Bull. Fac. Fish., Mie Univ.
No. 13 : 17−24
November 15. 1986

Effects of Temperature and Salinity on Egg Hatch of the Ayu *Plecoglossus altivelis*

Masaaki KASHIWAGI, Toshio IWAI, Hiroki YAMAMOTO and Yoshio SOKABE

Faculty of Fisheries, Mie University

Responses of eggs of the ayu *Plecoglossus altivelis* to a series of temperature and salinity combinations were measured for percents of total and viable hatch and time to 50% hatch.

Hatch occurred at almost all tested combinations of temperature range of 11.0 to 26.0℃ and chlorinity range of 0 to 8.0‰. Response surface analysis suggests that the maximum percents of total and viable hatch (*i.e.*,optimum) may be found at 19.0℃ combined with 0.9-1.1‰.

Time required to 50% hatch varied 6.5 and 26.2 days aftre fertilization, and was inversely and exponentially related to the temperature. The Q_{10} value was higher at the lower temperature. The effect of chlorinity on the time was statistically insignificant within the hatchable range of eggs.

Key words : temperature, salinity, fish egg, ayu, *Plecoglossus altivelis*

The ayu *Plecoglossus altivelis* is widely distributed in the inland water regions throughout Japan, and is a very important species for the fisheries and the culture located there.

It is well known that the adult fish spawn near the mouth of a river. Therefore, the variations of temperature and salinity caused by the tide of ebb and flow have a major effect on the survival of its early life stages. There are, however, few reports on the effects of these variables other than that which some workers noted in an individual case of temperature (HIGURASHI 1924, ITO *et al.* 1971) and salinity (ITO *et al.* 1967).

The purpose of this study is to determine the combined effects of temperature and salinity on the egg hatch of ayu.

Materials and Methods

Eggs and sperm were collected from the adult fish, 14 females and 13 males, caught near the mouth of Miya River, Mie Prefecture, on October 17, 1985. The eggs were fertilized *in situ* and then transported to our laboratory at a temperature of 19.2℃, the same as that of the river water at fertilization.

The experimental design was a 2 factor 6 × 9 factorial with a temperature range of 11.0 to 26.0℃ in 3℃ intervals and a chlorinity range of 0 to 8.0‰ in 1.0‰ intervals (**Table** 1). An average of 161 eggs (range ; 47-477) was put into each 50mm diameter glass beaker with 100ml water. One set of nine beakers with each chlorinity was placed in the six test temperature baths, constantly controlled within ±0.3℃. When the beakers were checked more than two times every day, any dead eggs were counted and removed. The percents of total and viable hatch and the time to 50% hatch were limited to the normal larvae hatched without any abnormality, such as the curvatures of tail or notochord and the lack of eyes. The time to 50% hatch was calculated by interpolation.

For the experimental data, statistical treatments followed the analysis of variance of a two-way lay out (BLISS 1967). And the relationship between data and two variables were calculated by the methods of orthogonal polynomial, and shown as the regression equation and response surfaces (ALDERDICE 1972).

Experimental chlorinities were obtained by dilution of ALLEN'S synthetic sea salts* with fresh water. The test water was changed every three days to keep the desired chlorinities. No aeration was used in any incubations.

Results

Percent of total hatch

Results are shown in Table 1. The percent of total hatch varied between 0 and 86.8% at all experimental combinations. Analysis of variance for this data proved to be significant for both temperature and chlorinity (**Table** 2). The relationship between total hatch (TH %), temperature (T ℃) and chlorinity (C ‰) was expressed by the second order equation :

$$TH = -119.9189 + 21.7520T - 0.5666T^2 + 6.2776C - 0.8047C^2 - 0.2393TC$$

From this equation, the response surface was constructed and shown as the isopleths of percent for a selected level such as 80,70,·········and 20% (**Fig.** 1). The highest percent was 89.8% at 19.0℃ and 1.1‰ (point S in **Fig.** 1). Changes in the two variables, both increases and decreases from these values reduced the percent of total hatch. Slight rotation of the surface axes indicated the possible existence of interaction effects of

* Formula for the salts : NaCl 28.17g, $MgCl_2$ 2.55g, KCl 0.77g, $CaCl_2$ 1.20g, $MgSO_4$ 3.50g, $NaHCO_3$ 0.22g, water to 1,000ml. Cl=20.127‰.

the two variables ; with decreases in temperature, a high percent of hatch was maintained only when coupled with increases in chlorinity.

Table 1. Total percent of ayu eggs incubated at 54 temperature and salinity combiations

Temp (℃)	Chlorinity (‰) 0		1		2		3		4		5		6		7		8	
	N	TH	N	TH	N	TH	N	TH	N	TH	N	TH	N	TH	N	TH	N	TH
26	194	80.4	129	86.8	95	54.7	231	65.4	140	57.9	289	39.1	104	24.0	134	0.7	131	0.0
23	228	64.5	233	80.3	214	79.9	341	71.8	120	66.7	477	61.2	127	72.4	122	66.4	90	25.0
20	158	77.8	167	82.0	185	74.6	165	81.2	73	72.6	61	73.8	175	77.7	189	73.5	163	52.8
17	145	80.7	169	80.5	47	85.1	54	72.2	148	74.3	110	70.9	108	72.2	275	82.5	91	65.9
14	196	75.0	90	72.2	297	81.8	132	80.3	104	65.4	360	68.6	57	68.4	136	75.7	136	56.6
11	202	64.9	161	53.4	244	63.1	71	62.0	86	65.1	84	40.5	130	46.2	142	8.5	184	0.0

N : number of eggs used. TH : percent of total hatch.

Table 2. Analysis of variance for data of percent of total hatch

Source	SS	DF	MS	F
Treatments	18,228.04	13	1,402.16	7.55**
Temperature	9,062.43	5	1,812.49	9.76**
Chlorinity	9,165.61	8	1,145.70	6.17**
Regression				
Linear	7,612.82	2	3,806.41	20.50**
Quadratic	10,473.84	3	3,491.28	18.80**
Remainder	141.38	48	2.95	0.02
Residual	7,428.74	40	185.72	
Total	41,973.09	53		

Asterisk : significant (** $p<0.01$) .

Percent of viable hatch

The percent of viable hatch varied between 0 and 83.7% (**Table** 3).The results of analysis of variance showed little difference from those of the total hatch (**Table** 4). The fitted equation was :

$$VH=-94.3245+18.6927T-0.4877T^2+5.4224C-1.4004C^2-0.1482TC$$

where VH=percent of viable hatch. The highest percent was 86.0% at 19.0℃ and 0.9‰ (**Fig.** 2). The highest percent salinity combinations for the maximum percents of total and viable hatch coincided with each other.

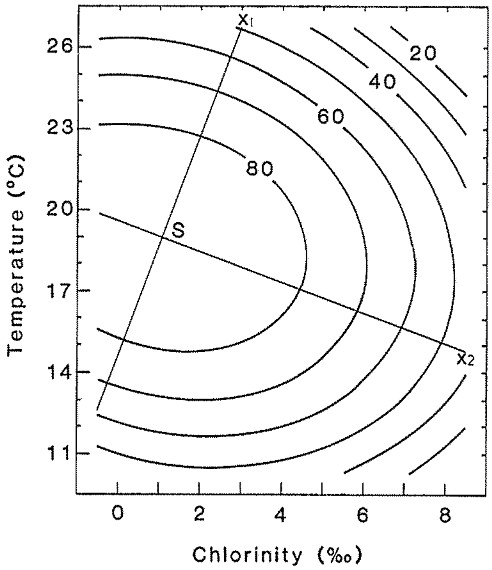

Fig. 1. Response surface showing the isopleths of percent of total hatch in rlation to temperature and chlorinity.

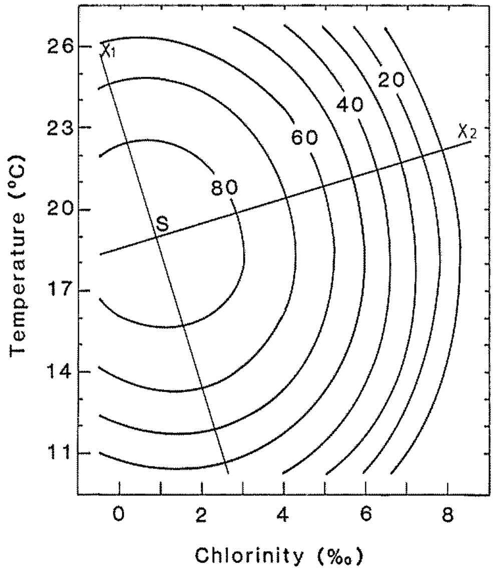

Fig. 2. Response surface showing the isopleths of percent of viable hatch in relation to temperature and chlorinity.

Table 3. Percent of viable hatch of ayu eggs incubated at 54 temperature and salinity combinations

T	Chlorinity (‰)								
(℃)	0	1	2	3	4	5	6	7	8
26	77.8	83.7	53.7	62.3	52.1	17.0	6.2	0.0	0.0
23	61.5	78.0	76.1	68.0	62.5	38.6	45.7	13.0	0.0
20	75.3	77.8	71.9	80.0	71.2	68.9	67.5	39.7	9.0
17	80.7	78.1	83.0	70.4	71.6	65.5	73.6	35.0	16.5
14	73.5	70.0	76.8	78.0	63.5	64.2	50.0	26.5	2.6
11	62.9	49.7	56.1	57.7	58.1	10.7	7.1	0.7	0.0

Table 4. Analysis of variance for data of percent of viable hatch

Source	SS	DF	MS	F
Treatments	37,117.83	13	2,855.22	22.47**
Temperature	6,859.20	5	1,371.84	11.30**
Chlorinity	30,258.63	8	3,782.33	31.16**
Regression				
Linear	26,151.98	2	13,075.99	102.91**
Quadratic	10,305.70	3	3,435.23	27.03**
Remainder	660.15	48	13.75	0.11
Residual	4,855.26	40	121.38	
Total	41,973.09	53		

Asterisk : significant (** $p<0.01$).

Time to 50% hatch

The date varied between 6.5 and 26.2 days at all experimental combinations where hatch occurred (**Table** 5). Analysis of variance for these data proved to be significant for temperature only (**Table** 6). The relationship between time (D days) and Temperature (T ℃) was expressed by the third order equation :

$$D=88.72240-9.06549T+0.35338T^2-0.00483T^3$$

The response surface in **Fig.** 3 showed the time was inversely and exponentially related to temperature, and the slope was steep at the lower temperatures. The point of bending was found at or in the vicinity of 18℃. From the equation, the Q_{10} values above and below the point was calculated at 2.09 and 3.14, respectively.

Table 5. Time (days) to 50% hatch of ayu eggs incubated at 54 temperature and salinity combinations

T	Chlorinity (‰)								
(℃)	0	1	2	3	4	5	6	7	8
26	6.6	6.6	6.5	7.4	6.7	7.7	7.3	7.4*	7.2*
23	8.0	7.9	7.7	9.3	7.8	9.3	8.0	7.8	8.5
20	10.1	10.5	10.4	10.3	10.1	10.1	10.7	10.5	10.9
17	12.2	13.1	12.8	12.5	12.6	12.8	12.6	13.3	12.7
14	18.8	17.0	19.2	16.8	17.9	18.6	16.7	18.7	17.9
11	25.7	24.7	25.4	24.9	26.2	24.7	24.4	26.3	25.5*

* Figures showing the replacement for missing values to calculate the analysis of variance for cross classification.

Table 6. Analysis of variance for data of time to 50% hatch

Source	SS	DF	MS	F
Treatments				
Temperature	2,147.84	5	429.57	1,204.45**
Chlorinity	2.79	8	0.35	0.98
Regression for temperature				
Linear	1,937.78	1	1,937.78	5,433.29**
Quadratic	198.41	1	198.41	556.33**
Cubic	9.94	1	9.94	27.87**
Remainder	1.70	2	0.85	2.38
Residual	14.27	40	0.36	
Total	2,164.90	53		

Asterisk : significant (** $p<0.01$).

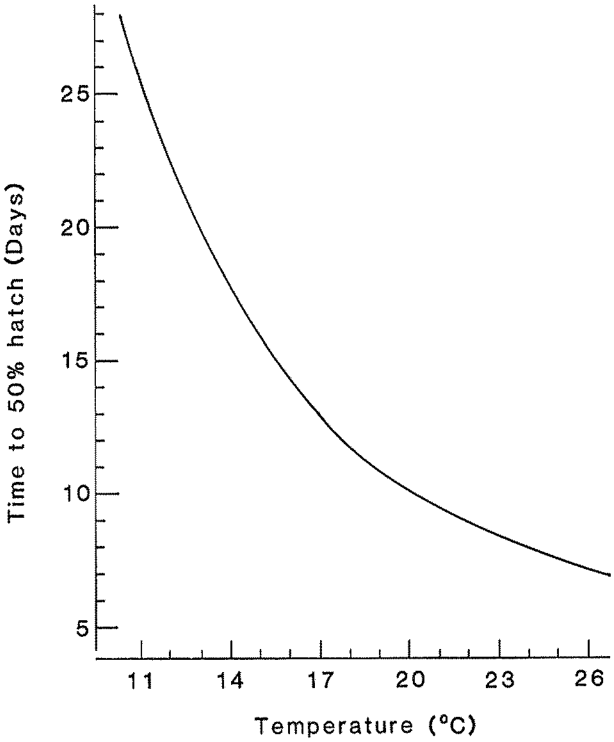

Fig. 3. Response surface showing the time to 50% hatch in relation to temperature.

Discussion

Responses of the teleosts egg to changing temperature and salinity varies widely with the speciese (KINNE 1963, 1964). In the present species, HIGURASHI (1924) and ITO *et al.* (1971) noted in regard to the effect of temperature that hatch occurred safely within the ranges of 10 to 19℃ and 12.5 to 20.3℃, respectively. And ITO *et al.* (1967) noted concerning salinity that hatch occurred only at the chlorinity lower than 8‰, and suggested that the best conditions for hatch were 0 to 1‰. Our results, in which the hatch occur at almost all tested combinations of temperature range of 11.0 to 26.0℃ and chlorinity range of 0 to 8‰, are well in accordance with their descriptions, and newly propose that both the maximum percents of total and viable hatch (*i.e.*, optimum) may be found to be at a temperature of 19.0℃ when combined with chlorinity of 0.9 to 1.1‰. The optimum temperature is nearly equal to the river water temperature at the time of fertilization

On the time to hatch, HIGURASHI (1924) reported that the days were 15, 20 and 30 after fertilization when the eggs were incubated at temperatures of 18, 15 and 10℃, respectively, and then suggested that the time was inversely and exponentially related to the temperatures. It is also a matter of common knowledge, as reviewed by BLAXTER (1969), that the Q_{10} values were higher at the lower temperature. The ayu eggs in the present study follow these relathionships. On the other hand, the effect of salinity on the time to 50% hatch was statistically insignificant within the hatchable range of eggs. The same effect has been reported on amago salmon *Oncorhynchus masou macrostomus* (LOPES *et al.* 1985) and Pacific herring *Clupea pallasi* (ALDERDICE and VELSEN 1971). while some acceleration or retardation of the time in changing salinitiies has also been reported on several fishes, such as English sole *Parophrys vetulus* (ALDERDICE and FORRESTER 1968), threeline grunt *Parapristipoma trilineatum* (KASHIWAGI *et al.* 1984), Atlantic herring *Clupea harengus* (HOLLIDAY and BLAXTER 1960), Pacific cod *Gadus macrocephalus* (FORRESTER and ALDERDICE 1965, ALDERDICE and FORRESTER 1971 b), petrale sole *Eopsetta jordani* (ALDERDICE and FORRESTER 1971 a), red sea bream (APOSTOLOPOULOS 1976) and yellowtail flounder *Limanda ferruginea* (LAURENCE and HOWELL 1981), it is generally seen that the effect of salinity is small in comparison with that of temperature (BLAXTER 1969, HOLLIDAY 1969, ROTHENTHALL and ALDERDICE 1976).

References

ALDERDICE, D. F., 1972. Factor combinations. Responses of marine poikilotherms to environmental factors acting in concert. In : KINNE, O. (ed.), *Marine Ecology*, Vol. I, Part 3 : 1659-1722. John Wiley & Sons, London.

——— and C. R. FORRESTER, 1968. Some effects of salinity and temperature on early development and survival of the English sole (*Parophrys vetulus*). *J. Fish. Res.Bd. Canada*, **25** (3) : 495-521.

——— and ———, 1971 a. Effects of salinity and temperature on embryonic development of the petrale sole (*Eopsetta jordani*). *Ibid.*, **28** (5) : 727-744.

——— and ———, 1971 b. Effects of salinity, temperature and dissolved oxygen on early development of the Pacific cod (*Gadus macrocephalus*). *Ibid.*, **28** (6) : 883- 902.

——— and F. P. J. VELSEN, 1971. Some effects of salinity and temperature on early development of Pacific herring (*Clupea pallasi*). *Ibid.*, **28** (10) : 1545-1562.

APOSTOLOPOULOS, J. S., 1976. Combined effect of temperature and salinity on the hatching rate, hatching time and total body length of the newly hatched larvae of the Japanese red sea bream *Pagrus major*. *La mer*, **14** (1) : 23-30.

BLAXTER, J. H. S., 1969. Development : eggs and larvae. In : HOAR, W. S. and D. J. RANDALL (eds.), *Fish Physiology*, Vol. 3 : 178-241. Acad. press., New York.

FORRESTER, C. R. and D. F. ALDERDICE. 1966. Effects of salinity and temperature on embryonic development of the Pacific cod (*Gadus macrocephalus*). *J. Fish. Res. Bd. Canada*, **23** (3) : 319-340.

HIGURASHI, T., 1924. An optimum temperature test for hatch of the ayu eggs. *J. Imp. Fish. Inst.*, **20** (4) : 129-131. (In Japanese)

HOLLIDAY, F. G. T., 1969. The effects of salinity on the eggs and larvae of teleosts. In :

HOAR, W. S. and D. J. RANDALL (eds.). *Fish Physiology*, Vol. 1 : 293-311. Acad. press., New York.

——— and J. H. S. BLAXTER, 1960. The effects of salinity on developing eggs and larvae of the herring. *J. Mar. Biol.Assoc. U. K.*, **39** (3) : 591-603.

ITO, T., T. IWAI, T. FURUICHI and N. HORIKI. 1967. Studies on the artificial production of ayu fish seedling-XLI The influence of salinity on the hatching of fertilized eggs. *Research Report of the Kisosansen Survey Team*, NO 4 : 733-830. (In Japanese).

ITO, T., T. TOMITA and T. IWAI, 1971. Studies on the artificial production of aẏu fish seedling-LXXI. Influence of water temperature upon the hatching of fertilized eggs of ayu fish. *Artificial Production of Ayu Fish, No.* 1 : 57-98. (In Japanese).

KASHIWAGI, M., N. YAMADA, Y. OKADA, F. NAKAMURA, S. KIMURA and T. IWAI, 1984. Some effects of temperature and salinity on developing eggs of the threeline grunt *Parapristipoma trilineatum* (Pisces : Haemulidae). *This Bull.* 11 : 1-13.

KINNE, O., 1963. The effects of temperature and salinity on marine and brackish water animals. I. Temperature. *Oceanogr. Mar. Biol. Ann. Rev.*, **1** : 301-340.

———, 1964. The effects of temperature and salinity on marine and brackish water animals. II. Salinity and temperature salinity combinations. *Ibid.*, **2** : 281-339.

LAURENCE, G. C. and W. H. HOWELL, 1981. Embryology and influence of temperature and salinity on early development and survival of yellowtail flounder *Limanda ferruginea*. *Mar. Ecol. Prog. Ser.*, **6** : 11-18.

LOPES, A. N. G., M. KASHIWAGI and T. IWAI, 1985. Effects of temperature and salinity on egg hatch of the amago salmon, *Oncorhynchus masou macrostomus*. *This Bull.*, 12 : 45-50.

ROTHENTHAL, H. and D. F. ALDERDICE, 1976. Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. *J. Fish. Res. Bd. Canada*, **33** (9) : 2047-2065.