TIME-TEMPERATURE RELATIONS IN THE INCUBATION OF THE WHITEFISH, COREGONUS CLUPEAFORMIS (MITCHILL)

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The incubation of whitefish eggs occurs in nature at extremely low temperatures that fluctuate within a very narrow range. In Lake Erie, whitefish spawn in late November when decreasing water temperatures reach about 6°C. Soon the lake freezes over. Thereafter, whitefish eggs in ice-covered waters develop at temperatures only slightly above freezing for a protracted period of about 4 months of incubation. The peak of hatching occurs in the spring as the water approaches 6°C. Such development is in sharp contrast to that of the majority of fresh-water species which develop at considerably higher and more widely fluctuating temperatures during spring and summer. In the study herein reported, whitefish development was followed from fertilization to hatching at constant temperatures of 0°, 0.5°, 2°, 4°, 6°, 8°, 10°, and 12°C. The resulting data yield information regarding the normality and rate of development at these several temperatures. Certain accelerative effects of temperature have been treated statistically and presented graphically.

Materials and Methods[1]

For this study, approximately 120,000 whitefish eggs have been incubated in three annual series, during the winters of 1934–35; 1935–36; 1937–38. At the height of the spawning season each of these years, eggs were stripped from females taken alive from commercial gill nets in Lake Erie. The eggs were at once fertilized with milt from live males, covered with water, and handled with special care by an experienced fisherman. Received from the fishing boat, the eggs were at once transported in clean lake water cooled with floating ice to the refrigeration cabinets in the Zoology Department, Ohio State University, at Columbus. Transferred to incubation jars, the eggs were incubated thereafter at constant temperatures as indicated.

In each case, eggs were placed in these cabinets when in the one- or two-celled stage. In the first two series, the eggs were gradually shifted during the first 24 hour period

[1] The author wishes to thank Dr. T. H. Langlois, and Mr. E. L. Wickliff of the Ohio State Division of Conservation for their cooperation in providing whitefish eggs for this study.

into their respective temperature cabinets, and appropriate corrections have been made in the tables in calculating the incubation time of the first few cleavages. The third year it was found practicable to place the eggs immediately in their incubation temperatures.

The whitefish egg is non-floating and measures 3 mm. in diameter. A thousand eggs occupy about 30 cc. of the bottom of a water-filled cylinder, and a quart jar carries about 30,000 eggs. Cone-shaped, 500 cc. graduated wide-mouthed flasks were filled to from ⅓ to ½ full of eggs, and covered by at least an equal volume of filtered lake water. A stream of air was introduced into the bottom of the flask by a vertical glass tube attached either to an individual air pump or to an air-pressure line which delivered 5 pounds pressure at the main valve. Air thus bubbling into the flask at the bottom both aerated the eggs and caused them to be rolled over one another, an action which automatically scarified the egg shell surface and kept it translucent.

In tests made previous to 1934, eggs kept in finger-bowls and not so aerated and agitated were definitely retarded in development in comparison with controls. They gradually acquired opaque shells which often supported growths of *Saprolegnia* and *Vorticella*. Such shells eventually disintegrated and sloughed off their outer membrane. Within, there was a gradual necrosis of the embryo, usually beginning at the tail. All such unaerated eggs died at temperatures above 5°C., and no fish hatched at even 2°, a temperature close to the optimum for incubating aerated eggs.

In the present study, only data from eggs so aerated are recorded. There was no evidence of injury from excessive aeration or agitation.

Filtered water taken some considerable distance from shore in Lake Erie and transported in glass carboys was the only water used in the incubation jars. Various samples tested had a pH value of from 8.0 to 8.2. At various times, this Lake Erie water taken from jars containing incubating eggs was found to range from 7.8 to 8.4 in pH readings. Hall (1925) found that a pH of 7.8 is optimum for whitefish incubation in early stages and that the optimum is lowered as the embryo becomes older. The writer regards the O_2/CO_2 ratio and the hydrogen ion concentration to have been nearly optimum and quite uniform throughout these tests, in view of the abundant aeration provided.

Controlled Temperature Cabinets.—The flasks containing incubating eggs were kept in controlled temperature air cabinets, described by Peterson (1934). Briefly, the six insulated air cabinets, each with an inner storage space of 27 cubic feet, are cooled by brine flowing through coils in such a way as to maintain a cabinet temperature 1–2°C. below that desired. An insulated brine tank is kept at −4 to −5°C. through the action of an ammonia compressor of ⅜ ton capacity. In each air cabinet an electric fan forces a draught of air over the coils and throughout the cabinet proper. The coils are separated from the air chamber by an insulated panel.

The air of the cabinets is maintained at the desired temperature by the action of an American Instrument Company sealed mercury "Metastatic" thermoregulator, Type C, with a sensitivity of 0.02°C. which operates light bulbs through a supersensitive relay. The relay operates on 0.007 amperes at 6 volts. The air in the cabinets is thus maintained at the desired temperature with variations of not more than 0.1°C. A continuous temperature record is provided by a Foxboro temperature recorder attached to each cabinet.

The coldest cabinet was maintained, as stated above, at an air temperature of 0°C. Still water, of course, freezes at this temperature. It was possible, however, to prevent

ice formation in the incubation jars through agitation produced by air bubbling through the water. A slight temperature depression caused by a light bulb burning out or a sticking brine valve, or if an air pump failed to force air through the incubation jar, would permit freezing. Such difficulties were encountered in both of two series of eggs incubated at this temperature. Freezing resulted in high mortality, and the remaining eggs were thereafter discarded. Hence, the data for the 0° series are incomplete.

The Handling of Eggs.—Daily observations and records were made of mortality and stage of development attained. All dead eggs were removed each day from the incubation jars. To do this, the eggs were transferred to finger-bowls, which were stacked in the cabinet until examined. The finger-bowls, removed singly, were kept half submerged in prechilled water kept cold with floating ice. Dead eggs were removed as rapidly as possible with pipettes specially drawn.

For stage determination, it was found that twenty-five eggs constituted a valid sample, *i.e.* by chance, among twenty-five eggs would be found essentially the same proportion of all stages present as would be found in the entire lot of eggs. All stages found in the sample were recorded daily. The midpoint of time required for a given jar of eggs to attain a certain stage was designated as that time at which 50 per cent or more of the sample had attained that stage.

Types and proportions of abnormalities in the various series were also noted. A discussion of these types will be published elsewhere.

Description of Stages

Throughout this study, stages of development are referred to by numbers which correspond to the numbers of similar stages in the 803-stage Ohio State University series, collected at the Ohio State Fish Hatchery at Put-in-Bay, Ohio, during the winter of 1926–27, and described by me in previous papers (Price, 1934 and 1935). Supplementing that description made solely from preserved material, certain features peculiar to living embryos have been used in the present study as diagnostic features.

It is noteworthy that two criteria often used for designating stages were found unreliable in this study. These are (1) embryo length and (2) the number of somites. Embryos incubated at the higher temperatures were uniformly shorter and smaller at a given stage of development otherwise than were embryos incubated in the lower range of temperature. At the higher temperatures likewise, many abnormal embryos were distorted in the midbody and tail regions, rendering a somite count futile. Embryo length and somite count as criteria were therefore discarded in favor of a combination of both structural and physiological features which proved to be more satisfactory.

Pigment appears in the eye, in the blood, and in chromatophores in a definite sequence. In just as precise a manner, the circulatory system developed in a consistent pattern, and the visible extent of the vitelline circulation seemingly varied within extremely narrow limits from one em-

TABLE I

Series of Stages Used in the Determination of the Degree of Development of Live Whitefish Embryos

Stage	Diagnostic characteristics
1-cell.....	Unsegmented blastodisc
2-cell.....	2-celled "
8-cell.....	8-celled "
16-cell.....	16-celled "
32-cell.....	32-celled "
No. 8 O.S.U.....	Blastodisc 4 cells deep. Early periblastic ridge.
16 "	Blastodisc 8–10 cells deep. Blastomeres small.
32 "	Formation of germ ring and sub-germinal cavity.
48 "	Blastoderm envelops upper ⅓ of yolk.
64 "	Blastoderm envelops upper ½ of yolk.
80 "	Blastoderm envelops upper ⅔ of yolk. Primary germ layers established.
96 "	Large yolk plug stage. Formation of embryonic streak.
112 "	Small yolk plug stage. Optic primordia appear.
128 "	Closure of blastopore. Three primary cerebral vesicles developed. Notochord extends forward to level of mesencephalon.
144 "	Embryo extends ½ distance around curvature of yolk, without torsion. Auditory anlagen visible.
160 "	Increased size of brain and optic vesicles. Two layered optic cup, with choroid fissure and lens primordium. Nasal pit anlagen appear.
176 "	Progressive development of features listed above.
192 "	Fourth ventricle visible. Optic stalk present. Tail begins to be undercut by tail fold.
224 "	First heart beat. Embryo ⅔ way around yolk. First anterior branchial pouch has perforated. Prominent fourth ventricle.
272 "	First curve of tail. First movements, spasmodic in mid-body regions.
320 "	Dorsal aortae traceable into tail region. First eye pigment visible.
368 "	First color of blood, detectable in heart region.
400 "	Embryo complete circle on yolk in series incubated at low temperatures. Heart U-shaped. Vitelline veins large and filled with erythrocytes. Semi-circular canals developing. Pectoral fins definitely raised above surface of lateral somatopleure. First eye pigment readily apparent.
448 "	Circulation very evident in extra-embryonic area. Eye pigment darker.
496 "	Pectoral fins definitely formed. Length slightly less than 8 mm. in case of normal embryos.
528 "	Circulation very clear throughout tail, body, and on sides of yolk. Hatching enzyme glands visible in throat region. Yolk vessels at periphery of splanchnocoele form single continuous loop from vitelline artery to vein. Two chambered heart.
592 "	Circulation in yolk vessels visible, in branch of vitelline vein lateral to heart on yolk. Spasmodic twisting of entire body, rotating yolk sac as it does so. No movements of pectoral fins. Estimated length of normal embryos 8.5 mm.

TABLE I—*Concluded*

Stage	Diagnostic characteristics
No. 632 O.S.U.....	Three semi-circular canals clearly visible through shell. Yolk with single large oil globule. Circulation in vitelline veins traceable from below oil globule. Eyes black. Caudal lobe on tail. No movement in pectoral fins. Embryo occasionally goes through spasmodic and violent twisting of entire body, changing the flexure from left to right and *vice versa*, in some cases moving entire yolk sac.
680 "	First evidence noted of pectoral fin movement. Pectoral fin spear-head shaped from dorsal view. Stellate chromatophores in double row along entire length of body. Heart beneath body.
776 "	Constant fin flutter stage. Pectoral fins in a state of rapid fluttering or twitching.
800 "	Stellate chromatophores appear for first time on head. Embryo extremely active.
	Hatching stage.

bryo to another of the same stage. The time of the initiation of characteristic movements, first spasmodic and general in the midbody region, followed by tail movements, then general body-twisting movements, and finally fin and eye movements all follow in as strict a chronological sequence as the development of morphological features. All these, taken together, form the basis for the designation of stages, in Table I.

RESULTS

I. Range of Temperature Tolerance.—Data given below (Tables II to IV) show that whitefish hatched alive at all temperatures tested between 0.5°C. and 10°C. Although certain development occurred at the extreme temperatures of 0°C. and 12°C., no fish hatched at these extremes. The effective range of temperatures is therefore limited to from 0.5–10°C., through which hatching occurred. The proportion of normal live fish hatched at intermediate temperatures suggests a range of 0.5–6°C. for normal development with an optimum at 0.5°C. A more complete statement follows.

The freezing of water at 0°C. imposes a natural limitation to development below that point. When freezing could be prevented at 0°C., development occurred although it was extremely retarded. (See Table IV.) Various tests showed that, upon being frozen, eggs in early cleavage stages underwent a disruption of the numerous scattered oil globules beneath the blastodisc. These globules then either coalesced within the yolk into one or two large globules, or were cast off into the perivitelline space. The blastodisc simultaneously became distorted and shortly thereafter opaque. If freezing were prevented at 0°C. until after gastrulation, it failed to cause any distortion visible externally, but general necrosis soon became evident.

In two years' tests, the writer reared eggs at 0°C. without freezing to stages 224 and 272 respectively, beyond gastrulation (stage 128). However, mortality in each case had reached at least 95 per cent. Whether eggs could be induced to hatch at this extreme temperature is seriously open to question. These results strongly indicate that 0°C. is the lowest temperature at which whitefish eggs will develop; *i.e.*, the physiological zero.

The *highest* temperature tested at which development occurred was 12°C. (1935–36 series). Development proceeded at a consistent rate, although mortality was extremely high from the beginning. In later stages, all surviving embryos were noticeably abnormal, the majority of them having short, twisted tails and distorted heads. A few hatched dead, but none

TABLE II

Mortality, Hatchability, and Abnormality of Whitefish Embryos Incubated at Constant Temperatures. 1934–35 Series

	Incubation temperatures					
	0.5	2.0	4	6	8	10
	°C.	°C.	°C.	°C.	°C.	°C.
Mortality, per cent						
(*a*) Prior to hatching stage	26.25	38.0	40.0	27.5	34.4	63.0
(*b*) During hatching stage	1.08	4.0	1.4	14.0	46.8	36.4
(*c*) Total	27.33	42.0	41.4	41.5	81.2	99.4
Eggs hatched alive, *per cent*	72.67	58.0	58.6	58.5	18.8	0.6
Estimated embryos alive at hatching stage that were *abnormal, per cent*	0	0	1	10	25	50

hatched alive. This temperature is therefore regarded as beyond the range of tolerance of the whitefish egg for effective incubation and survival.

The comparative survival and hatchability of whitefish eggs incubated throughout the effective temperature range of 0.5–10°C. is summarized in Table II from the 1934–35 series.

From these results, eggs incubated at 10°C. are seen to have a high mortality, 99 per cent total, and low hatchability, less than 1 per cent. The estimate of 50 per cent of the embryos alive at the hatching stage being abnormal is probably too low. In the other two series, the percentage of abnormals produced at this temperature was 95–100 per cent. At best, a temperature of 10°C. is extremely adverse to whitefish embryonic development.

The next lower 8° temperature, while less unfavorable, still produced a significant proportion of abnormal embryos, a high mortality, and a low hatch.

Temperatures from 2–6° yielded a quite uniform result in the percentage of incubated eggs hatched (58 per cent). Differences in the mortality records occur in the higher percentage of eggs dying at the time of hatching at 6°, although the total mortality for this series at 6° is approximately the same as for the 4° and 2° series. The 10 per cent of abnormal fishes alive at hatching time indicate 6° as the *maximum* temperature at which normal development characteristically occurs. Day old fishes hatched at this temperature swim vigorously in a tank of water, whereas those hatched at higher temperatures exhibit only occasional movements, lying most of the time quietly on the bottom of the tank.

The most favorable results were obtained in these tests in the 0.5°C. series. The relative proportion of fishes hatched, the lower mortality throughout, and the absence of abnormal fishes produced support this conclusion. In this connection, the typical lengths of newly hatched fishes incubated at various temperatures are interesting.

TABLE III

Lengths in Millimeters of Newly Hatched Whitefish, Incubated at Various Constant Temperatures (1934–35)

10°	8°	6°	4°	2°	0.5°
8–9.5		11–12		11–13	12–14

These results in length of hatched whitefish correspond to those obtained both by Hall (1925) at these same temperatures so far as tested, and at the lower temperatures with those typically secured at the Put-in-Bay, Ohio hatchery on Lake Erie. The above data seem to justify the conclusion that 0.5°C. is the *optimum* or at least very close to the optimum temperature of incubation of the whitefish egg.

From the above one may readily perceive that the whitefish is an unusually clear cut case of a species being precisely adjusted during its embryonic development, to a very narrow range of an environmental factor, 0.5–6°C. temperature, with the optimum for the species being extremely close not only to the limit for the medium (freezing point of water) but also to the physiological zero for many processes. Such a low temperature range obviously limits the occurrence and continued survival of the species to waters which maintain a winter time temperature close to the freezing point. Actually, whitefish do occur both in North America and in Europe, principally in lakes which, like the Great Lakes, are subject to continuous freezing in winter.

TABLE IV

Incubation of Whitefish Eggs in Three Annual Series; A = 1934–35 Series; B = 1935–36 Series; C = 1937–38 Series. Av. = Average Time in Days for the Three Series. K = Time Calculated from Equation

$$T = \frac{M}{A_1^t}\Big\}\begin{matrix}0^\circ\\6^\circ\end{matrix};\ T = \frac{M}{A_2^t}\Big\}\begin{matrix}6^\circ\\12^\circ\end{matrix}$$

Stage No.		Temperature of incubation							
		0°	0.5°	2°	4°	6°	8°	10°	12°
1-cell		0	0	0	0	0	0	0	0
2-cells	*A*	—		—					
	B	0.83		0.5					
	C	0.75		0.5					
	Av.	0.79		0.5					
4-cells	*A*	—		—	—	—			
	B	1.41		0.83	—	0.5			
	C	—		—	0.75	0.5			
	Av.	1.41		0.83	0.75	0.5			
8-cells	*A*	—		—		—	—		
	B	1.83		1.41		0.83	0.5		
	C	—		—		0.83	0.75		
	Av.	1.83		1.41		0.83	0.63		
16-cells	*A*			—				—	
	B			1.83				0.5	
	C			—				0.75	
	Av.			1.83				0.63	
32-cells	*A*	—					—		—
	B	2.83					0.83		0.5
	C	—					—		—
	Av.	2.83					0.83		0.5
Stage No. 8	*A*	—	3.4	2.83	2.1	—		—	—
	B	3.54	—	2.83	—	1.41		1.0	0.83
	C	3.87	—	—	—	—		—	—
	Av.	3.71	3.4	2.83	2.1	1.41	—	1.0	0.83
	K	3.82	3.52	2.77	2.01	1.43	1.18	0.99	0.83
16	*A*	—	4.4	3.83	3.6	2.0	1.83	1.6	—
	B	4.41	—	3.45	—	2.0	1.83	1.6	1.41
	C	6.5	—	4.5	4.0	3.8	—	—	—
	Av.	5.45	4.4	3.92	3.8	2.6	1.83	1.6	1.41
	K	5.34	5.05	4.26	3.4	2.58	2.0	1.64	1.35

TABLE IV—*Continued*

Stage No.		Temperature of incubation							
		0°	0.5°	2°	4°	6°	8°	10°	12°
32	*A*	—		7.33	6.0	3.75	3.3	2.5	—
	B	8.5		7.5	—	4.41	3.58	2.83	1.83
	C	12.0		8.5	5.58	5.0	—	—	—
	Av.	10.25	—	7.78	5.59	4.39	3.44	2.66	1.83
	K	10.24	9.53	7.68	5.76	4.4	3.38	2.54	1.9
64	*A*	—	17.4	13.83	10.6	6.25	5.0	4.35	—
	B	18.6	—	14.0	—	7.0	5.5	4.6	4.4
	C	21.6	—	15.0	12.5	10.0	—	—	—
	Av.	20.1	17.4	14.28	11.6	7.75	5.25	4.47	4.4
	K	20.08	18.6	14.78	10.87	7.5	5.84	4.84	4.01
128	*A*	—	28.4	23.33	15.6	9.62	7.3	6.6	—
	B	28.5	—	18.6	—	—	—	6.46	5.41
	C	(33.0)	—	23.6	15.5	13.0	9.16	—	—
	Av.	30.75	28.4	21.84	15.55	11.31	8.23	6.53	5.41
	K	30.60	28.15	21.91	15.68	11.05	8.55	6.70	5.25
224	*A*	—	(46.5)	42.83	23.8	16.55	(12.17)	—	—
	B	43.38	—	26.46	—	15.41	—	9.41	(8.0)
	C	55.5	—	38.5	23.75	19.83	15.3	10.5	—
	Av.	49.44	(46.5)	35.93	23.77	17.26	13.73	9.95	8.0
	K	49.92	45.65	34.93	24.44	17.25	13.66	10.28	7.9
272	*A*	—	54.5	48.0	27.6	19.3	13.67	10.0	—
	B	—	—	36.7	—	20.5	—	10.41	8.5
	C	60.5	—	41.5	(26.75)	(20.75)	(15.78)	(11.3)	—
	Av.	60.5	54.5	42.06	27.17	20.18	14.72	10.57	8.5
	K	60.15	54.79	41.43	28.52	19.7	14.80	11.05	8.24
400	*A*		63.5	60.3	33.6	22.17	17.5	13.6	—
	B		—	39.41	—	23.5	—	12.41	10.91
	C		—	(53.5)	34.75	—	18.33	13.08	—
	Av.		63.5	51.07	34.17	22.83	17.91	13.03	10.91
	K		69.09	51.08	34.15	22.75	17.63	13.68	10.62
448	*A*		71.5	67.0	37.6	24.75	20.8	15.6	—
	B		—	43.38	—	25.5	—	13.41	11.41
	C		—	55.5	38.5	25.75	20.16	—	—
	Av.		71.5	55.96	38.05	25.33	20.48	14.5	11.41
	K		75.6	56.16	37.78	25.6	19.64	14.93	11.35
528	*A*		78.7	73.0	43.6	27.25	24.34	—	—
	B		—	54.5	—	27.5	—	15.41	13.41
	C		—	60.5	42.5	27.75	(22.25)	16.25	—
	Av.		78.7	62.66	43.05	27.5	23.29	15.84	13.41
	K		86.37	63.43	42.02	27.98	21.81	16.92	13.12

TABLE IV—*Concluded*

Stage No.		Temperature of incubation							
		0°	0.5°	2°	4°	6°	8°	10°	12°
632	*A*		89.0	82.0	(52.6)	—	27.8	21.1	—
	B		—	—	—	30.5	—	17.4	15.41
	C		—	65.5	48.0	(37.0)	26.25	18.53	—
	Av.		89.0	73.75	50.3	33.75	27.02	19.01	15.41
	K		99.14	73.95	50.03	33.98	26.02	19.86	15.15
776	*A*		113.5	100.0	67.6	38.17	32.3	25.6	
	B		—	—	—	40.83	—	26.46	
	C		—	84.5	59.0	44.5	33.25	24.25	
	Av.		113.5	92.25	63.3	41.16	32.77	25.44	
	K		126.0	93.08	62.18	41.43	32.5	25.55	
Hatching	*A*		141.0	133.0	83.6	57.25	40.3	29.6	
	B		—	—	—	56.5	—	—	
	C		—	109.0	77.0	62.0	—	—	
	Av.		141.0	121.0	80.3	58.58	40.3	29.6	
	K		156.35	119.1	82.87	57.8	41.19	29.28	

II. Rate of Development.—The time in days required for whitefish eggs to attain the various stages described in Table I is listed in Table IV. Average values for the 3 years' tests are given. In some instances, the variation between different year groups was considerable. In no case, however, was there overlapping for the different year groups between successive stages in a given series, or in the time required for attaining the same stage at different temperatures. The variation is such, however, that the mean values of 3 years' tests are more consistent and probably more representative than are any one year's values alone.

One sees from Table IV that in whitefish eggs, the length of the developmental period up to any given stage varies inversely with the temperature, and that the spread in days between successive temperature series increases progressively with development. The relative difference in time between various temperature series remains, however, about the same. For instance, early development through gastrulation is complete by stage 128. This period increases from 6.53 days at 10°C. to 28.4 days at 0.5°C., an increase of 4.3 times. Likewise the total incubation period to hatching increases from 29.6 days to 141 days at these same temperatures, an increase of 4.7 times. Relatively therefore the differences are approximately the same.

In round numbers, a rise of 4°C. either halves the time or doubles the rate of attaining a given stage, and Q_{10} values fluctuate around 5.0 as a mean.

Chart 1 has been drawn by plotting the average observed time values of Table IV. The comparative slope of the lines indicates the relative rate of development at various temperatures. In general, the rate, $\frac{1}{\text{days}}$, of development is directly proportional to temperature.

No attempt has been made to smooth these lines. Some of the variations from the rectilinear are undoubtedly due to practical difficulties in determining end-points. However, the tendency of these lines to undulate somewhat regularly can scarcely be accounted for on this basis. They

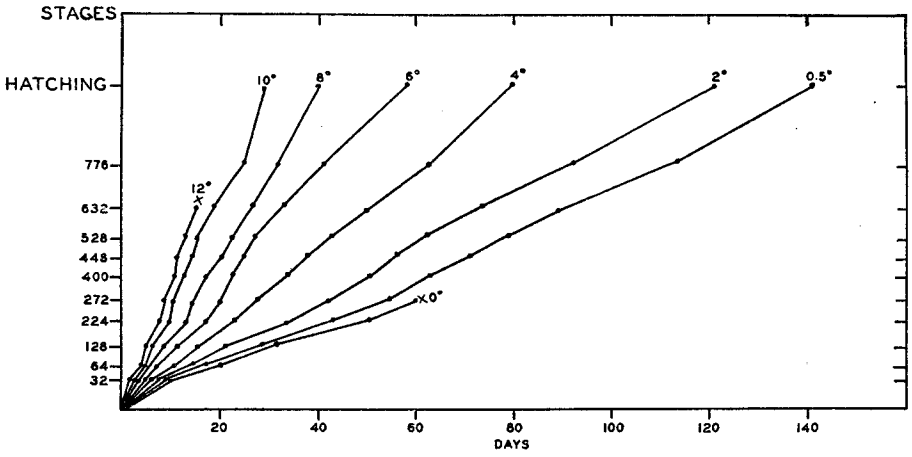

CHART 1. Velocity of development at different constant temperatures. Average time in days for each temperature for each stage.

somewhat suggest the rhythmic variation in growth rate of the chick (Schmalhausen *et al.*). Treated from another point of view, variations in rate during certain developmental periods are discussed at the end of this paper.

III. Determination of Thermal Increments.—Whether a single degree difference in incubation temperature results in the same proportionate amount of change in rate of development throughout the temperature range can be shown by the calculation of thermal increments or temperature characteristics.

For this purpose, stage 272 was arbitrarily selected as a test case. In the lower portion of Chart 2, the average observed number of days of incubation up to stage 272, as listed in Table IV, is plotted against temperatures. The logarithms of time (log T) plotted in the upper portion of this

chart rectify the natural number curves. By applying the method of least squares (Snedecor *et al.*) the two straight lines shown were fitted to these points (log T; C°). The equations used are:

$$T = \frac{M}{A_1^t}\Big\}\begin{matrix}0°\\6°\end{matrix}; \qquad T = \frac{M}{A_2^t}\Big\}\begin{matrix}6°\\12°\end{matrix} \tag{1}$$

$$\log T = \log M - t \log A \tag{2}$$

in which the time (T) to attain a given stage is inversely proportional to the constant (A) raised to an exponent, temperature (t). The constant

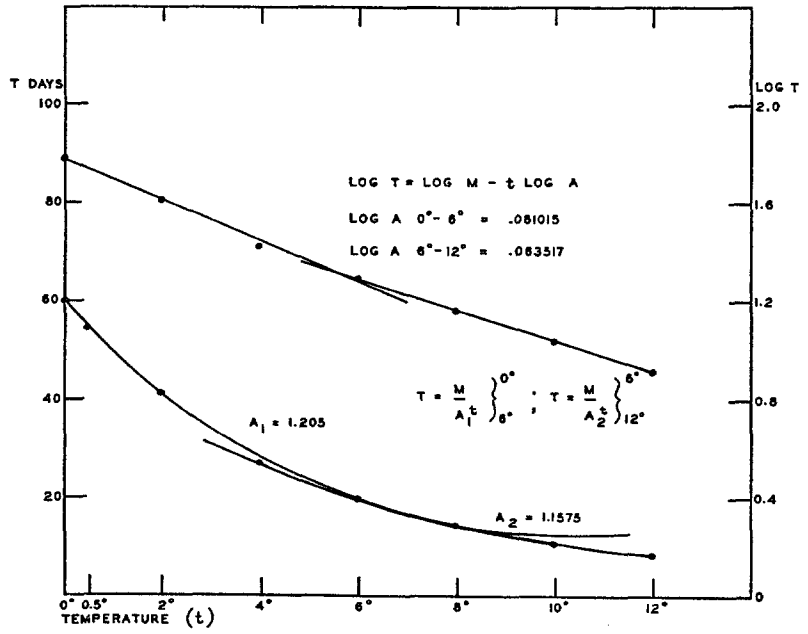

CHART 2. Incubation time as a function of temperature; stage 272

(M) is the Y-intercept at 0° ordinate. Since, within the temperature range given, values of A are constant for a given stage, the term A in equation (1) above serves as a temperature characteristic.

In fitting various curves to the points on these semi-log graphs, goodness of fit was measured by calculation of the standard error of estimate. A single straight line of equation (2) fitted to the semi-log curve from 0–12° had a standard error of estimate of 0.028. The combination of two straight lines as drawn on the semi-log graph has a standard error of estimate of only 0.0138, a reduction of more than half. Thus by resolving the semi-log curve for this stage into two straight lines, instead of one, estimated values

and the curves drawn from them more nearly agree with mean observed values. These lines intersect one another at the 6° locus. In equations (1) and (2), values of A derived from log A measure the slopes of these lines. They have one value below 6°C. and another value above it. Values of A regarded as temperature characteristics, express the relationship between time and temperature. In stage 272, $A_1 = 1.205$ between 0° and 6°, $A_2 = 1.1575$ between 6° and 12°. Equation (1) now becomes

$$T = \frac{M}{1.205^t} \Big\} \begin{matrix} 0° \\ 6° \end{matrix}; \qquad T = \frac{M}{1.1575^t} \Big\} \begin{matrix} 6° \\ 12° \end{matrix} \tag{3}$$

This is to say that below 6°, increasing the temperature 1° increases the speed of development 1.205 times; above 6°, increasing the temperature 1° increases the velocity 1.1575 times.

From the above result, 6° is a critical temperature, above and below which the temperature characteristics differ. In this particular case in question, curves which intersect just below 6° fit the observed points closer than do other curves drawn to intersect just above 6°, but neither differ significantly in their standard errors of estimate from that of the curves drawn intersecting at the 6° locus. The critical point in the temperature range for this stage then is 6° or slightly below.

Bělehrádek has studied time/temperature relations in a variety of circumstances, in which he has applied the empirical equations

$$y = \frac{a}{x^b} \tag{4}$$

$$\log y = \log a - b \log x \tag{5}$$

Here time, (y) is expressed as the reciprocal of the temperature, (x), raised to its exponent, b, the latter being regarded as a temperature characteristic. To express this relationship graphically, log y values are plotted against log x values. The constant a expresses the time at ± 1°, since log 1.00 is zero. The slope of the resulting straight line curve or curves expresses the value of b. For stage 272, a single straight line fitted to the log y/log x points yields a standard error of estimate of 0.063. Two lines intersecting at the 6° locus and fitted by the method of least squares yield a standard error of estimate of 0.0094, a reduction of 6.7 times over the error in the single line curve. In this case, equation (4) becomes

$$y = \frac{a}{x^{0.6644}} \Big\} \begin{matrix} 1° \\ 6° \end{matrix}; \qquad y = \frac{a}{x^{1.265}} \Big\} \begin{matrix} 6° \\ 12° \end{matrix} \tag{6}$$

From the above it is seen that equation (6) with a standard error of estimate

of 0.0094 can be made to fit the data just as closely over its range as does equation (3) with a standard error of estimate of 0.0138. Both equations indicate that 6° is a critical temperature. However, b values of equations (4) and (6) represent acceleration in log rate with reference to that rate at the base temperature of 1°. Obviously b in these equations is an exponent, expressing the power to which the temperature is raised as a function of time. It is not readily susceptible to a direct calculation in terms of natural numbers, as is the case with the natural number values of A in equations (1) and (3). This fact favors the use of the latter in deriving a temperature characteristic from an exponential equation. Further, equation (6) does not hold for the 0° temperature, and at 0.5°, there is a wide difference between calculated and observed values. For these reasons, equation (6) is discarded in this analysis in favor of equations (1) and (2) in expressing the time/temperature relations in the incubation of the whitefish.

Returning then to Chart 2, the lines as drawn express the time/temperature relations applying to stage 272. From either the chart directly or from the values of A given, it is seen that (1) the time required to attain stage 272 throughout the entire range from 0–12° through which whitefish eggs will develop is expressed as a combination of two negative exponential curves; (2) the mid-point in the temperature range, 6°, is a critical temperature. It will be recalled here that 6°C. is the temperature below which normal development typically occurs; above it, progressively abnormal development occurs; (3) below this critical temperature, the temperature characteristic, $A_1 = 1.205$, applies, meaning that for every degree rise in incubation temperature, the time required to attain this stage is decreased 1.205 times. Above 6°, the temperature characteristic, $A_1 = 1.1575$, applies, expressing a proportionate decrease in time per degree rise in incubation temperature.

Treating the data for the other stages studied, in a similar fashion, using the method of least squares for smoothing curves and calculating the values of A, results similar to the above were obtained. Two curves which intersect at the 6° locus give a closer fit to the data than does a single curve without such a break. The closeness of fit was satisfactory except at the 0.5° locus in later stages, where observed values were definitely lower than those calculated.

Calculated A values for the various stages are displayed in Table V. A values for temperatures below 6°C. have a mean of 1.193, while those for temperatures above 6°C. have a mean of 1.135. That these mean A values are significantly different is shown by the fact that their difference is more than five times the standard error of their difference.

Smoothed curves which show the time calculated from the above for whitefish eggs to attain certain significant stages are presented in Chart 3. Points indicated represent the average observed time of incubation at each temperature.

The estimated numbers of days for other stages based on similar calculations are listed in Table IV.

TABLE V

Temperature Characteristics of Whitefish Egg Incubation above or below the Critical Temperature, 6°C. A Values Express Acceleration Per Degree Rise in Temperature, from Fertilization to the Attainment of the Stage Given. For Stage Descriptions, See Table I

To stage	$A_{0-6°}$ values	$A_{6-12°}$ values
2-cell	1.257	—
4-cell	1.173	—
8-cell	1.141	—
8	1.173	1.092
16	1.119	1.103
32	1.154	1.155
64	1.165	1.098
128	1.181	1.130
224	1.195	1.140
272	1.205	1.157
400	1.223	1.135
448	1.219	1.146
528	1.228	1.135
632	1.215	1.144
776	1.223	1.128
Hatching	1.199	1.186
Mean =	1.19266	1.13486
Standard error of mean =	∓.00865	∓.00687
Difference between means =	5.235 × (standard error of their difference)	

The instantaneous responses to temperature during various phases of embryonic development are revealed when thermal increments are calculated for each phase independently. Thermal increments in terms of both the A values of the exponential equation above and μ values of Arrhenius' equation, as used by Crozier and others, have been determined for the following phases: From 1-cell stage to the 8-cell stage, designated as *early cleavage*; from 8-cell to stage 8, designated as *mid-cleavage*; stages 8–32, *late cleavage*; stages 32–128, *gastrulation*; stages 128–400, *organogenesis*; stages 400–776, subsequent *growth* to pre-hatching stage; stage 776 to mid-

hatching date, *hatching* period; fertilization to hatching, *total* incubation period.

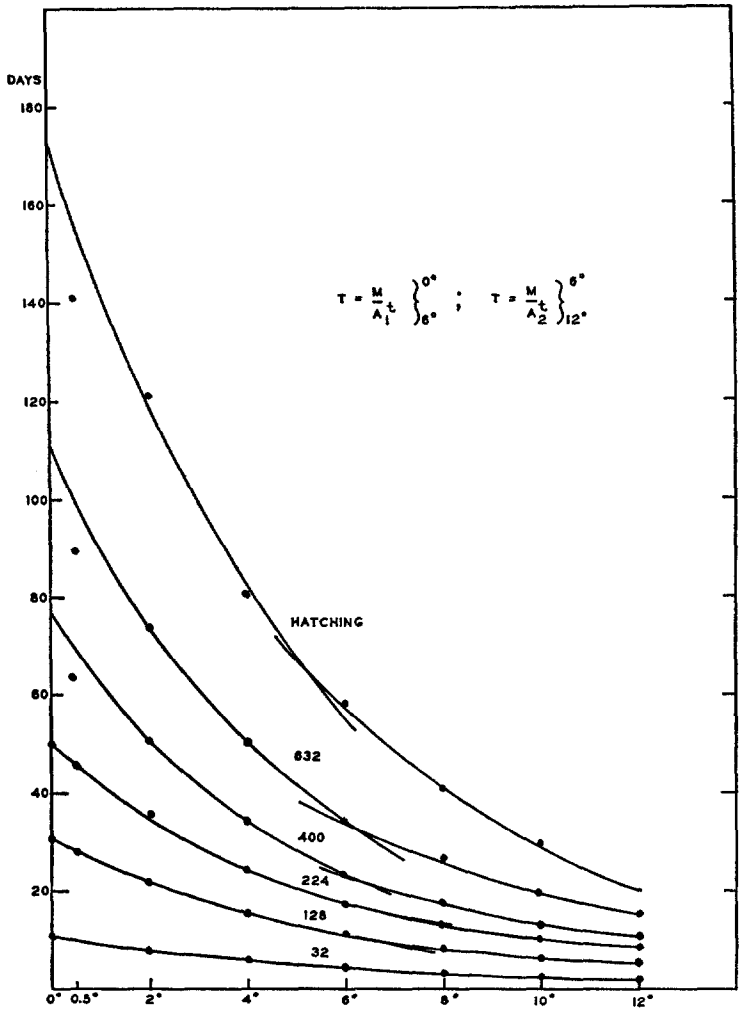

CHART 3. Length of incubation period of whitefish eggs at temperatures given, to attain certain selected stages.

Stage 32, end of cleavage	Stage 400, complete circle
Stage 128, closure of blastopore	Stage 632, growth
Stage 224, primary organogenesis	Hatching stage

The A and μ values for these several developmental phases are listed and plotted in Chart 4. Along the abscissae, the space occupied by each phase

shows its duration in per cent of the total incubation time. The proportionate part that each phase occupies of the whole is listed in Table VI.

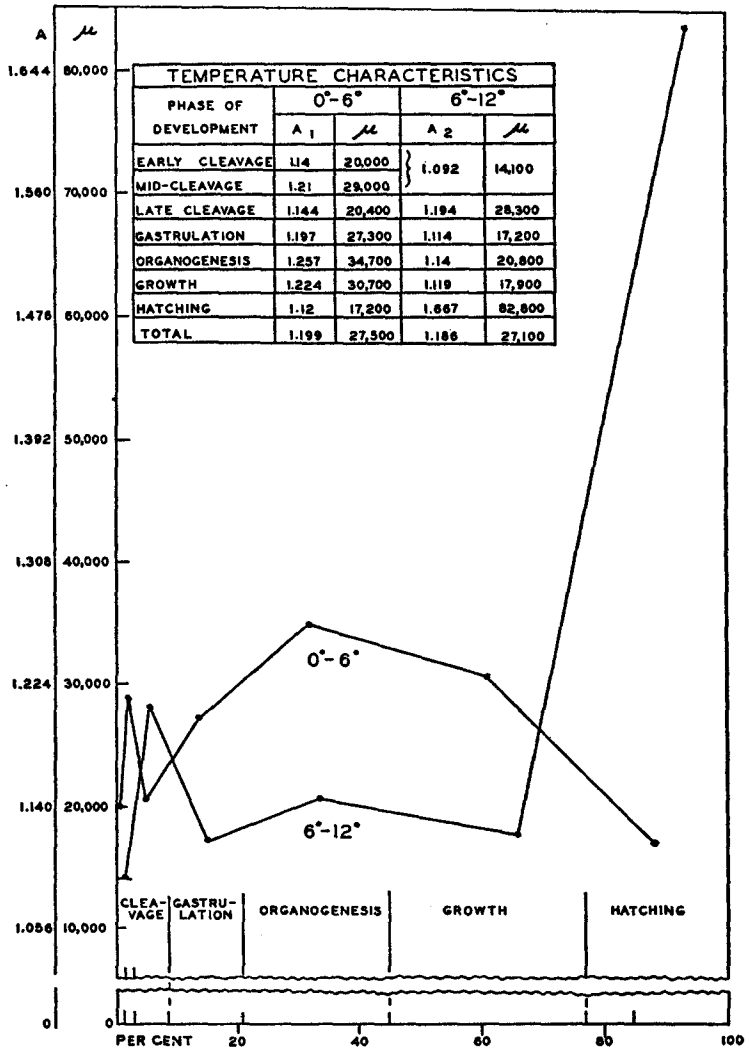

CHART 4. Instantaneous temperature characteristics for various periods of whitefish development.

Table VI shows that proportionate lengths of *pre-hatching* phases of development are not greatly altered by the temperature of incubation.

The mean length of the *hatching* period above 6°, however, is only slightly more than half (57.5 per cent) its mean length below that point. Obviously the hatching process is affected by temperature differently in the two portions of this range.

An especially interesting outcome shown in Chart 4, is the correspondence in fluctuations in A and μ values for these phases of development, since they describe the time/temperature relationship in different terms. As mentioned above, A values are a measure directly of the proportionate amount by which time of development is increased per degree Centigrade drop in temperature, or by which velocity is increased per degree rise in temperature, within a given range. Their utility is in interpolation. On the other hand, μ values have been interpreted to indicate the gram molecular energy of activation of the catalyst; *i.e.*, the "critical increment of the active substance" controlling the reaction. Their utility is in identifying, presumably, the character of the controlling reactions.

TABLE VI

Relative Duration of Various Stages

	0–6°	6–12°
	per cent	*per cent*
1-cell to 8-cell	1.25	3.24
8-cells to stage 8	1.16	
Stages 8– 32	4.37	5.77
Stages 32–128	12.00	13.92
Stages 128–400	23.37	23.88
Stages 400–776	34.65	39.84
Stages 776–hatching	23.20	13.35
	100.00	100.00

On either basis, the curves show similar trends, and that the whitefish embryo throughout its range of incubation temperatures responds in a cyclic manner to temperature differences. Peaks occur in both curves during the periods of cleavage and of organ formation. The peak during cleavage of the 6–12° curve is relatively 1.5 times higher above its origin than is the 0–6° curve. This is to say that the rate of change per degree Centigrade during cleavage is approximately 1.5 times greater in the higher temperature range than it is in the lower range at its maximal point. The sharpness of the peaks here may indicate a relative instability of the interacting factors. That the egg is highly sensitive to temperature effects during cleavage is borne out by the extreme mortality of eggs subject to adverse temperatures during this phase.

Beyond these points, the curves indicate that the rate of change per degree temperature is uniformly lower for the higher temperatures. The less acute and somewhat more regular angles of the curves may indicate an accumulative effect with age as to the organism's responsiveness to temperature.

One might be permitted the suggestion that up to the pre-hatching stage these curves represent the relative interaction to temperature of two separate sets of embryonic processes. The first set of processes consist of those which give a maximal response by way of rate of change to temperature differences early in development; *i.e.*, prior to gastrulation. The second set of processes are such that they give a maximal response during later stages.

The duration of the hatching period is primarily dependent upon processes that effect a rupture of the egg-shell membranes. From both observation and experiments, the hatching of whitefish eggs results from a combination of mechanical movements of the fish within the shell and the action of hatching enzymes. Temperature effects upon the rate of action of these factors may or may not be predictably similar to effects of temperature upon the speed of embryonic processes.

In Chart 4, the downward slope of the 0–6° line during the hatching period is directly opposed by the sharply rising 6–12° line. The magnitude of both the A and μ values during the hatching period in the upper temperature range indicates that hatching is probably controlled above 6° by factors unique to that process and not significant at temperatures below 6°.

The μ value of 82,800 does not at all correspond to such values for embryonic processes, as seen from the chart on the graph. Assuming that one or more catalytic agents are operating here as hatching enzymes, such enzymes must be extremely sensitive to temperature differences. This is supported by the fact that the hatching period lasts relatively only 57.5 per cent as long above 6° as it does below 6°. Likewise, a high mortality of embryos at the hatching period at high temperatures suggests an imbalance between interacting factors as affected by such temperatures.

SUMMARY

1. Whitefish eggs incubated in aerated lake water at controlled temperatures of 0°, 0.5°, 2°, 4°, 6°, 8°, 10°, and 12°C., failed to hatch at either 0° or 12°C. 0.6 per cent hatched alive at 10°C., 72.67 per cent hatched alive at 0.5°C., and an intermediate proportion hatched at intermediate temperatures.

2. The percentage of abnormal embryos which developed to the hatching stage varied directly with temperature between 4° and 12°, all embryos

being abnormal at 12°C.; but none were abnormal at either 0.5°, or 2°C. Normal development predominated from 0.5 to 6°C. The highest proportion of embryos to hatch alive was 72.67 per cent at 0.5°C., which is, hence, the optimum temperature.

3. Total incubation time ranged from 29.6 days at 10°C. to 141 days at 0.5°C.

4. The time (T) required to attain any given stage of development is expressed in equations

$$T = \frac{M}{A_1^t}\Big\}\begin{matrix}0°\\6°\end{matrix}; \qquad T = \frac{M}{A_2^t}\Big\}\begin{matrix}6°\\12°\end{matrix}$$

where temperature, t, is a negative exponent of the constant, A, whose value differs above or below 6°C., a critical temperature. Values of A above 6° fluctuate about 1.13; those of A below 6° fluctuate about 1.19 as a mean.

5. Applying Arrhenius' equation μ values for the total incubation period are 27,500 below 6° and 27,100 above it.

6. The relative magnitude of A values of the exponential equation and μ values of Arrhenius' equation show corresponding changes from one developmental period to another.

7. When plotted, thermal increments show cyclic variations, with maxima during periods of cleavage and of organogenesis. These may indicate the interaction of two separate sets of embryonic processes, which give a maximal response to temperature differences during these two separate periods.

8. Above 6°, μ values during the hatching process are distinct from those of developmental stages and are regarded as being due to the action of hatching enzymes.

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