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Some Effects of Temperature and Salinity on Developing Eggs of the Threeline Grunt, *Paraplistipoma trilineatum* (Pisces: Haemulidae)

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Responses of eggs of the threeline grunt, *Paraplistipoma trilineatum*, to a series of temperature-salinity combinations were measured for percent of total hatching and rate of development in a temperature range of 12 – 32°C and a salinity range of 29.0 – 36.2‰.

Hatching occurred at temperatures ranging from 18 to 28°C combined with salinities ranging from 31.0 to 36.2‰. Response surface analysis suggests that the maximum hatching percent (*i. e.*, optimum) may be found at 22.4°C and 34.2‰, and that the isopleth of 90% hatching is bounded on temperatures of 19.5 and 25.4°C and on salinities of 32.1 and 36.3‰. The threeline grunt eggs are considered to be stenothermal and stenohaline. The surface also indicates the possible existence of a low/low–high/high interaction between the two variables.

Time required to 50% hatching varied between 15.0 and 46.8 hrs (about 17 and 49 hrs after fertilization) for the combinations within which the range of hatching occurred. The time was inversely and exponentially related to temperature. The regression line for plots *log* time against temperatures consisted of the two straight components, intersecting in the vicinity of temperature at the time of spawning. The values of temperature characteristics such as Q_{10} and μ were higher at the lower temperatures. The effects of salinity on the time to hatching were small in comparison with those of temperature, though both the increasing and decreasing from 32.6 ‰ have a tendency to produce a progressive retardation of the development. Little interaction effect was seen between the two variables.

Of the time neccessary for the attainment of various developmental stages, each regression line in the relationship between time and temperature was essentially parallel to that of the time to 50% hatching. The rate of differentiation up to hatching seems to be governed by one process throughout.

Key words: temperature, salinity, fish egg, development, threeline grunt, *Paraplistipoma trilineatum*

Temperature and salinity are factors which may have a major effect on the development of estuarine and marine teleosts (KINNE 1964, BLAXTER 1969, ALDERDICE 1972). However, the effects of these variables on their early development have been studied in only a limited number of species. This paper presents such data for the threeline grunt, *Paraplistipoma trilineatum* (THUMBERG) (Pisces: Haemulidae).

The threeline grunt is distributed in the waters off central and sourthern Japan and furnishes an important catch for fisheries located there. Although great hopes are placed in this species as a new subject for artificial seed production, few studies have been made of its early development. Papers which have been published refer mainly to the external features of embryonic develompent at normal temperatures (KAMIYA 1922, YASUDA *et al.* 1962, MITO 1963).

The present study was designed to determine the percents of total hatching and rate of development of the threeline grunt eggs at various combinations of temperature and salinity.

Materials and Methods

The present work was carried out in June 1983 at our Fisheries Research Laboratory in Ago Bay, Mie Prefecture. Fertilized eggs were collected soon after natural spawning by adult fish, 49 females and 23 males, in an outdoor concrete tank (5.0m long × 2.0m wide × 1.0m depth).

First, a preliminary experiment was conducted on eggs collected on June 13, in 18 combinations of temperature and salinity, ranging from 12 to 32°C in 4°C intervals and from 29.0 to 36.2‰ in 3.6‰ intervals, respectively (Table 1). At spawning, water temperature and salinity in the tank were 21.8°C and 33.4‰. An average of 32 eggs were put into each 30mm diameter test tube with 50ml water. A set of the three tubes of each test salinity was placed in the six test temperature baths, constantly controlled by heaters and coolers with thermostats. After the tubes had deen inserted, about 15 minutes were required for water in the test tubes to reach the desired temperature. The eggs at the beginning of the experiment were at the 16−cell stage of development, about two hours having passed since their fertilization (Plate I−No. 5). The total hatching percent was determined for each combination.

Our second main experiment was done on eggs collected on June 20, in 24 combinations of temperature and salinity, ranging from 18 to 28°C in 2°C intervals and from 30.8 to 36.2‰ in 1.8‰ intervals, respectively (Table 2). The choice of these values was based on the results of the former experiment. The water temperature and salinity at the time of spawning were 21.5°C and 33.4‰. The egg diameter at the 16−cell stage was 0.84 ± 0.02mm. Two sets of four test tubes of each salinity, containing an average of 35 eggs each, were placed in the six test temperatures. When the tubes were checked every two hours any eggs which had sunk were considered dead and were

removed. Two replicates of the total hatching percent and time to 50% hatching were determined. Response surface techniques were employed to describe the relationship between temperature, salinity, hatching percent and time to 50% hatching (ALDERDICE 1972).

One other set was also provided in the main experiment to determine the attainment time of various developmental stages. For this, about 200 eggs were initially placed in each test tube, after which about ten were removed every two hours for observation and were not replaced.

The highest salinity of 36.2‰ in all main experiments was prepared by mixing natural sea water (33.4‰) and synthetic sea salts, and the lower salinities were obtained by dilution with fresh water. No aeration was used in any incubations.

Results

Percent of total hatching

Table 1 shows the results of the preliminary experiment in which hatching occurred

Table 1. Total hatching percent of threeline grunt eggs incubated at 18 temperature-salinity combinations

‰ Salinity		36.2		32.6		29.0	
Temperature(°C) D	E	N	H (%)	N	H (%)	N	H (%)
12	12.0	60	0.0	26	0.0	23	0.0
16	15.9	27	0.0	40	0.0	40	0.0
20	19.8	25	96.0	32	96.9	29	0.0
24	24.1	33	97.0	28	100.0	24	0.0
28	28.0	34	44.1	25	20.0	25	0.0
32	32.0	51	0.0	27	0.0	21	0.0

D; designed level. E; experimental level at beginning of experiment.
N; number of eggs used. H; hatching percent.

only at temperatures of 20, 24 and 28°C combined with salinities of 32.6 and 36.2‰. In the main experiment, the hatching occurred at almost all temperature-salinity combinations employed (Table 2). At the same combinations, the results coincide with each other (Teble 3). The results of the analysis of variance for the data in Table 2 indicate that the hatching does depend on temperature, salinity and their interaction (Table 4). It is evident that the development to hatching was inhibited at the lowest temperature (18 °C) combined with both the highest and lowest salinities (36.2 and 30.8‰), and at the highest temperature (28°C) with all salinities.

According to ALDERDICE and VELSEN (1971), the relationship between temperature, salinity and hatching percent can be expressed by a second order polynomial (linear model). Hence, in this work, the same calculating procedure was followed for the data in Table 2.

Table 2. Total hatching percent of threeline grunt eggs incubated at 24 temerature-salinity combinations

Temperature(°C) D	Temperature(°C) E	‰ Salinity 36.2 N	36.2 H (%)	34.4 N	34.4 H (%)	32.6 N	32.6 H (%)	30.8 N	30.8 H (%)
18	18.4±0.2	25	4.0	30	93.3	47	89.4	34	11.8
		28	0.0	25	76.0	37	89.2	29	3.4
20	19.7±0.7	32	100.0	48	97.9	38	97.4	43	72.1
		33	100.0	35	94.3	45	93.3	52	61.5
22	21.9±0.1	19	100.0	31	96.8	31	100.0	51	51.0
		20	100.0	32	93.8	39	61.5	61	41.0
24	24.2±0.8	33	97.0	25	92.0	21	100.0	50	50.0
		44	72.7	37	86.5	19	96.6	42	23.8
26	25.9±0.8	42	95.2	30	100.0	24	95.8	37	37.8
		30	66.7	21	90.5	37	56.8	33	24.2
28	28.4±1.0	35	48.6	23	17.4	35	17.1	39	12.8
		46	32.6	27	11.1	38	5.3	31	3.2

D; designed level. E; experimental level (mean ± s.d.).
N; number of eggs used. H; hatching percent.

Table 3. Analysis of variance for differences of data between Tables 1 and 2 at same temperature·salinity combinations*

Source	SS	DF	MS	F
Treatments	50.02	1	50.02	0.04^{NS}
Residual	12,915.86	10	1,291.59	
Total	12,965.88	11		

*Temperature; 20, 24 and 28°C. Salinity; 36.2 and 32.6‰.
NS; not significant.

Table 4. Analysis of variance for data in Table 2

Source	SS	DF	MS	F
Treatments	57.442.86	23	2,497.52	18.24**
Temperature	28,382.46	5	5,676.49	41.45**
x_1 linear	4,396.00	1	4,396.00	32.10**
x_1^2 quadratic	21,019.08	1	21,019.08	153.48**
Salinity	16,193.37	3	5,397.79	39.41**
x_2 linear	7,257.80	1	7,257.80	53.00**
x_2^2 quadratic	8,602.81	1	8,602.81	62.82**
Interaction	12,867.23	15	857.80	6.26**
$x_1 x_2$	429.13	1	429.13	3.13^{NS}
Residual	3,286.78	24	136.95	
Total	60,729.64	47		

**highly significant ($p<0.01$). NS; not significant.

The fitted equation was

$$Y = 104.98 - 2.80x_1 + 5.50x_2 - 2.10x_1^2 - 3.35x_2^2 + 0.55x_1x_2$$

where Y = percent of total hatching, x_1 = coded level of temperature: measured value − 23, and x_2 = coded level of salinity : (measured value − 33.5) / 0.9.

The response surface calculated from the equation is illustrated in Fig. 1. The maximum hatching (*i.e.*, optimum) was estimated to occur at 22.4°C and 34.2‰. The isopleth of 90% hatching was bounded on temperatures of 19.5 and 25.4°C and on salinities of 32.1 and 36.3‰. Slight rotation of the surface axes indicated the possible existence of interaction effects of the two variables : with increases (or decreases) in temperature, a high hatching percent is maintained only when coupled with increases (or decreases) in salinity.

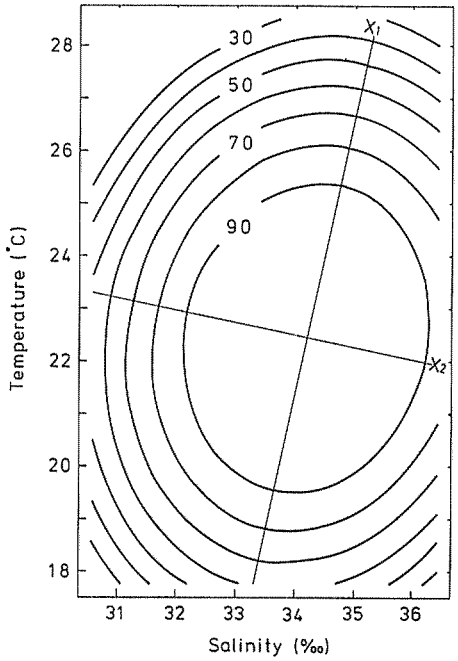

Fig. 1. Response surface showing the isopleths of total hatching percent in relation to temperature and salinity. The X_1 − and X_2 − axes are the geometric axes of the surface.

Rate of development

Time to 50% hatching: The data varied between 15.0 and 46.8 hours (about 17 and 49 hrs after fertilization) at the 24 temperature-salinity combinations (Table 5). The time

Table 5. Time to 50% hatching of threeline grunt eggs incubated at 24 temperature-salinity combinations

‰ Salinity		36.2		34.4		32.6		30.8	
Temperature(°C) D	E	N	Hours	N	Hours	N	Hours	N	Hours
18	18.4±0.2	0	47.0*	19	46.8	42	45.4	1	46.5*
		1	46.9*	28	46.6	33	45.2	4	46.5
20	19.7±0.7	33	38.2	33	37.3	42	35.8	32	37.2
		32	37.4	47	37.0	37	35.8	31	37.1
22	21.9±0.1	19	28.6	30	27.4	24	27.1	26	27.2
		20	27.1	30	26.7	31	26.9	25	26.9
24	24.2±0.1	32	23.5	32	22.8	28	22.4	10	22.9
		32	23.2	23	21.6	21	22.4	25	21.3
26	25.9±0.8	40	19.2	19	19.6	21	19.7	14	19.4
		20	18.9	30	19.4	23	18.7	8	17.3
28	28.4±1.0	15	17.0	4	16.0	6	16.0	5	17.5
		17	16.4	3	15.3	2	15.0	1	15.1*

D; design. E; experimental level (mean ± s.d.). N; number of larvae hatched.
* Replacement for missing values to calculate analysis of variance for cross classification.

depended significantly on temperature and salinity but insignificantly on their interaction (Table 6). The fitted second order polynomial was

$$Y = 24.74 - 3.00x_1 + 0.13x_2 + 0.25x_1^2 + 0.06x_2^2$$

Table 6. Analysis of variance for data in Table 5

Source	SS	DF	MS	F
Treatments	5,390.34	23	234.36	447.68**
Temperature	5,376.60	5	1,075.32	2,054.10**
x_1 linear	5,049.60	1	5,049.60	9,645.85**
x_1^2 quadratic	309.43	1	309.43	591.08**
Salinity	7.27	3	2.42	4.63**
x_1 linear	4.16	1	4.16	7.94**
x_2^2 quadratic	2.71	1	2.71	5.17**
Interaction	6.47	15	0.43	0.82^{NS}
Residual	10.47	20	0.52	
Total	5,400.81	43		

*significant ($p<0.05$). **highly significant ($p<0.01$). NS; not significant.

where Y = time to 50% hatching (hrs), and x_1 and x_2 = coded levels of temperature and salinity the same as the case of total hatching percent mentioned above. The time associated with the maximum hatching at 22.4°C and 34.2‰ was calculated to be 26. 8hrs (about 29 hrs after fertilization).

The response surface is illustrated in Fig. 2. The time to 50% hatching was inversely and exponentially related to temperature: the higher temperature, the shorter the time and the higher the density of isopleths. Generally, the relationship between the time to hatching (T) and temperature (θ) can be expressed by a linear equation: $\log T = a - b\theta$, where a and b are constants (HIGURASHI and TAUCHI 1925). In the case of the present data, the regression line at each salinity was not the simple but the two straight components, intersecting at or in the vicinity of 22°C (Table 7 and Fig. 3−H_{50}). The values of temperature characteristics such as Q_{10} and μ were higher at the lower than at the higher temperatures (Table 7). The temperature intersected was nearly equal to that at the time of spawning.

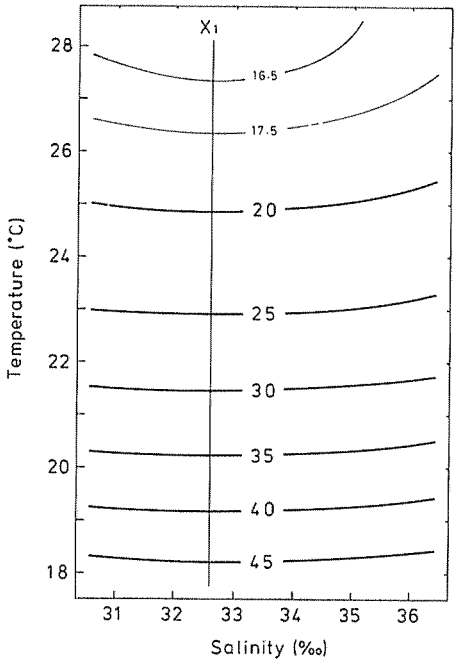

Fig. 2. Response surface showing the isopleths of time to 50% hatching (hrs) in relation to temperature and salinity. The X_1 − axis is the geometric axis of the surface.

Table 7. Values of constants and temperature characteristics for relationship between time to 50% hatching and temperature

Temperature	Below 22°C				Above 22°C			
Salinity (‰)	Constants*		Characteristics		Constants*		Characteristics	
	a	b	Q_{10}	μ	a	b	Q_{10}	μ
36.2	2.767	0.060	3.98	22,931	2.134	0.035	2.24	13,389
34.4	2.902	0.067	4.68	25,621	2.228	0.036	2.29	13,755
32.6	2.823	0.063	4.27	24,099	2.249	0.037	2.23	14,114
30.8	2.873	0.066	4.57	25,226	2.136	0.033	2.14	12,630

* log (time) = $a - b$ (temperature).

On the effects of salinity, the surface in Fig. 2 showed that the time to hatching was the shortest at 32.6‰ in all temperatures, and tended to lag behind gradually with both the increases and decreases in salinity. However, the changes in salinity obviously were of less influence than those in temperature.

Time of attainment of various developmental stages: The external features of normal embryonic development of the fish are shown in Plate I. While the findings in Plate I are of little difference from those in the reports by KAMIYA (1922), YASUDA *et al.* (1962) and MITO (1963) who have observed the same spcies, our findings offer a more detailed description of the development.

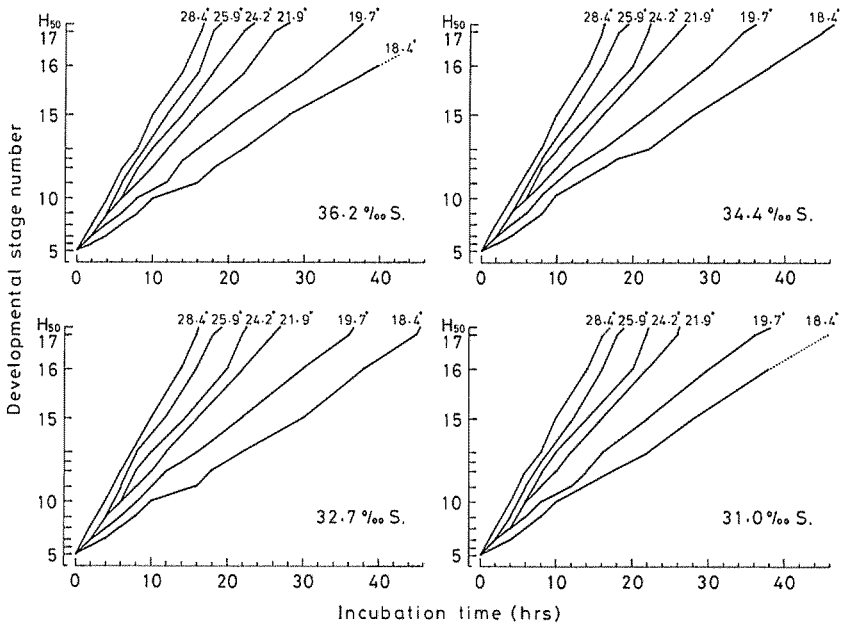

Fig. 3. Rate of development of threeline grunt eggs incubated at the 24 temperature-salinity combinations. The earliest time of attainment of each stage is plotted. The external features of each developmental stage number are shown in Plate I.

Time neccessary for attainment of various developmental stages at the 24 temperature-salinity combinations are displayed in Fig. 3. The regression lines for the relatioship between the time and temperature are illustrated in Fig. 4. In Figs. 3 and 4, the results of time to 50% hatching above-mentioned are also shown. All lines were essentially parallel with each other.

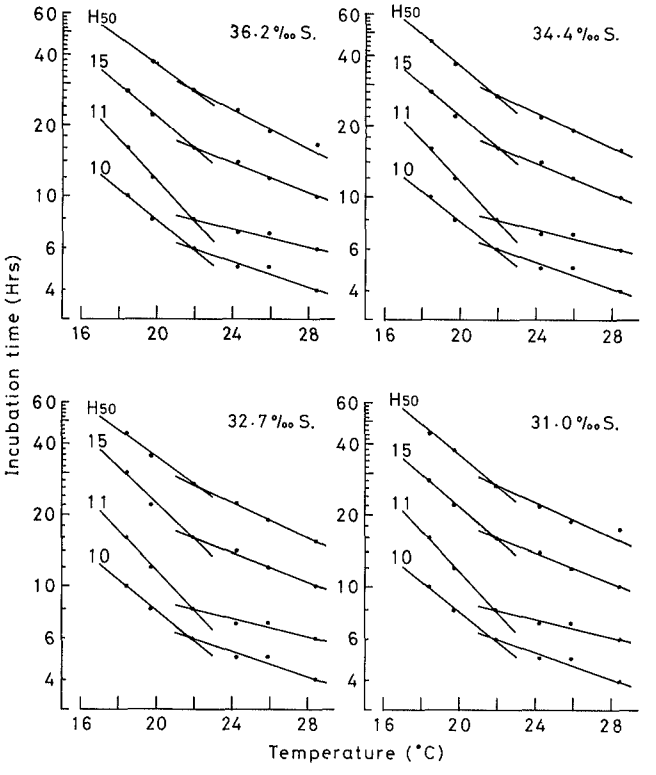

Fig. 4. Relationship between time of attainment of various developmental stages and temperature at each salinity. The stage number: 10; middle gastrula, 11; late gastrula, 15; pigments appearance, H_{50}; 50% hatching.

Discussion

Tolerance of teleost eggs to changing temperature and salinity varies widely with the species (KINNE 1963, 1964). In the present study, hatching of the threeline grunt eggs occurred at a temperature range of 18−28°C combined with a salinity range of 31.0−36.2‰. The isopleth of 90% hatching was bounded on temperatures of 19.5 and 25.4°C and on salinities of 32.1 and 36.3‰. These tolerable ranges are considerably narrower than those of red sea bream *Pagrus major* (APOSTOLOPOULOS 1976), which live in the same water region as the threeline grunt. On the basis of descriptions by KINNE (1963, 1964), it can be concluded that the threeline grunt eggs are stenothermal and stenohaline. The interaction between the two variables, that a high percent hatching

is maintained at the higher (or lower) temperatures only combined with higher (or lower) salinities, is equal to the "beneficial effect of low/low-high/high combination" of factors reviewed by KINNE (1964) and ALDERDICE (1972).

KAMIYA (1922), YASUDA *et al.* (1962) and MITO (1963) noted for the same species that the hours to hatching were 22.5, 28.5 and 38 hrs after fertilization when the eggs were incubated at temperatures of 24.8－27.5°, 21.8－22.3° and 17.2－20.3°C, respectively. Our results are well in accordance with their results at each comparable temperature. It is a matter of common knowledge, as reviewed by BLAXTER (1969), that the time to hatching of teleost eggs is inversely and exponentially related to the incubation temperature within the thermal tolerance limits of the species. The threeline grunt eggs also follow this relationship.

The form of the regression line for plots of *log* time against temperatures varies in kind with the fish species, and generally, the values of temperature characteristics such as Q_{10} and μ are higher at the lower than at the higher temperatures (BLAXTER 1969). The phenomenon that the two straight lines intersect at a certain temperature has been reported formerly on Atlantic mackerel *Scomber scombrus* (WORLEY 1933) and recently on bastard halibut *Paralichtys olivaceus* (YASUNAGA 1976). However, the cause or physiological mechanism of this phenomenon has yet to be clarified. Much depends upon future multilateral studies.

Changing salinities, both increasing and decreasing from 32.6‰, have the tendency to produce a progressive retardation of development of the threeline grunt. The same effect has been reported on English sole *Parophrys vetulus* (ALDERDICE and FORRESTER 1968), while some acceleration in high salinities has been done on Atlantic herring *Clupea harengus* (HOLLIDAY and BLAXTER 1960), Pacific cod *Gadus macrocephalus* (FORRESTER and ALDERDICE 1965, ALDERDICE and FORRESTER 1971b), petrale sole *Eopsetta jordani* (ALDERDICE and FORRESTER 1971a), the red sea bream (APOSTOLOPOULOS 1976) and yellowtail flounder *Limanda ferruginea* (LAURENCE and HOWELL 1981). On Pacific herring *Clupea pallasi,* no clear relation has been observed between time and salinity (ALDERDICE and VELSEN 1971). In any case, it is certain that the effects of salinity on the rate of development are small in comparison with those of temperature (BLAXTER 1969, HOLLIDAY 1969, ROTHENTHALL and ALDERDICE 1976). Further studies may be needed to determine the synergestic effects of salinity combined with other factors (*e. g.,* dissolved oxygen) as suggested by KINNE and KINNE (1962) and KINNE (1964).

On the time neccessay for attainment of various stages, our results showing that each regression line for the relationship between the time and temperature is essentially parallel to that of the time to hatching, agreed with those obtained on the Atlantic mackerel (WORLEY 1933), the bastard halibut (YASUNAGA 1975) and the yellowtail flounder (LAURENCE and HOWELL 1981), and strongly support WORLEY's suggestion that the rate of differentiation up to hatching is governed by one process throughout.

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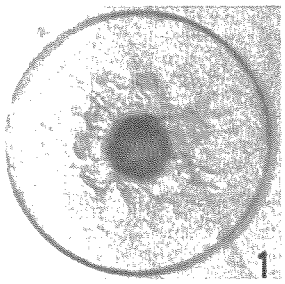
Explanation of Plate I

Embryonic development of threeline grunt eggs incubated at temperature of 23.3 ± 0.5°C and salininty of 33.4‰

Stage and Micrograph Number	Developmental stage	Time after fertilization
1.	Fertilized egg	
2.	2-cell	50 min
3.	4-cell	1 hr 10 min
4.	8-cell	1 hr 30 min
5.	16-cell	2 hrs
6.	Early morula	3 hrs
7.	Late morula	4 hrs 30 min
8.	Blastula	6 hrs 30 min
9.	Early gastrula	7 hrs 30 min
10.	Middle gastrula	8 hrs 30 min
11.	Late gastrula	10 hrs 30 min
12.	Embryonic body formation	11 hrs 30 min
13.	KUPFFER'S vesicle appearance	12 hrs 30 min
14.	Myotomes formation	13 hrs 30 min
15.	Pigments apperance	15 hrs 30 min
16.	Heart pulsation commencement	20 hrs 30 min
17.	Newly hatched larva*	24 hrs

* total length; 2.22 ± 0.07mm.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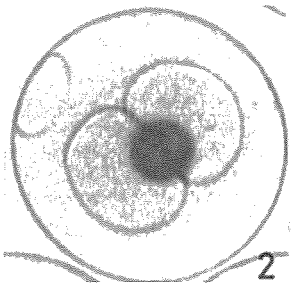
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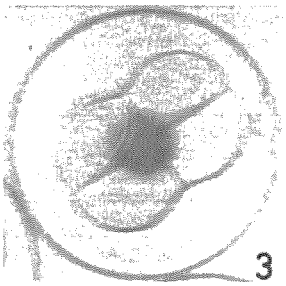
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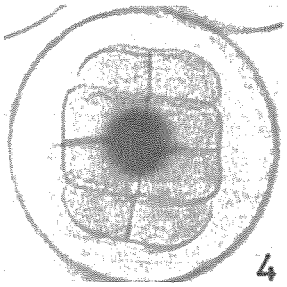
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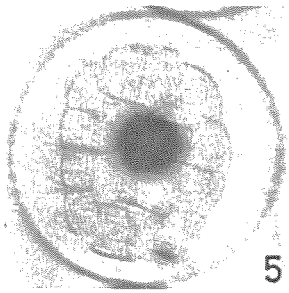
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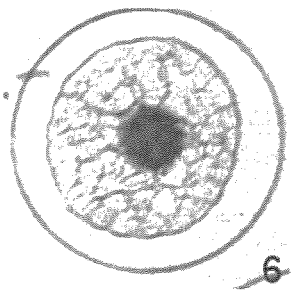
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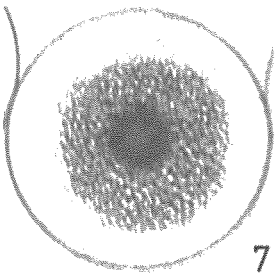
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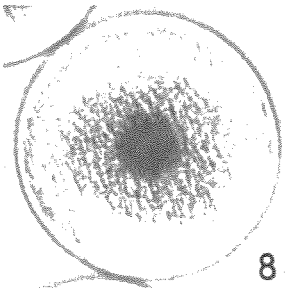
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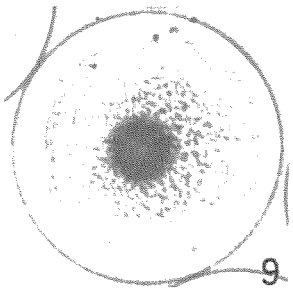
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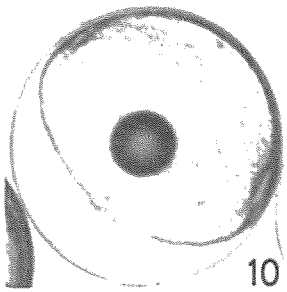
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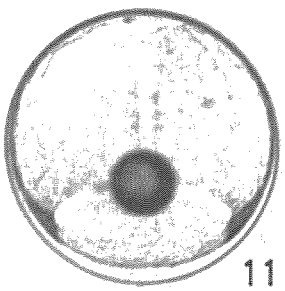
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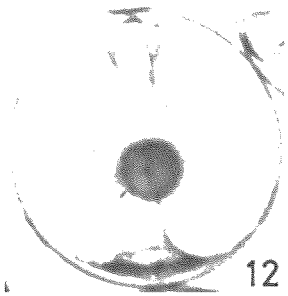
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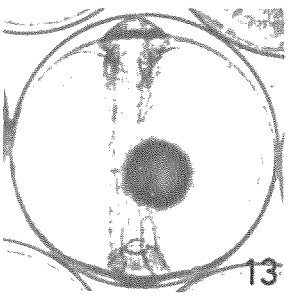
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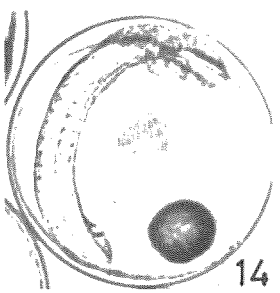
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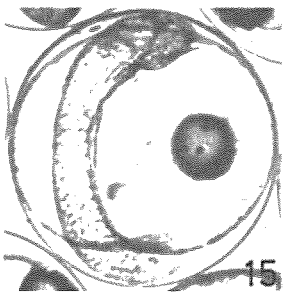
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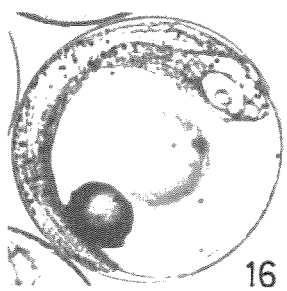
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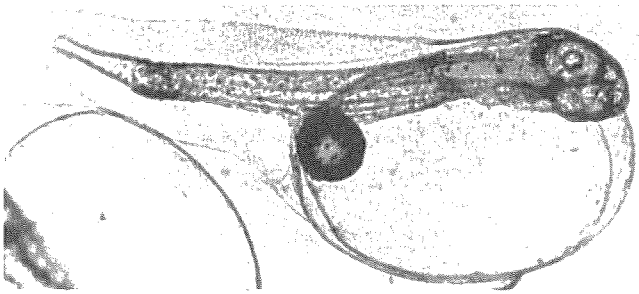
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