EFFECT OF TEMPERATURE ON THE BODY FORM AND MORTALITY IN THE DEVELOPMENTAL AND EARLY LARVAL STAGES OF THE ALASKA POLLACK, *THERAGRA CHALCOGRAMMA* (PALLAS)

Ikusô HAMAI*, Kenichiro KYÛSHIN* and Tetsuichiro KINOSHITA*

The Alaska pollack, *Theragra chalcogramma* (PALLAS), is one of the most abundant and important commercial fish in the Hokkaido waters; as a matter of fact the annual yield from 1963 to 1967 reached 392–521 thousand metric tons, which was approximately one-third of the total catch of all kinds of fish. This species approach the coast in a great mass during the spawning season from late December to March in the coastal areas of the Japan Sea and Funka Bay of Hokkaido.

The biological and biometrical investigations on the discrimination of the unit stock in the Alaska pollack have been carried out for a long time. However, the studies on the quantitative effects of the environmental factors limiting the abundance and survival, or influencing the biometrical characteristics have not yet been published to date. The present study was undertaken to determine the effect of temperature on the development, survival and body form during the embyronic and larval stages in this species.

Before going any further, the authors wish to express appreciation to the members of Shikabe Fisheries Cooperative Association for their aid in collecting the materials.

Materials and methods

1) Materials

The eggs and milt of the Alaska pollack were taken from two ripe females and one ripe male captured by a demersal gill net in the coastal waters off Shikabe at the entrance of Funka Bay, Hokkaido on March 1st, 1961, when surface water temperature was about 2°C. Artificial fertilization was accomplished on board by the ordinary dry method, and the fertilized eggs were transported to the laboratory in the thermos jar to keep temperature out of rise. The water temperature was 4°–5°C in the jar during the transport. The adults used for fertilization were identified as the B-type of the Alaska pollack on the basis of the measurements

* *Laboratory of Biology of Fish Population, Faculty of Fisheries, Hokkaido University*
(北海道大学水産学部資源生物学講座)

Table 1. Adults used in artificial fertilization of eggs

Adult fishes	Male	Female	
		Material 1	Material 2
Body length (cm)	44.3	40.2	45.7
Average weight of a pair of otoliths (mg)	332.5	245.0	362.5
Ridge on the concave of otolith	Observable	Observable	Observable

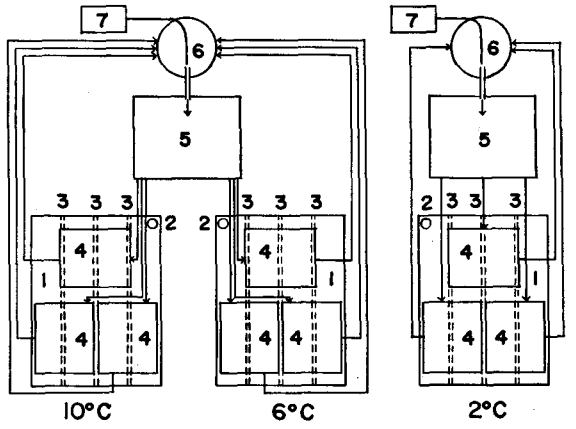

Fig. 1. Diagrammatic surface view of the closed water circulation system 1: tap water bath (46×35×30 cm) 2: thermostat 3: heater 4: glass incubator (16×19×19 cm) 5: glass reservoir (22×29×29 cm) 6: beaker (5 L) 7: air compressor

of otolith according to the previous report of Kyûshin, Kinoshita and Hayashi (1961) (Table 1).

2) Incubation of eggs

Immediately on arrival at the laboratory, the eggs were washed with sea water and the eggs of material 1 were transferred separately to the glass incubators placed in two closed water circulation systems and also to those containing 3 liters of sea water in still water condition. Material 2 was kept only in the closed circulation system. They were incubated at three constant water temperatures, 10°, 6° and 2°C, keeping both materials at the same temperature.

In the closed circulation system the water was lifted up by air bubbling from a five-liter beaker to a reservoir and it flowed over through a glass pipe to the incubator, and then came back to the beaker successively by the difference in water level (Fig. 1). The incubators for 10° and 6°C were set in the same closed circulation system. The total capacity of sea water was about 34 liters in the system for 10° and 6°C and about 27 liters in the 2°C system. The rate of water

Table 2. Number of eggs incubated and conditions of sea water during the embryonic period

Exp. section	Material*	Initial number of eggs	Percentage of hatching	Temperature (°C)		Dissolved oxygen (cc/L)		Chlorinity(‰)	
				Mean	Range	Mean	Range	Mean	Range
10°C	1 (A)	3632	5.7	10.03	8.2-14.4	6.08	5.28-6.44		
	1 (B)	1845	35.8	9.91	7.8-13.1	6.08	5.77-6.39	18.75	18.72-18.78
	1 (B)	1941	31.8	9.91	7.8-13.1				
	2 (A)	4411	0.9	10.01	8.2-14.4	6.19	5.59-6.49		
	2 (A)	4994	0.3	10.08	8.3-14.5			18.96	18.72-19.15
6°C	1 (A)	3933	74.7	6.54	5.1- 8.2	6.34	5.81-6.71		
	1 (B)	1805	82.4	6.61	5.4-10.6	6.13	6.86-7.78	18.79	18.76-18.82
	1 (B)	3241	83.5	6.61	5.4-10.6				
	2 (A)	4776	71.7	6.58	5.2- 8.1	6.28	5.81-6.50		
	2 (A)	4338	72.4	6.67	5.5- 8.2			18.98	18.72-19.15
2°C	1 (A)	6705	90.9	2.43	1.8- 4.7	7.31	6.86-7.78		
	1 (B)	2684	90.5	2.40	0 - 6.7	6.69	6.31-6.93	18.80	18.75-18.87
	1 (B)	2451	94.2	2.40	0 - 6.7	6.81	6.72-6.89	18.86	18.80-18.91
	2 (A)	7183	83.2	2.54	1.8- 4.6	7.39	7.00-7.84		
	2 (A)	3962	89.1	2.46	1.8- 4.7			18.85	18.82-18.89

* A: Incubated in closed circulation system
B: Incubated in still sea water

circulation in each incubator was about 25 cc/min., thus each incubator in these circulation systems could keep about 3 liters of sea water continuously.

One-half of the water in both the circulation systems and the incubators in still-water condition was changed for fresh sea water every four days. Since the incubators were immersed in the tap water baths the temperature of which was regulated by means of the thermostat and the heater, the water temperature of the incubators was indirectly adjusted as desired. About 1800–7200 eggs were incubated in each incubator at the beginning of the experiment (Table 2).

The water temperature was recorded daily and was on the average 9.9°–10.1° C for the 10°C experimental section; 6.5°–6.7°C for the 6°C section and 2.4°–2.5° C for the 2°C section for each set of incubators. But in the 10°C experimental section, the water temperature rose only once to 14.5°C due to a defect of the thermostat between the 3rd and the 4th day after fertilization. Observations of the chlorinity and the dissolved oxygen in the water were made at regular intervals of three days. The chlorinity varied from 18.75–18.98‰ on the average for each set of incubators and the dissolved oxygen tended towards a lower percentage at higher temperatures (Table 2).

Dead eggs were removed from the incubators, counted daily and preserved in a 3 % formalin solution of sea water.

Table 3. Number of larvae reared and conditions of sea water in the larval stage

Exp. section	Material	Number of rearing vessel	Water volume of one vessel(L)	Number of larvae	Mean Temperature (°C)	Mean dissolved oxygen (cc/L)	Mean chlorinity (‰)	Remark
10°C	1	2(B)	3	618, 661	9.84, 9.70	5.52, 5.05	18.98, 18.99	
	2	1(C)	1	95	6.28			*N. closterium*
	2	1(C)	1	99	6.28			Non fed
	2	1(C)	1	206	6.48			Non fed
	2	3(C)	1	186-207	6.31-6.47	5.75	19.10	
6°C	2	3(C)	1	401-435	6.33-6.47	5.88	19.12	
	1	1(B)	3	557	6.39	5.60	18.92	
	2	2(C)	1	809, 828	6.32, 6.41	5.60	19.10	
	1	1(B)	3	1575	6.42	5.13	19.18	
	2	1(A)	10	2319	5.77	5.27	19.12	
	2	1(A)	10	2436	6.17	4.95	18.95	Chloromycetin
	1	1(E)	0.5	101	2.50			
	2	2(C)	1	197, 202	2.32, 2.54	7.08, 6.96	18.79, 18.74	
	1	1(E)	0.5	201	2.48		18.58	
	2	4(C)	1	395-405	2.31-2.53	6.41-6.97	18.68-18.79	
	1	2(D)	1	396, 405	2.45, 2.50			
	1	1(E)	0.5	392	2.48			
2°C	2	1(C)	1	498	2.57	6.62	18.76	
	2	2(C)	1	798, 801	2.30, 2.30	5.96, 6.63	18.57, 18.67	
	2	2(B)	3	727, 832	2.80, 2.83	6.58, 5.90	18.65, 18.73	
	2	1(B)	3	99 8	2.38	4.05	18.82	
	1	1(B)	3	1042	2.36	6.14	18.71	
	1	1(B)	3	2966	2.77	5.17	18.60	
	1	1(E)	0.5	99	2.46	6.97	18.80	Chloromycetin
	1	1(E)	0.5	188	2.45	6.22	18.90	Chloromycetin
	1	1(E)	0.5	390	2.45	5.54	18.61	Chloromycetin
	1	1(D)	1	391	2.45		18.93	Chloromycetin

A: Glass vessel 22×29×29 (cm)
B: Glass vessel 14×18×16 (cm)
C: Glass vessel 10.5×14.5×13 (cm)
D: Beaker (1L)
E: Beaker (0.5L)

3) Rearing of larvae

When hatching began, the newborn larvae were separated at once into the various size of rearing vessels with different densities distinguishing the material (Table 3). The transfer of larvae from each incubator was made once a day for the 10°C experiment, but once in three days for the 6°C experiment and once in four days for the 2°C experiment, because the number of hatched larvae was smaller in the lower temperature. They were reared at the same temperature as during the incubation period without any change of water and aeration. Temperature, dissolved oxygen and chlorinity of the water are shown in Table 3. The dissolved oxygen showed a rising tendency at a lower temperature and in a lower larval density.

The nauplii of *Artemia salina* and *Nitzschia closterium* which were artificially cultured in the laboratory were provided as food for the larvae, and the diets were offered every two days after the larval mouth and the anus had completely opened. Some larvae were kept in vessels without food for the starvation experiment. As a special experiment, the rearing by use of sea water containing chloromycetin of 50 p.p.m. was carried out in the 6°C and the 2°C experimental sections to examine the effect of bacterial contamination of water on the survival of larvae (Table 3). A great effort was made to keep the vessels clean by removing dead bodies of *Artemia*, excrements and other dirts using a glass siphon every day. Dead larvae were also removed daily, counted and preserved.

4) Observations of the stages of the embryonic development and the measurements of embryos and larvae

During incubation, microscopic observations were frequently made, randomly sampling small numbers of eggs to determine the stage of development. Four living eggs from each material in each experimental section were sampled to measure the egg diameter, the diameter of the optic vesicle and the diameter of the auditory vesicle at a particular developmental stage, that shows the closure of the blastopore, the formation of the lens, the pulsation of the heart and the fully circled embryo.

In the larval stages the body length, the eye diameter, the diameter of the auditory vesicle and the largest horizontal length of the yolk sac were measured every two days for the 10°C experimental section, every three or five days respectively for the 6°C and the 2°C sections (Fig. 2). Eight living larvae were sampled randomly from each material for these measurements. After observation and measurements, the eggs and larvae were preserved in a 3 % formalin sea water solution for further studies of the embryonic and larval development.

Results

1) The rate of the embryonic development

The first observation at the laboratory was made fourteen hours after fertilization, when the development reached nearly the morula stage. The embryonic development at different temperatures was examined according to the graphycal method used by Worley (1933). This is shown in Fig. 3, in which the stages have been spaced on the ordinate in such a way as to show the 6°C development (material 2 in circulation system) as a straight line which passes through the origin at an angle of 45 degrees against the abscissa. The development at the other temperatures was then plotted against these same ordinates. As it is evident from the figure, a linear relation was approximately obtained for each temperature in each material and water condition. This means that the effect of

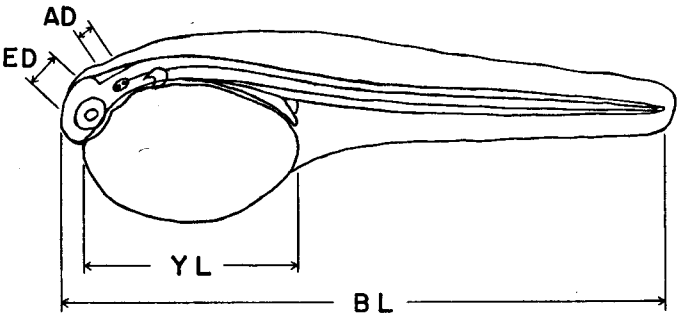

Fig. 2. Diagram to show the measurements of body parts of the newly hatched larvae in the Alaska pollack

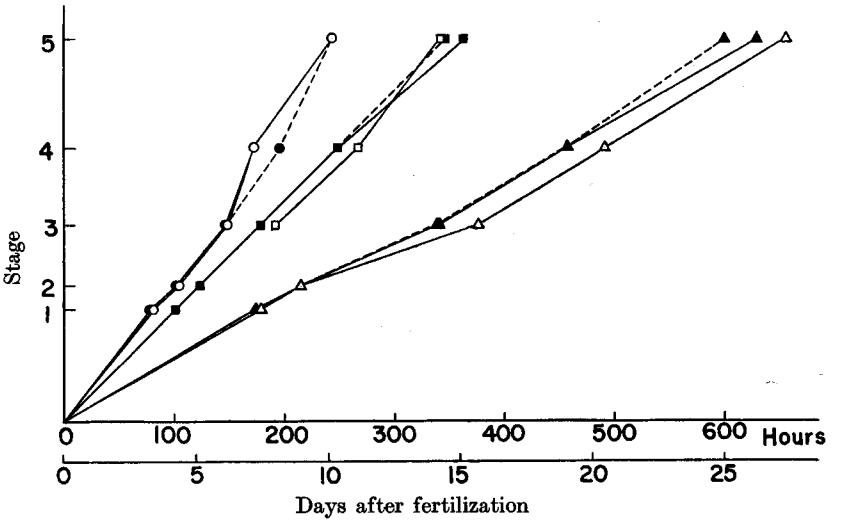

Fig. 3. The velocity of embryonic development at different temperatures
Stage 1: closure of the blastopore Stage 2: formation of the lens Stage 3: pulsation of the heart Stage 4: fully circled embryo Stage 5: hatching

●	10°C circulating water	○	10°C still water
■	6°C circulating water	□	6°C still water
▲	2°C cicrulating water	△	2°C still water
———	material 1	----------	material 2

different temperatures on the rate of development was relatively uniform in all stages. The slope in the regression of the stage on time was 1.30–1.35, 0.95–1.00 and 0.50–0.55 in the 10°, 6° and 2°C development respectively; the rate of development is higher at higher temperatures and differs little between materials and between water conditions at the same temperature.

The rate of development is related to temperature as follows; $\log 1/t = -\frac{\mu}{2}\frac{1}{T} + C$, where t is the time in days required for the eggs to reach a stage, T is

the absolute temperature on the average, μ is Arrhenius' temperature characteristic and C is the constant. The temperature characteristic was 13200 at the closure of the blastopore, 16000 at the formation of the lens, 18400 at the beginning of the heart pulsation, 19900 at the accomplishment of the fully circled embryo and 19700 at the hatching, therefore it shows a tendency to increase slightly in later developmental stages. The incubation time from fertilization to 50 % hatch was 10 days at 10°C, 13.8–14.4 days at 6°C and 24.5–27.4 days at 2°C in each incubator. The percentage of hatches was as high as 83–94 % at 2°C, but it was lower in higher temperature (Table 2). The strikingly low percentage of hatching in the 10°C circulation system was undoubtedly due to the effect of the temporal rise in temperature as high as 14.5°C given to the survival of eggs. The high egg density in circulating water condition also resulted in a low percentage of hatching as compared with the low density in still water.

2) Development and growth of larvae

The newly hatched larvae floated upside-down at the surface of the water due to the buoyancy of their large yolk sac. Although still inactive, the larvae were able to swim straight for a short distance by fluttering their tail and beating their pectoral fins one or two days after hatching. The mouth became completely functional and the anus opened about four, six and nine days after hatching at the temperature of 10°, 6° and 2°C respectively. The largest horizontal length of the yolk sac, measured, at hatching, 1.25–1.65 mm on the average; it gradually diminished with the consumption of the yolk (Fig. 4). The yolk was almost absorbed 10, 15 and 22 days after hatching at the respective experimental temperature, but the yolk sac still remained in the body cavity as a cord-like one. In this experiment the larvae died out before the differentiation of caudal, dorsal, anal and ventral fins, although they were reared beyond the yolk-sac stage with success.

The average body length of the newly hatched larvae was 4.62 mm at 10°C, 4.60 mm at 6°C and 4.72 mm at 2°C. Growth curves of the larvae fed with *Artemia* nauplii are shown in Fig. 5. As it is evident from Fig. 5, the growth rate was rapid during the yolk-sac stage and decreased thereafter. Growth was apparently better at a high temperature; the time which the larvae required to attain the body length of 5.6 mm was 13 days at 10°C, 16 days at 6°C and 29 days at 2°C. The average increase in body length was about 1.1–1.5 mm during the experiment. Because of the insufficient data with regard to the larvae which were fed with *Nitzschia closterium*, it could not be confirmed whether this phytoplankton is useful for the growth of larvae or not.

3) Changes in body form

The diameter of the eye and the auditory vesicle were measured to study their development under various temperatures. The optic vesicle differentiated just

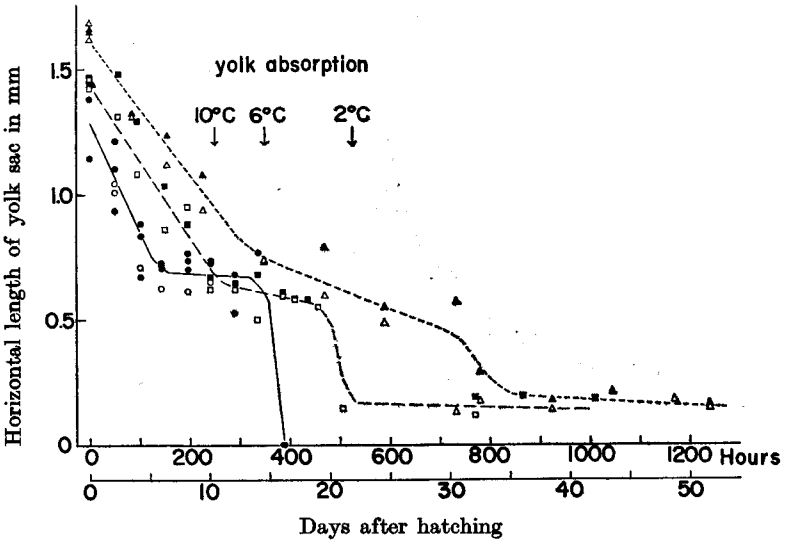

Fig. 4. Changes in the horizontal length of the yolk-sac with the lapse of time after hatching

10°C ● material 1 ○ material 2 6°C ■ material 1 □ material 2 2°C ▲ material 1 △ material 2

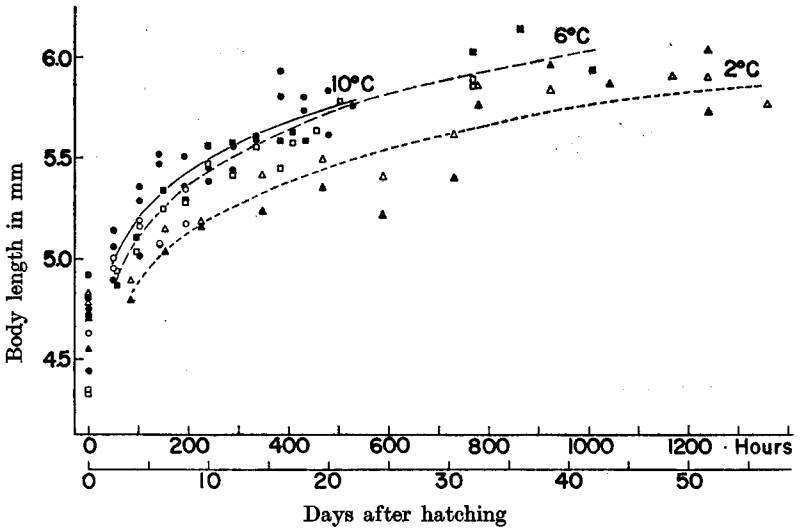

Fig. 5. Growth curves of the Alaska pollack larvae
Notations are the same as those in Fig. 4.

about the stage at which the epibolic growth of the embryo extended semicircularly to the equatorial position. Since the auditory vesicle was observable at the stage of the blastopore closure under the proper light, its outlines not being very clear, the diameter was measured after the stage of the heart pulsation.

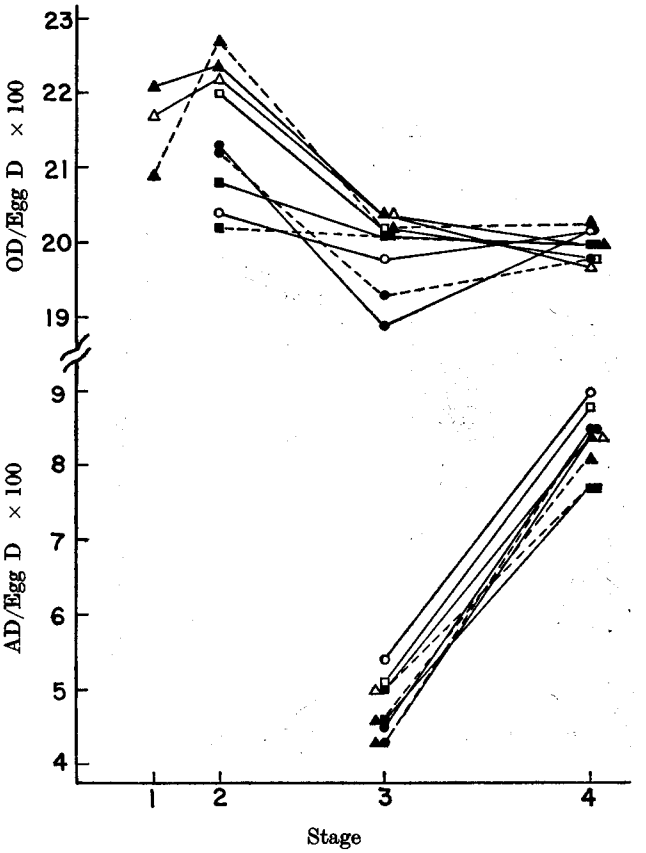

Fig. 6. Changes in percentage of the diameters of both the optic and the auditory vesicles to egg diameter during the embryonic period
Stages and notations are the same as those in Fig. 3 respectively.

To avoid the possible bias attributed to the egg diameter, the ratios of these vesicular diameters to the egg diameter were used for the following analysis (Fig. 6). As for the water circulation system, the three-way analysis of variance for temperature, material and developmental stage as the factors, shows that the three main factors are insignificant for the optic vesicle but the developmental stage is highly significant for the auditory vesicle (Table 4). Similar results were obtained from the analysis of variance within each material except for a result of the optic vesicle only, in material 1, in which the developmental stage was highly significant; that is, the auditory vesicle was significantly affected by the stages (Table 5). The egg diameter ranged from 1.37 mm to 1.55 mm, but significant differences could not be recognized between materials and also between developmental stages (Table 6). Accordingly, the above results mean that the temperature and the water condition have no reasonable influence upon the development of the

Table 4. Analysis of variance on both optic and auditory vesicles in the embryonic period in closed water circulation, using vaules of arcsine transformation for percentage of these diameters to egg diameter
T, S and M represent temperature, developmental stage and material respectively.

1) Optic vesicle

Source of variance	Sum of squares	df	Mean square	Variance ratio		Remark
T	2.6222	2	1.3111	1.06	insig.	
S	14.9079	2	7.4540	6.03	insig.	
M	0.0342	1	0.0342	0.27	insig.	
T×S	4.9425	4	1.2356	9.29	(P<0.005)	
Collective error	5.6773	44	0.1290			(Error)+(T×S×M)+(S×M)+(T×M)
S×M	0.0658	2	0.0329	0.25	insig.	
T×M	0.2900	2	0.1450	1.09	insig.	
Collective error	5.3215	40	0.1330			(Error)+(T×S×M)
T×S×M	0.9861	4	0.2465	2.05	insig.	
Error	4.3354	36	0.1204			
Total	28.1841	53				

T: 10°C, 6°C, 2°C
S: Formation of lens. pulsation of heart, fully circled embryo
M: Material 1, material 2

2) Auditory vesicle

Source of variance	Sum of squares	df	Mean square	Variance ratio		Remark
T	0.1080	2	0.0540	0.21	insig.	
S	115.4570	1	115.4570	452.06	(P<0.005)	
M	0.0864	1	0.0864	0.34	insig.	
Collective error	4.8527	19	0.2554			(Error)+(T×S×M)+(T×S)+(S×M)+(T×M)
T×S	0.4757	2	0.2379	0.82	insig.	
S×M	0.0001	1	0.0001	0.00	insig.	
T×M	0.3037	2	0.1519	0.52	insig.	
Collective error	4.0732	14	0.2909			(Error)+(T×S×M)
T×S×M	1.0021	2	0.5011	1.96	insig.	
Error	3.0711	12	0.2559			
Total	120.5041	23				

S: Pulsation of heart, fully circled embryo
T, M: The same as those in optic vesicle

auditory and the optic vesicles in the embroynic stage before the fully circled embryo.

Combining the data from different environmental conditions and different materials, the average diameter of the optic vesicle was calculated as 307 μ at the closure of the blastopore, 309 μ at the formation of the lens, 286 μ at the pulsation of the heart and 285 μ at the fully circled embryo. The outline of an

Table 5. Analysis of variance on both optic and auditory vesicles in the embryonic period, using values of arcsine transformation for percentage of these diameters to egg diameter

T, S and W represent temperature, developmental stage and water condition respectively.

1) Material 1

a. Optic vesicle

Source of variance	Sum of squares	df	Mean square	Variance ratio		Remark
T	1.8310	2	0.9155	1.29	insig.	
S	19.0427	2	9.5214	13.40	($P<0.005$)	
W	0.2115	1	0.2115	0.30	insig.	
Collective error	8.5240	12	0.7103			(T×S×W)+(T×S)+(S×W)+(T×W)
T×S	4.1803	4	1.0451	1.32	insig.	
S×W	0.4380	2	0.2190	0.28	insig.	
T×W	0.7460	2	0.3730	0.47	insig.	
T×S×W	3.1597	4	0.7899	6.41	($P<0.005$)	
Error	4.4351	36	0.1232			
Total	34.0443	53				

T: 10°C, 6°C, 2°C

S: Formation of lens, pulsation of heart, fully circled embryo

W: Circulating water, still water

b. Auditory vesicle

Source of variance	Sum of squares	df	Mean square	Variance ratio		Remark
T	0.6501	2	0.3251	0.37	insig.	
S	108.2050	1	108.2050	442.92	($P<0.005$)	
W	2.8290	1	2.8290	3.23	insig.	
Collective error	4.1531	17	0.2443			(Error)+(T×S×W)+(T×S)+(S×W)
T×S	1.0243	2	0.5122	2.38	insig.	
S×W	0.1122	1	0.1122	0.52	insig.	
T×W	1.7541	2	0.8771	4.07	($0.05>P>0.025$)	
Collective error	3.0166	14	0.2155			(Error)+(T×S×W)
T×S×W	0.0748	2	0.0374	0.15	insig.	
Error	2.9418	12	0.2452			
Total	117.5913	23				

S: Pulsation of heart, fully circled embryo

T, W: The same as those in optic vesicle

2) Material 2

a. Optic vesicle

Source of variance	Sum of squares	df	Mean square	Variance ratio	
T	6.4909	2	3.2453	2.03	insig.
S	12.4174	2	6.2087	3.88	insig.
T×S	6.4051	4	1.6013	10.92	($P<0.005$)
Error	6.5948	45	0.1466		
Total	31.9079	53			

T, S: The same as those in optic vesicle of material 1

Table 5. (Continued)

b. Autitory vesicle

Source of variance	Sum of squares	df	Mean square	Variance ratio
T	0.3195	2	0.1598	0.06 insig.
S	148.6368	1	148.6368	57.74 (0.025>P>0.01)
T×S	5.1482	2	2.5741	11.54 (P<0.005)
Error	6.6942	30	0.2231	
Total	160.7987	35		

T, S: The same as those in auditory vesicle of material 1

Table 6. Analysis of variance on egg diameter
T, S, W and M represent temperature, developmental stage, water condition and material respectively.

1) Material 1

Source of variance	Sum of squares	df	Mean square	Variance ratio	Remark
T	11032.26	2	5516.13	2.73, 0.95 insig.	
S	5435.50	2	2717.63	1.35 insig.	
W	337.50	1	337.50	0.06 insig.	
T×S	8077.97	4	2019.49	2.76 (0.05>P>0.025)	
S×W	152.44	2	76.22	0.10 insig.	
T×W	11620.78	2	5810.39	7.94 (P<0.005)	
Collective error	29286.11	40	732.15		(Error)+(T×S×W)
T×S×W	3410.11	4	852.53	1.19 insig.	
Error	25876.00	36	718.78		
Total	65942.32	53			

T: 10°C, 6°C, 2°C
S: Formation of lens, pulsation of heart, fully circled embryo
W: Circulating water, still water

2) Material 1 and 2 in closed water circulation

Source of variance	Sum of squares	df	Mean square	Variance ratio	Remark
T	5616.00	2	2808.00	2.96 insig.	
S	2355.11	2	1177.56	1.24 insig.	
M	510.30	1	510.30	0.54 insig.	
Collective error	45541.93	48	948.79		(Error)+(T×S×M)+(T×S)+(S×M)+(T×M)
T×S	4390.56	4	1097.64	1.12 insig.	
S×M	1936.15	2	968.08	0.99 insig.	
T×M	125.48	2	62.74	0.06 insig.	
Collective error	39089.74	40	977.24		(Error)+(T×S×M)
T×S×M	1033.07	4	258.27	0.24 insig.	
Error	38056.67	36	1057.13		
Total	54023.34				

M: Material 1, material 2
T, S: The same as those in Material 1

optic vesicle was oval in lateral view in its early developmental stage, but was gradually transformed after the formation of the lens into an approximately circular form towards the time of the heart pulsation. The diminution of the diameter occurred in this transition period. On the other hand, the auditory vesicle, which was approximately oval in lateral view throughout the embryonic development, showed a rapid increase in diameter from 70 μ at the pulsation of the heart to 120 μ at the fully circled embryo (Fig. 6).

The changes in body form in the larval stage were examined by the allometric equation, that is $\log y = \log b + k \log x$, where x is the body length in μ and y is the diameter of the eye or auditory vesicle in μ. The equilibrium constant k and another constant $\log b$ obtained by the least squares method are shown in Table 7. When comparing the regressions between materials and between temperatures by the method of the analysis of covariance, we found that the equilibrium constants and the adjusted means were not significantly different in regard to both body parts in general, the only exception being in the adjusted means of the auditory vesicle between temperatures. The results calculated from the data combined with the materials agreed approximately with the above results (Table 8, Fig. 7). The equilibrium constants of 1.76–1.95 for the eye diameter show tachyauxesis and those of 2.93–3.39 for the diameter of the auditory vesicle demonstrate strong tachyauxesis.

4) Survival of the eggs and larvae

The survival curves for the eggs and larvae showed a similar pattern in the same water temperature and the same food supply, independently from the material as well as from the egg and larval density. Combining the data from the same condition, the survival curves are summarized in Fig. 8, in which the data obtained from the incubation period in the 10°C water circulation system have been excluded from the curve, since the extremely high mortality has been attributed to an accidental rise in water temperature at the mid-stage of embryonic development. The curves are generally composed of two phases. The first is for the survivor from fertilization to the end of the yolk-sac stage. The second is for that after the yolk absorption. The curves in both phases vary lineally and the reduction coefficient is calculated by the method of the least square (Table 9). The coefficient is smaller in the stage before the yolk absorption than after and is also smaller at a lower temperature. The survival rate at the yolk absorption period was 20, 57 and 59 % for the 10°, 6° and 2°C temperature respectively. On the other hand, the majority of larvae reared without food or with *Nitzschia closterium* died before the end of the yolk absorption or just after it. Consequently, rearing the larvae beyond the end of the yolk-sac stage with *Nitzschia closterium* proved to be unsuccessful. The survival rate was remarkably high in the larvae

Table 7. Equilibrium constant k and another constant log b in allometric equation of length of body parts to body length in the larval stage and comparison between temperatures

1) Eye

Exp. section and material			10°C	6 °C		2 °C		k
			1	1	2	1	2	
10°C	1	F		0.89	0.52	1.17	0.09	1.948
		df		1, 30	1, 30	1, 28	1, 28	
		P		0.25~0.50	0.25~0.50	0.25~0.50	>0.50	
6 °C	1	F	0.06		0.35	0.00	1.03	1.727
		df	1, 31		1, 22	1, 20	1, 20	
		P	>0.50		>0.50	>0.50	0.25~0.50	
	2	F	2.01	0.67		0.20	0.18	1.827
		df	1, 31	1, 23		1, 20	1, 20	
		P	0.10~0.25	0.25~0.50		>0.50	>0.50	
2 °C	1	F	0.43	0.07	0.09		0.59	1.731
		df	1, 29	1, 21	1, 21		1, 18	
		P	>0.50	>0.50	>0.50		0.25~0.50	
	2	F	0.32	0.54	3.12	0.80		1.897
		df	1, 29	1, 21	1, 21	1, 19		
		P	>0.50	>0.50	0.05~0.10	0.25~0.50		
log b			−4.723	−3.895	−4.267	−3.909	−4.536	

Comparison between k

Comparison between adjusted means

2) Auditory vesicle

Exp. section and material			10°C	6 °C		2 °C		k
			1	1	2	1	2	
10°C	1	F		1.77	1.85	0.18	1.66	2.926
		df		1, 30	1, 30	1, 28	1, 28	
		P		0.10~0.25	0.10~0.25	>0.50	0.10~0.25	
6 °C	1	F	10.54		0.07	3.66	0.00	3.376
		df	1, 31		1, 22	1, 20	1, 20	
		P	< 0.005		>0.50	0.05~0.10	>0.50	
	2	F	2.94	1.59		3.16	0.06	3.476
		df	1, 29	1, 23		1, 20	1, 20	
		P	0.05~0.10	0.10~0.25		0.05~0.10	>0.50	
2 °C	1	F	14.62	0.39	1.89		3.30	2.778
		df	1, 29	1, 21	1, 21		1, 18	
		P	<0.005	>0.50	0.10~0.25		0.05~0.10	
	2	F	27.57	4.32	8.87	1.22		3.382
		df	1, 29	1, 21	1, 21	1, 19		
		P	<0.005	0.05~0.10	0.005~0.01	0.25~0.50		
log b			−8.538	−10.247	−10.608	−8.015	−10.287	

Comparison between k

Comparison between adjusted means

Table 8. Equilibrium constant k and another constant $\log b$ in allometric equation of length of body parts to body length in the larval stage from combined data of materials
Figures in brackets represent 95% confidence interval of equilibrium constant.

Body part	Exp. section	k	$\log b$	Adjusted mean	Comparison between k	Comparison between adjusted means
Eye	10°C	1.948 (1.693~2.203)	−4.723	2.562	F=1.64 df : 1, 43 0.10<P<0.25	F=0.83 df : 1, 44 0.25<P<0.50
	6°C	1.759 (1.593~1.925)	−4.015	2.564	F=0.84 df : 1, 39 0.25<P<0.50	F=0.02 df : 1, 40 P>0.50
	2°C	1.787 (1.716~1.858)	−4.120	2.562	F=0.05 df : 1, 44 P>0.50	F=0.48 df : 1, 45 0.25<P<0.50
Auditory vesicle	10°C	2.926 (2.365~3.487)	−8.538	2.403	F=2.01 df : 1, 43 0.10<P<0.25	F=8.69 df : 1, 44 0.005<P<0.01
	6°C	3.387 (3.020~3.754)	−10.285	2.384	F=0.05 df : 1, 39 P>0.50	F=28.30 df : 1, 40 P<0.005
	2°C	3.000 (2.639~3.361)	−8.852	2.368	F=2.45 df : 1, 44 0.10<P<0.25	F=5.23 df : 1, 45 0.01<P<0.05

reared in the sea water containing chloromycetin as compared with the ordinary sea water.

Discussion

As mentioned at the beginning of this paper, the important object of the present experimental study is to elucidate the effect of temperature on the body form and the survival rate in the early stages of development and growth of the Alaska pollack with a view to explaining the geographical discrimination of subpopulation. Temperature had no influence upon the auditory veiscle during the embryonic development before the stage of the fully circled embyro, but a high temperature evidently operated after this stage to make the diameter larger. This suggests that a period sensitive to temperature for the growth of the auditory vesicle comes in the later stage of the embryonic development. On the contrary, the eye was not affected by the temperature throughout the embryonic and larval stages. Such a different influence of the external condition on the growth of the body parts in fish was also observed in the relative growth of *Salmo gairdnerii* (Martin, 1949) and in the rearing experiment of the greenling, *Hexagrammos*

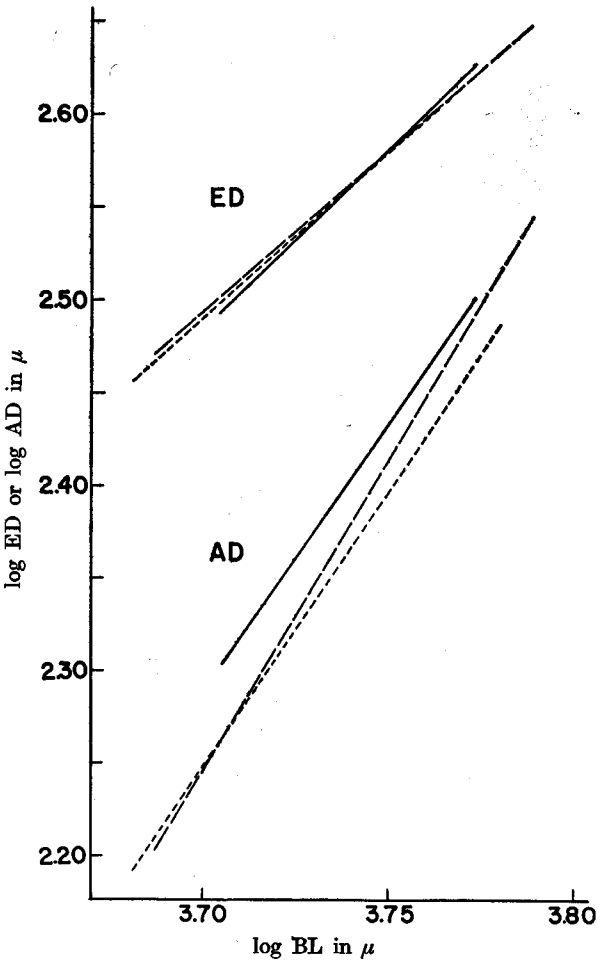

Fig. 7. Relative growth of the diameters of both the eye and the auditory vesicle against body length

—— 10°C — — 6°C ---- 2°C

otakii (Hamai and Kyûshin, 1966). As characteristics of the discrimination of subpopulation of the Alaska pollack, it is proposed that the size and weight of otoliths and the eye diameter in adult fish are different geographycally (Kyûshin, Kinoshita and Hayashi, 1961; Hashimoto and Koyachi, 1969). The relation of these facts to the present experiment must be pursued in detail in future studies.

The daily percentage of mortality was as high as 74–99 % in the 10°C section of the water circulation system, but in this case it occurred probably that the eggs were suddenly exposed to a high unadapted temperature such as 14.5°C due to an accident of the apparatus. Hence, it was concluded that 10°C was the upper limit of temperature for the survival of eggs and larvae. In general, the water temperature for the developing eggs and larvae of the Alaska pollack is about

Table 9. Reduction coefficient calculted from the survival curve in the embryonic and larval stages and comparison between temperatures

Stage	Exp. section	Reduction coefficient	Comparison between reduction coefficients
Fertilization ~ yolk absorption	10°C	0.0288	F=65.05 df : 1, 16 P<0.005
	6°C	0.0062	F=30.73 df : 1, 24 P<0.005
	2°C	0.0018	F=27.33 df : 1, 26 P<0.005
After yolk absorption	10°C	0.1444	F=0.57 df : 1, 8 0.25<P<0.50
	6°C	0.1288	F=8.71 df : 1, 8 0.01<P<0.025
	2°C	0.0866	F=56.36 df : 1, 8 P<0.005

2°–6°C in the Funka Bay area, and the mortality due to the temperature of 10°C or more is not normally a factor limiting the abundance of population during the early stages of development and growth of this species.

The unusually high survivals were achieved by the larvae reared in sea water containing chloromycetin. It is suspected that the antibiotic employed probably preserved larvae from bacterial infection, though its direct or indirect action could not be pursued. Similar results of the marvellous efficacy of antibiotics on egg and larval survivals have been reported by Oppenheimer (1955), Shelbourne (1963) and Hamai and Kyûshin (1966).

Although the dissolved oxygen in the water tended lower at a higher temperature, its effect was not analysed, but it was confirmed by the present study that the development, growth, survival and change of body form in the early life stages of the Alaska pollack were closely concerned with water temperature. The quantitative determination with respect to an independent effect of dissolved oxygen or combined effects of dissolved oxygen and temperature is left for further study.

Experimental studies on a larger scale in the laboratory and long-term field investigations are required to make clear the factors in natural conditions which

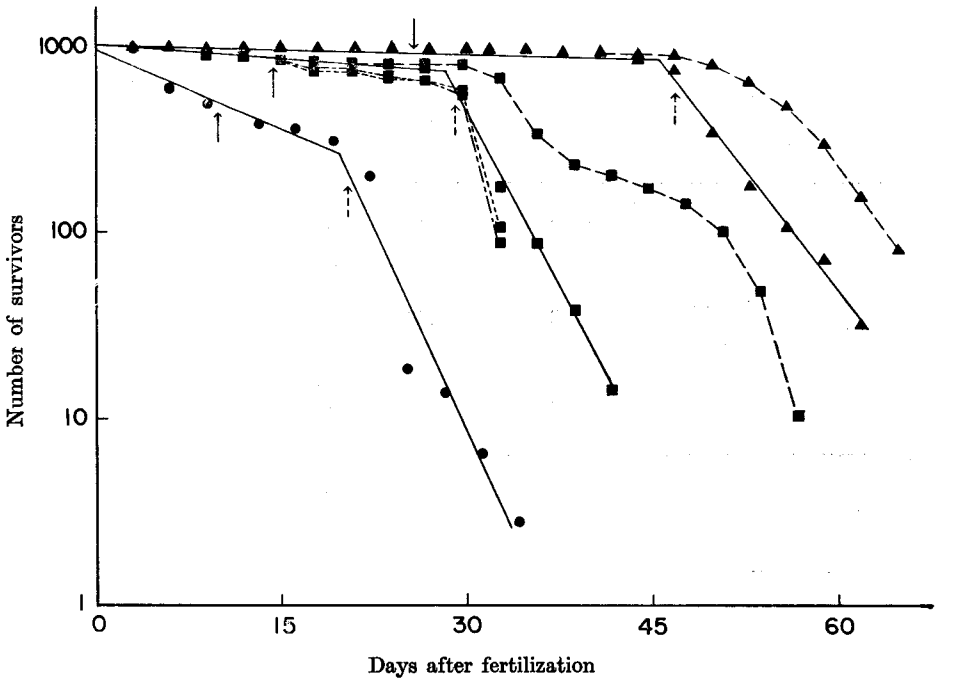

Fig. 8. Survival curves for various experimental contitions, original number of egg being 1000
← hatching ←- yolk absorption —●— 10°C —■— 6°C --■-- 6°C non-fed —■— 6°C *N. closterium* —■— 6°C chloromycetin —▲— 2°C —▲—2°C chloromycetin

influence the rate of development, survival rate and form changes throughout all the life stages of the Alaska pollack.

Summary

The observations and results regarding the effect of temperature on the development, survival and body form of the eggs and larvae in the Alaska pollack, *Theragra chalcogramma* (PALLAS) are summarized as follows;

1. Fertilized eggs were incubated and hatched larvae were reared at three constant water temperatures of 10°, 6° and 2°C in circulating or still water conditions. The larvae were fed with *Artemia salina* nauplii and *Nitzschia closterium*.

2. The rate of the embryonic development was higher at a higher temperature. Arrhenius' temperature characteristics showed the values from 13200 to 19900 in the embryonic period and had a tendency to increase in the later developmental stages.

3. Incubation time required from fertilization to 50 % hatching ranged from 10 days at 10°C to 24.5–27.4 days at 2°C. Percentage of hatching was as high

as 83–94 % at 2°C and was lower in the higher temperatures.

4. The average body length of the newly hatched larvae was about 4.60–4.72 mm. Growth was better at a higher temperature.

5. High temperatures operated on the auditory vesicle to make its diameter larger in the larval stage, but no significant influence of temperature upon the development of the eye was observed throughout the embryonic and larval stages.

6. Survivals were better at a lower temperature. The survival rate during the yolk absorption period ranged from 20% at 10°C to 59 % at 2°C.

7. No contribution from *Nitzschia closterium* as food for the larval survival was recognized. The effect of chloromycetin on the raising up of the survival rate in the larval stage was confirmed.

References

1) Hamai, I. and Kyûshin, K. (1966). Effect of temperature on the form and mortality during the embryonic and early larval stages in the greenling, *Hexagrammos otakii* JORDAN *et* STARKS. *Bull. Fac. Fish. Hokkaido Univ.*, **17** (1), 1–32.

2) Hashimoto, R. and Koyachi, S. (1959). Biology of the Alaska pollack, *Theragra chalcogramma* (PALLAS), distributed on the fishery grounds of the Tohoku Districts and the Pacific coast of Hokkaido, southward from the Erimo ground. 1. The morphological differentiation of the three types and the comparison with the other fishery grounds groups. *Bull. Tôhoku Reg. Fish. Res. Lab.*, (29), 37–92.

3) Kyûshin, K. Kinoshita, T. and Hayashi, K. (1961). On the population of the Alaska pollack in the Pacific coastal area west of Cape Erimo of Hokkaido. *Jour. Hokkaido Fish. Sci. Inst.*, **18** (3), 14–20.

4) Martin, W. R. (1949). The mechanics of environmental control of body form in fish. *Univ. Toronto Studies Biol. Ser.*, (58), 1–73.

5) Oppenheimer, C. H. (1955). The effect of marine bacteria on the development and hatching of pelagic fish eggs and control of such bacteria by antibiotics. *Copeia*, 1955, (1), 43–49.

6) Shelbourne, J. E. (1963). A marine fish-rearing experiment using antibiotics. *Nature*, **198** (4875), 74–75.

7) Worley, L. G. (1933). Development of the eggs of the mackerel at different constant temperatures. *Jour. Gen. Physiol.*, **16** (5), 841–857.