EFFECT OF INCUBATION TEMPERATURE ON MORTALITY OF EMBRYOS OF THE LARGEMOUTH BASS, MICROPTERUS SALMOIDES (LACÉPÈDE)

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University for the Degree of

Master of Science

by

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BIOGRAPHICAL SKETCH

Theo Rudolf Badenhuizen was born in Amsterdam, Holland, on October 12, 1938. He attended public schools in Delft (Holland) and Johannesburg (South Africa). In 1955 he graduated from Potchefstroom Boys High School in South Africa. He enrolled at the University of Witwatersrand in Johannesburg in 1958 and received the Bachelor of Science degree in Biology in 1961 and the Bachelor of Science (Honours) degree in Zoology in 1963. From 1963 to 1966 he worked as a Fisheries Research Officer in Zambia and in Cape Town, South Africa. On December 27, 1966 he married Gillian Pullinger. In February 1967 he enrolled at Cornell University as a candidate for the Master of Science degree in Fisheries Biology. He has two publications in fisheries research and is a member of the American Fisheries Society.

ACKNOWLEDGMENTS

This study was carried out under the auspices of the New York Cooperative Fishery Unit, led by Dr. A. W. Eipper who helped design and execute the project. Dr. C. A. Carlson, Assistant Unit Leader, gave much help and encouragement in various ways.

Mr. W. H. Swallow and Dr. D. S. Robson of the Cornell University Biometrics Unit provided statistical assistance.

Colleagues at the Cornell Fishery Laboratory helped with the project at various times. Photographs of the project were taken by Mr. D. M. Payne, Photographer of the Department of Conservation.

My wife Gillian typed the drafts and helped prepare the tables.

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INTRODUCTION

Purpose of research

The purpose of this study was to determine the influence of various incubation temperatures on mortality of embryos of the largemouth bass (Micropterus salmoides Lacépède) at various developmental stages. This work was a continuation and expansion of research done by Kelley (1968) and Swallow (unpublished),[1/] and sought to determine how the effects of temperature on mortality were influenced by acclimation, embryo age, and/or stage of embryological development.

Effect of temperature on fish

The dominant environmental influence of temperature on living organisms has long been recognized by biologists. It has often been stated by fishery biologists that temperature has a profound effect on development and mortality during early life stages of fishes, including largemouth bass. Only little definite information regarding the direct effect of temperature on fish embryos has been obtained from limited field observations and laboratory experiments. Adverse incubation temperatures may be a fundamental cause of the large early mortality observed in many fishes and may, in addition, indirectly affect subsequent development and year class strengths in fish populations.

[1/] Swallow, W. H.: The relation of incubation temperature to the mortality of fish embryos. M.S. Thesis, 1968, Cornell Univ., 45 p.

LITERATURE REVIEW

Physiology of temperature

According to Fry (1947), temperature is a controlling factor and exerts a dominant environmental influence on living organisms. The effects of temperatures on living matter have been comprehensively described by Bělehrádek (1935), and provided considerable insight into the physiological processes affected by temperature. Luyet and Gehenio (1938, 1940) offered explanations of the processes involved in cold-death as well as heat-death. Briefly, cold-death seems to be caused by the transformation of cell protoplasm from a sol (hydration) to a gel (dehydration) which results in an unequal slowing down of various metabolic processes, whereas heat-death seems to be caused by a number of secondary effects, such as dehydration, and protein denaturation. The maintenance of a protoplasmic sol in adverse temperatures is a manifestation of acclimation, and acclimation to cold regimes is accompanied by increased susceptibility to warm regimes and vice versa (Luyet and Gehenio, 1940). Heat acclimation is a far more rapid process than cold-acclimation according to Doudoroff (in Brown, 1957).

Temperature and developing embryos

Many useful references on this topic have been obtained from Raney and Menzel's (1967) comprehensive bibliography. Relevant literature concerning the effects of temperature on developing fish embryos has been extensively reviewed by Swallow (footnote 1, p. 1).

Falling temperature and/or nest-desertion associated therewith has led to very high mortality in nature in embryos of the smallmouth bass Micropterus dolomieu (Reighard, 1905; Meehan, 1911; Beeman, 1924; Tester, 1930; Rawson, 1945) and largemouth bass (Leary, 1901; Lydell, 1902; Reighard, 1905; Bennett, 1962; Kelley unpublished,[1/] 1968). In the laboratory, fish embryo mortality at high and low temperature extremes has been found in various fish species including the largemouth bass (Gray, 1928; Johnson and Brice, 1953; Combs and Burrows, 1957; Combs, 1965; Swift, 1965; Kelley, 1968).

At low temperature extremes, "monsters" have been induced in the mummichog, Fundulus heteroclitus (Stockard, 1921) and chromosome mosaics in the threespine stickleback, Gasterosteus aculeatus (Swarup, 1958).

Lower incubation temperatures produced larger fish embryos, and higher temperatures resulted in smaller embryos, according to the observations of Gray (1928) and Merriman (1935).

The review of Swallow (op. cit.) has pointed out two instances when parabolic mortality curves have been produced in fish embryos subjected to a range of incubation temperatures, with progressively higher mortality at temperatures above and below some optimum.

The developmental rate of fish embryos is directly related to temperature, according to Walford (1938) and Hayes (1949), and so is the hatching success (Nakai, 1928a; Yamamoto, 1937; Kowalska, 1959; Kelley, 1968). Ranges of tolerance to temperatures, varying among different fish species, have been found by Higurashi and Nakai (1926),

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1/ Kelley, J. W.: The largemouth bass, Micropterus salmoides (Lacépède), of Belgrade Stream, Kennebec County, Maine. M.S. thesis, University of Maine, 134 p.

Nakai (1928b) and Kelley (1968).

Within this tolerance zone, optimum temperatures for fish embryo development have been reported by Reighard (1905), Uvarov (1931), Carr (1942), Webster (1945 and 1954), Kowalska (1959) and Kelley (1968). Uvarov (1931) defined the optimum temperature for development as the point at which the greatest number of individuals develop in the shortest time. Kowalska (1959), working with brown trout, Salmo trutta, found that the number of degree-days till hatching was not constant, but was maximum at optimum temperature. Optimum temperature for development of the largemouth bass embryos is above 15 C (Reighard, 1905; Sprecher, 1938; Kramer and Smith, 1962) and might even be in the region of 23 to 26 C (Carr, 1942).

There is considerable evidence that year class strength is established when fish are still very young and, presumably, in their most temperature-sensitive stage, according to Hjort (1926), Fry and Watt (1955) and Kramer and Smith (1962).

Critical temperature-sensitive periods during embryonic development have been observed in a number of fish species. The review of Swallow (op. cit.) gave many examples of research showing that critical periods may occur immediately after fertilization, at blastopore closure and/or at hatching, and of other studies in which evidence of such a critical stage was completely lacking.

MATERIALS AND METHODS

Temperature control apparatus

The temperature control equipment in the aquarium room at the Cornell Fishery Laboratory, originally described by Regier and Swallow (1968) and used by Kelley (1968), was supplemented in 1967 and 1968 to provide 10 constant and 10 variable controlled-temperature aquaria (Figure 1). The capacity of the water cooling system was increased by providing two extra 1 H. P. Min-O-Cool refrigeration units, by insulating coolant pipes with fiberglass, by increasing the lengths of aluminum cooling coils inside each aquarium and by air-conditioning the aquarium room somewhat. It was difficult to find a satisfactory coating material that would prevent electrolytic corrosion of the cooling coils without reducing the cooling capacity too much. A flexible epoxy resin proved to be the best of several materials tried.

The experimental design called for temperatures ranging (in individual aquaria) from 10 to 30 C. All the equipment was pretested and adjusted to maintain temperatures accurate to within 0. 5 C of those desired throughout all experimental programs. During some of the warmer days of June, when air temperatures were 25 to 31 C and maximum demands were being made on the cooling system, the lowest temperature that could be maintained in those aquaria programmed for 10 C was 12. 5 C. However a maximum rate of change of about 3. 0 C per hour was possible with the variable temperature units, which was well above the 1. 0 C per hour required by the experimental design.

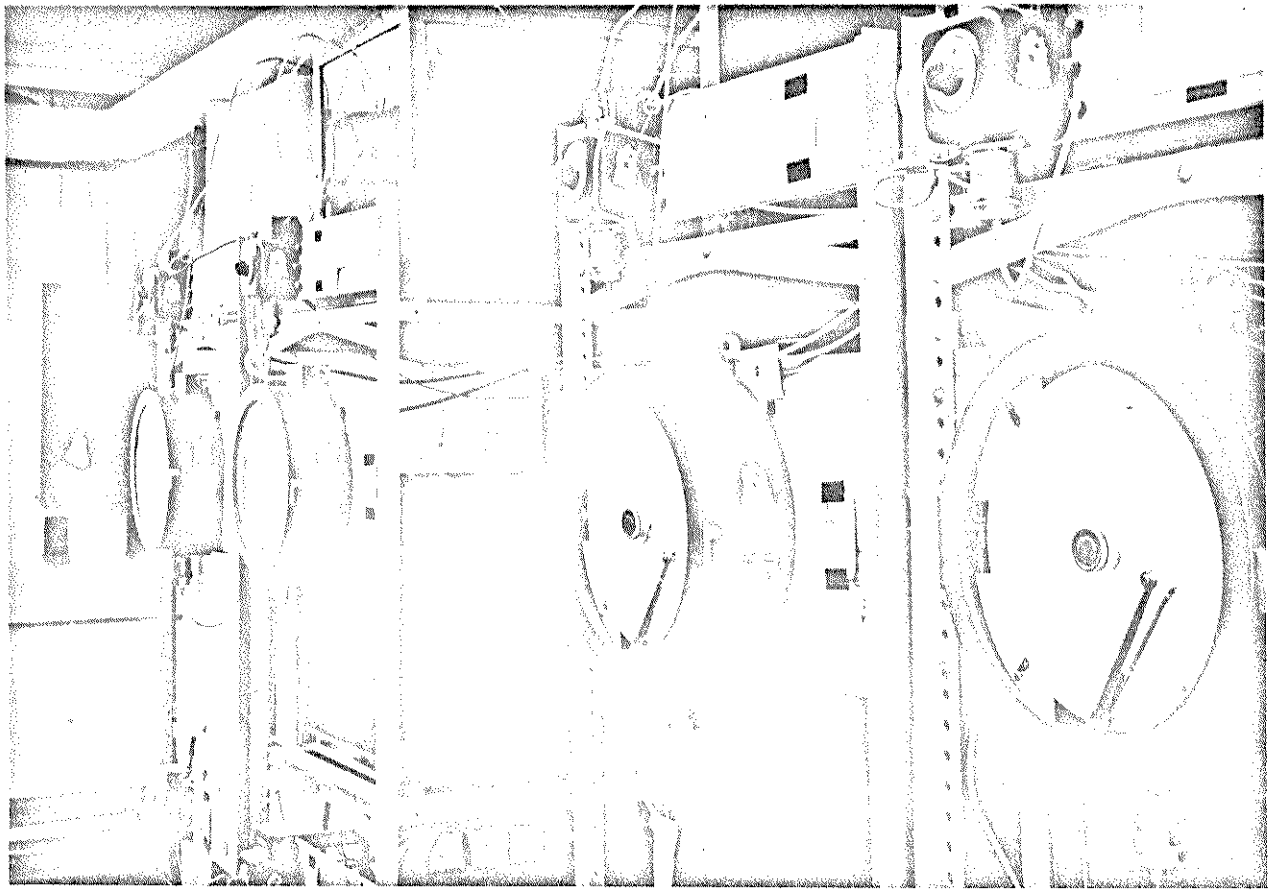

Figure 1. Some of the 50 gallon aquaria with temperature control, sensing, and recording equipment used in the bass embryo incubation experiments.

Obtaining and fertilizing eggs

One pair of spawning bass provided all the eggs and sperm used in a given experiment. In 1967 six pairs of bass were obtained in the act of spawning between May 20 and June 14 at widely different times of the day (0900 to 2100) using a simple electric shocker (Figure 2). The two shocker electrodes were 4.5 m poles, each having a section of exposed metal conduit pipe 1.5 m long, and insulated 1.5 m sections at either end. House current (110 v a-c) was supplied by depressing a waterproofed "deadman" foot switch. Pond temperatures were taken daily at about 1600 with an electrical resistance thermometer. The behavior of bass in six laboratory ponds was carefully observed and when impending spawning was indicated by intensive courtship activities, the electrodes were placed about 2-1/2 m apart in the water on either side of the nest, which was usually within 1-1/2 m of the bank. When both bass were on the nest, the current was applied for 5 to 10 sec to stun the fish. After the current was turned off, the fish remained under electro-narcosis just long enough to be dip-netted and placed in a live car. In all cases, recovery seemed complete by the time the bass reached the live car, and there was no external evidence of any adverse after-effects.

A portable "blind" of black polyethylene tacked to a hinged wooden frame was used effectively in some cases to conceal the observer while waiting for the bass to return to the nest. A waiting period of 15 min to over 1 hr was often necessary. In other instances however, the bass returned to the nest 5 to 15 min after the electrodes were placed in position and appeared undisturbed by the presence of the observer standing 1 to 2 m back from the pond edge.

Figure 2. Electric shocker and accessory equipment.

In addition to the above technique, during the 1968 spawning season, an 'unripe'[1/] female bass was seined from the laboratory pond on May 28, at the height of the spawning season, and injected with a pituitary extract (dissolved in 5 ml distilled water) from a freshly-killed female carp (Pickford and Atz, 1957). This bass was ripe on May 30. Another female bass was obtained and injected with 5 ml male carp pituitary extract on June 6, 1968, at the end of the spawning season, and never ripened at all (upon examination the eggs of the ovary were found to be atrophied).

All pairs of bass collected in 1967 and 1968 were taken into the laboratory 1 to 3 hr after shocking. Sperm could not be obtained by stripping, even when the male was anaesthetized, catheterized, or when, under deep anaesthesia, the body cavity was opened and stripping of the testes by direct contact was attempted. Accordingly, an artificial fertilization technique suggested by Dr. F. E. Hester (personal communication) was employed. The male was killed with a blow on the head and the testes were carefully dissected out, avoiding contamination by blood. Several incisions were made in the testes, which were then wrapped in a double layer of moist cheesecloth. At the same time eggs were carefully stripped from the female into a clean, moistened, plastic dish floating in a water bath which kept the temperature between 20 and 23 C. The testes contents were extracted by twisting and squeezing the cheesecloth so that the exudate dripped onto the eggs.

Gentle mixing of eggs and sperm was done with one finger held firmly against the bottom of the dish. A few drops of water were added

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[1/] The word 'ripe' is used here to denote a female from which eggs could be expressed by gentle manual pressure on the ventral surface.

to the eggs and more water was gradually added over 15 min until the dish was half full. The eggs were rinsed and left to water-harden for about 1.5 hr with occasional gentle swirling.

Incubation of embryos

After completion of water-hardening, 20 eggs were counted into each incubating container. These containers were similar to those used by Kelley (1968) except that the bottoms were lined with blue terry cloth (Figure 3). In 1967 the containers were kept on epoxy-coated hardware cloth trays in the aquaria, but in 1968 each container was provided with a handle of stainless steel wire and suspended from one of three rods spanning the aquarium (Figure 4). During loading, containers were kept in trays containing about 1 inch of water at 20 to 23 C. It took two operators about 2 hr to load the 60 to 70 containers required for an experiment. In 1967, two devices were found reasonably practical for the exacting operation of counting out lots of 20 embryos into each container. One of these consisted of a 20 ml wide-mouth pipette equipped with a three-way neoprene manipulator bulb (made by Spectronics Corp., Westbury, N. Y.). The other was a 10 ml syringe connected to a 13 cm glass tube tapered to 2.5 mm internal diameter with a spring inserted in the barrel of the syringe so it would suck up embryos slowly and evenly as the plunger was released. In 1968 eggs were spread out on a submersed glass tray with a black background and transferred into the containers, held beneath the tray, with a 'scoop' consisting of fine nylon mesh stretched across a 1-inch wire loop. Both tray and containers were immersed in water in a plastic basin.

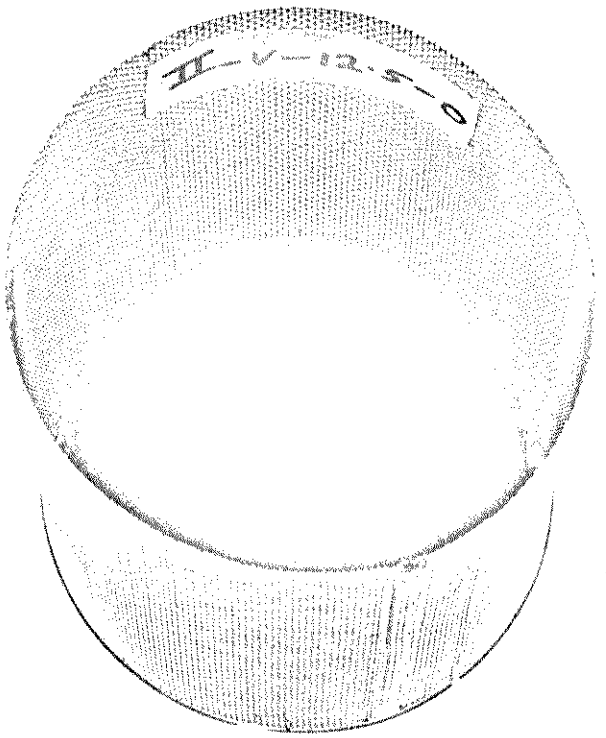

Figure 3. Container used for incubating largemouth bass embryos.

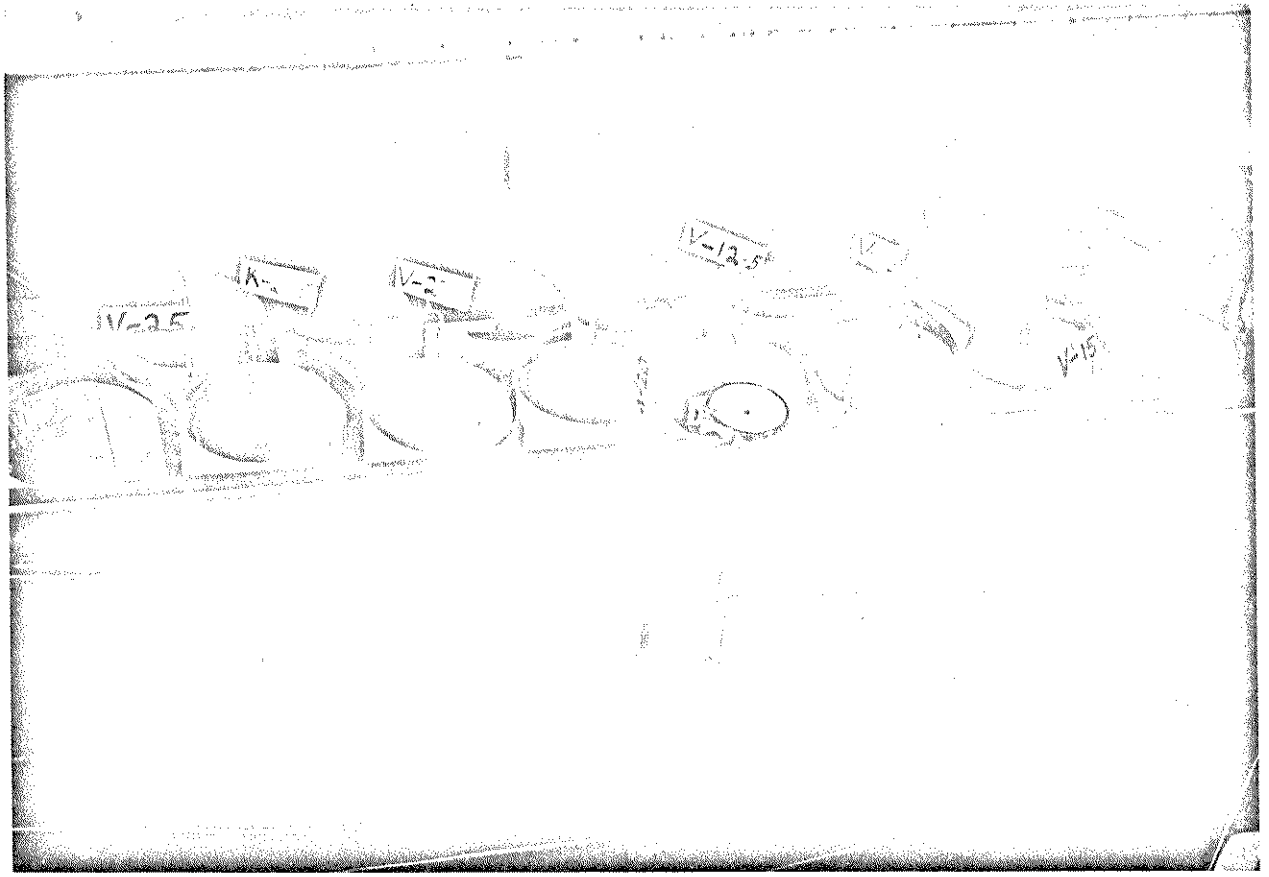

Figure 4. Side view of aquarium unit showing heating and cooling coils and embryo incubating containers in position.

Design and conduct of experiments

Experiments were designed so that lots containing 20 embryos each were transferred from 20 C to eight different incubation temperatures ranging from 10 to 30 C at 2.5 C intervals. Control lots were kept at 20 C during the entire experiment.

Embryos started the temperature program 3 to 5 hr after fertilization and lots were then transferred to the different final incubation temperatures at 5 hr intervals up to 30 hr after the start of the experiment.

Some of the lots were transferred to their final incubation temperatures directly, others reached there via an acclimation rate of 1.0 C per hour achieved in intermediate aquaria with variable temperature controls. In the 1967 experiments the variable temperature control templates of Kelley (1968), which effected an acclimation rate of 0.3 C per hour, were used in addition. Figures 7 through 14 in the Results section show schematic diagrams detailing the actual temperature transfers made in each experiment.

A detailed schedule was prepared which listed in chronological order the many steps involved in executing the temperature program sequence in each experiment.

Three experiments were carried out between May 28 and June 29, 1967 and two experiments between May 30 and June 10, 1968. The data from one of the 1967 experiments were discarded because total mortality occurred in all lots within two days. Only the range 20 to 30 C was covered in the second 1968 experiment (Experiment 4) because the other incubating facilities were still occupied by embryos of the previous experiment.

Aquaria and embryo containers were disinfected between experiments by soaking overnight in 10% formalin or the bactericide Hyamine 3500 (manufactured by Rohm & Haas Co., Philadelphia, Pa.). After this disinfection treatment the aquaria and containers were thoroughly rinsed and soaked in water before re-use.

Each container was inspected and embryo mortality recorded beginning 24 hr after the start of a given experiment (except for Experiment 1, in which observations were started after 48 hr.). In general, eggs were inspected and aquarium temperatures checked every 10 to 12 hours during the first two days of an experiment. After the second day, each lot was inspected and the aquarium temperatures checked once a day. Dead embryos and larvae were removed from the containers every second day with specially fabricated wire loop forceps. In 1967 these embryos were preserved in 5% formalin but this was found unsatisfactory for subsequent clearing and examination to determine the developmental stage at time of death. This purpose was achieved successfully in 1968 by preserving, clearing and fixing the opaque dead embryos directly in 5% acetic acid solution. Small samples of living embryos from 20 C were collected every hour for 30 hrs after fertilization, and their developmental stage checked to serve as a reference with the stages outlined in Carr (1942). Time of hatching and number of hatched embryos was always recorded for each lot.

Separate index cards listing all the information obtained in each experiment were made out for each lot of 20 embryos.

RESULTS

Physico-chemical analyses of laboratory ponds

Temperatures taken in two laboratory ponds during April to June 1967 and 1968 (Figures 5 and 6 respectively) showed close agreement, although the pond nearest the intake from Cascadilla Creek (Pond I) was consistently cooler than the pond farthest removed (Pond A). Spawning occurred during days of rising temperature in both years, but started earlier in 1968 than in 1967. In both years spawning occurred first in Pond I (May 20, 1967; May 6, 1968), at surprisingly low temperatures and well ahead of the main spawning peaks in Pond A (May 28 - 30, 1967; May 20 - 30, 1968). The surface temperature range during the 1967 spawning season was 13.5 to 29 C and during the 1968 season was 11.5 to 28.5 C. In the initial part of the seasons it is possible that minimum temperatures may sometimes have been lower than indicated, especially during the night.

Results of the physico-chemical analyses of water samples taken from Pond I and Pond A on May 23, 1967 (Table 1) revealed no real difference between the two ponds except in the alkalinity relationships which fluctuated widely at the time in Pond A due to the effects of photosynthesis from abundant aquatic plants in this shallow pond.

Data presentation with schematic diagrams

Schematic diagrams showing per cent mortality in each lot at hatching and three days after hatching for two experiments in 1967 and two experiments in 1968 are given in Figures 7 through 14.

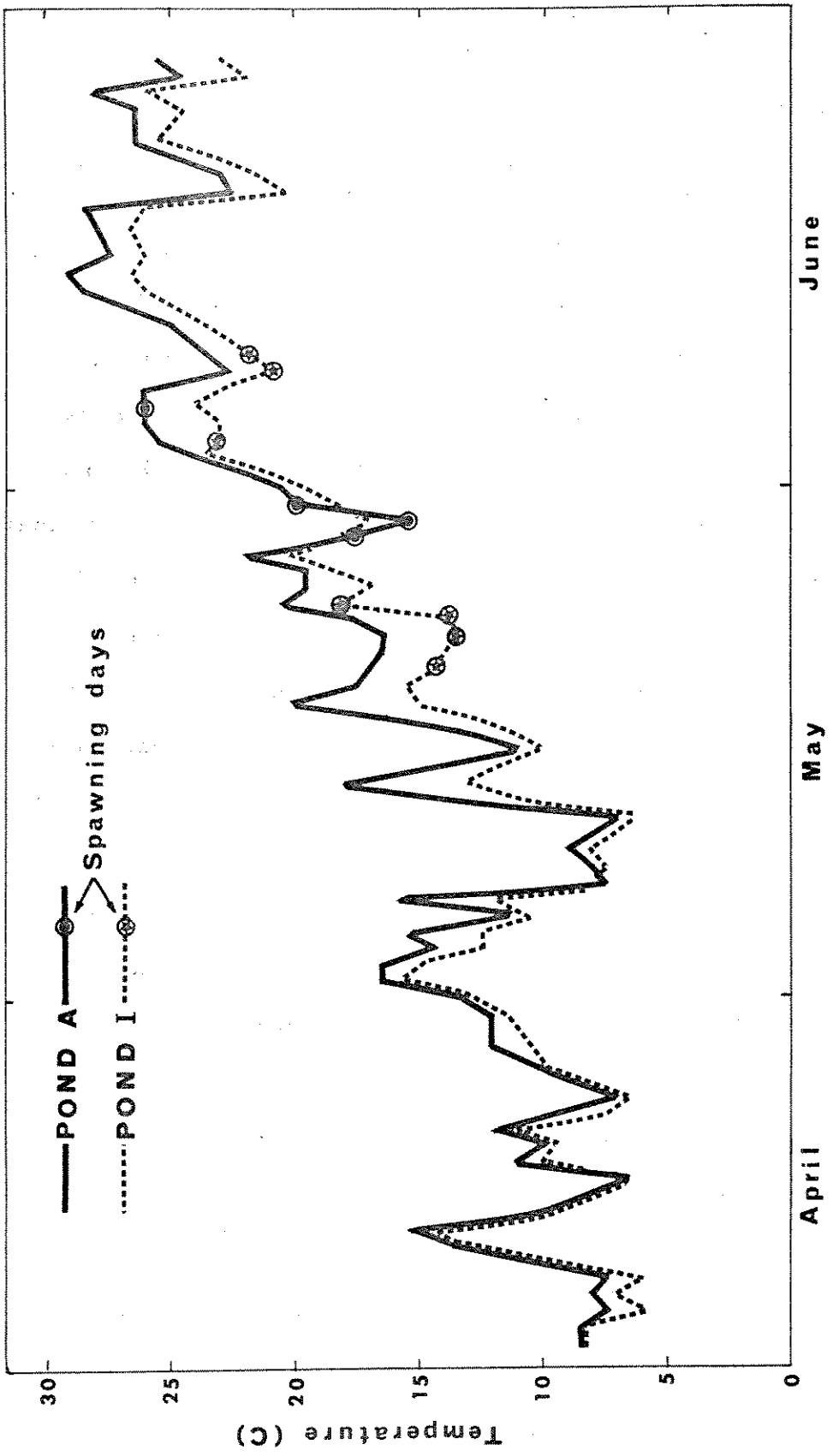

Figure 5. Surface temperatures (taken daily around 1600) in Ponds A and I during the 1967 spawning season.

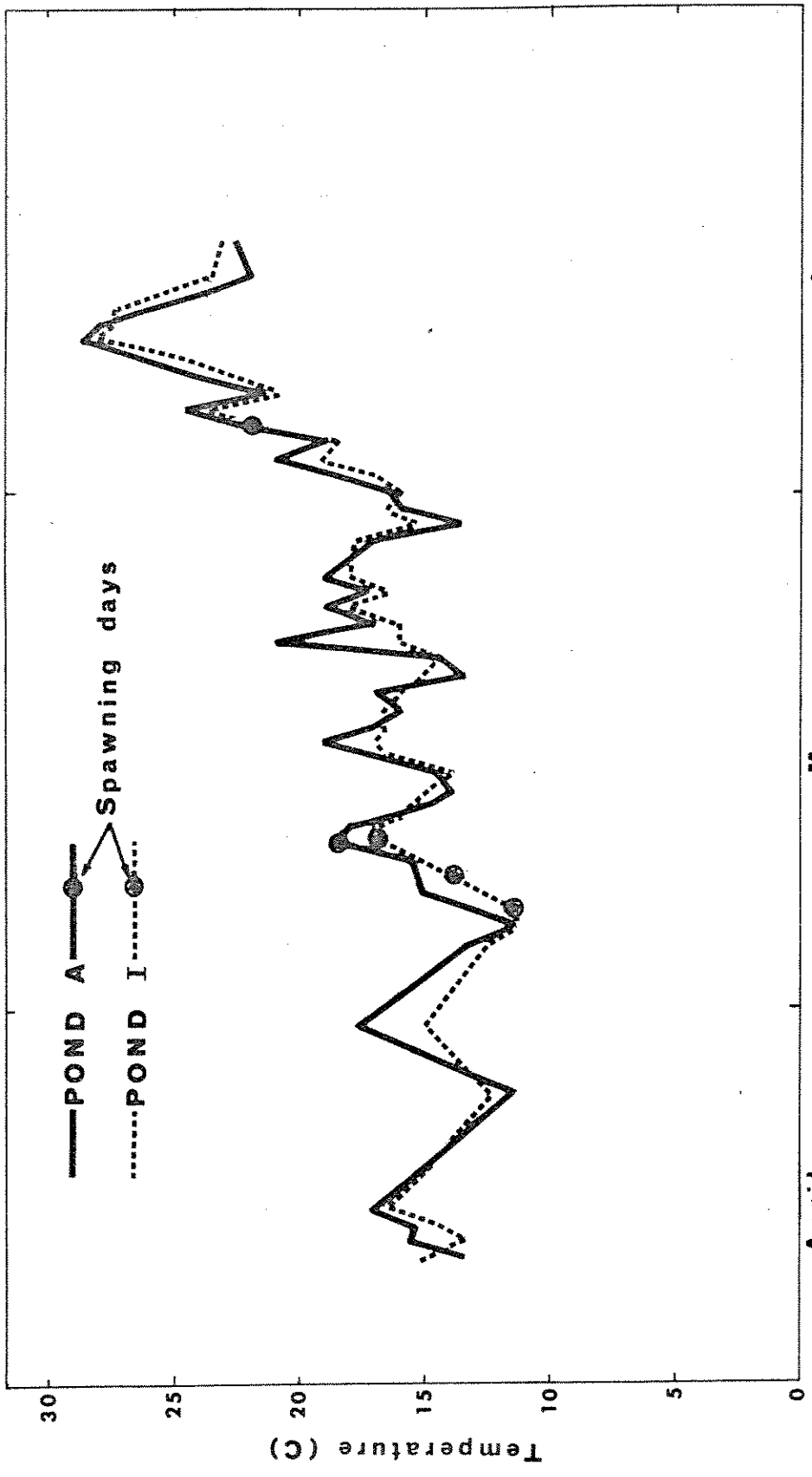

Figure 6. Surface temperatures (taken daily around 1600) in Ponds A and I during the 1968 spawning season.

Table 1. Physico-chemical analyses[1/] of water samples taken from hatchery ponds A and I on May 23, 1967, three days after initial spawning in Pond I, but 5 days before the first spawning occurred in Pond A.

Description (samples taken at 1100)		Pond A	Pond I
Temperature (C)		17.0	14.5
Dissolved oxygen (ppm)		16.0	12.5
Oxygen saturation (%)		140	120
Bicarbonate alkalinity	(as ppm $CaCO_3$)	0	85.5
Carbonate alkalinity	(as ppm $CaCO_3$)	68.4	0
Hydroxide alkalinity	(as ppm $CaCO_3$)	17.1	0
Total alkalinity	(as ppm $CaCO_3$)	85.5	85.5
Total hardness	(as ppm $CaCO_3$)	102.6	102.6
Free carbon dioxide	(as ppm)	0	5
pH		10.3	9.2
Conductivity (μ-mhos at 25 C)		198.5	185.8

[1/] Carried out by Mr. C. S. Schofield at the Cornell Fishery Laboratory.

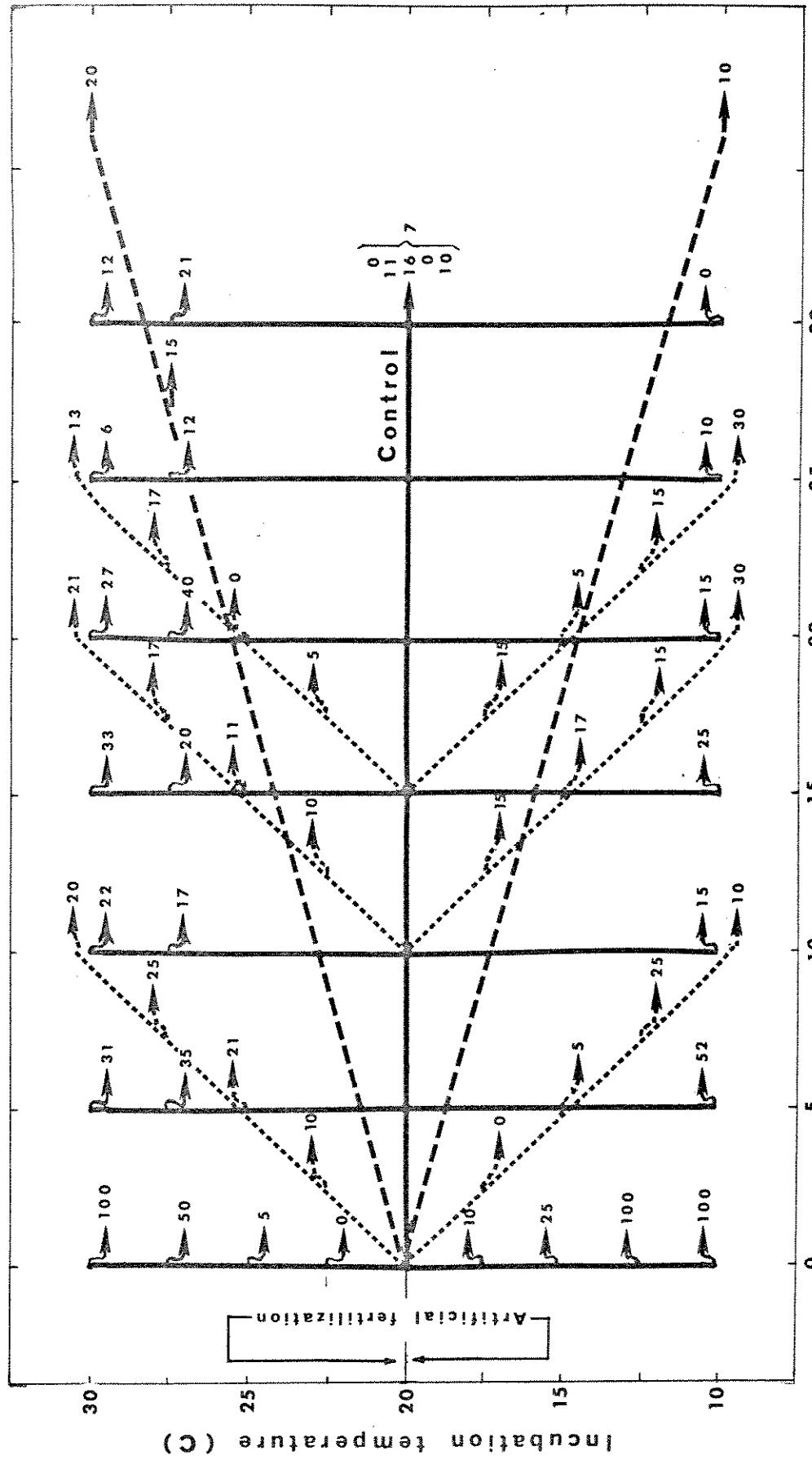

Figure 7. Schematic diagram of embryo transfers to various temperatures, showing per cent mortality to hatching in experiment 1, 1967. Dotted and solid lines denote transfers with and without acclimation, respectively.

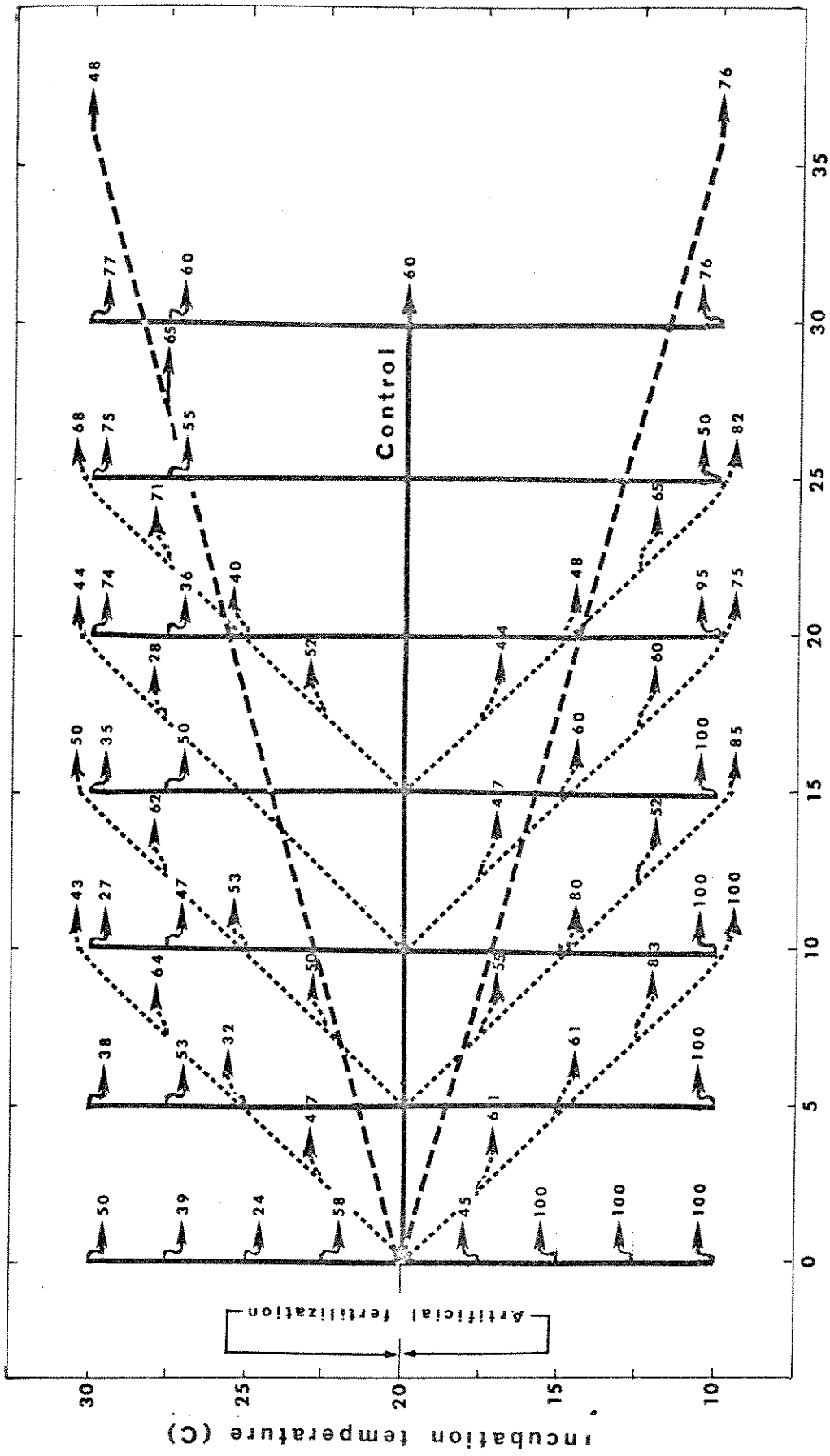

Figure 8. Schematic diagram of embryo transfers to various temperatures, showing per cent mortality to hatching in experiment 2, 1967. Dotted and solid lines denote transfers with and without acclimation, respectively.

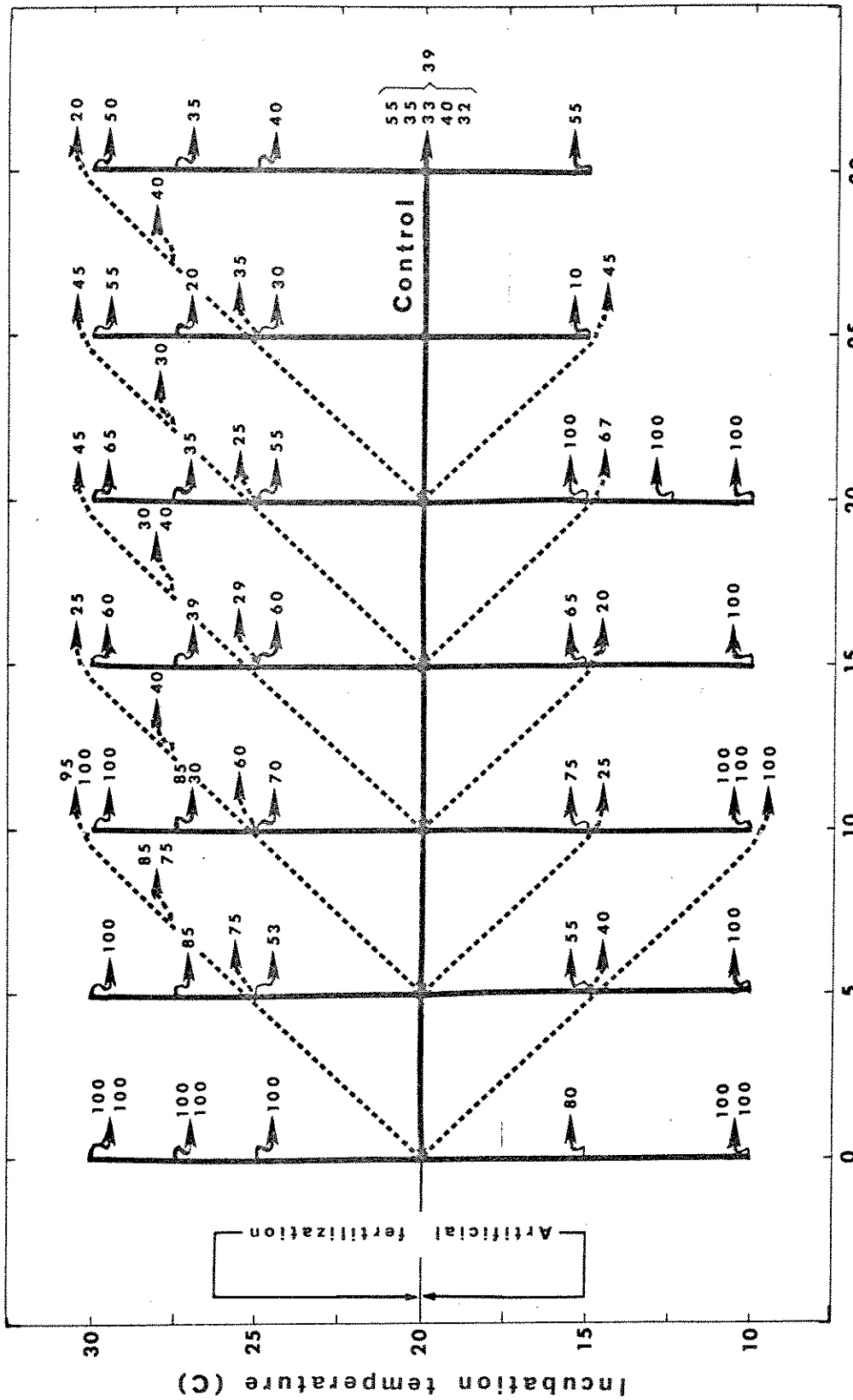

Figure 9. Schematic diagram of embryo transfers to various temperatures, showing per cent mortality to hatching in experiment 3, 1968. Dotted and solid lines denote transfers with and without acclimation, respectively.

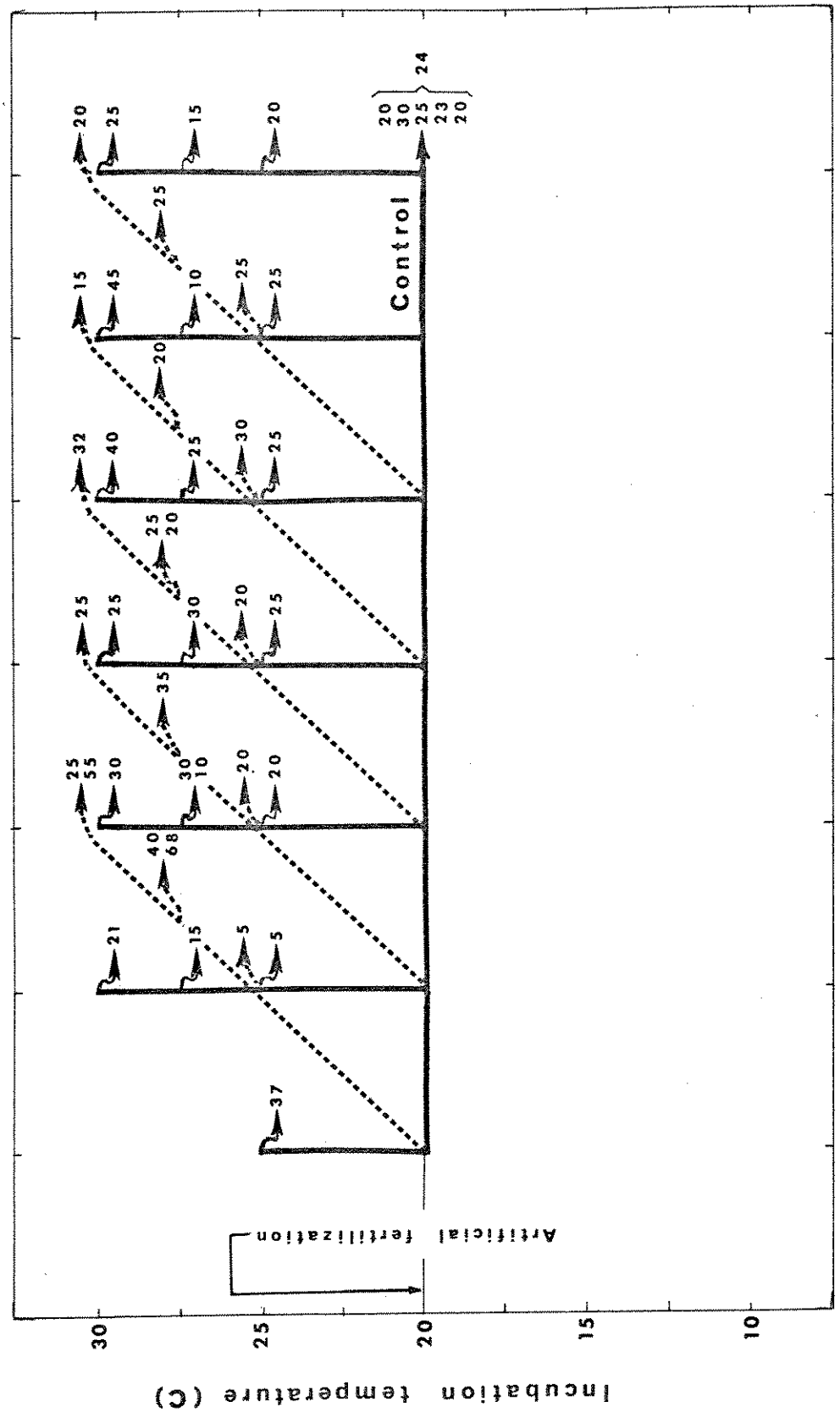

Figure 10. Schematic diagram of embryo transfers to various temperatures, showing per cent mortality to hatching in experiment 4, 1968. Dotted and solid lines denote transfers with and without acclimation, respectively.

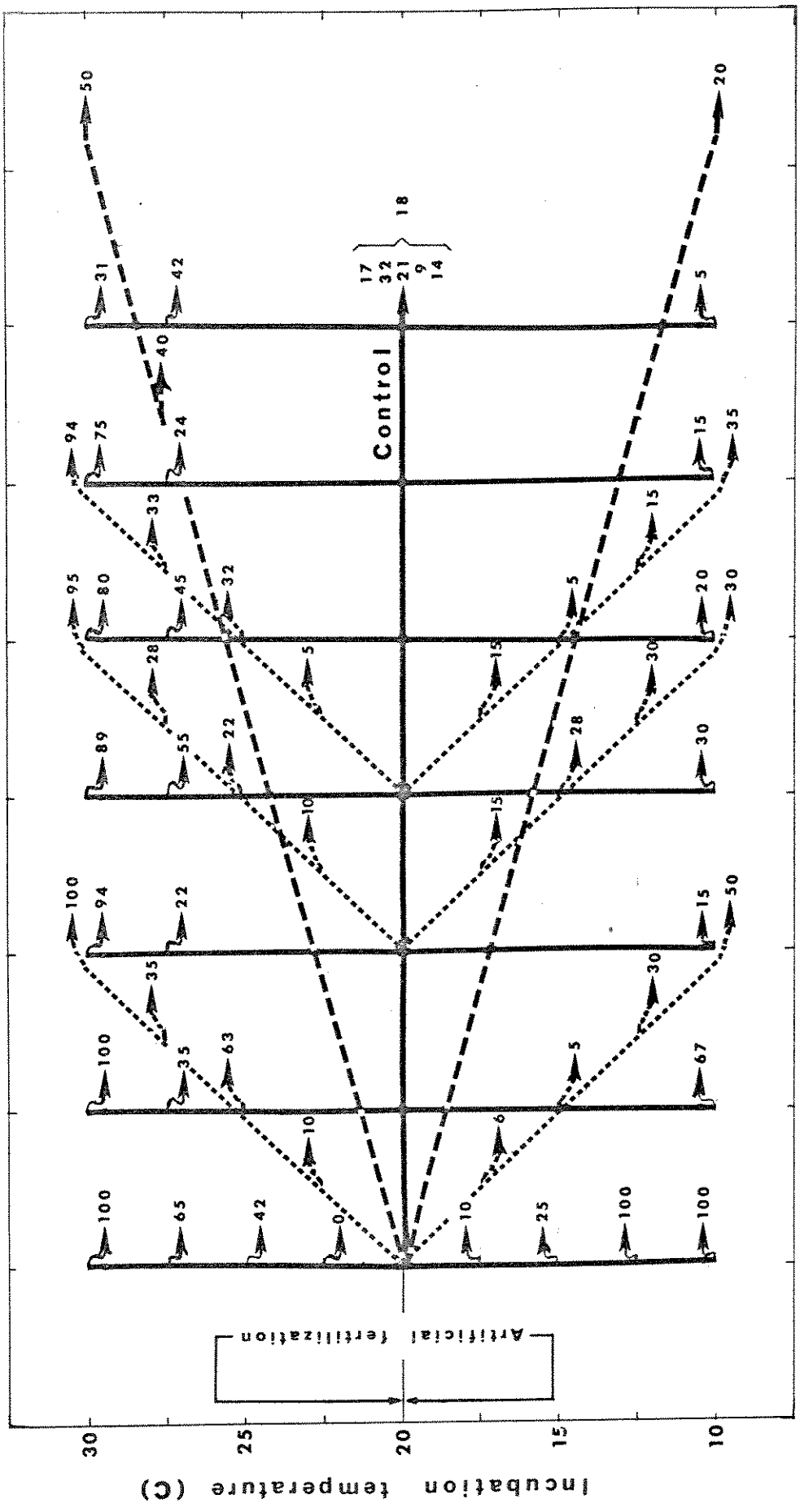

Figure 11. Schematic diagram of embryo transfers to various temperatures, showing per cent mortality to three days after hatching in experiment 1, 1967. Dotted and solid lines denote transfers with and without acclimation, respectively.

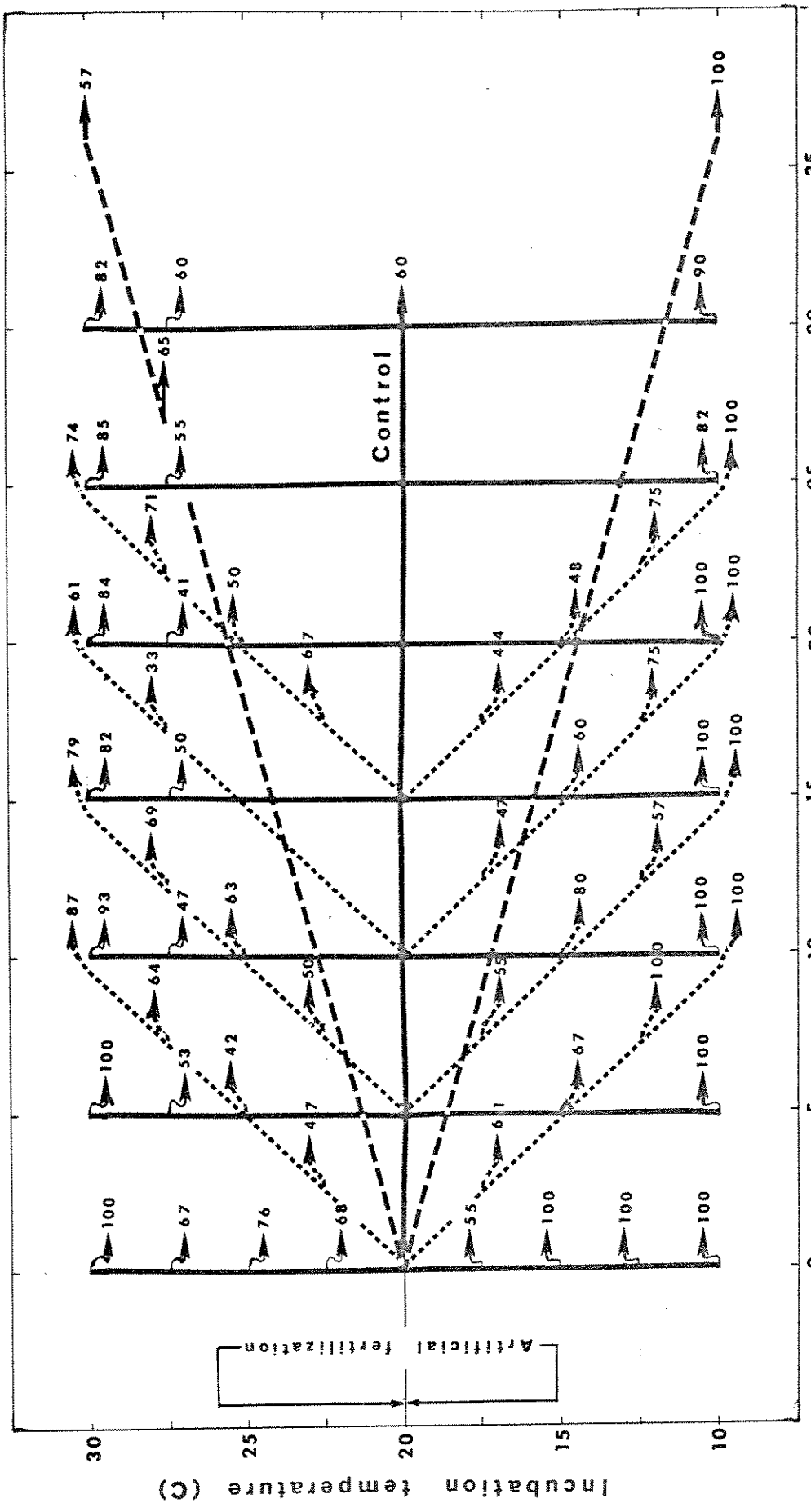

Figure 12. Schematic diagram of embryo transfers to various temperatures, showing per cent mortality to three days after hatching in experiment 2, 1967. Dotted and solid lines denote transfers with and without acclimation, respectively.

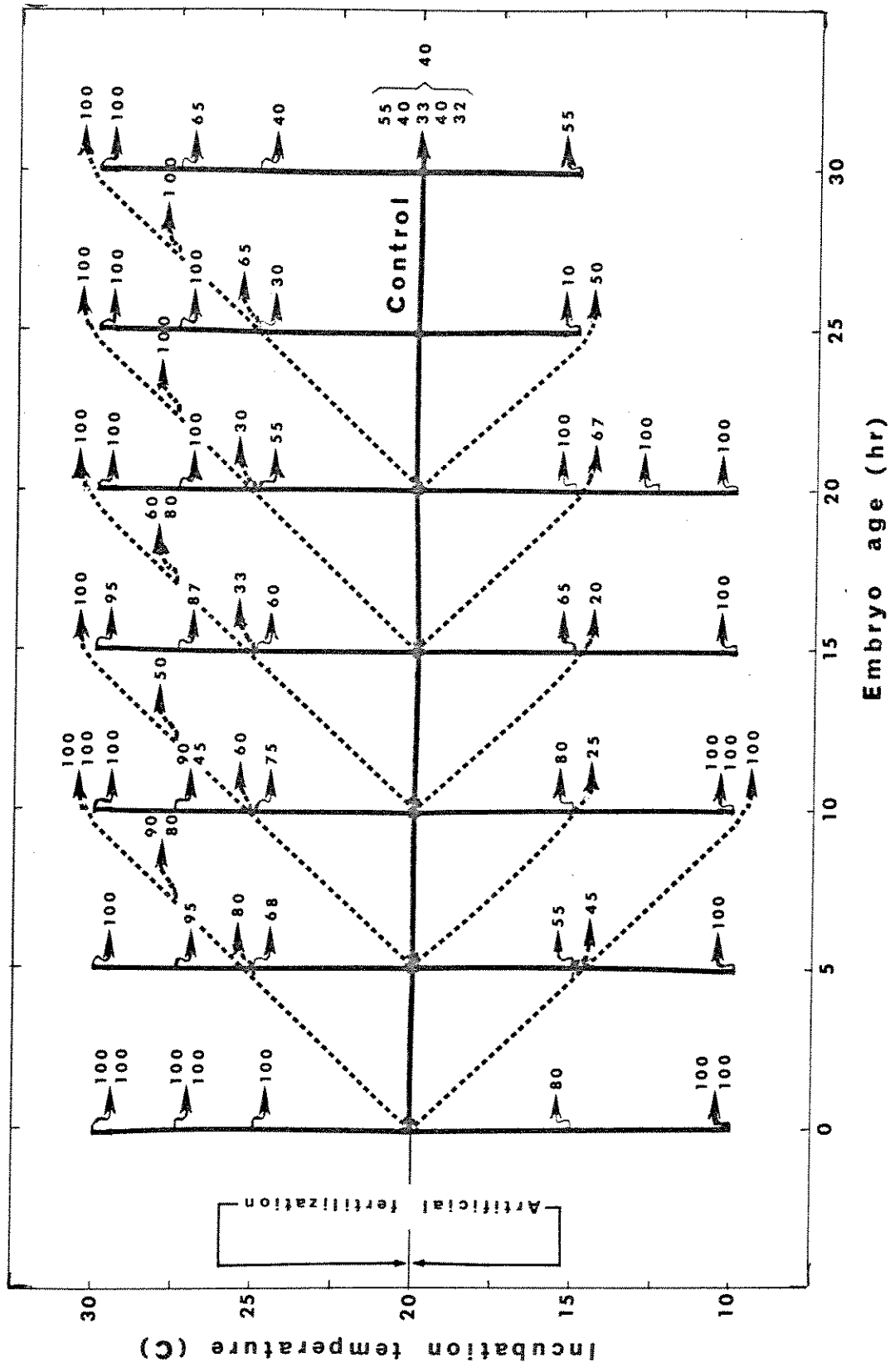

Figure 13. Schematic diagram of embryo transfers to various temperatures, showing per cent mortality to three days after hatching in experiment 3, 1968. Dotted and solid lines denote transfers with and without acclimation, respectively.

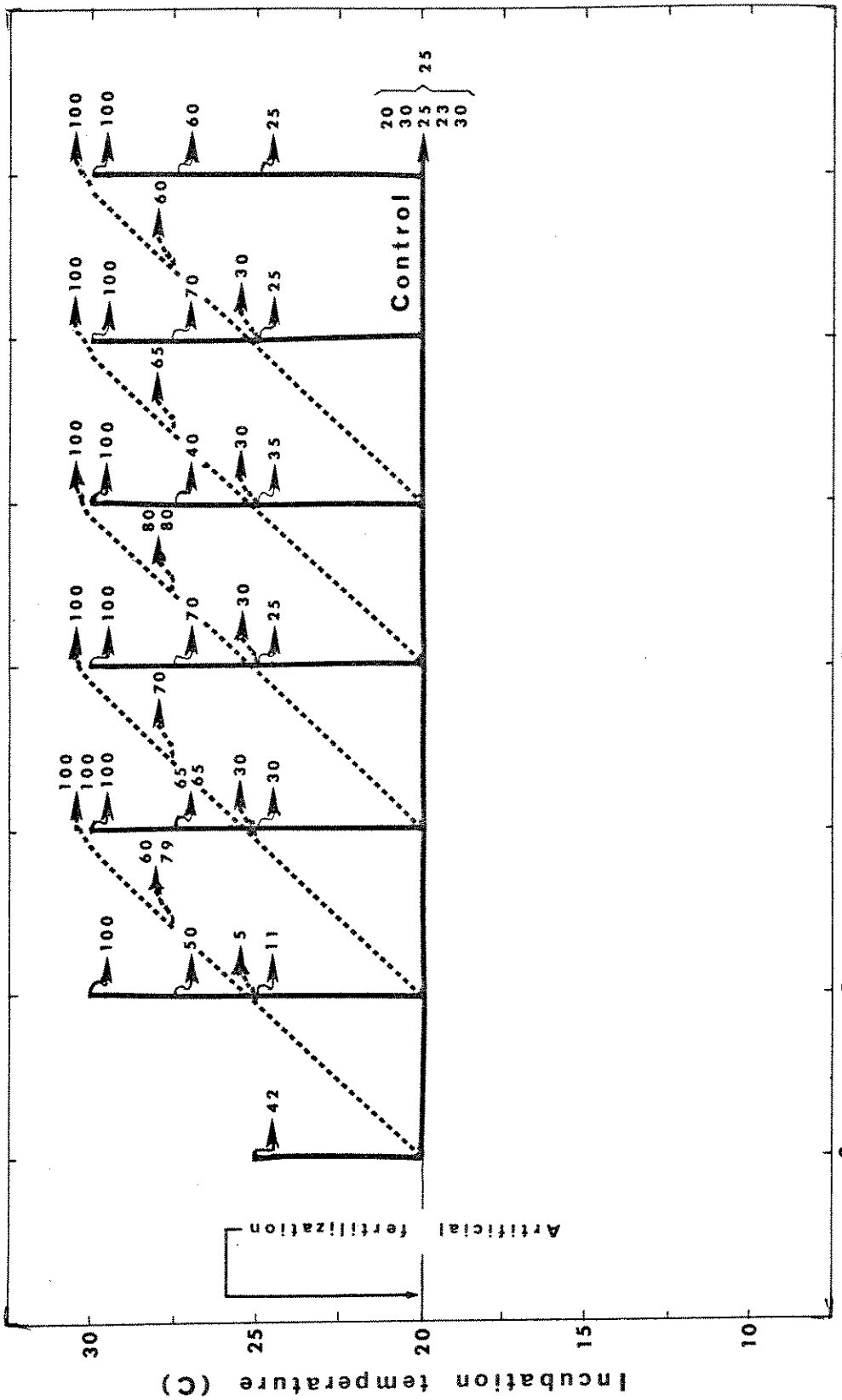

Figure 14. Schematic diagram of embryo transfers to various temperatures, showing per cent mortality to three days after hatching in experiment 4, 1968. Dotted and solid lines denote transfers with and without acclimation, respectively.

These diagrams show the temperature programs, on a time scale, to which each embryo lot was exposed, up to the level of final incubation temperature. Solid lines represent the sudden temperature transfer of non-acclimated embryos and dotted lines the gradual temperature transfer of acclimated embryos. Beyond this point the diagrams do not indicate the actual time elapsed to hatching. For example, in Figure 7 development at the 10 C level is only shown to a maximum of 36 hr after the start of the experiment, but the hatching point occurred nine days later.

Invalid and inaccurate data were omitted from these diagrams and from subsequent analysis. Factors responsible for such data included rapid disintegration of dead embryos at temperatures above 25 C, failure of the cooling system during the experiment which affected the lower incubation temperatures (10 and 12.5 C), and mistakes made in executing the temperature program.

It is evident that in each experiment there was substantial variation between the mortalities of individual lots, even those which were included at a constant temperature of 20 C and comprised the control lots. This variation is further demonstrated in Figure 15, which compares the ranges and means of mortalities at hatching and three days after hatching within the control lots at 20 C of the four experiments. In the control lots there was little additional mortality after hatching except in the first experiment.

Adjustment of mortality data

To estimate that portion of the total mortality rate (in a given egg lot) attributable to the temperature treatment, the standard

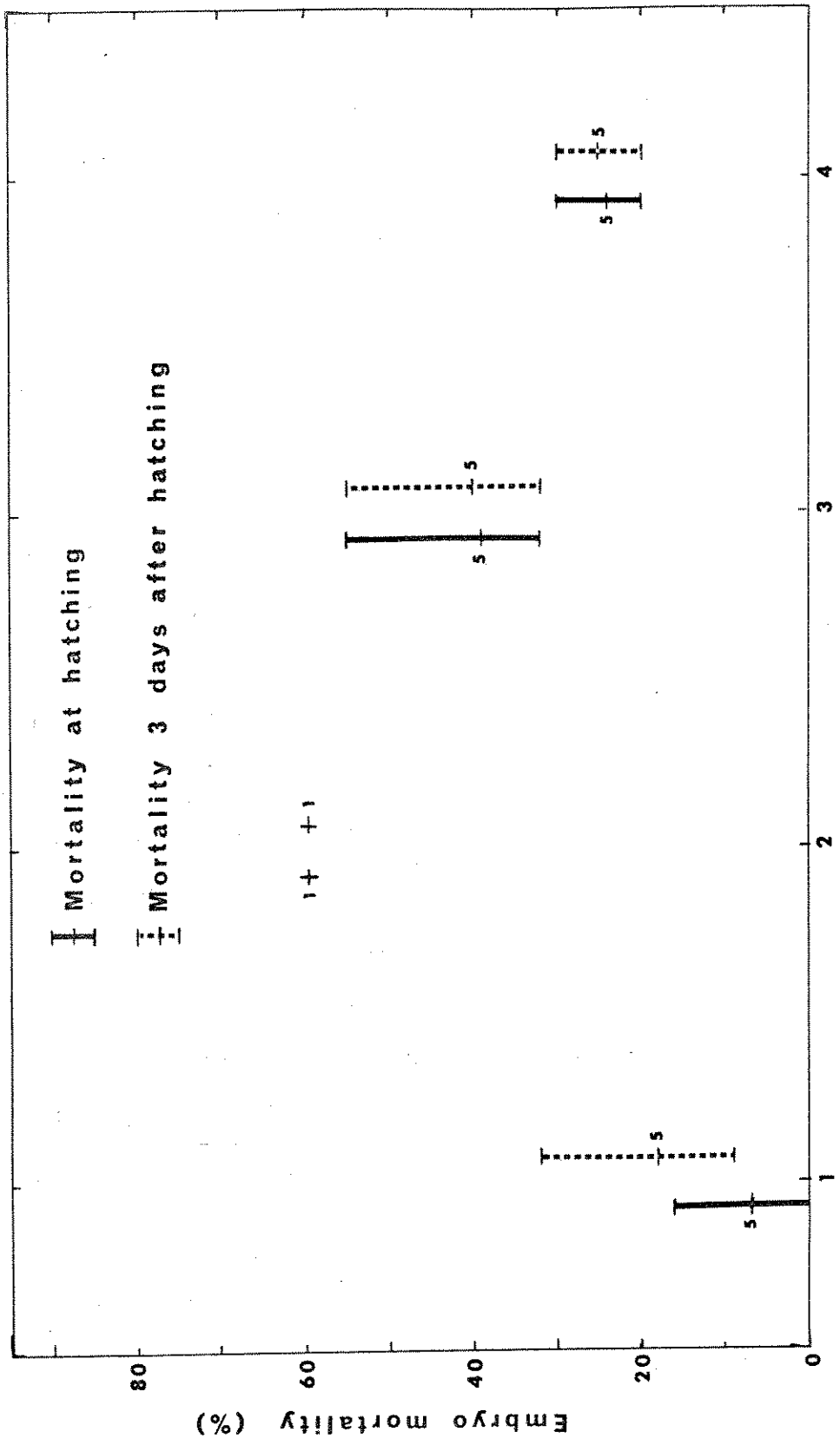

Figure 15. Embryo mortality of control lots kept at 20 C, showing the ranges and means. Number of observations are given for each mean.

exponential approach described by Ricker (1958) was used, with the same assumptions:[1/]

a = total mortality rate of a given lot.

m = mortality rate attributable to the temperature treatment (Ricker's angling mortality rate).

n = mortality rate attributable to factors other than treatment - i.e., mortality in the control lots of the experiment under consideration, incubated at 20 C throughout (Ricker's natural mortality rate).

$$a = m + n - mn$$

whence

$$m = \frac{a - n}{1 - n}$$

For subsequent analysis, treatment mortalities (m) of all the egg lots shown in Tables 2 through 5 were computed by the above formula, as illustrated in the following example:

As shown in Figure 7 (Experiment 1), total mortality (a) of the egg lot incubated at 20 C for 5 hr and then transferred directly (without acclimation) to a 27.5 C temperature bath was 35%. Mean mortality of the four control lots in this experiment was 7%. Treatment mortality (m) of the lot under consideration was

$$\frac{.35 - .07}{1.00 - .07} = \frac{.28}{.93} = .30$$

Tables 2 through 5 give the treatment mortality rates of non-acclimated and acclimated embryos at hatching and three days after

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[1/] William Swallow (unpublished description, 1968) called attention to a similar approach proposed by Abbott (1925) to estimate the effectiveness of insecticide treatments.

Table 2. Per cent treatment mortalities of non-acclimated largemouth bass embryos, to time of hatching in the four experiments of 1967-1968.

Age (hr) at transfer[1/]	Experiment No.	Final incubation temperature (C) 10	12.5	15	17.5	22.5	25	27.5	30	N	$\bar{x}$
	1	100	100	19	3	0	0	46	100		
0	2	100	100	100	0	0	0	0	0		
	3	100		67			100	100	100	22	52
	4						17				
	1	48						30	26		
	2	100						0	0		
5	3	100		26			23	75	100	14	38
	4						0	0	0		
	1	9						11	16		
	2	100						0	0		
10	3	100		59			51	30	100	14	34
	4						0	0	1		
	1	19						14	28		
	2	100						0	0		
15	3	100		43			34	0	34	14	28
	4						1	8	1		
	1	9						35	21		
	2	87						0	35		
20	3	100	100	100			26	0	42	15	38
	4						1	1	21		
	1	3						5	0		
	2	0						0	37		
25	3			0			0	0	26	13	8
	4						1	0	28		
	1	0						15	5		
	2	40						0	43		
30	3			26			2	0	18	13	12
	4						0	0	1		
	N	19	3	9	2	2	16	27	27	105	
	$\bar{x}$	64	100	49	2	0	16	14	29		

[1/] From initial (20 C) to final incubation temperature.

Table 3. Per cent treatment mortalities of acclimated largemouth bass embryos, to time of hatching, in the four experiments of 1967-1968.

Age (hr) at transfer[1]	Experiment No.	Final incubation temperature (C) 10	12.5	15	17.5	22.5	25	27.5	30	N	$\bar{x}$
0	1	3	19	0	0	3	15	19	14	24	26
	2	100	57	3	3	0	0	10	0		
	3	100		2			59	67			
	4						0	40			
5	1									15	12
	2	63	0	50	0	0	0	5	0		
	3			0			34	2	0		
	4						0	14	1		
10	1	25	9	11	9	3	4	11	15	21	7
	2	37	0	0	0			0	0		
	3			0			0	0	10		
	4						0	0	10		
15	1	25	9	0	9	0	0	11	6	23	10
	2	55	13	0	0	0	0	27	20		
	3			46			0	0	10		
	4						8	0	0		
20	1									7	2
	2										
	3			10			0	2	0		
	4						1	1	0		
	N	8	7	12	7	6	16	17	17	90	
	$\bar{x}$	51	16	10	3	1	8	12	10		

[1] Age at beginning of acclimation (at 1 C/hr) from initial 20 C to final (constant) incubation temperature.

Table 4. Per cent treatment mortalities of non-acclimated largemouth bass embryos, to three days after hatching, in the four experiments of 1967-1968.

Age (hr) at transfer[1/]	Experiment No.	Final incubation temperature (C) 10	12.5	15	17.5	22.5	25	27.5	30	N	$\bar{x}$
0	1	100	100	19	3	0	29	57	100	22	62
	2	100	100	100	0	20	40	17	100		
	3	100		67			100	100	100		
	4						23				
5	1	59						30	100	14	62
	2	100						0	100		
	3	100		26			46	91	100		
	4						0	33	100		
10	1	9						11	92	14	59
	2	100						0	83		
	3	100		66			58	46	100		
	4						7	53	100		
15	1	19						45	86	14	58
	2	100						0	55		
	3	100		41			33	78	91		
	4						0	60	100		
20	1	9						35	76	15	62
	2	100						0	60		
	3	100	100	100			26	100	100		
	4						13	20	100		
25	1	3						7	69	13	43
	2	55						0	63		
	3			0			0	100	100		
	4						1	60	100		
30	1	0						29	16	13	38
	2	75						0	55		
	3			26			2	41	100		
	4						0	47	100		
	N	19	3	9	2	2	16	27	27	105	
	$\bar{x}$	70	100	49	2	10	24	39	87		

[1/] From initial (20 C) to final incubation temperature.

Table 5. Per cent treatment mortalities of acclimated largemouth bass embryos, to three days after hatching in the four experiments of 1967-1968.

Age (hr) at transfer[1/]	Experiment No.	Final incubation temperature (C)								N	$\bar{x}$
		10	12.5	15	17.5	22.5	25	27.5	30		
	1	39	19	0	0	3	54	21	100		
	2	100	100	17	3	0	0	10	67		
0	3	100		8			66	75	100	24	44
	4						0	59	100		
	1										
	2	100	0	50	0	0	7	23	47		
5	3			0			34	17	100	15	36
	4						7	60	100		
	1	25	15	12	9	3	5	12	93		
	2	100	37	0	0			0	3		
10	3			0			0	50	100	21	30
	4						7	73	100		
	1	25	9	0	9	0	17	18	92		
	2	100	37	0	0	17	0	27	35		
15	3			46			0	100	100	23	34
	4						7	53	100		
	1										
	2										
20	3			17			41	100	100	7	59
	4						7	47	100		
	N	8	7	12	7	6	16	17	17	90	
	$\bar{x}$	74	31	12	3	4	16	44	84		

[1/] Age at beginning of acclimation (at 1 C/hr) from initial 20 C to final (constant) incubation temperature.

hatching for each egg lot in each experiment, as well as the number of observations and mean treatment mortality rate at each incubation temperature and age of transfer.[1/] These tables show that the available data are mainly concentrated at the temperature extremes and rather scanty with many gaps in the median temperature range.

Multiple regression analyses of treatment mortalities

The standard arcsin transformation was applied to the percentage treatment mortalities to normalize the data for statistical analysis. It was evident from Tables 2 through 5 that there were too many gaps in the data to perform a four-way factorial analysis of variance which would have revealed interaction effects. Instead, two multiple regression analyses were carried out, one for mortality to hatching and one for mortality to three days after hatching, utilizing the following variables:

Dependent Y = arcsin transformation of per cent treatment mortality rate

Independent X_1 = method of temperature transfer (i. e. with or without acclimation)

X_2 = embryo age (hours) at time temperature change initiated

X_3 = final incubation temperature (C)

To facilitate the calculations for the multiple regressions, the following coding system was used for the independent variables:

[1/] Since by definition there were no treatment mortalities in any of the (20 C) control lots, these lots are not included in Tables 2 through 5.

X_1:	Non-acclimation				Acclimation			
Code	0				1			
X_2 (hr):	0	5	10	15	20	25	30	
Code	0	1	2	3	4	5	6	
X_3 (C):	10	12.5	15	17.5	22.5	25	27.5	30
Code	-4	-3	-2	-1	1	2	3	4
$(Code)^2$	16	9	4	1	1	4	9	16

As the experiments showed that the relationship between temperature and mortality was parabolic (see Figure 16) the codes for temperature (X_3) were squared to obtain a straight-line regression of temperature on mortality.

The mechanics of the multiple regression analyses were carried out following the abbreviated Doolittle method as given by Steel and Torrie (1960, p. 290-299). Results of the analysis of mortality to hatching are presented in Table 6, and of mortality to three days after hatching in Table 7. Further details of the multiple regression analyses are provided in Appendix I.

The F tests in both tables showed that there is a highly significant reduction in sums of squares attributable to regression. The multiple correlation coefficients (R) were also highly significant in both cases, indicating that treatment mortality was highly correlated with acclimation, age and temperature.

The coefficient of determination (R^2) was low (26.08) in Table 6 and much higher (83.50) in Table 7, showing a considerable increase

Table 6. Analysis of multiple regression of treatment mortality (to hatching) on incubation temperature, acclimation and embryo age.

Significance of regression:

Source	d. f.	S. S.	M. S.	F
Regression on 3 variables	3	42,939	14,313	22.47**
Residual	191	121,687	637	
Total	194	164,626		

Equation $Y = 29.11 - 17.86X_1 - 5.518X_2 + 1.6569X_3$

No. of observations	195
Multiple correlation coefficient ($R_{y.123}$)	0.5107**
Coefficient of determination ($100R^2_{y.123}$)	26.08
Significance for partial regression coefficients (Student's t tests):	
t_1 (acclimation)	-4.687**
t_2 (age)	-5.333**
t_3 (temperature)	4.880**
Standard partial regression coefficients:	
b'_1 (acclimation)	-0.3080
b'_1 (age)	-0.3467
b'_1 (temperature)	0.3122

** Highly significant differences, at less than 1% level.

Table 7. Analysis of multiple regression of treatment mortality (to three days after hatching) on incubation temperature, acclimation and embryo age.

Significance of regression:

Source	d.f.	S.S.	M.S.	F
Regression on 3 variables	3	185,256	61,752	321.6**
Residual	191	36,595	192	
Total	194	221,851		

Equation $Y = 17.58 - 8.441X_1 - 3.664X_2 + 4.151X_3$

No. of observations	195
Multiple correlation coefficient ($R_{y.123}$)	0.9138**
Coefficient of determination ($100R^2_{y.123}$)	83.50

Significance for partial regression coefficients (Student's t tests):

t_1 (acclimation)	-4.0356**
t_2 (age)	-6.4477**
t_3 (temperature)	22.26**

Standard partial regression coefficients

b'_1 (acclimation)	-0.1255
b'_1 (age)	-0.1983
b'_1 (temperature)	0.6739

**Highly significant differences, at less than 1% level.

in the amount of reduction in the sum of squares of the treatment mortality to three days after hatching attributable to the combined effect of acclimation, embryo age at transfer and temperature.

Student's t tests on the partial regression coefficients showed that in both cases the effects on treatment mortality due to acclimation, embryo age and incubation temperature are all highly significant.

The standard partial regressions in Table 6 are similar to each other, indicating that acclimation, age and temperature contributed almost equally to the treatment mortality to hatching. In Table 7, however, it appears that temperature was the major determinant of treatment mortality to three days after hatching, and hence this increased effect of temperature is probably mainly responsible for the increase in the coefficient of determination three days after hatching noted above. The ranking of the importance of the effects of acclimation, age and temperature probably has little significance except in the case of the effect of temperature on treatment mortality to three days after hatching in Table 7.

Graphic analysis of factors affecting treatment mortality

Figure 16 shows the parabolic relationship between final incubation temperature and mean treatment mortality to hatching and to three days after hatching, for both non-acclimated and acclimated embryos. Treatment mortality is lowest in the median temperature range (around 20 C) and increases towards 10 and 30 C. Treatment mortality to three days after hatching is higher than at hatching, the difference increasing towards the 10 and 30 C temperature extremes. Treatment mortality

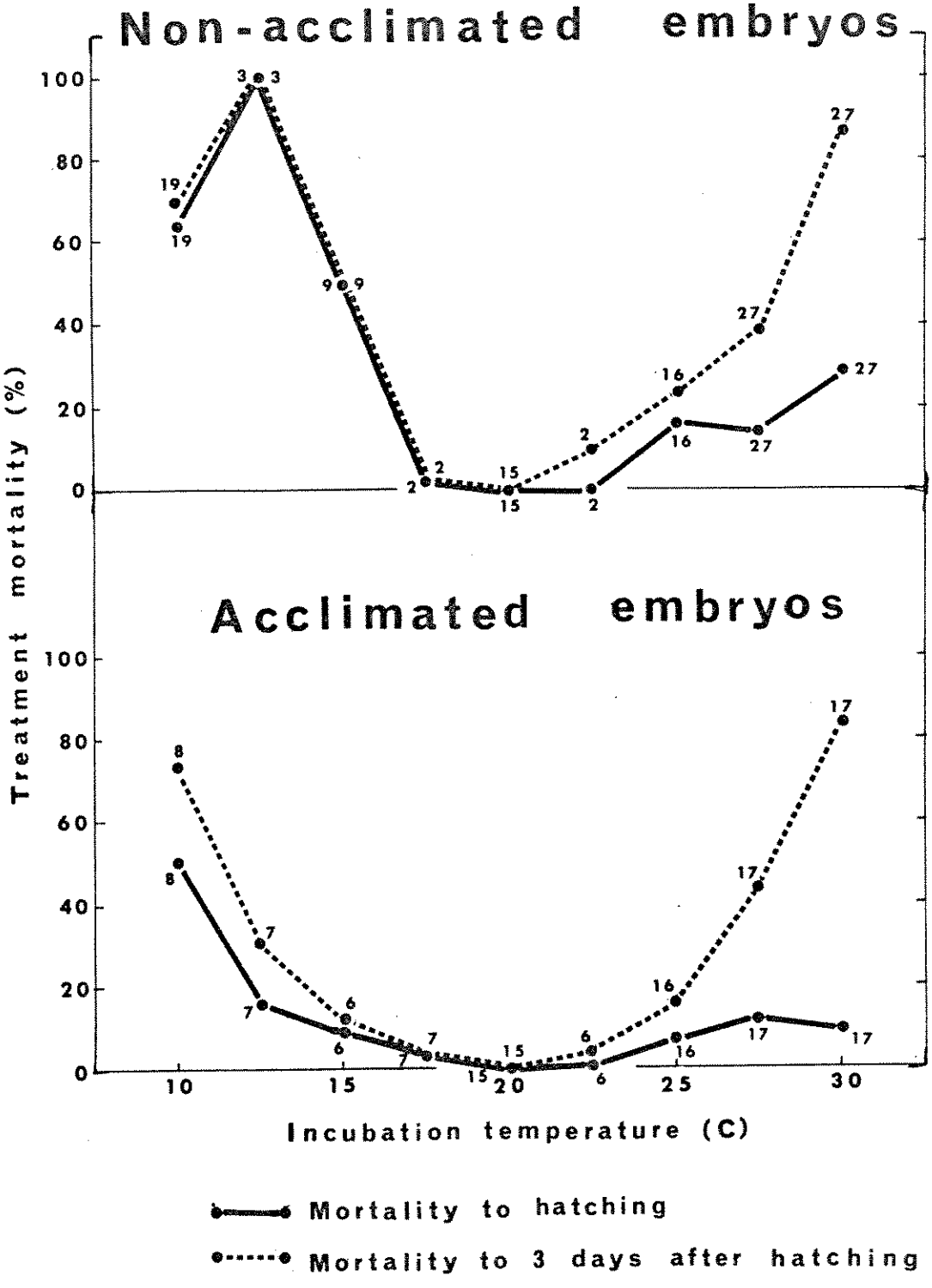

Figure 16. Graphs showing relationship between mean embryo treatment mortalities and final incubation temperatures. Number of observations are given for each mean. Combined data for various final incubation temperatures, regardless of when transferred.

towards the temperature extremes is higher and more irregular in non-acclimated than in acclimated embryos. The higher treatment mortality of non-acclimated embryos at 12.5 C than at 10 C is probably due to the paucity of observations (3) at 12.5 C.

Figure 17 shows the relationship between embryo age at the initiation of transfer from 20 C to the final incubation temperature and mean treatment mortality to hatching and to three days after hatching, for both non-acclimated and acclimated embryos. Treatment mortality in non-acclimated embryos decreases slightly to 15 hours, and then fluctuates, whereas in acclimated embryos treatment mortality decreases to 10 hours and then fluctuates in the case of treatment mortality to three days after hatching. The data are too variable and not precise enough to suggest that the discrepancy between 10 and 15 hr lies in the time 'reference point' chosen for the acclimated embryos. The overall significant trend in all cases, however is a decrease of treatment mortality with increasing age at transfer, as shown in the multiple regression analyses. There is no clearcut evidence of a critical temperature-sensitive age beyond immediately after fertilization.

Variability of comparable treatment mortalities between experiments

Table 8 indicates by means of chi-square tests that there is a highly significant difference in every case tested between the comparable treatment mortalities of the four experiments. Only those cases were considered where the data of all four experiments overlapped, which occurred at 25, 27.5 and 30 C incubation temperatures and at all embryo ages at transfer (0 to 30 hr) to those temperatures. Unfortunately data was too incomplete for testing at any of the other lower temperatures.

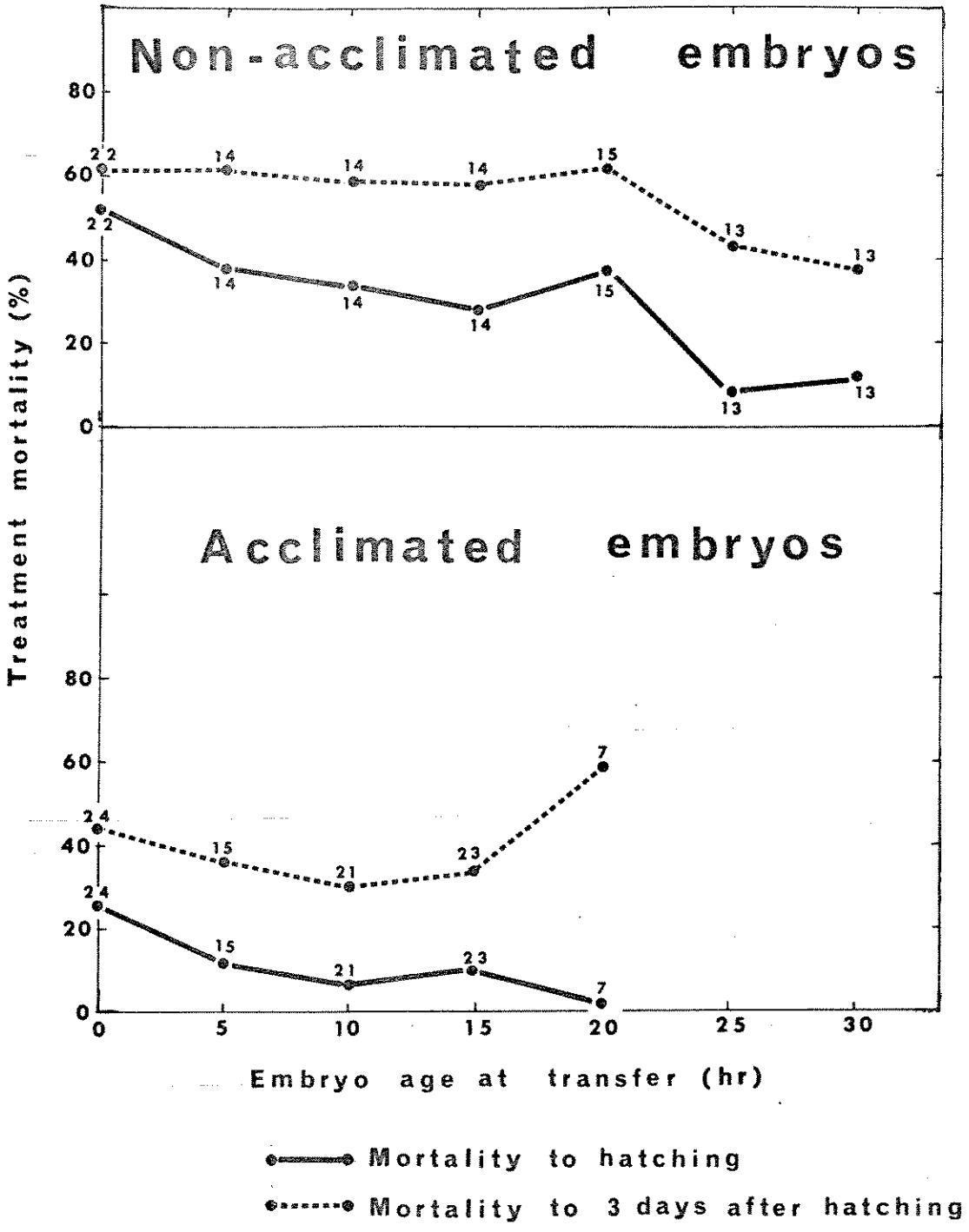

Figure 17. Graphs showing relationship between mean embryo treatment mortalities and embryo age at transfer. Number of observations are given for each mean.

Table 8. Comparison of mean treatment mortalities between the four experiments at those incubation temperatures (25, 27.5 and 30 C) at which there were completely replicated data from all four experiments and at all ages of transfer (0 to 30 hr) to final incubation temperature.

Stage	Treatment	Experiment No.	Per cent treatment mortality		X^2
			Observed	Expected 1/	
To hatching	Non-acclimated embryos (n = 8)	1	12	14	26**
		2	7	14	
		3	30	14	
		4	7	14	
		Total	56	56	
	Acclimated embryos (n = 13)	1	16	18	42**
		2	9	18	
		3	41	18	
		4	6	18	
		Total	72	72	
To three days after hatching	Non-acclimated embryos (n = 8)	1	51	51	34**
		2	18	51	
		3	74	51	
		4	61	51	
		Total	204	204	
	Acclimated embryos (n = 13)	1	48	58	36**
		2	27	58	
		3	88	58	
		4	69	58	
		Total	232	232	

** Highly significant differences, at less than 1% level.

1/ Expected no. = $\frac{\text{total}}{4}$

Daily mortality during development

Tables 9 through 13 give the daily mortalities during development of non-acclimated embryos transferred to 12.5 and 27.5 C at 0 and 30 hr age, acclimated embryos transferred to 12.5 and 27.5 C at 0 and 15 hr age, and the control lots kept at 20 C. These mortalities included deaths of unfertilized eggs. These cases serve as a good illustration of the considerable variability between individual experiments with regard to daily mortality patterns and trends. Experiment 1 data were excluded because no observations of mortality were made during the first two days after fertilization.

Despite the variability, the mean daily mortality rates at 12.5 C for non-acclimated and acclimated embryos plotted in Figure 18, showed that most of the mortality occurred before hatching. One observation per day was not precise enough, however, to detect any well-defined delay in mortality due to acclimation or increased age at transfer.

In Figure 19 (transfers to 27.5 C) there does appear to be a delay in mortality between embryos transferred at 0 hr and those transferred at 15 and 30 hr. As in Figure 18, there seems little appreciable difference between non-acclimated and acclimated embryo mortality patterns. In embryos transferred later to 27.5 C (at 15 and 30 hr) there occurred two peak mortalities, one at two days and one at five days after fertilization. The second peak occurred two days after hatching.

Figure 20 shows the plotted mean daily mortality rates of non-acclimated and acclimated embryos transferred to 12.5 and 27.5 C, compared to the means of the control lots kept at 20 C. Greatest mortality of most embryo lots occurred before hatching and the greatest initial mortality occurred on the first day after fertilization at 27.5 and

Table 9. Numbers of deaths and (in parentheses) percentages of all deaths that occurred each day during development of non-acclimated embryos transferred to 12.5 C temperature baths at ages 0 and 30 hr.

Mortality at age 0 hr transfer

Day	Experiment 2	Experiment 3 (a)	Experiment 3 (b)	Mean %
1	1 (5)	3 (30)	1 (10)	12
2	0	6 (60)	6 (60)	29
3	7 (33)	1 (10)	0	20
4	13 (62)	0	2 (20)	36
5		0	0	0
6		0	0	0
7		H[1/] 0	H 0	H 0
8		0	0	0
9		0	0	0
10		0	1 (10)	3
11		0	0	0
Total deaths	21	10	10	100

Mortality at age 30 hr transfer

Day	Experiment 3
1	7 (87)
2	1 (13)
3	0
4	0
5	0
6	H 0
7	0
8	0
9	0
10	0
11	0
Total deaths	8 (100)

[1/] H = hatching point.

Table 10. Numbers of deaths and (in parentheses) percentages of all deaths that occurred each day during development of acclimated embryos transferred to 12.5 C temperature baths at ages 0 and 15 hr.

Mortality at age 0 hr transfer

Day	Experiment 2	Experiment 3 (a)	Experiment 3 (b)	Mean %
1	0	3 (25)	3 (33)	14
2	4 (19)	5 (42)	3 (33)	29
3	5 (24)	0	2 (22)	17
4	0	1 (8)	0	3
5	8 (38)	0	0	19
6	1 (5)	0	1 (11)	5
7	0	H 1 (8)	H 0	H 2
8	0	0	0	0
9	0	1 (8)	0	2
10	H[1] 0	0	0	H 0
11	0	1 (8)	0	2
12	3 (14)			7
Total deaths	21	12	9	100

Mortality at age 15 hr transfer

Day	Experiment 2	Experiment 3	Mean %
1	0	7 (78)	30
2	4 (27)	2 (22)	25
3	8 (53)	0	33
4	1 (7)	0	4
5	0	0	0
6	0	0	0
7	0	0	0
8	H 0	H 0	H 0
9	0	0	0
10	1 (7)	0	4
11	1 (7)	0	4
Total deaths	15	9	100

[1] H = hatching point.

Table 11. Numbers of deaths and (in parentheses) percentages of all deaths that occurred each day during development of embryos kept at 20 C (control lots).

Day	Experiment 2	Experiment 3 (a)	Experiment 3 (b)	Experiment 3 (c)	Experiment 3 (d)	Experiment 3 (e)	Experiment 4 (a)	Experiment 4 (b)	Experiment 4 (c)	Experiment 4 (d)	Experiment 4 (e)	Mean %
1	0	9 (82)	4 (57)	5 (83)	3 (33)	5 (83)	0	2 (33)	1 (14)	0	1 (17)	36
2	3 (17)	2 (18)	3 (43)	1 (17)	4 (45)	0	4 (100)	2 (33)	0	1 (20)	1 (17)	25
3	7 (39)	H 0	H 0	H 0	H 1 (11)	H 1 (17)	0	H 2 (33)	2 (29)	3 (60)	H 2 (33)	H 21
4	H[1/] 2 (11)	0	0	0	1 (11)	0	H 0	0	H 2 (29)	H 1 (20)	2 (33)	H 9
5	0	0	0	0	0	0	0	0	1 (14)	0	0	1
6	0	0	0	0	0	0	0	0	1 (14)	0	0	1
7	0											0
8	0											0
9	0											0
10	0											0
11	0											0
12	1 (5)											1
13	4 (22)											5
14	1 (5)											1
Total deaths	18	11	7	6	9	6	4	6	7	5	6	100

[1/] H = hatching point.

Table 12. Numbers of deaths and (in parentheses) percentages of all deaths that occurred each day during development of non-acclimated embryos transferred to 27.5 C temperature baths at ages 0 hr and 30 hr.

Mortality at age 0 hr transfer

Day	Experiment 2	Experiment 3 (a)	Experiment 3 (b)	Experiment 4 (a)	Experiment 4 (b)	Mean %
1	5 (28)	20 (100)	20 (100)	19 (95)	19 (95)	85
2	H[1/] 5 (28)			H 1 (5)	H 0	H 6
3	0				1 (5)	1
4	1 (5)					1
5	1 (5)					1
6	0					0
7	0					0
8	2 (11)					2
9	4 (22)					4
Total deaths	18	20	20	20	20	100

Mortality at age 30 hr transfer

Day	Experiment 2	Experiment 3	Experiment 4	Mean %
1	2 (11)	4 (27)	0	12
2	9 (50)	3 (20)	3 (18)	30
3	H 1 (6)	H 0	H 0	H 2
4	0	0	0	0
5	0	6 (40)	9 (53)	30
6	0	2 (13)	5 (29)	14
7	0			0
8	0			0
9	0			0
10	6 (33)			12
Total deaths	18	15	17	100

[1/] H = hatching point.

Table 13. Numbers of deaths and (in parentheses) percentages of all deaths that occurred each day during development of accli- embryos transferred to 27.5 C temperature baths at ages 0 and 15 hr.

Mortality at age 0 hr transfer

Day	Experiment 2	Experiment 3 (a)	Experiment 3 (b)	Experiment 4 (a)	Experiment 4 (b)	Mean %
1	5 (36)	16 (80)	11 (55)	13 (68)	5 (25)	54
2	H[1/] 4 (29)	H 1 (5)	H 4 (20)	H 0	H 3 (15)	H 13
3	0	0	0	0	0	0
4	0	2 (10)	1 (5)	2 (11)	4 (20)	10
5	0	1 (5)	4 (20)	1 (5)	2 (10)	8
6	1 (7)	0	0	3 (16)	6 (30)	11
7	0					0
8	0					0
9	0					0
10	4 (29)					4
Total deaths	14	20	20	19	20	100

Mortality at age 15 hr transfer

Day	Experiment 2	Experiment 3	Experiment 4	Mean %
1	1 (5)	5 (25)	0	10
2	14 (74)	1 (5)	3 (16)	31
3	H 0	H 0	H 1 (5)	H 2
4	0	1 (5)	8 (42)	16
5	0	13 (65)	7 (37)	34
6	0			0
7	0			0
8	0			0
9	0			0
10	4 (21)			7
Total deaths	19	20	19	100

[1/] H = hatching point.

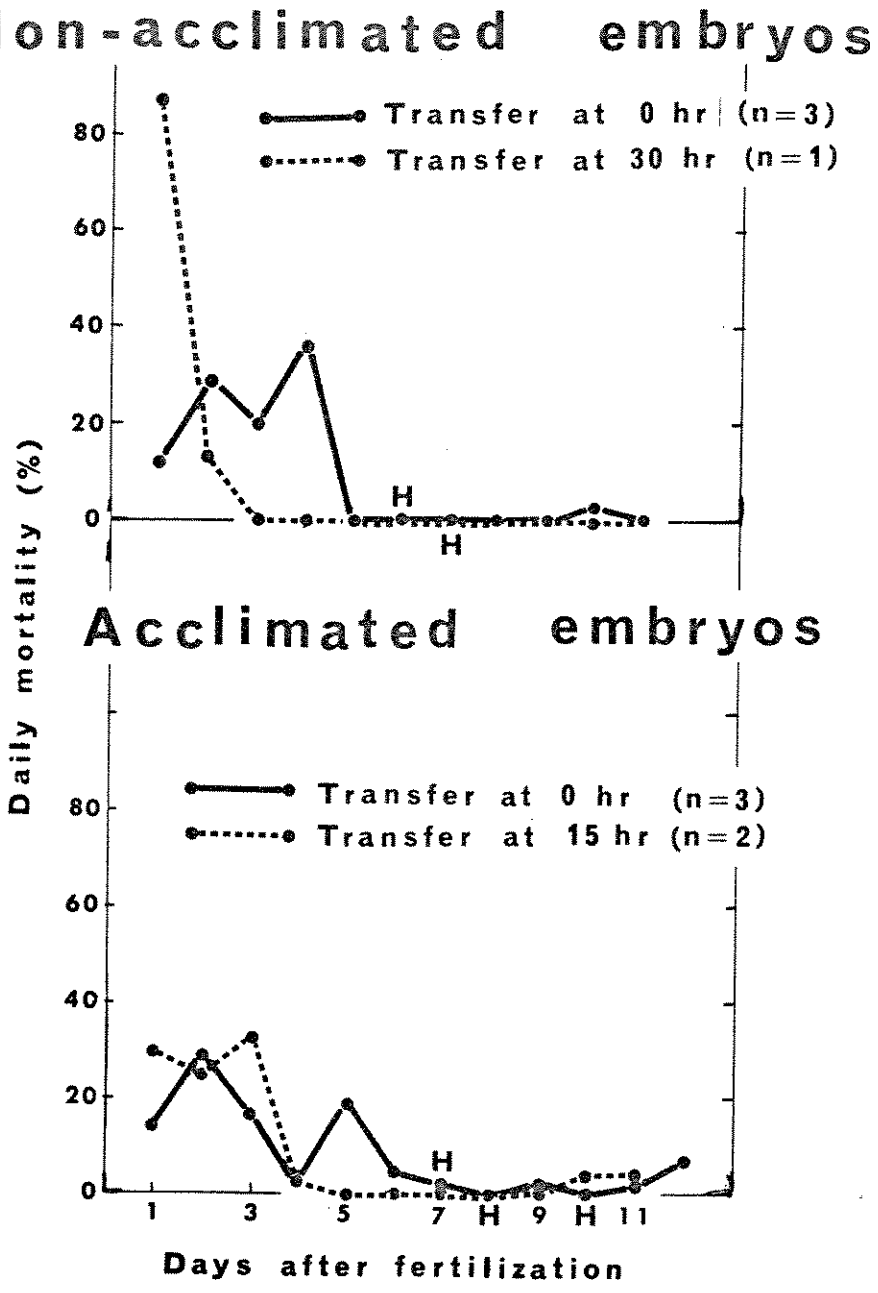

Figure 18. Mean percentage daily mortality of embryos transferred to 12.5 C from 20 C after 30 hours (non-acclimated), 15 hours (acclimated) and 0 hours (both). Data from last columns of Tables 9 and 10. **H** = hatching point.

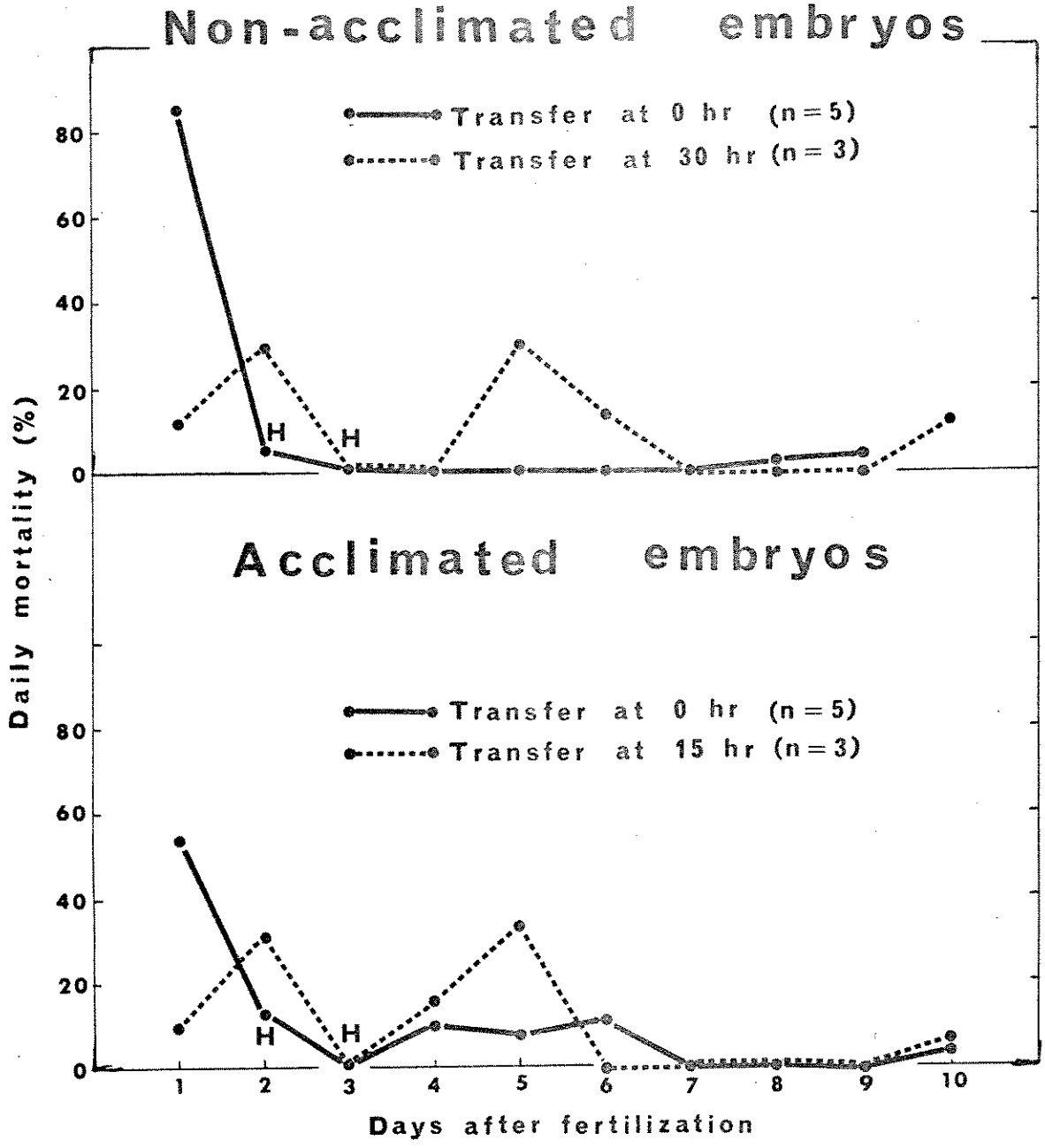

Figure 19. Mean percentage daily mortality of embryos transferred to 27.5 C from 20 C after 30 hours (non-acclimated), 15 hours (acclimated) and 0 hours (both). Data from last columns of Tables 12 and 13. H = hatching point.

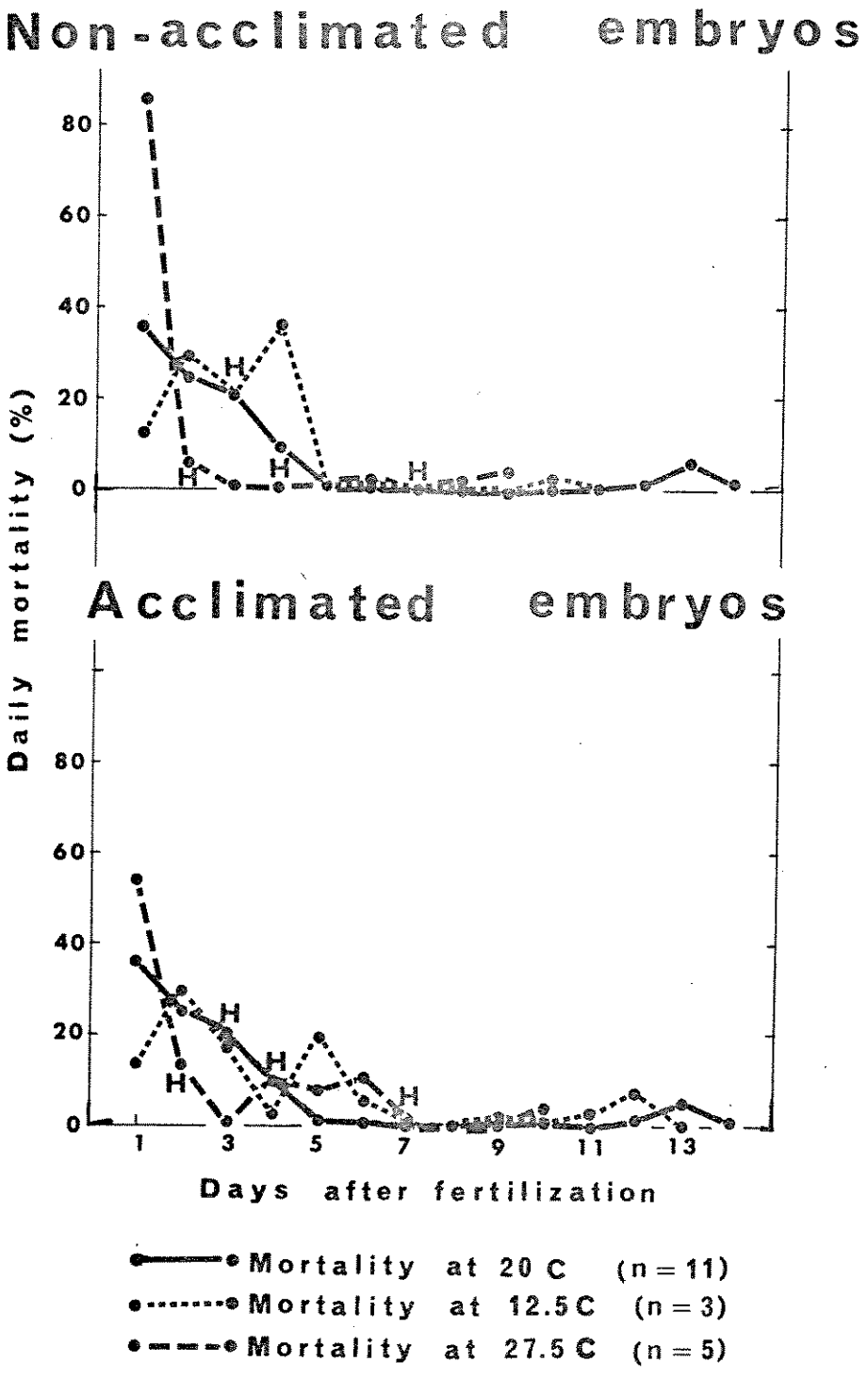

Figure 20. Mean percentage daily mortality of embryos transferred to 12.5 C, 20 C (control) and 27.5 C temperature baths at age 0 hours. Data from last columns of Tables 10 through 13. **H** = hatching point.

20 C incubation temperatures. At 12.5 C most of the mortality occurred from the second to the fifth day after fertilization.

It could not be determined to what extent the daily mortality rates observed were influenced by any delay in the appearance of death symptoms or the presence of unfertilized eggs.

Embryological examinations of eggs

During the 1967 experiments extensive collections were made of live and dead eggs at various stages of development. While it was possible to distinguish with the help of Carr (1942) the more advanced stages after gastrulation in live eggs even after they were subsequently preserved in 5% formalin, the opaque dead eggs could not be cleared after preservation in formalin, although various clearing agents were tried.

During the 1968 experiments dead embryos were successfully cleared if they were immediately put in 5% acetic acid instead of formalin. Dead eggs in both experiments were cleared in this way immediately after removal from the incubation containers and examined under a binocular dissecting microscope with 45X magnification.

Numerous unfertilized eggs were examined while still fresh and clear and it was noted that their contents were homogeneous without a polar cap. In contrast, a polar cap was distinctly visible in most eggs examined immediately after fertilization. This feature remained visible even in eggs preserved in formalin or acetic acid.

On this basis, out of 210 dead eggs examined from Experiment 3 (1968) 2 days after fertilization, 6 unfertilized eggs (2.85%) were detected. No unfertilized eggs were detected in Experiment 4.

No stages could be clearly distinguished in any of the dead eggs examined during Experiments 3 and 4. Most of these seemed to be in some blastodisc stage with some doubtful exceptions: one early embryo at 20 C in 12 to 16 hr stage in Experiment 3, and one 11 hr stage with yolk plug at 25 C in Experiment 4, following the classification of Carr (1942). Stages prior to age 20 hr also could not be clearly distinguished in samples taken hourly from the normally-developing embryos at 20 C. At the hatching stage and beyond, mortality of larvae while hatching and completely hatched was easily recognized, and these stages are correctly reflected in the daily mortality rates.

From the above observations, it would seem that, judging from the supposition that only a blastodisc stage was detectable, any mortality noted beyond the first day or two after fertilization could be attributed largely to delayed death symptoms, which supports the contention of Johnson and Brice (1953).

Relationship between treatment mortality and degree-days

No well-defined correlation was found between treatment mortality to hatching of acclimated and non-acclimated embryos in Experiments 1 and 2 (1967) and number of degree-days to hatching, either when a base of 0 C or one of base 20 C was used (Figures 21 and 22 respectively). Detailed data are given in Appendix II.

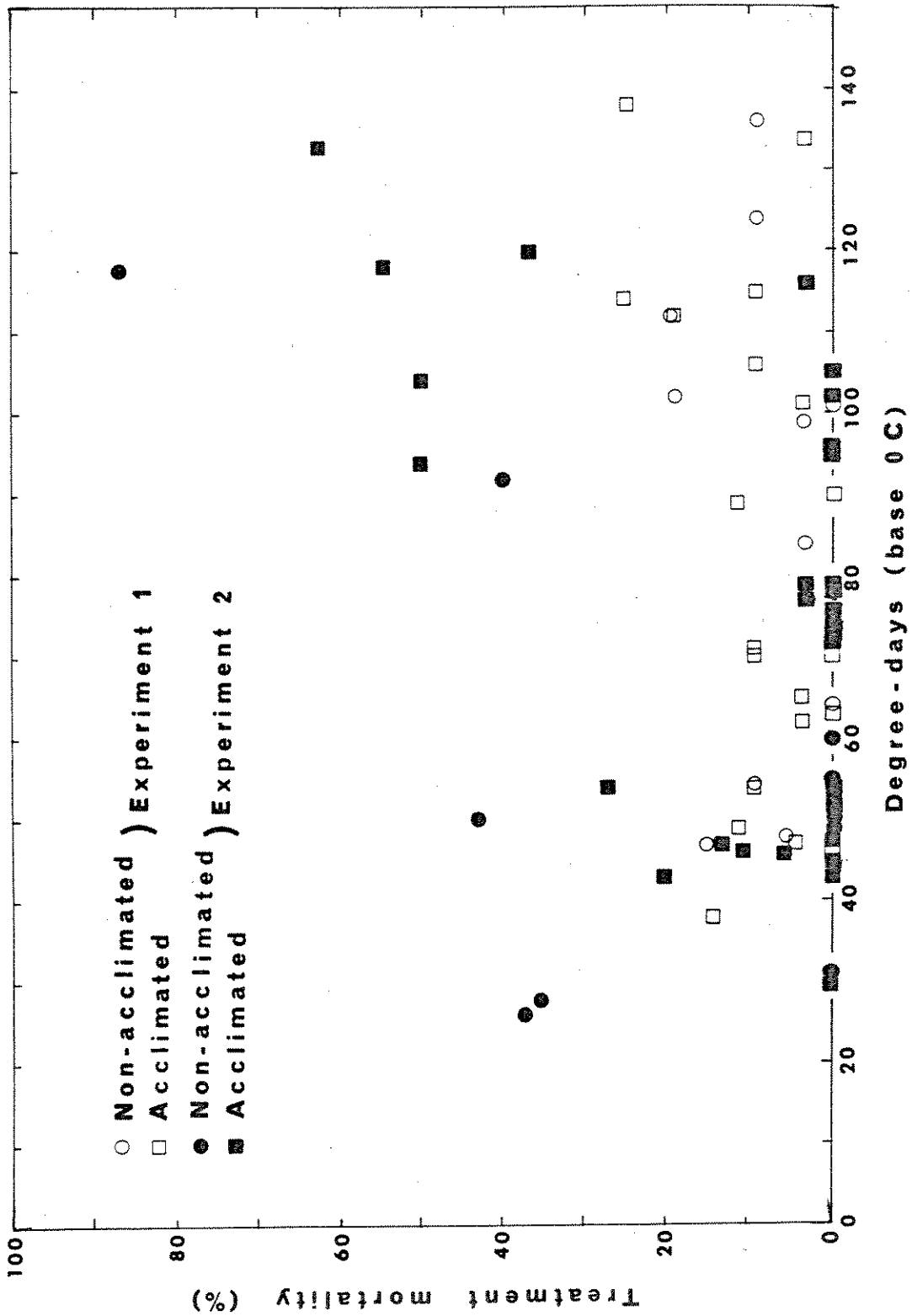

Figure 21. Percentage treatment mortality to hatching of embryos in experiments 1 and 2 (1967) plotted against degree-days to hatching, with base at 0 C.

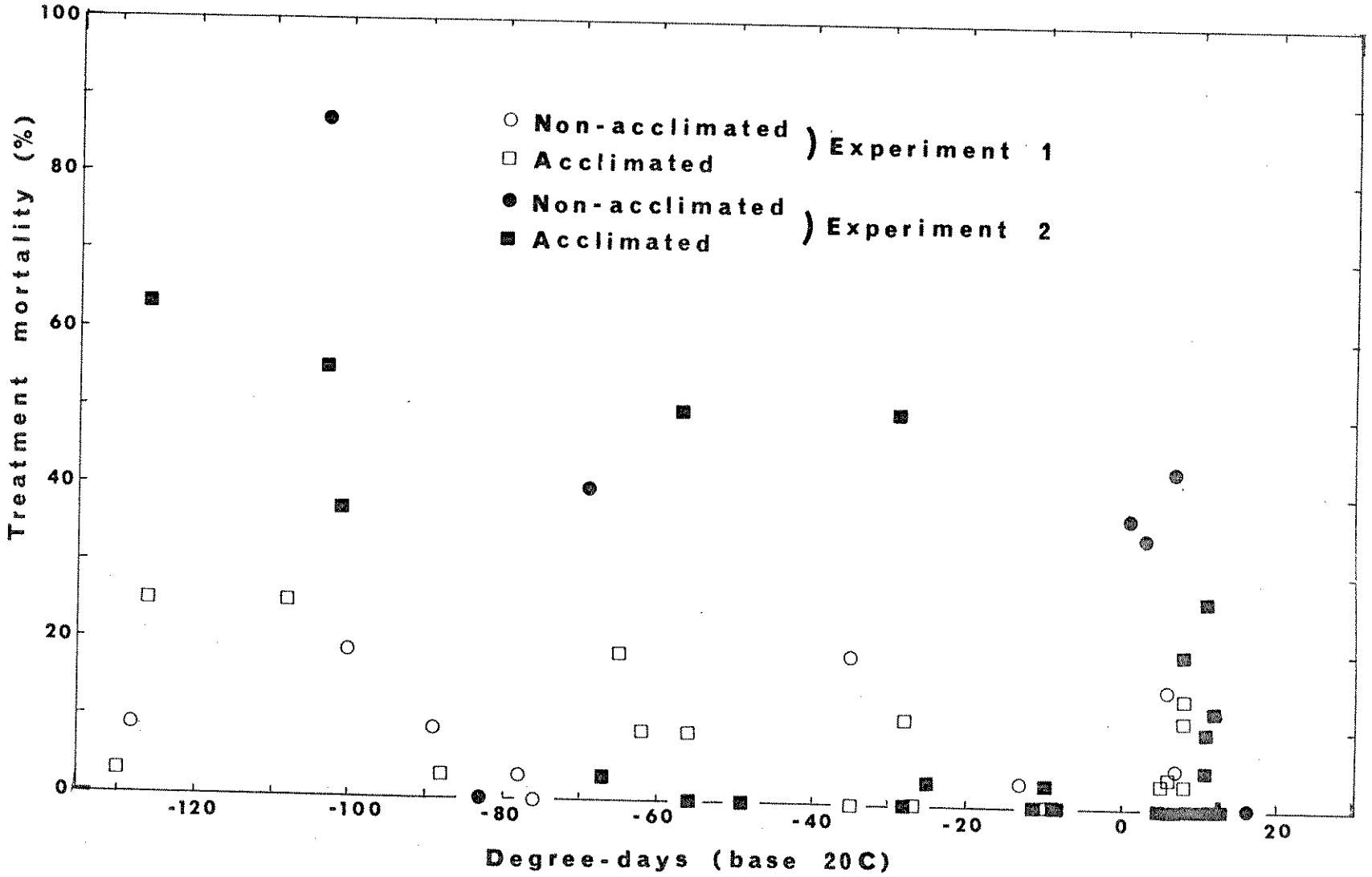

Figure 22. Percentage treatment mortality to hatching of embryos in experiments 1 and 2 (1967) plotted against degree-days to hatching, with base at 20 C.

DISCUSSION

Utility and limitations of data obtained

A striking feature in all four experiments was the significantly variable mortality obtained (Table 8), even in the case of the control lots at 20 C (Figure 15). This might have been caused by unsuitable spawning periods resulting in decreased egg viability or by handling during capture of the adult bass, the artificial fertilization procedure, the loading and transferring of the lots into the aquaria, and the examination of the embryos during the experiment.

Despite the compensatory adjustment made to consider treatment mortality rather than total mortality, and because the variability was by no means eliminated by this adjustment, the data is of limited value quantitatively and should rather be considered from a qualitative aspect, i.e. the examination of trends or directions of change.

Interpretation of data

The pond temperatures in 1967 (Figure 5) showed that the spring warm-up was delayed somewhat and the bass spawned about one week later than in 1966. However spawning still took place in temperatures considered suitable (i.e. above 15 C and still rising). In 1968 the pond temperatures (Figure 6) were about the same as in 1967 but the bass started spawning in colder temperatures, about one week earlier than in 1966. However, even in eggs spawned at 11.5 C in Pond I, only slight mortality was noted, provided the male remained to tend the nest. This might indicate that the bass used in the 1967 experiments, especially in

Experiment 2 towards the end of the spawning season, may have been affected in the gonads by the delayed spawning period, as noted also by Beeman (1924) who, however also contradicted himself by stating that the last spawning of the season was the most vigorous. This is borne out by the mortality observed in the control lot in Experiment 2, which was highest of the control lots in all four experiments in 1967 and 1968. Nakai (1927 and 1928b) also stressed the importance of the prime spawning season for getting eggs in good condition. The lower mortality of the control lots in the other three experiments indicate that these were not affected to such a degree by any possible unsuitability of the spawning seasons.

The physico-chemical analyses in 1967 (Table 1) revealed little that could be considered as a hindrance to the successful bass-spawning which occurred in these ponds during that season.

Despite the low coefficient of determination found by the multiple regression analysis of treatment mortality to hatching (Table 6), which showed that only 26% of the mortality was attributable to the treatment effects (and left 74% unexplained), all three independent variables, namely acclimation, age and temperature, were found to have a highly significant effect on the treatment mortality. These variables affected the mortality about equally, and ranking of the effects has little significance in this case. This low coefficient of determination provided another reason why the data could hardly be considered quantitatively. However, the directions of change (whether the independent variables caused increased or decreased mortality) were significantly detectable and could be predicted. These trends were accentuated in the multiple regression analysis carried out for treatment mortality to three days

after hatching (Table 7) which showed a considerable increase in the coefficient of determination (83.5) mainly attributable to a considerably heightened temperature effect which far exceeded the combined effects of age and acclimation. In this case the ranking of temperature as the most important effect after hatching, was of considerable significance, depressing the effects of age and acclimation which still however contributed about equally. It should be kept in mind that most of the mortality after hatching occurred at incubation temperatures above 25 C (Figure 16), and that therefore the effects at the temperature range 25 to 30 C contributed most to the regression.

It is clear that treatment mortality decreased with acclimation as well as with embryo age at the time of temperature fluctuation (Figure 17). There was a parabolic relationship between treatment mortality and incubation temperature with rapidly increasing mortality towards the extremes (10 and 30 C) and decreasing mortality towards 20 C. An exception occurred in acclimated embryos, which had but a relatively slight increase in treatment mortality at hatching in incubation temperatures above 25 C. Thus an acclimation rate of 1 C per hour was sufficient to practically overcome the ill-effects of the warmer incubation temperatures. This might have some significance in nature where pond temperatures seldom increase at a greater rate than 1 C per hour. There is some indication that there might be a zone of tolerance for embryos ranging from 15 to 25 C, hence the embryos from the earliest stage onwards could probably well withstand any of the fluctuations which occurred during and after the spawning seasons of 1967 and 1968 (Figures 5 and 6).

However, largemouth bass embryos did increase their resistance to adverse temperatures with increasing age, showing that they were most susceptible to mortality immediately after fertilization (Figure 17), which is supported by the observations made on stage of development at time of death. This initial mortality is further shown in the daily mortalities (Figures 18 to 20) which may be confounded, however, up to the hatching stage by delayed death symptoms as mentioned earlier. None of the above evidence indicates any separate critical temperature-sensitive periods apart from the initial high mortality observed immediately after fertilization. Rather the evidence points more towards a gradual lessening of the initially high mortality rate with increasing embryo age.

Comparison of findings with other research work

Mortality in most of the 20 C control lots of embryos produced by artificial fertilization was higher than that in comparable groups of naturally-fertilized embryos reported by Kelley (1968).

Mortalities at hatching obtained in largemouth bass embryos incubated at optimum temperatures (around 20 C) by previous workers has been compared with the present study in the following table:

Method	Author	Percent mortality Range (mean)
Natural fertilization	Kramer and Smith (1962)	6-100
	Kelley (1968)	0- 9.2 (3.5)
Artificial fertilization	Sprecher (1938)	(51)
	Carr (1942)	(14.3)
	Present study: (Experiments 1, 2, 4)	7-60 (19.5)
(Hormone induced)	(Experiment 3)	32-55 (39)

The above table shows that mortality, even of naturally-fertilized eggs, varied considerably.

The high initial mortality immediately following artificial fertilization, showing that at this period the embryos are most sensitive to temperature and/or handling, has also been found by Bonnett (1939), Combs (1965) and Swallow[1/] and called a critical period by them. The evidence from the 1967-68 experiments did not show that mortality was increased at the stage of blastopore closure, labelled a critical period by Hayes and Armstrong (1942), Hayes (1949) and Franklin and Smith (1963). There was no evidence to support a critical period at hatching recorded in various fish species by Kowalska (1959), Hubbs (1966) and other workers mentioned by Swallow.[1/]

The parabolic relationship between temperature and mortality found in the present study supports the findings of Combs and Burrows (1957), Swift (1965a) and Swallow (unpublished[2/]). This work also supports Kelley (1968) who found increased embryo mortality at temperature extremes of 10 and 30 C and decreased mortality with acclimation (which was confounded with embryo age in his experiments). The gradually decreasing mortality with increasing age noted here agrees with the findings of other workers, as reported by Swallow.[1/]

From the above findings and discussion, it appears that temperature influences on largemouth bass embryo mortality during the incubation period are unlikely to account entirely for high mortalities

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[1/] Swallow, W. H.: The relation of incubation temperature to the mortality of fish embryos. M. S. Thesis, 1968, Cornell Univ., 45 p.

[2/] Swallow, W. H.: The effects of incubation temperature on survival of largemouth bass eggs - literature review and preliminary (1965) experiments. In N. Y. Coop. Fish. Unit Ann. Report for 1966, Cornell Univ. (mimeo)

observed in nature, where incubation temperatures (in this latitude) seldom if ever reach the high or low levels found to be critical in these experiments. This has already been suggested by Kelley (1968). It is probable that many factors contribute to the mortality during the entire period from ovum to adulthood, but that these factors, and their interactions, may have their greatest impact during the egg stage when most mortality has been shown to occur. The importance of a prime spawning season on egg viability as stressed by Beeman (1924) and Nakai (1928b), was also suspected as a possible additional factor causing variability in embryo mortality in the present study. However, excessive mortality was not observed in naturally spawned eggs during the 1967-1968 seasons.

It seems further entirely possible that the delayed death symptoms propounded by Johnson and Brice (1953), also served in this case as a confounding factor in determining the time of embryo mortality.

The increased mortality after hatching, found at temperatures above 25 C, may well be due to the secondary effect of starvation from the increased metabolic rate causing a rapid depletion of yolk energy reserves, as suggested by Gray (1928).

The lack of correlation found between minimum mortality and maximum degree-days at the optimum temperature for hatching, did not support the findings of Kowalska (1959), working with trout.

Suggestions for future work

Temperatures in the laboratory ponds should be continuously monitored with a recording thermometer, in order to determine the extent of diel temperature fluctuations during the spawning season

which may have an important influence on embryo development and mortality rate.

During the spawning season, bass could be injected with gonadotropic hormone in order to produce spawning at will in both females and males. This would facilitate obtaining eggs for laboratory experiments at suitable intervals during the normal spawning season.

The present scope of research should be expanded to include more factors likely to cause mortality, such as siltation, oxygen concentration, waterflow and circulation, of pollution by metabolites, and predation. These factors should be examined at non-lethal temperature levels more likely to occur in the natural environment, as it is obvious that temperatures outside the 12.5 to 27.5 C range are unrealistic at this latitude during the spawning season and will kill embryos directly.

More detailed field observations are required to determine the cause of mortality at non-lethal temperatures when the guarding male bass leaves the nest for varying lengths of time. Microscopic examination of the egg surface under varying environmental conditions and at various stages during the incubation period may reveal harmful encrustations. Water above a bass nest could even be artificially stirred while the male bass is excluded, to observe the effect on mortality. The effect of siltation may be important as clean, artificially fertilized eggs survive well in the laboratory in the absence of a guarding male bass.

A simple field experiment could easily be set up to study the effect of the exclusion of the male bass guarding a nest with eggs by means of a wire mesh screen device placed around the nest for varying

time periods. The percentage embryo mortality caused by various periods of exclusion of the male bass could then be determined, and, together with observations on oxygen concentration and silt accumulation around the eggs, could lead to a much better insight into the factors responsible for embryo mortality when the guarding male bass deserts the nest, as observed by Kelley (1968) and others.

Subsequent to or coincident with this field trial, the problem could be tackled in the laboratory using silt-laden pond water and mechanical stirrers operated at different speeds inside test aquaria. In these experiments, temperature regulation would not be essential as long as temperatures remain within the range of 15 to 25 C, which would considerably simplify the apparatus involved.

In the laboratory well-balanced replications of observations at all levels within experiments with two replications per treatment and 20 embryos per replicate are essential so that adequate statistical analysis is possible, preferably by means of a factorial analysis of variance which would reveal the effect of interactions of factors.

SUMMARY

Objectives: 1) To determine if embryo age, acclimation, or both factors produce the mortality differences between non-acclimated and acclimated bass embryos observed in the 1966 experiments at temperatures above 25 C and below 12.5 C.

2) To observe the effects of sudden and gradual temperature shifts of various magnitudes on the mortality of largemouth bass embryos of various known ages.

3) To determine the developmental stage at which non-acclimated and acclimated bass embryos are most critically affected by temperature changes.

Methods: A total of four experiments were carried out during the spawning seasons (May-June) of 1967 and 1968 using artificially-fertilized eggs obtained from one pair of bass for each experiment. Embryo containers with lots of 20 eggs in each were initially kept at 20 C (the control temperature), then transferred to nine different incubation temperatures (ranging from 10 C to 30 C at 2.5 C intervals) either suddenly or gradually (with acclimation rates of 0.3 C and 1 C/hr) at 5 hr intervals up to 35 hrs after fertilization, and then kept at their final incubation temperatures until at least three days after hatching. Mortality was recorded daily in most cases.

Results: Multiple regression analyses indicated that temperature, acclimation and age had significant effects on treatment mortality, and chi-square tests showed a significant difference between comparable treatment mortalities of the four experiments.

Conclusions: The variability of the data preclude definite quantitative conclusions, but the following trends were established:

1) Treatment mortality had a parabolic relationship with temperature, being highest at 10 and 30 C and lowest around 20 C.

2) Mortality appeared highest just after fertilization (the most critical period) and declined gradually with increasing age, with no indications of any other critical period.

3) Mortality decreased with acclimation.

It seems unlikely that temperature effects alone adequately explain the early mortality observed in largemouth bass in nature, and further experiments on the effect of siltation interacting with temperature, oxygen concentration, and water movement are suggested.

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APPENDICES

APPENDIX I

A. Multiple regression analysis of treatment mortality to hatching

Table 1a. Enumeration of per cent treatment mortality to hatching (with arcsin transformation applied) as the dependent variable (Y) and coded values of acclimation, age and temperature as the independent variables (X_1, X_2, and X_3 respectively).

Y	X_1	X_2	X_3	Y	X_1	X_2	X_3	Y	X_1	X_2	X_3
90	0	0	16	0	0	0	4	13	0	5	9
90	0	0	16	0	0	0	4	0	0	5	9
90	0	0	16	90	0	0	4	0	0	5	9
44	0	1	16	24	0	0	4	0	0	5	9
90	0	1	16	29	0	1	4	23	0	6	9
90	0	1	16	0	0	1	4	0	0	6	9
17	0	2	16	46	0	2	4	0	0	6	9
90	0	2	16	0	0	2	4	0	0	6	9
90	0	2	16	36	0	3	4	90	0	0	16
26	0	3	16	6	0	3	4	0	0	0	16
90	0	3	16	31	0	4	4	90	0	0	16
90	0	3	16	6	0	4	4	31	0	1	16
17	0	4	16	0	0	5	4	0	0	1	16
69	0	4	16	6	0	5	4	90	0	1	16
90	0	4	16	8	0	6	4	0	0	1	16
10	0	5	16	0	0	6	4	24	0	2	16
0	0	5	16	43	0	0	9	0	0	2	16
0	0	6	16	0	0	0	9	90	0	2	16
39	0	6	16	90	0	0	9	6	0	2	16
90	0	0	9	33	0	1	9	32	0	3	16
90	0	0	9	0	0	1	9	0	0	3	16
90	0	4	9	60	0	1	9	36	0	3	16
26	0	0	4	0	0	1	9	6	0	3	16
90	0	0	4	19	0	2	9	27	0	4	16
55	0	0	4	0	0	2	9	36	0	4	16
31	0	1	4	33	0	2	9	40	0	4	16
50	0	2	4	0	0	2	9	27	0	4	16
41	0	3	4	22	0	3	9	0	0	5	16
90	0	4	4	0	0	3	9	37	0	5	16
0	0	5	4	0	0	3	9	31	0	5	16
31	0	6	4	16	0	3	9	32	0	5	16
10	0	0	1	36	0	4	9	13	0	6	16
0	0	0	1	0	0	4	9	41	0	6	16
0	0	0	1	0	0	4	9	25	0	6	16
0	0	0	1	6	0	4	9	6	0	6	16

Table 1a (continued). Enumeration of variables.

Y	X_1	X_2	X_3	Y	X_1	X_2	X_3	Y	X_1	X_2	X_3
10	1	0	16	17	1	2	1	13	1	1	9
90	1	0	16	0	1	2	1	8	1	1	9
90	1	0	16	17	1	3	1	22	1	1	9
53	1	1	16	0	1	3	1	19	1	2	9
30	1	2	16	10	1	0	1	0	1	2	9
37	1	2	16	0	1	0	1	0	1	2	9
30	1	3	16	0	1	1	1	0	1	2	9
48	1	3	16	10	1	2	1	19	1	3	9
26	1	0	9	0	1	3	1	31	1	3	9
49	1	0	9	0	1	3	1	0	1	3	9
0	1	1	9	23	1	0	4	0	1	3	9
17	1	2	9	0	1	0	4	8	1	4	9
0	1	2	9	50	1	0	4	6	1	4	9
17	1	3	9	0	1	0	4	22	1	0	16
21	1	3	9	0	1	1	4	0	1	0	16
0	1	0	4	36	1	1	4	74	1	0	16
10	1	0	4	0	1	1	4	6	1	0	16
8	1	0	4	12	1	2	4	0	1	1	16
45	1	1	4	0	1	2	4	0	1	1	16
0	1	1	4	0	1	2	4	6	1	1	16
19	1	2	4	0	1	3	4	23	1	2	16
0	1	2	4	0	1	3	4	0	1	2	16
0	1	2	4	0	1	3	4	18	1	2	16
0	1	3	4	16	1	3	4	18	1	2	16
0	1	3	4	0	1	4	4	14	1	3	16
43	1	3	4	6	1	4	4	27	1	3	16
18	1	4	4	26	1	0	9	18	1	3	16
0	1	0	1	18	1	0	9	0	1	3	16
10	1	0	1	55	1	0	9	0	1	4	16
0	1	1	1	39	1	0	9	0	1	4	16

Table 2a. Sums, means and unadjusted and adjusted sums of squares and crossproducts (Y = treatment mortality at hatching).

No. of observations = 195

	Y	X_1	X_2	X_3
sums	4,660	90	441	1,851
means	23.90	0.4615	2.262	9.492

	Y	X_1	X_2	X_3
Y	275,988	1,358	8,330	54,260
X_1		90	154	741
X_2			1,647	4,490
X_3				23,415

	y	x_1	x_2	x_3
y	164,626	-792	-2,208	10,026
x_1		49	-49	-113
x_2			650	304
x_3				5,845

Table 3a. Abbreviated Doolittle method for multiple regression (Y = treatment mortality to hatching).

Instruction		A matrix x_1	x_2	x_3	G matrix y	Check
1	x_1	49	-49	-113	-792	-905
	x_2		650	304	-2,208	-1,303
	x_3			5,845	10,026	16,062
2	A_1	49	-49	-113	-792	-905
	B_1	1	-1	-2.3061	-16.16	-18.47
3	A_2		601	191	-3,000	-2,208
	B_2		1	0.3178	-4.9916	-3.6738
4	A_3			5,524	9,153	14,677
	B_3			1	1.6569	2.6569

End of forward solution

backward	C_1	0.022788	0.001550	0.000360
	C_2		0.001681	-0.000057
solution	C_3			0.000181

	C_1	C_2	C_3
Instruction	7	6	5

Table 4a. The multiple regression equation and F test of significance of regression for treatment mortality (Y) to hatching.

Partial regression coefficients:

$b_3 = 1.6569$

$b_2 = -4.9916 - (0.3178 \times 1.6569) = -5.5182$

$b_1 = -16.16 - (-1 \times -5.5182) - (-2.3061 \times 1.6569) = -17.8572$

Check for b's:

$\Sigma x_1 y = (-17.8572 \times 49) + (-5.5182 \times -49) + (1.6569 \times -113)$

$= -792$

Regression SS

$= (-17.8572 \times -792) + (-5.5182 \times -2,208) + (1.6569 \times 10,026)$

$= 42,939$

Multiple regression equation:

$b_o = 23.90 - (-17.86 \times 0.4615) - (-5.5182 \times 2.262) - (1.6569 \times 9.492)$

$= 29.11$

$Y = 29.11 - 17.86X_1 - 5.5182X_2 + 1.6569X_3$

Significance of regression:

	d. f.	SS	MS	F
Regression on three variables	3	42,939	14,313	22.47**
Residual	191	121,687	637	
Total	194	164,626		

Table 5a. Multiple correlation coefficient, coefficient of determination and significance of partial regression coefficients for treatment mortality (Y) to hatching.

Multiple correlation coefficient:

$$R_{y.123} = \sqrt{\frac{42,939}{164,626}} = 0.5107^{**}$$

Coefficient of determination:

$$100R^2_{y.123} = 100(0.5107)^2 = 26.08$$

Standard errors of b's:

$$s_{b_1} = \sqrt{0.022788 \times 637} = 3.81$$

$$s_{b_2} = \sqrt{0.001681 \times 637} = 1.0348$$

$$s_{b_3} = \sqrt{0.000181 \times 637} = 0.3395$$

Significance of partial regression coefficients (Student's t tests):

$$t_1 = \frac{-17.8572}{3.81} = -4.6869^{**}$$

$$t_2 = \frac{-5.5182}{1.0348} = -5.3326^{**}$$

$$t_3 = \frac{1.6569}{0.3395} = 4.8804^{**}$$

Table 6a. Standard deviations and standard partial regression coefficients for treatment mortality (Y) to hatching.

Standard deviations of variables:

$$s_y = \sqrt{\frac{164,626}{194}} = 29.13$$

$$s_1 = \sqrt{\frac{49}{194}} = 0.5025$$

$$s_2 = \sqrt{\frac{650}{194}} = 1.8303$$

$$s_3 = \sqrt{\frac{5,845}{194}} = 5.489$$

Standard partial regression coefficients (ranks in parentheses):

$$b_1' = (-17.8572)\frac{0.5025}{29.13} = -0.3080\ (3)$$

$$b_2' = (-5.5182)\frac{1.8303}{29.13} = -0.3467\ (1)$$

$$b_3' = (1.6569)\frac{5.489}{29.13} = 0.3122\ (2)$$

B. Multiple regression analysis of treatment mortality to three days after hatching.

Table 7a. Enumeration of per cent treatment mortality to three days after hatching (with arcsin transformation applied) as the dependent variable (Y) and coded values of acclimation, age and temperature as the independent variables (X_1, X_2 and X_3 respectively).

Y	X_1	X_2	X_3	Y	X_1	X_2	X_3	Y	X_1	X_2	X_3
90	0	0	16	43	0	1	4	90	0	0	16
90	0	0	16	0	0	1	4	90	0	0	16
90	0	0	16	50	0	2	4	90	0	0	16
50	0	1	16	15	0	2	4	90	0	1	16
90	0	1	16	35	0	3	4	90	0	1	16
90	0	1	16	0	0	3	4	90	0	1	16
17	0	2	16	31	0	4	4	90	0	1	16
90	0	2	16	21	0	4	4	74	0	2	16
90	0	2	16	0	0	5	4	66	0	2	16
26	0	3	16	6	0	5	4	90	0	2	16
90	0	3	16	8	0	6	4	90	0	2	16
90	0	3	16	0	0	6	4	68	0	3	16
17	0	4	16	49	0	0	9	48	0	3	16
90	0	4	16	24	0	0	9	73	0	3	16
90	0	4	16	90	0	0	9	90	0	3	16
10	0	5	16	33	0	1	9	61	0	4	16
48	0	5	16	0	0	1	9	51	0	4	16
0	0	6	16	73	0	1	9	90	0	4	16
60	0	6	16	35	0	1	9	90	0	4	16
90	0	0	9	19	0	2	9	56	0	5	16
90	0	0	9	0	0	2	9	53	0	5	16
90	0	4	9	43	0	2	9	90	0	5	16
26	0	0	4	47	0	2	9	90	0	5	16
90	0	0	4	42	0	3	9	24	0	6	16
55	0	0	4	0	0	3	9	48	0	6	16
31	0	1	4	62	0	3	9	90	0	6	16
54	0	2	4	51	0	3	9	90	0	6	16
40	0	3	4	36	0	4	9	39	1	0	16
90	0	4	4	0	0	4	9	90	1	0	16
0	0	5	4	90	0	4	9	90	1	0	16
31	0	6	4	27	0	4	9	90	1	1	16
10	0	0	1	15	0	5	9	30	1	2	16
0	0	0	1	0	0	5	9	90	1	2	16
0	0	0	1	90	0	5	9	30	1	3	16
27	0	0	1	51	0	5	9	90	1	3	16
33	0	0	4	33	0	6	9	26	1	0	9
39	0	0	4	0	0	6	9	90	1	0	9
90	0	0	4	40	0	6	9	0	1	1	9
29	0	0	4	43	0	6	9	23	1	2	9

Table 7a (continued). Enumeration of variables.

Y	X_1	X_2	X_3	Y	X_1	X_2	X_3	Y	X_1	X_2	X_3
37	1	2	9	0	1	3	1	0	1	2	9
17	1	3	9	24	1	3	1	45	1	2	9
37	1	3	9	47	1	0	4	59	1	2	9
0	1	0	4	0	1	0	4	25	1	3	9
24	1	0	4	54	1	0	4	31	1	3	9
16	1	0	4	0	1	0	4	90	1	3	9
45	1	1	4	15	1	1	4	47	1	3	9
0	1	1	4	36	1	1	4	90	1	4	9
20	1	2	4	15	1	1	4	43	1	4	9
0	1	2	4	13	1	2	4	90	1	0	16
0	1	2	4	0	1	2	4	55	1	0	16
0	1	3	4	15	1	2	4	90	1	0	16
0	1	3	4	24	1	3	4	90	1	0	16
43	1	3	4	0	1	3	4	43	1	1	16
24	1	4	4	0	1	3	4	90	1	1	16
0	1	0	1	15	1	3	4	90	1	1	16
10	1	0	1	40	1	4	4	75	1	2	16
0	1	1	1	15	1	4	4	10	1	2	16
17	1	2	1	27	1	0	9	90	1	2	16
0	1	2	1	18	1	0	9	90	1	2	16
17	1	3	1	60	1	0	9	74	1	3	16
0	1	3	1	50	1	0	9	36	1	3	16
10	1	0	1	29	1	1	9	90	1	3	16
0	1	0	1	24	1	1	9	90	1	3	16
0	1	1	1	51	1	1	9	90	1	4	16
10	1	2	1	20	1	2	9	90	1	4	16

Table 8a. Sums, means and unadjusted and adjusted sums of squares and crossproducts (Y = treatment mortality to three days after hatching).

No. of observations = 195

	Y	X_1	X_2	X_3
sums	8,717	90	441	1,851
means	44.70	0.4615	2.262	9.492

	Y	X_1	X_2	X_3
Y	611,523	3,320	19,007	106,847
X_1		90	154	741
X_2			1,647	4,490
X_3				23,415

	y	x_1	x_2	x_3
y	221,851	-703	-706	24,103
x_1		49	-49	-113
x_2			650	304
x_3				5,845

Table 9a. Abbreviated Doolittle method for multiple regression (Y = treatment mortality to three days after hatching).

Instruction		A matrix x_1	A matrix x_2	A matrix x_3	G matrix y	Check
1	x_1	49	-49	-113	-703	-816
	x_2		650	304	-706	199
	x_3			5,845	24,103	30,139
2	A_1	49	-49	-113	-703	-816
	B_1	1	-1	-2.3061	-14.35	-16.65
3	A_2		601	191	1,409	-617
	B_2		1	0.3178	-2.3444	-1.0266
4	A_3			5,524	22,930	28,454
	B_3			1	4.1509	5.1509
End of forward solution						
backward solution	C_1		0.022788		0.001550	0.000360
	C_2				0.001681	-0.000057
	C_3					0.000181
			C_1		C_2	C_3
Instruction			7		6	5

Table 10a. The multiple regression equation and F test of significance of regression (Y = treatment mortality to three days after hatching).

Partial regression coefficients:

$b_3 = 4.1509$

$b_2 = -2.3444 - (0.3178 \times 4.1509) = -3.6636$

$b_1 = -14.35 - (-1 \times -3.6636) - (-2.3061 \times 4.1509) = -8.4412$

Check for b's:

$\Sigma x_1 y = (-8.4412 \times 49) + (-3.6636 \times -49) + (4.1509 \times -113)$

$= -703$

Regression SS

$= (-8.4412 \times -703) + (-3.6636 \times -706) + (4.1509 \times 24,103)$

$= 185,256$

Multiple regression equation:

$b_o = 44.70 - (-8.4412 \times 0.4615) - (-3.6636 \times 2.262) - (4.1509 \times 9.492)$

$= 17.58$

$Y' = 17.58 - 8.4412X_1 - 3.6636X_2 + 4.1509X_3$

Significance of regression:

Source	d.f.	SS	MS	F
Regression on three variables	3	185,256	61,752	321.6**
Residual	191	36,595	192	
Total	194	221,851		

Table 11a. Multiple correlation coefficient, coefficient of determination and significance of partial regression coefficients (Y = treatment mortality to three days after hatching).

Multiple correlation coefficient:

$$R_{y.123} = \sqrt{\frac{185,256}{221,851}} = .9138^{**}$$

Coefficient of determination:

$$100R^2_{y.123} = 100(0.9138)^2 = 83.50$$

Standard errors of b's:

$$s_{b_1} = \sqrt{0.022788 \text{ X } 192} = 2.0917$$

$$s_{b_2} = \sqrt{0.001681 \text{ X } 192} = 0.5682$$

$$s_{b_3} = \sqrt{0.000181 \text{ X } 192} = 0.1865$$

Significance of partial regression coefficients (Student's t tests):

$$t_1 = \frac{-8.4412}{2.0917} = -4.0356^{**}$$

$$t_2 = \frac{-3.6636}{0.5682} = -6.4477^{**}$$

$$t_3 = \frac{4.1509}{0.1865} = 22.26^{**}$$

Table 12a. Standard deviations and standard partial regression coefficients (Y = treatment mortality to three days after hatching).

Standard deviations of variables:

$$s_y = \sqrt{\frac{221,851}{194}} = 33.81$$

$$s_1 = \sqrt{\frac{49}{194}} = 0.5025$$

$$s_2 = \sqrt{\frac{650}{194}} = 1.8303$$

$$s_3 = \sqrt{\frac{5,845}{194}} = 5.489$$

Standard partial regression coefficients (ranks in parentheses):

$$b_1' = (-8.4412)\frac{0.5025}{33.81} = -0.1255\ (3)$$

$$b_2' = (-3.6636)\frac{1.8303}{33.81} = -0.1983\ (2)$$

$$b_3' = (4.1509)\frac{5.489}{33.81} = 0.6739\ (1)$$

APPENDIX II

The relationship between treatment mortality and degree-days to hatching.

Table 13a. The number of days, degree-days (base 0 C and 20 C) and per cent treatment mortality to hatching for each lot of non-acclimated embryos in Experiment 1 (1967).

Incubation temperature (C)	Age at transfer (hr)	Days to hatching	Degree-days to hatching		Treatment mortality (%)
			base 0 C	base 20 C	
10	10	13.21	136	-128	9
	15	10.62	112	-100	19
	20	10.67	124	-89	9
	25	8.87	99	-78	3
	30	8.83	101	-76	0
15	0	6.83	102	-35	19
17.5	0	4.83	84	-13	3
20[1/]	0	2.87	57	0	0
22.5	0	2.87	64	7	0
27.5	15	2.04	51	10	14
	20	2.04	49	8	35
	25	2.04	48	7	5
	30	2.04	47	6	15

[1/] Mean values of control lots kept at 20 C throughout Experiment 1.

Table 14a. The number of days, degree-days (base 0 C and 20 C) and per cent treatment mortality to hatching for each lot of acclimated embryos in Experiment 1 (1967).

Incubation temperature (C)	Age at transfer (hr)	Days to hatching	Degree-days to hatching		Treatment mortality (%)
			base 0 C	base 20 C	
10	0[2/]	9.46	101	-88	3
	0	13.21	134	-130	3
	10	13.21	138	-126	25
	15	10.67	114	-108	25
12.5	0	8.87	112	-65	19
	10	8.87	115	-62	9
	15	8.08	106	-56	9
15	0	6.83	102	-35	0
	10	5.83	89	-28	11
	15	5.83	90	-27	0
17.5	0	4.00	70	-10	0
	10	4.00	71	-9	9
	15	4.00	70	-10	9
20[1/]	0	2.87	57	0	0
22.5	0	2.87	65	8	3
	10	2.87	62	5	3
	15	2.87	63	6	0
25	10	2.04	47	6	4
	15	2.04	46	5	0
27.5	0[2/]	2.04	54	13	9
	15	2.04	49	8	11
30	0[2/]	1.50	38	8	14

[1/] Mean values of control lots kept at 20 C throughout Experiment 1.

[2/] Lots acclimated at 0.3 C per hour. All other lots acclimated at 1.0 C per hour.

Table 15a. The number of days, degree-days (base 0 C and 20 C) and per cent treatment mortality to hatching for each lot of non-acclimated embryos in Experiment 2 (1967).

Incubation temperature (C)	Age at transfer (hr)	Days to hatching	Degree-days to hatching		Treatment mortality (%)
			base 0 C	base 20 C	
	20	11.04	118	-103	87
10	25	9.25	102	-83	0
	30	8.04	92	-69	40
17.5	0	4.33	76	-11	0
20[1/]	0	3.29	66	0	0
22.5	0	2.25	50	5	0
25	0	1.25	31	6	0
	10	1.75	45	10	0
	15	2.21	55	11	0
27.5	20	2.21	60	16	0
	25	2.21	52	8	0
	30	2.17	49	6	0
	15	1.25	30	5	0
	20	1.25	28	3	35
30	25	1.25	26	1	37
	30	2.17	50	7	43

[1/] Control lot kept at 20 C throughout experiment.

Table 16a. The number of days, degree-days (base 0 C and 20 C) and per cent treatment mortality to hatching for each lot of acclimated embryos in Experiment 2 (1967).

Incubation temperature (C)	Age at transfer (hr)	Days to hatching	Degree-days to hatching		Treatment mortality (%)
			base 0 C	base 20 C	
10	0[2]	11.04	117	-104	100
	5	12.96	133	-126	63
	10	11.04	120	-101	37
	15	11.04	118	-103	55
12.5	0	9.17	116	-67	57
	5	8.04	104	-57	0
	10	8.04	105	-56	0
	15	7.25	96	-49	13
15	0	6.21	90	-25	3
	5	6.17	94	-29	50
	10	6.17	95	-28	0
	15	6.17	95	-28	0
17.5	0	4.33	77	-10	3
	5	4.33	76	-11	0
	10	4.33	78	-9	0
	15	4.38	79	-8	0
20[1]	0	3.29	66	0	0
22.5	0	3.29	74	8	0
	5	3.29	73	7	0
	15	3.29	72	6	0
25	0	1.75	43	8	0
	5	2.25	54	9	0
	15	2.21	51	7	0
27.5	0[2]	1.75	47	12	13
	0	1.75	46	11	10
	5	1.75	46	11	5
	10	1.83	45	8	0
	15	2.17	54	11	27
30	0	1.08	30	8	0
	5	1.75	48	13	0
	10	1.83	48	11	0
	15	1.75	43	8	20

[1] Control lot kept at 20 C throughout experiment.

[2] Lots acclimated at 0.3 C per hour. All other lots acclimated at 1.0 C per hour.