	10.80	*5
.04% thiourea	30°					1	40	22	1		64	11.36	*6
(3) Parent: b													
Fresh water control	26°						21	66	5		92	11.83	
0.8 PPM thyroxine	26°					13	37	4			54	10.83	*7
.04% thiourea	26°						2	23	5		30	12.10	*8

Note:
1. Lower than control ($P<.01$).
2. Tends to be higher than control ($P<.05; >.02$).
3. Not different from control ($P>.05$).
4. Higher than control ($P<.01$).
5. Lower than control ($P<.01$).
6. Not different from control ($P>.05$).
7. Lower than control ($P<.01$).
8. Tends to be higher than control ($P<.05$ ~ $.02$).

Appendix VII continued. Frequency distribution fo pectoral rays in experiment XI: Effect of thyroxine and thiourea.

(c) Chorion pricked eggs reared in the solution up to hatching.

Treatment	Temp (°C)	Pectoral rays 5	6	7	8	9	10	11	12	13	Number	Mean	Remarks
Parent: V													
Fresh water control	26°						2	42	14		58	11.21	
3.2 PPM thyroxine	26°				10	18	12	4			44	9.23	*1
.04% thiourea	26°							15	26	1	42	11.67	*2

(d) Larvae reared in the solutions after hatching.

Parent: V													
Fresh water control	26°						6	52	12		70	11.09	
3.2 PPM thyroxine	26°	23	28	3							54	5.63	*3
.05% thiourea	26°						1	21	45	1	68	11.68	*4

Note:
1. Lower than control ($P<.01$); 475 hours in thyroxine and hatched in water; higher than 3.2 PPM in expt. XI a(VII).
2. Higher than control ($P<.01$).
3. Lower than 3.2 PPM means in expt. XI a(VII), and XIc.
4. Higher than control ($P<.01$).

Appendix VIII. Frequency distribution of anal rays in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	Temp (°C)	Anal rays 17	18	19	20	21	22	Number	Mean	Remarks
(1) Parent: G										
.0025% thiourea	24°	1	11	8	1			21	18.43	
.005% "	24°	1	18	15				34	18.41	
.01% "	24°	3	9	14	1			27	18.48	
.02% "	24°	1	12	2	1			16	18.19	
.04% "	24°	1	3	7	1			12	18.67	
.05% "	24°	1	13	3				17	18.12	
(2) Parent: M										
Fresh water control	24°			5	10			15	19.67	
0.1 PPM thyroxine	24°		1	12	21	8	2	44	19.95	*[1]
0.2 PPM "	24°		1	5	7	3		16	19.75	
0.4 PPM "	24°		4	14	9	1	1	29	19.34	*[2]

Note: 1. Not different from control (P>.05).

2. Not different from control (P>.05).

Appendix VIII continued. Frequency distribution of anal rays in experiment XI: Effect of thyroxine and thiourea.

(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	Temp (°C)	Anal rays 17	18	19	20	21	22	Number	Mean	Remarks
(3) Parent: O										
Fresh water control	24°		6	28	15	6		55	19.38	
0.2 PPM thyroxine	24°		2	3	2	1		8	19.25	
0.4 PPM "	24°			6	10	3	1	20	19.95	*[1]
0.8 PPM "	24°		7	4	3	1		15	18.87	*[2]
1.6 PPM "	24°	1		5	2			8	19.00	*[3]
.01% thiourea	24°			6	12	1		19	19.74	*[4]
.02% "	24°		1	2	14	4		21	20.00	*[5]
.04% "	24°		1	8	11	1	3	24	19.88	*[6]
(4) Parent: P										
Fresh water control	24°		3	13	6	1		23	19.22	
0.2 PPM thyroxine	24°		4	16	3			23	18.96	*[7]
0.4 PPM "	24°		13	6	1			20	18.40	*[8]
0.8 PPM "	24°		9	5	1			15	18.47	*[9]
.01% thiourea	24°	1	7	18	4			30	18.83	
.02% "	24°		2	12	2	1		17	19.12	
.04% "	24°		3	8	4			15	19.07	

Note:
1. Tends to be higher than control ($P<.02; >.01$).
2. Not different from control ($P=.05$).
3. Not different from control ($P>.05$).
4. "
5. Higher than control ($P<.01$).
6. Not different from control ($P>.05$).
7. "
8. Lower than control ($P<.01$).
9. "

Appendix VIII continued. Frequency distribution of anal rays in experiemtn XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	Temp (°C)	Anal rays 17	18	19	20	21	22	Number	Mean	Remarks
(5) Parent: Q										
Fresh water control	24°		5	10	9	2		26	19.31	
0.8 PPM thyroxine	24°	1	8	7	5		2	21	18.76	Tends to be lower than control (P<.05).
.02% thiourea	24°		5	8	5	2		20	19.20	

Treatment	Temp (°C)	Anal rays 16	17	18	19	20	21	22	23	Number	Mean	Remarks
(6) Parent: S												
Fresh water control	24°				5	17	7	2		31	20.19	
0.2 PPM thyroxine	24°				10	23	10	1		44	20.04	
0.4 PPM "	24°				13	26	16	3		58	20.16	
0.8 PPM "	24°				4	13	12	1		30	20.33	
1.6 PPM "	24°				8	10	3	1		22	19.86	
.01% thiourea	24°				6	10	9	4	1	30	20.47	
.02% "	24°				1	3	16	8	2	30	21.23	*1
.04% "	24°					4	16	7	3	30	21.30	*2

*1. Higher than control (P<.01)
*2. Higher than control (P<.01)

Appendix VIII (Cont'd). Frequency distribution of anal rays in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching.

Treatment	Temp (°C)	Anal rays 16	17	18	19	20	21	22	Number	Mean	Remarks
(7) Parent: V											
Fresh Water (control)	26°			8	34	26	2		70	19.31	
0.4 PPM thyroxine	26°		4	14	26	7	1		52	18.75	*1
0.8 PPM "	26°		1	20	34	7	1		63	18.79	*2
3.2 PPM "	26°	1	9	19	6	1			36	17.92	*3
.05 % thiourea	26°		1	13	27	18	1		60	19.08	
(8) Parent: Y											
Fresh Water (control)	26°		2	18	37	12	1		70	18.89	
0.4 PPM thyroxine	26°			9	32	8	1		50	19.02	
0.8 PPM "	26°			10	32	7	1		50	18.98	
.02 % thiourea	26°			10	21	11			42	19.02	

*1. Lower than control ($P<.01$).
*2. Lower than control ($P<.01$).
*3. Lower than control ($P<.01$); 489 hrs. in thyroxine and hatched in water.

Appendix VIII (Cont'd). Frequency distribution of anal rays in experiment XI: Effect of thyroxine and thiourea.
(b) Eggs fertilized and reared in the solutions up to hatching.

Treatment	Temp (°C)	Anal rays 16	17	18	19	20	21	22	Number	Mean	Remarks
(1) Parent: Y											
Fresh Water (Control)	26°		2	18	37	12	1		70	18.89	
0.8 PPM thyroxine	26°		1	13	38	22	2	1	77	19.18	*1
.02% thiourea	26°			10	26	13	1		50	19.10	*2
(2) Parent: á											
Fresh Water (Control)	30°	4	16	11	2				33	17.37	
0.8 PPM thyroxine	30°	4	16	10	3				33	17.37	
.04% thiourea	30°	2	5	15	9	1			32	18.06	*1
(3) Parent: b											
Fresh Water (Control)	26°				19	19	6	2	46	19.80	
0.8 PPM thyroxine	26°		2	5	17	1	2		27	18.85	*1
.04% thiourea	26°		1	1	8	3	2		15	19.27	*2

*1. Tends to be higher than control ($P<.05$).
*2. Not different from control ($P>.05$).

*1. Tends to be higher than control ($P<.02$).

*1. Lower than control ($P<.01$).
*2. Not different from control ($P>.05$).

Appendix VIII (Cont'd). Frequency distribution of anal rays in experiment XI: Effect of thyroxine and thiourea.
(c) Chorion pricked eggs reared in the solutions up to hatching.

Treatment	Temp (°C)	Anal rays 15	16	17	18	19	20	21	Number	Mean	Remarks
Parent: V											
Fresh Water (Control)	26°			4	13	5	4	3	29	18.62	
3.2 PPM thyroxine	26°			1	10	3	2		16	18.37	*1
.02% thiourea	26°				3	13	5		21	19.09	*2

*1. Not different from control (P>.05).
*2. Not different from control (P>.05).

(d) Larvae reared in the solutions after hatching.

Parent: V											
Fresh water (Control)	26°				7	15	12	1	35	19.20	
3.2 PPM thyroxine	26°	8	9	7	2	1			27	16.22	
.05% thiourea	26°				4	10	12	8	34	19.71	*1

*1. Tends to be higher than control (P<.02).

Appendix IX. Frequency distribution of dorsal rays in experiment XI: Effect of thyroxine and thiourea
(a) Fertilized eggs reared in the solutions up to hatching

Treatment	Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean	Remarks
(1) Parent: G								
.0025% thiourea	24°		18	3		21	6.14	
.005% "	24°		25	9		34	6.26	
.01% "	24°		22	4		26	6.15	
.02% "	24°		12	4		16	6.25	
.04% "	24°		4	8		12	6.67	
.05% "	24°		13	4		17	6.23	
(2) Parent: M								
Fresh Water (Control)	24°		9	6		15	6.40	
0.1 PPM thyroxine	24°		16	29		45	6.64	
0.2 PPM "	24°		7	9		16	6.56	
0.4 PPM "	24°		15	13	1	29	6.51	
(3) Parent: O								
Fresh Water (Control)	24°		37	18		55	6.33	
0.1 PPM thyroxine	24°		4	4		8	6.50	
0.4 PPM "	24°		10	9	1	20	6.55	
0.8 PPM "	24°		9	6		15	6.40	

Appendix IX. (Cont'd). Frequency distribution of dorsal rays in experiment XI: Effect of thyroxine and thiourea
(a) Fertilized eggs reared in the solutions up to hatching

Treatment	Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean	Remarks
(3) Parent: O (Cont'd)								
1.6 PPM thyroxine	24°		5	3		8	6.37	
.01% thiourea	24°		5	14		19	6.74	*1
.02% "	24°		8	13		21	6.62	*2
.04% "	24°		4	19	1	24	6.88	*3
(4) Parent: P								
Fresh Water (Control)	24°	1	20	2		23	6.04	
0.2 PPM thyroxine	24°		20	3		23	6.13	
0.4 PPM "	24°		15	5		20	6.25	
0.8 PPM "	24°		15			15	6.00	
.01% thiourea	24°	1	20	9		30	6.27	
.02% "	24°		10	7		17	6.41	*4
.04% "	24°		11	4		15	6.23	

*1. Higher than control ($P<.01$).
*2. Not different from control.
*3. Higher than control ($P<.01$).
*4. Higher than control ($P<.01$).

Appendix IX (Cont'd). Frequency distribution of dorsal rays in experiment XI: Effect of thyroxine and thiourea
(a) Fertilized eggs reared in the solutions up to hatching

Treatment	Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean	Remarks
(5) Parent: Q								
Fresh Water (Control)	24°		22	4		26	6.15	
0.8 PPM thyroxine	24°		19	2		21	6.09	
.02% thiourea	24°	1	13	6		20	6.25	
(6) Parent: S								
Fresh Water (Control)	24°		21	10		31	6.32	
0.2 PPM thyroxine	24°		30	14		44	6.32	
0.4 PPM "	24°		49	9		58	6.16	
0.8 PPM "	24°		22	8		30	6.27	
1.6 PPM "	24°		18	4		22	6.18	
.01% thiourea	24°		24	6		30	6.20	
.02% "	24°		17	13		30	6.43	
.04% "	24°		18	12		30	6.40	
(7) Parent: V								
Fresh Water (Control)	26°	4	53	13		70	6.13	
0.4 PPM thyroxine	26°	2	42	8		52	6.11	

Appendix IX (Cont'd). Frequency distribution of dorsal rays in experiment XI: Effect of thyroxine and thiourea

(a) Fertilized eggs reared in the solutions up to hatching

Treatment	Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean	Remarks
(7) Parent: V (Cont'd)								
0.8 PPM thyroxine	26°	4	53	6		63	6.03	
3.2 PPM "	26°		29	7		36	6.19	
.05% thiourea	26°	1	45	13	1	60	6.23	
(8) Parent: Y								
Fresh Water (Control)	26°		50	20		70	6.29	
0.4 PPM thyroxine	26°		33	17		50	6.34	
0.8 PPM "	26°		28	22		50	6.44	
.02% thiourea	26°		19	23		42	6.55	*1

*1. Higher than control ($P<.01$).

(b) Eggs fertilized and reared in the solutions up to hatching

(1) Parent: Y								
Fresh Water (Control)	26°		50	20		70	6.29	
0.8 PPM thyroxine	26°		44	31	2	77	6.45	
.02% thiourea	26°		30	20		50	6.40	

Appendix IX (Cont'd). Frequency distribution of dorsal rays in experiment XI: Effect of thyroxine and thiourea

(b) Eggs fertilized and reared in the solutions up to hatching

Treatment	Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean	Remarks
(2) Parent: ♂								
Fresh Water (Control)	30°	1	32			33	5.97	
0.8 PPM thyroxine	30°	1	30	2		33	6.03	
.04% thiourea	30°		27	5		32	6.16	
(3) Parent: ♂								
Fresh Water (Control)	26°	1	33	11	1	46	6.26	
0.8 PPM thyroxine	26°	1	24	2		27	6.04	
.04% thiourea	26°		14	1		15	6.07	

(c) Chorion pricked eggs reared in the solutions up to hatching

Parent: V								
Fresh Water (Control)	26°	6	16	7		29	6.03	
3.2 PPM thyroxine	26°		18	3		21	6.14	
.02% thiourea	26°		13	8		21	6.38	

(d) Larvae reared in the solutions after hatching

Parent: V								
Fresh Water (Control)	26°	3	28	4		35	6.03	

Appendix IX (Cont'd). Frequency distribution of dorsal rays in experiment XI: (d) Larvae reared in the solutions after hatching

Parent: V (Cont'd)	Temp (°C)	Dorsal rays 5	6	7	8	Number	Mean A	Remarks
3.2 PPM thyroxine	26°	13	13	1		27	5.56	*1
.05% thiourea	26°	2	22	10		34	6.24	

*1. Lower than control ($P<.01$).

Appendix X. Frequency distribution of total caudal rays in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching

Treatment	Temp (°C)	Total Caudal rays 19	20	21	22	23	24	25	26	No.	Mean	Remarks
(1) Parent: G												
.0025% thiourea	24°			1		11	8	1		21	23.38	
.005% "	24°					8	21	5		34	23.91	
.01% "	24°			2	5	8	8	2		25	23.12	
.02% "	24°			1	1	8	6			16	23.19	
.04% "	24°			1		7	3	1		12	23.25	
.05% "	24°				1	4	10	2		17	23.76	
(2) Parent: M												
Fresh Water (Control)	24°					6	5	4		15	23.87	
0.1 PPM thyroxine	24°			1	2	16	14	8	2	43	23.72	
0.2 PPM "	24°				2	8	6			16	23.25	*1
0.4 PPM "	24°			2	7	14	6			29	22.83	*2
(3) Parent: O												
Fresh Water (Control)	24°			1	14	20	17	1		54	23.13	*1

*1. Tends to be lower than control ($P<.05$).
*2. Lower than control ($P<.01$).

*1. One fish with 27 rays.

Appendix X. (Cont'd). Frequency distribution of total caudal rays in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching

Treatment	Temp (°C)	Total Caudal rays 19	20	21	22	23	24	25	26	No.	Mean	Remarks
(3) Parent: O (Cont'd)												
0.2 PPM thyrozine	24°					4	2	1	1	8	23.75	
0.4 PPM "	24°					14	6			20	23.30	
0.8 PPM "	24°				2	8	4	1		15	23.27	
1.6 PPM "	24°		1		1	4	2			8	22.75	
.01% thiourea	24°			1	4	7	6	1		19	23.10	
.02% "	24°				1	11	6	3		21	23.52	
.04% "	24°				1	11	9	3		24	23.58	
(4) Parent: P												
Fresh Water (Control)	24°					3	11	9		23	24.26	
0.2xPPM thyroxine	24°				2	6	10	5		23	23.78	*1
0.4 PPM "	24°				3	7	8	2		20	23.45	*2
0.8 PPM "	24°				2	4	6	3		15	23.67	*3
.01% thiourea	24°	1		1	3	6	16	3		30	23.43	*4
.02% "	24°					6	9	2		17	23.76	*5

*1. Tends to be lower than control ($P<.05$).
*2. Lower than control ($P<.01$).
*3. Tends to be lower than control ($P<.05$).
*4. Lower than control ($P<.01$).
*5. Tends to be lower than control ($P<.05$).

Appendix X. (Cont'd). Frequency distribution of total caudal rays in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching

Treatment	Temp (°C)	Total Caudal rays 19	20	21	22	23	24	25	26	No.	Mean	Remarks
(4) Parent: P (Cont'd)												
.04% thiourea	24°				1	5	6	3		15	23.73	*1
*1. Tends to be lower than control (P<.05).												
(5) Parent: Q												
Fresh Water (Control)	24°				1	13	10	2		25	23.50	
0.8 PPM thyroxine	24°					9	12			21	23.57	
.02% thiourea	24°			2	1	7	6	3	1	20	23.50	
(6) Parent: S												
Fresh Water (Control)	24°			1	13	10	4	2		30	22.81	
0.2 PPM thyroxine	24°			3	18	12	9	2		44	22.75	
0.4 PPM "	24°			8	15	16	17	1	1	58	22.84	
0.8 PPM "	24°			4	11	9	4	1	1	30	22.67	
1.6 PPM "	24°			3	8	9	1	1		22	22.50	
.01% thiourea	24°			4	13	8	3	1		29	22.48	
.02% "	24°			1	9	10	7	3		30	23.07	
.04% "	24°		1	4	6	10	8	1		30	22.77	

Appendix X. (Cont'd). Frequency distribution of total caudal rays in experiment XI: Effect of thyroxine and thiourea.
(a) Fertilized eggs reared in the solutions up to hatching

Treatment	Temp (°C)	Total Caudal rays 19	20	21	22	23	24	25	26	No.	Mean	Remarks
(7) Parent: V												
Fresh Water (Control)	26°			5	8	41	13	2		69	23.50	
0.4 PPM thyroxine	26°			3	13	24	8	4		52	22.96	*1
0.8 PPM "	26°			6	12	34	10	1		63	22.82	*2
3.2 PPM "	26°		1	9	6	19	1			36	22.28	*3
.05% thiourea	26°			2	12	26	14	5	1	60	23.17	*4

*1. Lower than control ($P<.01$).
*2. Lower than control ($P<.01$).
*3. Lower than control ($P<.01$); 489 hrs. in thyroxine and hatched in water.
*4. Tends to be lower than control ($P<.05$).

(8) Parent: Y												
Fresh Water (Control)	26°	1	2	23	23	16	5			70	21.94	
0.4 PPM thyroxine	26°		1	8	16	17	6	2		50	22.50	*1
0.8 PPM "	26°		1	13	19	12	5			50	21.16	*2
.02% thiourea	26°		1	11	20	8	2			42	21.97	

*1. Higher than control ($P<.01$).
*2. Not different from control ($P>.05$).

Appendix X. (Cont'd). Frequency distribution of total caudal rays in experiment XI: Effect of thyroxine and thiourea.
(b) Eggs fertilized add reared in the solutions up to hatching

Treatment	Temp (°C)	Total Caudal rays 19	20	21	22	23	24	25	26	No.	Mean	Remarks
(1) Parent: Y												
Fresh Water (Control)	26°	1	2	23	23	16	5			70	21.94	
0.8 PPM thyroxine	26°		1	25	17	26	7			*77	22.11	*1
.02% thiourea	26°	1		11	21	14	3			50	22.12	
*1. One with 18 rays.												
(2) Parent: a												
Fresh Water (Control)	30°				3	15	10	4	1	33	23.54	
0.8 PPM thyroxine	30°			2	2	13	5	11		33	23.63	
.04% thiourea	30°			2		16	5	6	2	*32	23.72	*1
*1. One with 27 rays.												
(2) Parent: b												
Fresh Water (Control)	26°	1	3	19	10	13				46	21.67	
0.8 PPM thyroxine	26°	1	2	17	3	4				27	21.26	
.04% thiourea	26°		1	11	1	2				15	21.27	

Appendix X. (Cont'd). Frequency distribution of total caudal rays in experiment XI: Effect of thyroxine and thiourea

(c) Chorion pricked eggs reared in the solution up to hatching

Treatment	Temp (°C)	Total Caudal rays 19	20	21	22	23	24	25	26	No.	Mean	Remarks
Parent: V												
Fresh Water (Control)	26°			5	10	12	2			29	22.38	
3.2 PPM thyroxine	26°	1	1	6	4	6	3			*22	21.79	*1
.02% thiourea	26°			2	6	9	4			21	22.71	*2

*1. One with 12 rays. Not different from control (P>.05).
*2. Not different from control (P>.05).

(d) Larvae reared in the solutions after hatching

Treatment	Temp (°C)	Total Caudal Rays 13	14	15	16	17	18	19	20	21	22	23	24	No.	Mean	Remarks
Parent: V																
Fresh Water (Control)	26°									5	11	12	6	35	22.40	
3.2 PPM thyroxine	26°	1	1	5	4	6	1		3	2	4			27	17.63	
.05% thiourea	26°							1	5	17	3	4	2	33	21.24	*1

*1. Lower than control (P<.01).