Abstract.— Eggs of walleye pollock, *Theragra chalcogramma*, from Shelikof Strait, Alaska, were reared at three temperatures: 3.8°, 5.7°, and 7.7°C. Development was divided into 21 stages. A piecewise regression model with midpoints of each stage describes the relation between time to each stage of development and temperature. Preserved eggs of each stage are described, illustrated, and photographed. Midpoint of hatch was 393 hours at 3.8°C, 303 hours at 5.7°C, and 234 hours at 7.7°C. Mean length of larvae at hatch increased linearly with temperature.

We compared rate of development, time to 50% hatch, and morphological development with other studies of walleye pollock eggs. Rate of development and time to 50% hatch were similar among populations of eastern North Pacific walleye pollock. Western North Pacific walleye pollock required longer incubation times than eastern North Pacific walleye pollock. Morphological development of Shelikof Strait eggs differs from development of western North Pacific walleye pollock eggs: optic vesicles, myomeres, eye lenses, heart, and otic capsules appear earlier than in Shelikof Strait eggs, and eye pigment appears later. The differences in development may be exacerbated by the condition of the eggs in which they were examined (e.g. preserved vs. live). Developmental differences between stocks are discussed with the conclusion that model components for egg mortality and spawning biomass must be based on specimens collected in the area of interest.

Embryonic development of walleye pollock, *Theragra chalcogramma*, from Shelikof Strait, Gulf of Alaska*

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Walleye pollock, *Theragra chalcogramma*, is the most abundant member of the family Gadidae in the subarctic Pacific Ocean and Bering Sea, supporting the largest single-species commercial fishery in the world (Megrey, 1991). In the Gulf of Alaska, Shelikof Strait is the principal spawning area (Kendall and Picquelle, 1990) and has been the site of intensive research to understand processes leading to recruitment variability of walleye pollock (Schumacher and Kendall, 1991).

Age determination of fertilized eggs is a basis for investigating biotic and abiotic impacts on the earliest life-history stage and thus for understanding interannual variability in walleye pollock recruitment. Age of walleye pollock eggs has been crucial to several studies. Egg mortality and spawning biomass are estimated by modeling age-specific egg abundance over time (Picquelle and Megrey, 1993; Bates[1]). Patterns in horizontal or vertical distribution and abundance of walleye pollock eggs in the western Gulf of Alaska have been described by grouping developmental stages into broad age groups (Kendall and Kim, 1989; Kendall and Picquelle, 1990).

Egg age is an independent variable in the models used to estimate egg production and mortality. Therefore, increasing the accuracy in measuring egg ages should improve estimates of these values. In past studies, walleye pollock eggs have been incubated in the laboratory to develop temperature-specific equations that estimate duration of development or age of the eggs, to describe morphological development, to observe effects of light on egg buoyancy and hatching rate, and to obtain larvae for experiments (Table 1). Although these incubation studies provide pertinent data on ontogeny of walleye pollock, none can be used with accuracy to determine age of eggs

[1] Bates, R. D. 1987. Estimation of egg production, spawner biomass, and egg mortality for walleye pollock, *Theragra chalcogramma*, in Shelikof Strait from ichthyoplankton surveys during 1981. U.S. Dep. Commer., NOAA, Nat. Mar. Fish. Serv., Northwest Alaska Fish. Cent., 7600 Sand Point Way N.E., Bin C15700, Bldg. 4, Seattle, WA 98115-0070. Proc. Rep. 87-20, 192 p.

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Table 1

Summary of *Theragra chalcogramma* egg incubation studies.

Reference	Temperature (°C)	Source and region	Stages[1]	Regression equation	Morphological description	Illustrations	Photographs
Gorbunova, 1954	3.4, 8.2 (means)	western Pacific Ocean	None	No	Yes	Yes	No
Yusa, 1954	6.0–7.0	Ishikari Bay, Japan	27[2]	No	Yes	Yes	Yes
Hamai et al., 1971	2.4–2.5, 6.5–6.7, 9.9–10.1, (means)	Funka Bay, Japan	4	Stage-specific equation to predict age (d) at any stage	No	No	No
Hamai et al., 1974	5.0 (mean)	Funka Bay, Japan	6	No	No	No	No
Matarese 1983, unpubl.[3]	5.0	N. Gulf of Alaska	21	Stage-specific equation to predict age (h) at any stage[4]	No	No	No
Haynes and Ignell, 1983	2.0, 5.0, 6.0, 8.0, 11.0	Stephens Passage, SE Alaska	7	General equation to predict age (h) at any stage	No	No	No
Nakatani and Maeda, 1984	−1.0, 0.0, 2.0, 4.0, 7.0, 10.0, 13.0	Funka Bay, Japan	5	To 50% hatch	No	No	No
Paul, 1984, unpubl.[5]	5.0	N. Gulf of Alaska	21	No	No	No	No
Bailey and Stehr, 1986	5.6, 8.5	Puget Sound, Washington	None	No	No	No	No
Olla and Davis, 1993	6.0	Shelikof Strait, Alaska	None	No	No	No	No

[1] Prior to hatch.
[2] Reported as intervals of time.
[3] A. C. Matarese, Alaska Fisheries Science Center, National Marine Fisheries Service, 7600 Sand Point Way NE., Seattle, WA 98115.
[4] In Bates 1987 (Footnote 1).
[5] A. J. Paul, University of Alaska Fairbanks, Institute of Marine Science, Seward Marine Center Lab, P.O. Box 730, Seward, AK 99664.

from Shelikof Strait. Eggs need to be obtained from the study area and incubated at a range of temperatures occurring in the area. Categorizing the continuous process of egg development into a large number of stages should increase the precision of the egg-age estimate.

The first objective of our study was to incubate Shelikof Strait walleye pollock eggs at the mean water temperature for Shelikof Strait, bracketing that temperature to include upper and lower extremes. Egg development times were used to produce a stage duration table and a regression model to estimate egg age based on water temperature and developmental stage. Morphological development is described for 21 developmental stages. These descriptions are accompanied by illustrations and photographs to facilitate identification of body structures and stage hallmarks.

The second objective was to compare our rates of egg development to other walleye pollock incubation studies. Morphological development is included in this comparison.

Methods

Incubation

Adult walleye pollock were collected with a rope trawl off Cape Kekurnoi (57°42.5'N, 155°16.2'W) in Shelikof Strait, Alaska, on 7 April 1989 from the NOAA research vessel *Miller Freeman*. Eggs from one female and milt from three or four males were hand stripped into glass petri dishes, gently mixed, and left undisturbed for one minute. Eggs were then rinsed, transferred to 3°C (surface water temperature) seawater in glass jars (3.8 L), and held two hours. Floating eggs with a perivitelline space were assumed to be fertilized (Blaxter, 1969; Alderdice, 1988). Viable eggs were poured into eighteen 0.5-L jars filled with 3°C seawater. Eggs were not counted but apportioned similarly among the jars at a concentration of about one egg/mL. Six capped jars were held in each of three water bath incubators onboard the *Miller Freeman*. Initial incubation temperatures were set to include the range of temperatures in the area. Mean water temperature at depths of 150–200 m in Shelikof Strait, where most eggs are found (March–May) (Kendall and Kim, 1989), is 5°C; extremes of 3.6° and 5.9°C have been reported (Reed and Schumacher, 1989). Incubators were sealed to minimize light and movement and placed in separate refrigerators adjusted to 3°, 5°, and 7°C. One-half of the water in the jars was replaced every day with seawater of the same temperature. Eggs were preserved in phosphate buffered formalin (5%)[2] or Stockard's solution[3] (Velsen, 1980). Stockard's solution cleared the chorion and darkened embryonic tissue, easing examination of embryonic development. Phosphate buffered formalin did not darken embryonic tissue as much as Stockard's solution, yielding better definition of some structures like somites and otic capsules. Live, newly hatched larvae were measured (standard length in millimeters) and preserved (5% buffered formalin). Detailed examination and morphological description of embryos were completed after eggs were returned to the laboratory.

During the first 24 hours after fertilization, eggs were sampled at 2–3 hour intervals. After 24 hours, intervals were increased to about 6 hours. When an interval was less or greater than 6 hours, the subsequent sampling time was adjusted to return to the original 6–hour schedule. Data were not recorded for three sampling times late in development because intervals were inadvertently extended to 12 hours (236, 258, and 282 hours).

At each interval, 10 to 50 eggs were sampled from one jar per incubator; only one jar was sampled to ensure there would be enough eggs and larvae left to sample near the end of the incubation period. Jars were sampled in rotation throughout the duration of the experiment until no eggs remained. When eggs began to hatch, all jars were checked and newly hatched larvae were removed in addition to eggs scheduled to be sampled. Dead eggs were removed from the designated sample jar at each interval. Water bath temperatures were recorded for every sampling interval. Frequent opening of refrigerators during the initial short sampling intervals increased temperatures in the refrigerators despite thermostat adjustments. Water bath temperatures stabilized after 48 hours to 3.8°, 5.7°, and 7.7°C.

Morphological descriptions

Eggs were examined with the aid of a dissecting microscope (6–50× magnification) and described according to a 21-stage scheme adapted from Naplin and Obenchain (1980) (Table 2). Morphological terms follow Trinkaus (1951) with one exception: the term "blastodisc," in this paper, includes the germinal area from the time of cytoplasm polarization until embryonic shield formation (Markle and

Table 2

Stages of embryonic development of *Theragra chalcogramma* (adapted from Naplin and Obenchain, 1980).

Stage	Developmental stage
1	Precell
2	2 cell
3	4 cell
4	8 cell
5	16 cell
6	32+ cell
7	Blastodermal cap
8	Early germ ring
9	Germ ring 1/4 down yolk
10	Germ ring 1/2 down yolk
11	Germ ring 3/4 down yolk
12	Late germ ring
13	Early middle (blastopore closure)
14	Middle middle (appearance of pigment)
15	Late middle (tail bud thickens)
16	Early late (tail bud lifts from yolk)
17	Tail 5/8 around yolk
18	Tail 3/4 around yolk
19	Tail 7/8 around yolk
20	Full circle around yolk
21	Tail 1-1/8 around yolk

[2] 50 mL 37% formaldehyde, 4.0 g sodium phosphate monobasic, 6.5 g sodium phosphate dibasic, made up to 1 L with distilled water.

[3] 50 mL 37% formaldehyde, 40 mL glacial acetic acid, 60 mL glycerin, and 850 mL distilled water.

Waiwood, 1985, in part). Eggs preserved in Stockard's solution were photographed with a Nikon F2 camera fitted with a PB6 200-mm bellows extension and a 24-mm 1:2.8 reverse-mounted lens. This configuration produced a 47× magnification. Reflected light was supplied by two synchronized flash units. Other photographs (stages 5 and 6) were taken with a single-lens reflex adapter (0.32×) on a Wild M-8 dissecting microscope with transmitted light. At 50×, the phototube and adapter increased magnification to 66×.

Analysis

Endpoint, midpoint, and duration of stage (in hours) were estimated for eggs incubated at each temperature. For stages 1–20, stage endpoint was determined by the presence of two stages during a sampling time; if stages n and n+1 were present, the time at which the eggs were sampled was considered a transition and therefore the endpoint for stage n. If there was no transition, the endpoint for stage n was the midpoint between the last sampling time during which stage-n eggs were present and the first time stage-n+1 eggs were observed. Duration and midpoint of stage n were determined as

$$\text{Duration Stage } n = \text{Endpoint Stage } (n) - \text{Endpoint Stage } (n-1);$$

$$\text{Midpoint Stage } n = \text{Endpoint Stage } (n-1) + \frac{\text{Duration Stage } n}{2}.$$

Endpoint of stage 21 was the sampling interval when the last embryo had hatched. With the midpoints and time of 50% hatch, a piece-wise least-squares linear regression model (SAS, 1985) was derived to estimate age (hours) of eggs at a specific stage incubated at any temperature within the limits of this experiment.

Differences in mean lengths of larvae hatched from the three temperature groups were analyzed by a Student-Newman-Keuls test. Lengths of larvae hatching at stages 20 and 21 were analyzed by a two-way analysis of variance (ANOVA) by using stage and temperature.

We chose five representative developmental stages and compared time to midpoint of each stage among incubation studies. Comparison with Hamai et al. (1971, 1974) was possible for only three stages. We grouped data on time to 50% hatch into western and eastern North Pacific studies and performed a log-transformed analysis of covariance to test for differences in time to 50% hatch between these two areas with incubation temperature as the covariate.

Results

Incubation rates

Temperatures of the three water baths increased at the beginning of sampling (Fig. 1). Temperature spikes that occurred after 288 hours in the 5.7°C jars and after 396 hours in the 3.8°C jars, were associated with the appearance of large numbers of larvae; water baths may have warmed when refrigerators were opened frequently to measure larvae.

Eggs developed at similar rates among incubation temperatures for the first 36 hours through stage 6 (Fig. 2). After stage 6, at about 36 hours, when temperatures had stabilized, development rates began to diverge. Duration of stages 7–21 was variable (Table 3). Usually the duration of a stage was longer at cooler temperatures. However, this was not always the case, and stages 12 and 20 required similar amounts of time regardless of temperature. At all temperatures, hatching began during stage 20; the percentage of eggs hatched by the beginning of stage 21 was 35% at 3.8°, 40% at 5.7°, and 8.1% at 7.7°C. Four larvae from the 7.7°C group hatched after 192 hours; another 18 hours elapsed before other larvae hatched at this temperature. These early larvae were not included in this analysis because we assumed that the hiatus in hatching times indicated that early hatching was anomalous, i.e. hatching may have been mechanically induced. After hatching began, time required for 50% hatch decreased as temperature increased: 48, 36, and 24 hours at 3.8°, 5.7°, and 7.7°C. The elapsed time between hatching of the first and last larvae was 72 hours at 3.8°C and 60 hours at both 5.7° and 7.7°C.

Eggs developed normally at 5.7° and 7.7°C; however, curvature of the spine was observed in some late-stage embryos incubated at 3.8°C. These abnormal eggs hatched, but most larvae were not measured because of curvature. Mean length at hatch of all larvae increased with incubation temperature: 4.15 (SD 0.380, n=100), 4.29 (SD 0.272, n=192), and 4.55 mm (SD 0.303, n=84) at 3.8°, 5.7°, and 7.7°C (Fig. 3). Mean lengths of larvae from the three temperature groups were significantly different (P<0.05). In addition, larval lengths increased as the hatching period progressed at all temperatures. Length of larvae hatching at stages 20 and 21 was significantly different at all temperatures (P<0.01); larvae that hatched at stage 21 were 9–13% longer than larvae that hatched at stage 20.

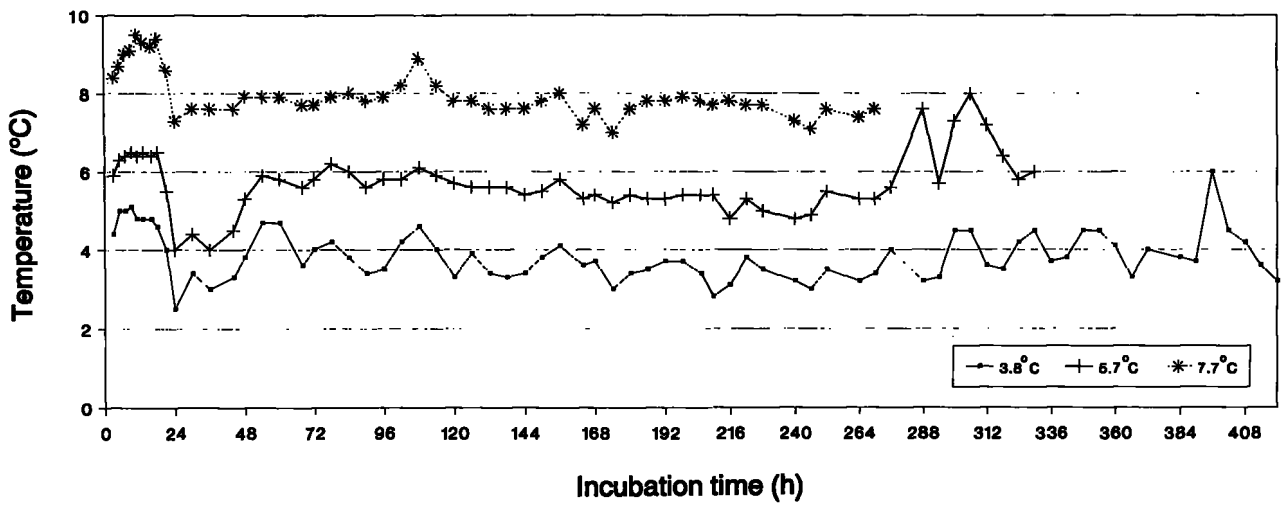

Figure 1

Temperatures recorded in water baths during incubation of *Theragra chalcogramma* eggs.

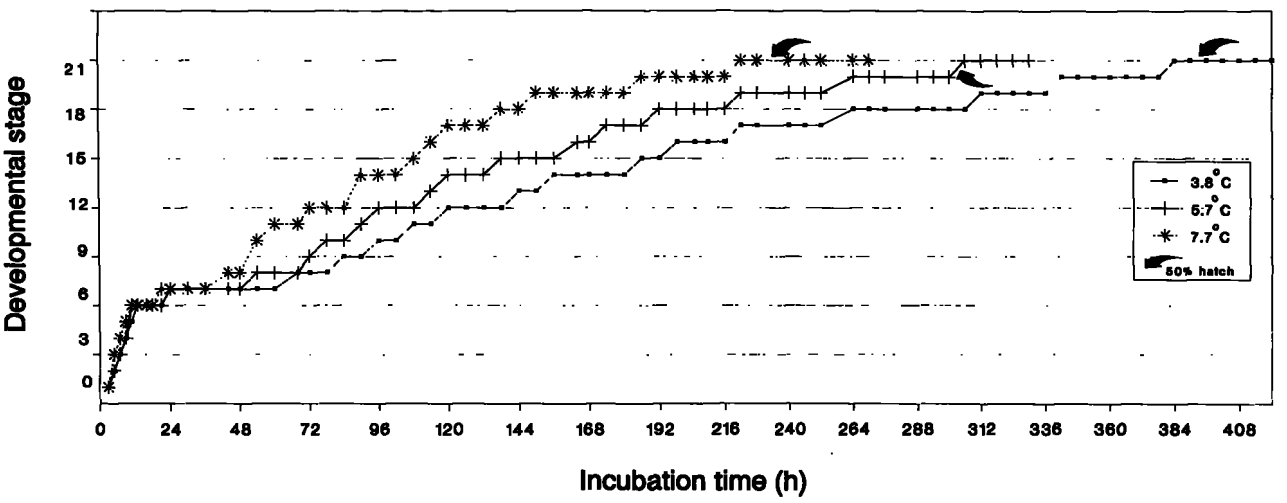

Figure 2

Development of *Theragra chalcogramma* eggs incubated at 3.8°, 5.7°, and 7.7°C. Points represent occurrence of stages at scheduled sampling times.

The piece-wise regression model (SAS, 1985) has two separate components and is discontinuous between stages 6 and 7 (Fig. 4). This type of model was necessary because of the rapid divergence of developmental rates at all temperatures after stage 6; it was not possible to fit one equation to the entire incubation time. The two components are described by the following equations:

component 1: stages 1–6

$$\text{Age} = 3.27 - 0.13\,(\text{stage})\,(\text{temperature}) + 0.47\,(\text{stage}^2);$$

component 2: stages 7–21

$$\text{Age} = 17.82 + 7.05(\text{stage}) - 0.656\,(\text{stage})\,(\text{temperature}) + 0.043(\text{stage}^3) - 0.0032\,(\text{temperature})\,(\text{stage}^3),$$

Table 3

Endpoint, midpoint, and duration in hours (h) of stage of development of *Theragra chalcogramma* eggs incubated at 3.8°, 5.7°, and 7.7°C.

	3.8°C			5.7°C			7.7°C		
Stage	Endpoint (h)	Midpoint (h)	Duration (h)	Endpoint (h)	Midpoint (h)	Duration (h)	Endpoint (h)	Midpoint (h)	Duration (h)
1	4.00	2.00	4.00	4.00	2.00	4.00	3.50	1.75	3.50
2	6.00	5.00	2.00	6.00	5.00	2.00	4.00	3.75	0.50
3	8.00	7.00	2.00	7.00	6.50	1.00	5.00	4.50	1.00
4	10.25	9.12	2.25	9.00	8.00	2.00	7.00	6.00	2.00
5	12.50	11.37	2.25	10.25	9.62	1.25	10.25	8.62	3.25
6	22.50	17.50	10.00	22.50	16.37	12.25	19.50	14.87	9.25
7	64.00	43.25	41.50	51.00	36.75	28.50	40.00	29.75	20.50
8	78.00	71.00	14.00	68.00	59.50	17.00	48.00	44.00	8.00
9	90.00	84.00	12.00	75.00	71.50	7.00	54.00	51.00	6.00
10	105.00	97.50	15.00	87.00	81.00	12.00	57.00	55.50	3.00
11	120.00	112.50	15.00	93.00	90.00	6.00	68.00	62.50	11.00
12	138.00	129.00	18.00	108.00	100.50	15.00	84.00	76.00	16.00
13	153.00	145.50	15.00	114.00	111.00	6.00	87.00	85.50	3.00
14	180.00	166.50	27.00	135.00	124.50	21.00	102.00	94.50	15.00
15	195.00	187.50	15.00	164.00	149.50	29.00	111.00	106.50	9.00
16	219.00	207.00	24.00	174.00	169.00	10.00	117.00	114.00	6.00
17	252.00	235.50	33.00	189.00	181.50	15.00	132.00	124.50	15.00
18	312.00	282.00	60.00	219.00	204.00	30.00	144.00	138.00	12.00
19	336.00	324.00	24.00	258.00	238.50	39.00	180.00	162.00	36.00
20	378.00	357.00	42.00	300.00	279.00	42.00	219.00	199.50	39.00
21	414.00	393.00[1]	36.00	330.00	303.00[1]	30.00	270.00	234.00[1]	51.00

[1] 50% hatch.

where age of the egg is expressed in hours. The value of R^2 is 0.96 for component 1 and 0.99 for component 2.

We compared our rates of egg development to other walleye pollock incubation studies in the 5–7°C range (Table 4). There was a significant difference between regression equations of incubation time to 50% hatch and temperature for western versus eastern North Pacific studies ($P<0.01$), but the slopes were not different ($P=0.18$). Based on the 95% confidence interval about the parameter estimates, time to 50% hatch of western North Pacific walleye pollock tended to be 1.2 to 1.3 times longer on average than that of the eastern North Pacific fish at a specific temperature.

Morphological descriptions

Walleye pollock eggs are pelagic and have a smooth, clear chorion and homogeneous yolk. No oil globules are present. Preserved eggs range from 1.2 to 1.8 mm in diameter, although most are 1.35–1.45 mm (Matarese et al., 1989). Appearance of the egg varies with type of preservative. There was little or no shrinkage of yolk material in Stockard's solution, whereas yolk of formalin-preserved eggs decreased in volume and the yolk membrane frequently collapsed. This effect of formalin preservation was helpful in determining how much of the tail had lifted away from the yolk in late-stage embryos.

Development of walleye pollock eggs and embryos, from fertilization to just before hatching, was divided into the following 21 stages (Table 2):

Precell (stage 1) Cytoplasm at the animal pole forms a blastodisc; bands of cytoplasm extend from below the equator to the blastodisc (Fig. 5A), which is without distinct margins (Fig. 6A). When intact, the yolk membrane almost touches the inner wall of the chorion. The perivitelline space is most visible over the blastodisc.

2 cells (stage 2) The first cell division of the blastodisc is in the horizontal plane. Cell material may not be equally divided (Figs. 5B and 6B).

4 cells (stage 3) The second cleavage is perpendicular to, and in the same plane as, the first. Cells are roughly equal in size and form a square (Figs. 5C and 6C).

8 cells (stage 4) The third cleavage is perpendicular to the second cleavage (parallel to first cleavage). Each cell divides in half in the horizontal

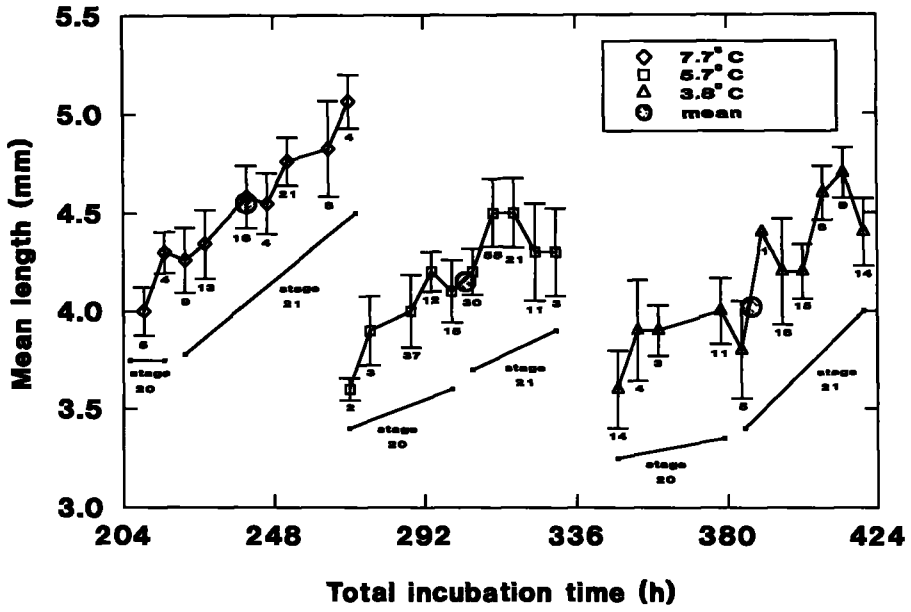

Figure 3

Mean hatch lengths at each sampling interval during the hatching period of *Theragra chalcogramma* larvae incubated at 3.8°, 5.7°, and 7.7°C. Stage of development at hatch is also shown. Shaded circle indicates overall mean length for each temperature. Vertical bars are standard deviations; numbers indicate sample size.

plane. Cells form a rectangle with the four cells in the center smaller than those at the corners of the rectangle (Figs. 5D and 6D).

16 cells (stage 5) The fourth cleavage is perpendicular to the third; this is the last stage in which cell division is restricted to the horizontal plane. Most eggs have a square or rectangular block of cells with four cells on each side; all cells are in contact with yolk through this stage (Figs. 5E and 6E).

32 cells (stage 6) Initially, the single layer of cells has a flat, irregular square or rectangular shape. Cell division continues in horizontal and vertical planes, transforming the blastodisc into a hollow cap of cells on the yolk resembling a raspberry (Figs. 5F and 6F). Cells increase in number but the size of the blastodisc remains constant. The perivitelline space widens between yolk and chorion.

Blastodermal cap (stage 7) The blastodisc progresses through two steps: at first, cell size decreases from continued cleavage; cell material appears granular and the blastodisc resembles a flattened dome on the yolk surface. Then, the base of the cell mass sinks below the yolk surface; the periblast extends beyond the equator of the blastodisc, giving the appearance of a "flying saucer" in lateral view (Figs. 5G and 6G).

Early germ ring (stage 8) The center of the blastodisc flattens and the periphery (germ ring) thickens in preparation to overgrow the yolk (epiboly). The blastocoel, visible on one side of the blastodisc, appears grainy and pale (Fig. 5H). The margin between blastocoel and blastodisc is indistinct (Fig. 6H).

Germ ring 1/4 around yolk (stage 9) The blastodisc, now the embryonic shield, expands as the germ ring begins to overgrow the yolk. The margin of the future anterior end of the embryo is slightly curved and sharply defined. Cell material covering the blastocoel appears less grainy than in the previous stage. After preservation, this thin cellular layer appears concave in lateral view. The germ ring margin is thin and flattened, extending 1/4 around yolk (Figs. 5I and 6I).

Germ ring 1/2 around yolk (stage 10) The germ ring envelopes half the yolk and the anterior margin of the embryonic shield is sharply curved and thick (Figs. 5J and 6J). The beginning of neural development is visible; a neural keel extends from the anterior margin of the embryonic shield to 2/3 its length (Fig. 5K).

Germ ring 3/4 around yolk (stage 11) Head and upper body region begin to differentiate but no distinct brain lobes are apparent. Optic vesicles develop. Prospective head and body mesoderm outlines the hour-glass shape of the developing embryo (Fig. 7A). The notochord is visible ventrally. The germ ring has progressed 3/4 down the yolk (Fig. 6K).

Late germ ring (stage 12) Myomere differentiation begins; separate myomeres are not visible. The midbody expands dorsoventrally; prospective head and body mesoderm forms a narrow outline of the embryo. The blastopore is open and the germ ring envelopes more than 7/8 of the yolk (Figs. 7B and 8A).

Early middle stage (stage 13) The blastopore is closed. The notochord and 7–12 incomplete myomeres are visible. Tail margin is indistinct and

flat; the medial portion of the tail bud is thicker (Figs. 7C and 8B). The body of the embryo appears flattened. Although not distinguishable in preserved specimens, Kupffer's vesicle is visible in the live egg.

Middle middle stage (stage 14) Embryos have 14–16 myomeres. Differentiation begins in eyes and mid- and hindbrain. Forebrain very small and underdeveloped. The tail bud margin is defined but still flattened (Fig. 8C). The entire length of the body is thicker. Small melanophores are scattered along the dorsum between the hindbrain and 4/5 of body length (Fig. 7D).

Late middle stage (stage 15) About 20–25 myomeres are visible. Eye lenses are formed. The liver appears as a slight bulge in the body wall, and the gut area is delineated. The tail bud is thick and appears lifted from the yolk surface with the margin attached (Fig. 7E). Pigment is darker than in the previous stage and dendritic, extending from midbrain to tip of tail bud and confined mostly to the dorsum. Nares, mid- and hindbrain, and pectoral bud anlagen are visible dorsally (Fig. 8D).

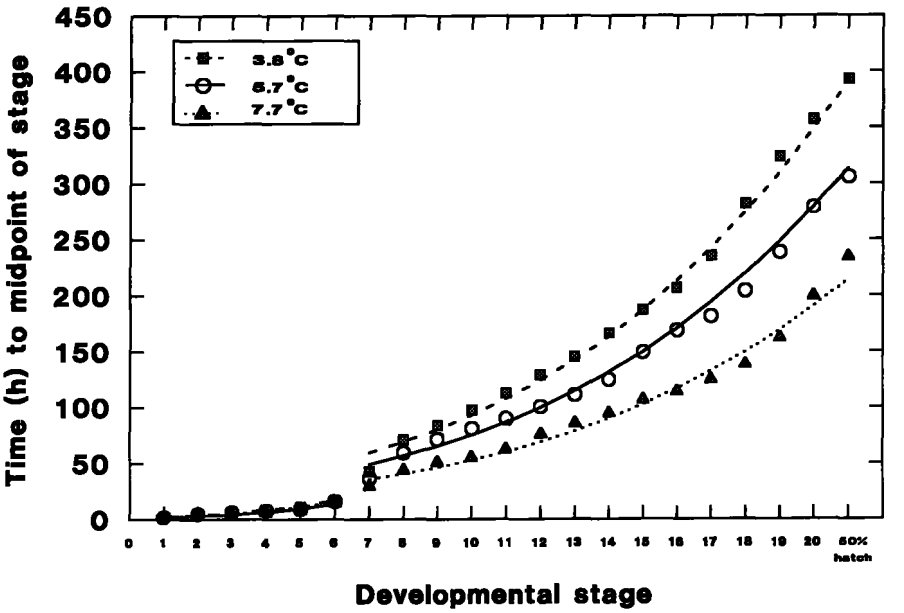

Figure 4

Time (h) to midpoint of stage of *Theragra chalcogramma* eggs incubated at 3.8°, 5.7°, and 7.7°C. Fitted lines are results of regression model; symbols are observed values.

Early late stage (stage 16) Heart tissue begins to expand when the embryo has about 24–36 myomeres. Forebrain differentiates from midbrain. The tail bud lifts from the yolk surface (Figs. 7F and 8E) and pigment forms two parallel rows dorsoposteriorly.

Tail 5/8 around yolk (stage 17) The embryo has 27–36 myomeres. More of the tail lifts from the

Table 4

Comparison of time (h) to estimated midpoint of five developmental milestones of *Theragra chalcogramma* embryos incubated at 5–7°C.

	Eastern North Pacific incubation studies				Western North Pacific incubation studies			
Stage	Matarese, 1983, unpubl.[1] (5.0°C)	Paul, 1984, unpubl.[2] (5.0°C)	Haynes and Ignell, 1983[3] (6.0°C)	This study (5.7°C)	Hamai et al., 1971[4] (6.5–6.7°C)	Yusa, 1954 (6.0–7.0°C)	Nakatani and Maeda, 1984[5] (7.0°C)	Hamai et al., 1974 (5.0°C)
Blastodermal cap	37.5	39.5	35	36.8		28.5	31	
Blastopore closure	114	118	105	108	100	102	148	139
Tail 3/4	211.7	217	161	204		234	192	
Tail full circle	274.5	264	250	279	250	270	216	330
50% hatch	349	320	285	303	345	288+	298	411

[1] A. C. Matarese, Alaska Fisheries Science Center, National Marine Fisheries Service, 7600 Sand Point Way N.E., Seattle, WA 98115.
[2] A. J. Paul, University of Alaska Fairbanks, Institue of Marine Science, Seward Marine Center Lab, P.O. Box 730, Seward, AK 99664.
[3] Values except for hatch estimated from Table 3 in Haynes and Ignell (1983). 50% hatch from Table 7.
[4] Values except for hatch estimated from Fig. 3 in Hamai et al. (1971).
[5] Values except for hatch estimated from Fig. 5 in Nakatani and Maeda (1984).

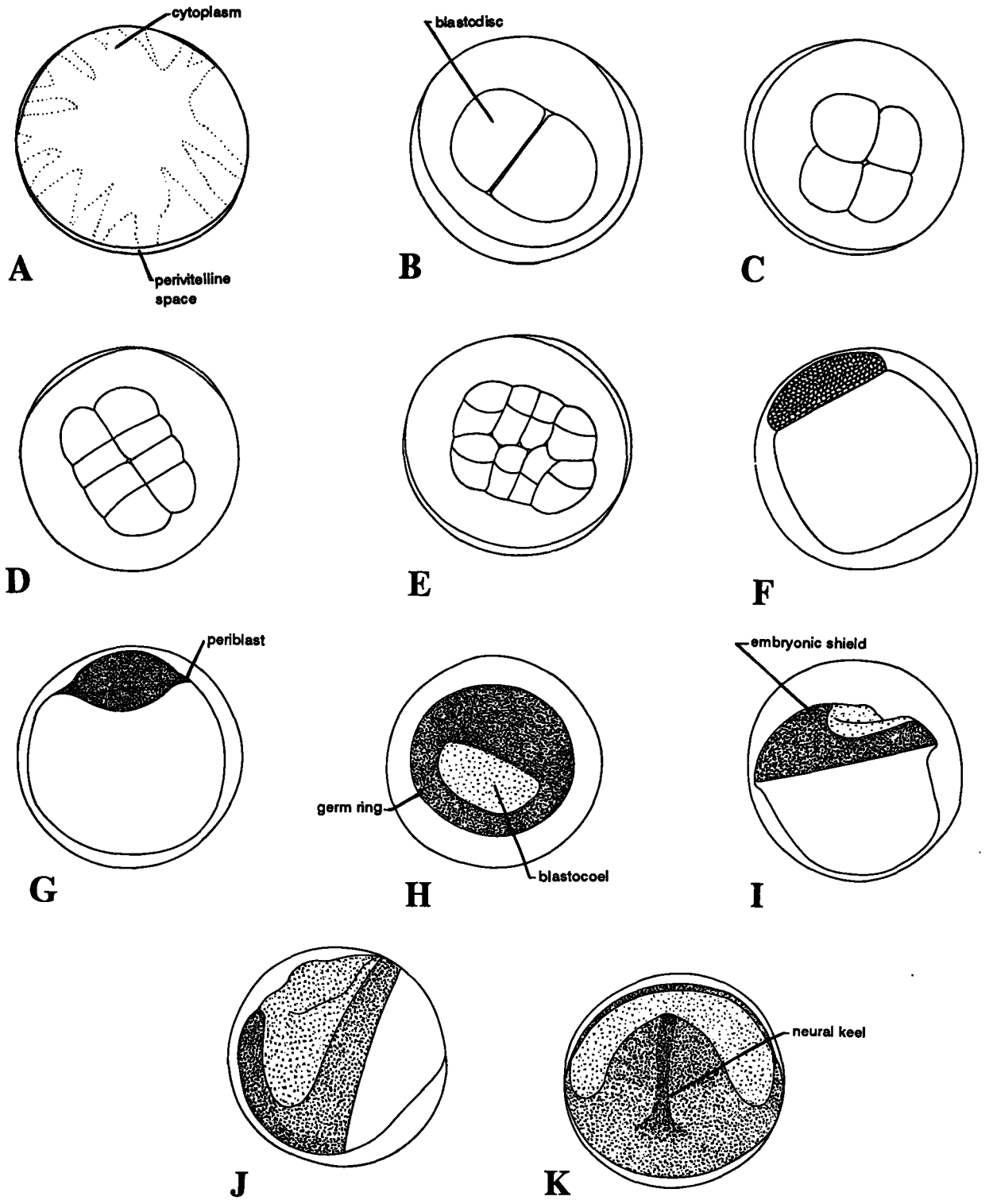

Figure 5

Illustrations of preserved *Theragra chalcogramma* eggs. (**A**) Stage 1 (precell); (**B**) Stage 2 (2 cell); (**C**) Stage 3 (4 cell); (**D**) Stage 4 (8 cell); (**E**) Stage 5 (16 cell); (**F**) Stage 6 (32 cell); (**G**) Stage 7 (blastodermal cap); (**H**) Stage 8 (early germ ring); (**I**) Stage 9 (germ ring 1/4, lateral view); (**J**) Stage 10 (germ ring 1/2, lateral view); (**K**) Stage 10 (dorsal view).

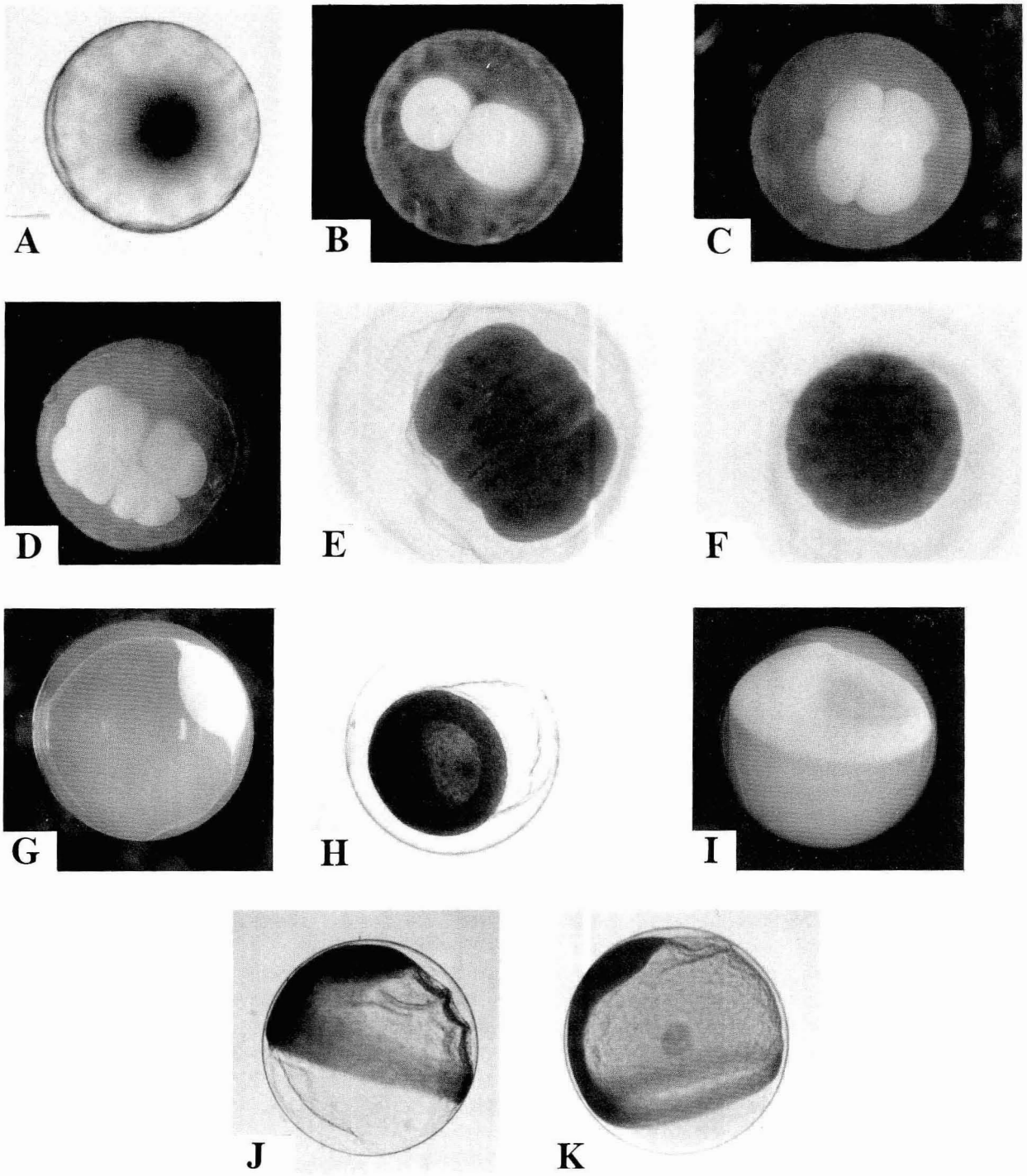

Figure 6

Photographs of preserved *Theragra chalcogramma* eggs. (**A**) Stage 1 (precell); (**B**) Stage 2 (2 cell); (**C**) Stage 3 (4 cell); (**D**) Stage 4 (8 cell); (**E**) Stage 5 (16 cell); (**F**) Stage 6 (32 cell); (**G**) Stage 7 (blastodermal cap); (**H**) Stage 8 (early germ ring); (**I**) Stage 9 (germ ring 1/4); (**J**) Stage 10 (germ ring 1/2); (**K**) Stage 11 (germ ring 3/4).

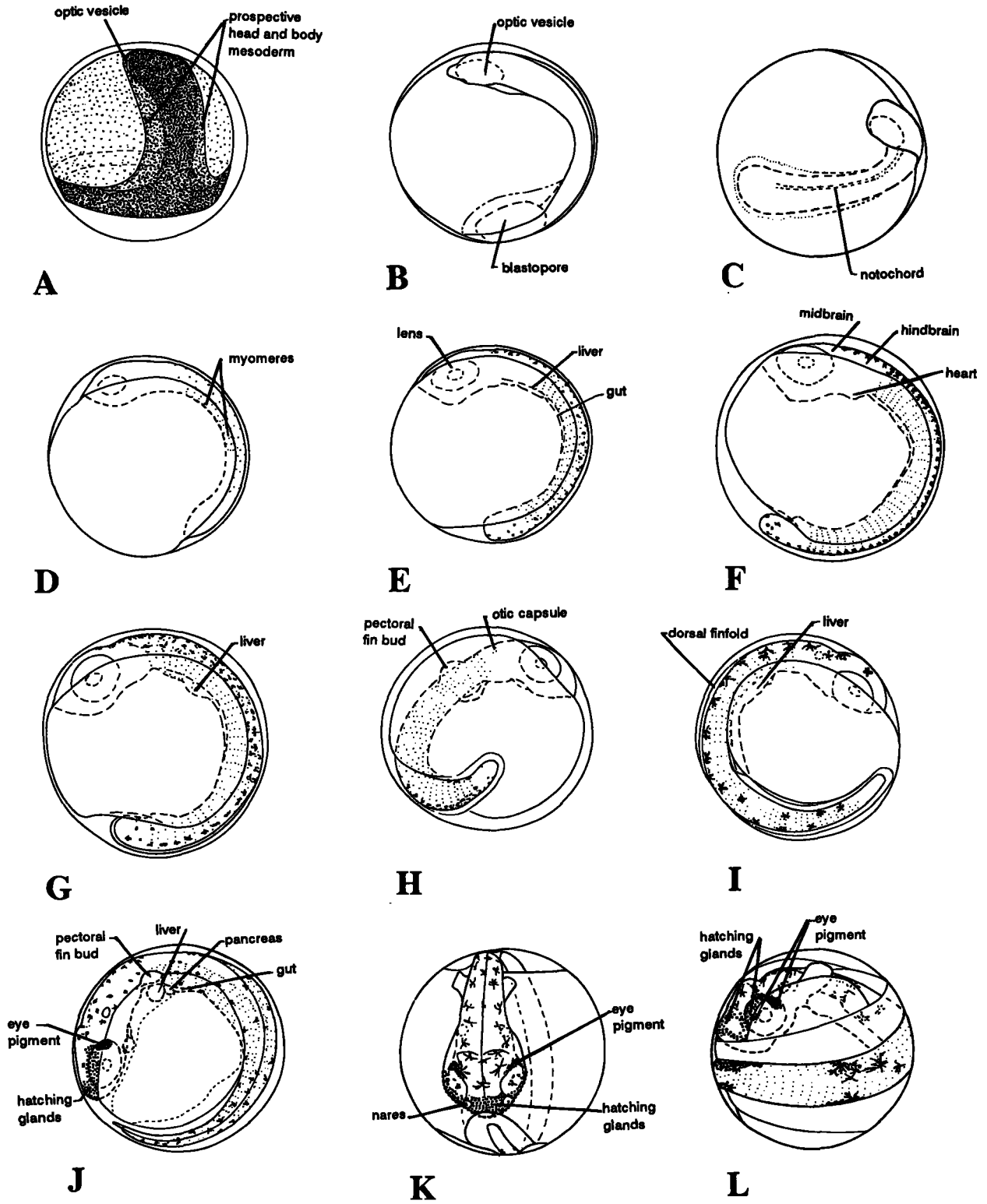

Figure 7

Illustrations of preserved *Theragra chalcogramma* eggs. (**A**) Stage 11 (germ ring 3/4); (**B**) Stage 12 (blastopore almost closed); (**C**) Stage 13 (early middle); (**D**) Stage 14 (middle middle); (**E**) Stage 15 (late middle); (**F**) Stage 16 (early late); (**G**) Stage 17 (tail 5/8 circle); (**H**) Stage 18 (tail 3/4 circle); (**I**) Stage 19 (tail 7/8 circle); (**J**) Stage 20 (tail full circle, lateral view); (**K**) Stage 20 (dorsal view); (**L**) Stage 21 (tail 1-1/8 circle).

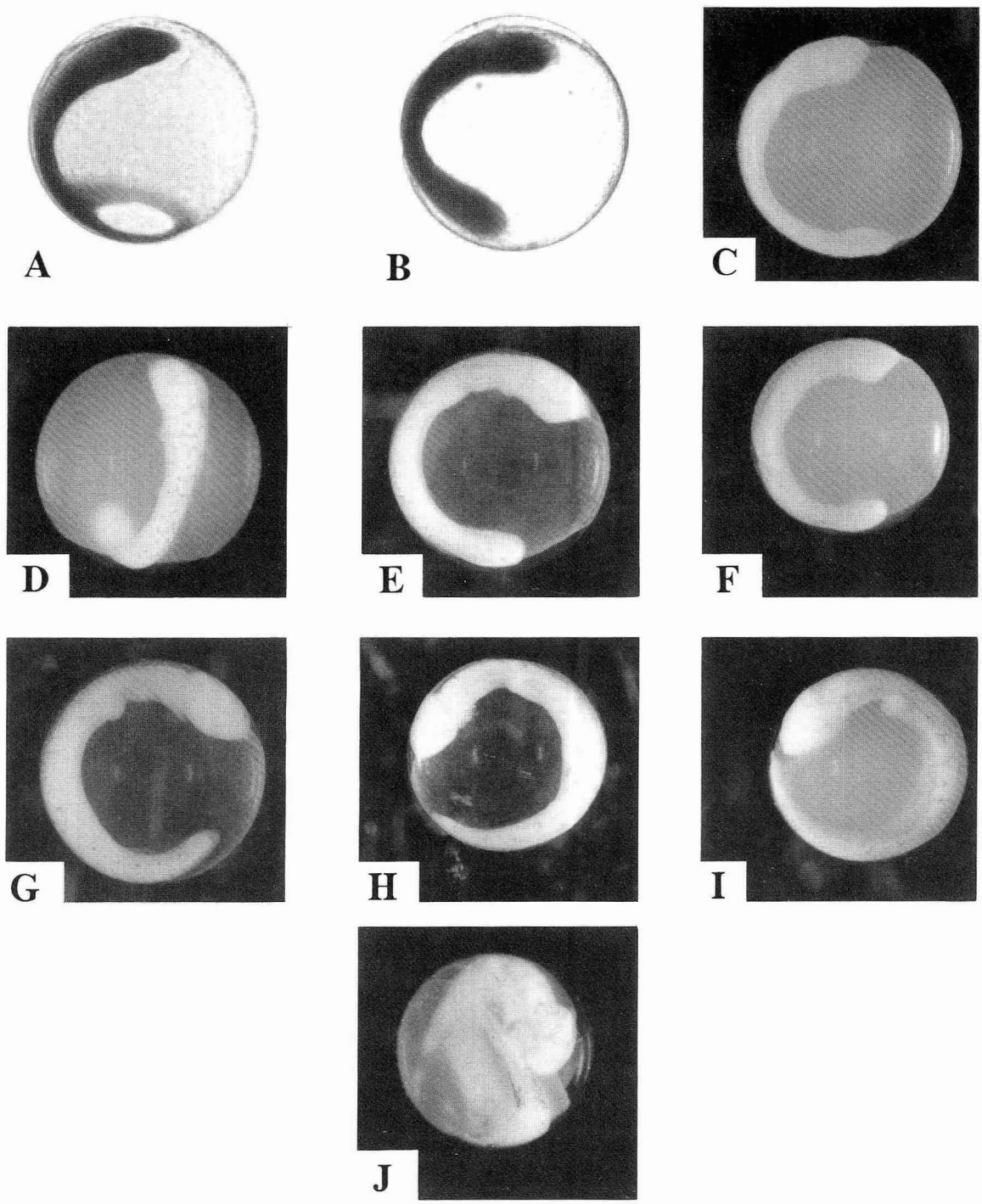

Figure 8

Photographs of preserved *Theragra chalcogramma* eggs. (**A**) Stage 12 (blastopore almost closed); (**B**) Stage 13 (early middle); (**C**) Stage 14 (middle middle); (**D**) Stage 15 (late middle); (**E**) Stage 16 (early late); (**F**) Stage 17 (tail 5/8 circle); (**G**) Stage 18 (tail 3/4 circle); (**H**) Stage 19 (tail 7/8 circle); (**I**) Stage 20 (tail full circle); (**J**) Stage 21 (tail 1-1/8 circle).

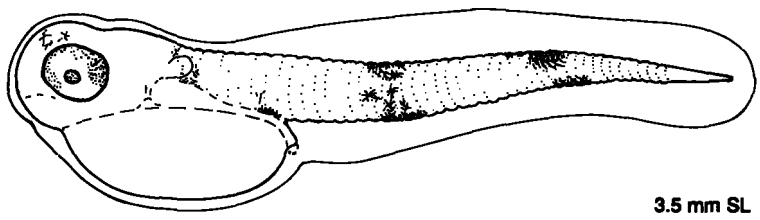

Figure 9
Illustration of preserved *Theragra chalcogramma* yolk-sac larva (Matarese et al., 1989).

yolk surface (Fig. 8F). The dorsal finfold is formed on the posterior 1/3 of the body and pigment on the head extends at least to the posterior margin of the eye (Fig. 7G). The liver is prominent and the heart is beating in the live egg.

Tail 3/4 around yolk (stage 18) The embryo has 36–41 myomeres. The tip of the tail is tapered and curves away from the longitudinal axis of the embryo (Fig. 7H). The dorsal finfold extends to midbody and pectoral fin buds are prominent. Otic capsules are formed. Large stellate melanophores are scattered over the dorsum, extending just to the midlateral surface; posterior to the anus, two rows of melanophores are seen dorsally and a few are found along the ventral midline (Fig. 8G). The tip of the tail is unpigmented.

Tail 7/8 around yolk (stage 19) When the embryo has 44–48 myomeres, the dorsal finfold extends anteriorly 2/3 body length, inserting just posterior to the pectoral fin buds and centered over the liver (Figs. 7I and 8H). Pigment on the head extends to the middle of the eye. At midbody, pigment is scattered on either side of the dorsal midline, extending to just above the lateral midline. Postanal pigment migrates toward the dorsal and ventral midlines.

Tail full circle around yolk (stage 20) The embryo has 48–49 myomeres and the pancreas is visible adjacent to the liver (Fig. 7J). The embryo now encircles the yolk and the tail tip may reach from near the snout to as far back as the posterior margin of the eye (Fig. 8I). Hatching glands, similar to those of other teleosts (Yamagami, 1988), are discernible on the surface of the snout and may extend over the dorsal surface of the eye (Figs. 7J and 7K). The posterior portion of the eye is pigmented. Postanal pigment migrates and begins to form the postanal bars found in yolk-sac larvae (Matarese et al., 1989) (Fig. 9).

Tail 1 1/8 times around yolk (stage 21) The embryo has 49–50 myomeres and the tail tip elongates, extending beyond the posterior margin of the eye (Fig. 8J). The urinary bladder is visible posterior to the anus (1/3 body length; not shown on figure) and the dorsal finfold extends to midbrain. Head pigment extends to the anterior margin of the eye (Fig. 7L). The dorsal half of the eye is pigmented. Most body pigment coalesces to three areas: dorsally, on gut; a bar at 1/2 body length; and a bar at 3/4 body length. In the postanal bars, most pigment is along dorsal and ventral midlines; some pigment extends onto the lateral body. Pigment is scattered on the preanal body.

Discussion

Time from first hatch to 50% hatch was inversely related to temperature. Hatch times reflected the effects of temperature described by Yamagami (1988), who demonstrated that the hatching enzyme secreted by the embryo solubilizes the chorion more rapidly at higher temperatures. The first larvae to hatch were stage 20. Early hatching may have been an artifact of rearing conditions. However, hatching glands were present at this stage, which, with the appearance of eye pigment, may correspond to a level of development that would enable these larvae to survive. Early hatching may occur naturally with some frequency. Within batches of walleye pollock larvae from Puget Sound that had been incubated in the laboratory, larvae hatching early grew to an equivalent size as larvae hatching later (larvae hatched on day 1 were the same length at day 3 as larvae hatched on day 3). Those early hatched larvae also began to feed at the same time as larvae hatched later.[4]

Rate of development and time to 50% hatch were similar among studies of walleye pollock from the eastern North Pacific, specifically the Gulf of Alaska (Matarese, unpubl. data; Haynes and Ignell, 1983; and this study; Paul[5]). From data on time (days) to 50% hatch for all temperatures reported in all incubation studies (Fig. 10), incubation times of western North Pacific walleye pollock are longer than eastern North Pacific walleye pollock.

This finding appears to conflict with Haynes and Ignell's (1983) comparison with Yusa's (1954) study in which they report similar rates of development

[4] Olla, B. Mark O. Hatfield Marine Science Center, Oregon State University, 2030 Marine Science Drive, Newport, OR 97365-5297. Pers. commun. 18 August 1992.

[5] Paul, A. J. University of Alaska Fairbanks, Institute of Marine Science, Seward Marine Center Lab, P.O. Box 730, Seward, AK 99664. Unpubl. data.

for eastern and western stocks. However, their comparison was made with midpoints of stages calculated from a regression model instead of observed midpoints. Also, Yusa (1954) reported a temperature range of 6–7°C instead of a mean; our interpretation of Haynes and Ignell's (1983) classification and calculation of Yusa's (1954) data suggests incubation temperatures were always above 6.5°C (see their Table 6 and our Table 4). Finally, Haynes and Ignell (1983) monitored midpoints of stages more closely than midpoint of hatch and did not specifically refer to 50% hatch.[6] We assumed the values reported as observed midpoints of hatch (their Table 7) were close to 50% hatch. Yusa's (1954) study could not be compared with ours with regard to time to 50% hatch.

Time of hatch is often a result of how eggs are treated during incubation and may vary with different batches.[7] However, walleye pollock eggs from Japanese waters are larger than those from the Gulf of Alaska (mean=1.4–1.6 mm and 1.3–1.4 mm, respectively; Bailey and Stehr, 1986). At similar temperatures, larger eggs take longer to develop (Pepin, 1991). The difference in incubation time emphasizes the need to collect data from fish specific to the area of interest. This will reduce the sources of variation in development time for laboratory-reared eggs; failure to identify and improve these sources would compromise the usefulness of models predicting egg age based on water temperature.

Development is a continuous process. The sampling intervals and arbitrary designation of stage endpoints break development into subjective units. Using the 21-stage scheme, we did not see a clear decrease in each stage duration with an increase in temperature. However, this will not affect the usefulness of our results. When stages are grouped to encompass a greater degree of morphological development, as in Haynes and Ignell (1983) and Picquelle and Megrey (1993), development time is inversely related to temperature. A greater number of stages within a group increases the accuracy of prediction of egg age. A large number of stages also allows others greater flexibility in grouping those stages.

Our regression model predicts temperature-specific development time for purposes of computing rates of egg production and egg mortality. There is no biological basis upon which the regression is predicated because stages that are assigned to the eggs are arbitrary; stages are ordinal data that are based on morphological criteria without consideration for development time. An alternative method to estimate development time from temperature is to fit a separate regression for each stage. The disadvantage of this alternative method is that many parameters are fitted with few data points.

Two studies describing morphological development, Gorbunova (1954) and Yusa (1954), have been published. Gorbunova (1954) was not comparable to our study. We compared our descriptions of morphological development with Yusa (1954). We assigned stages to descriptions of hourly morphological devel-

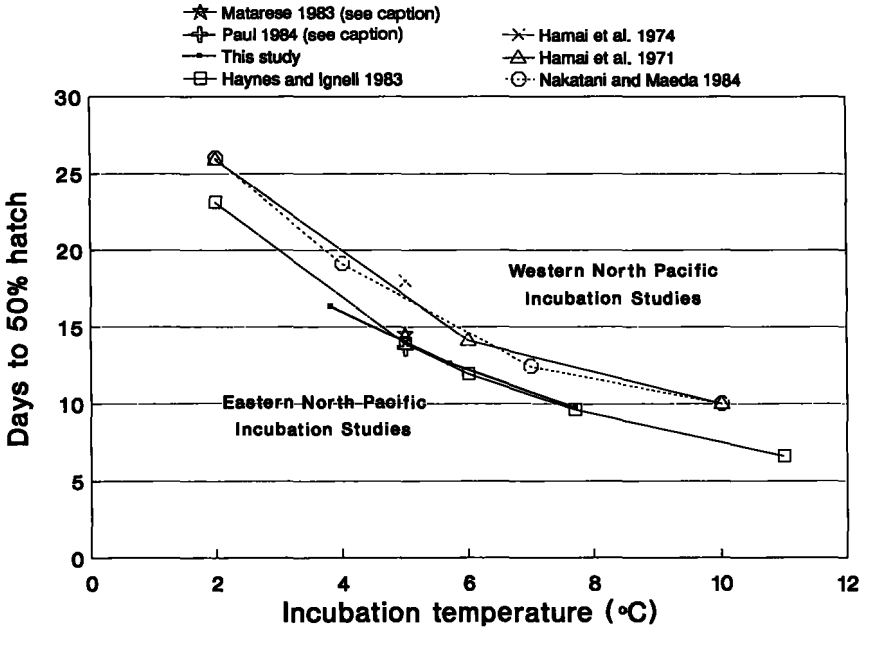

Figure 10

Days to 50% hatch for *Theragra chalcogramma* eggs at various temperatures of incubation. (A. C. Matarese, unpubl. data, Alaska Fisheries Science Center, National Marine Fisheries Service, 7600 Sand Point Way N.E., Seattle, WA 98115. A. J. Paul, unpubl. data, University of Alaska Fairbanks, Institute of Marine Science, Seward Marine Center Lab, P.O. Box 730, Seward, AK 99664.)

[6] Haynes, E., National Marine Fisheries Service, Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801-8626. Pers. commun. April 1991.

[7] Paul, A. J., University of Alaska Fairbanks, Institute of Marine Science, Box 730, Seward, AK 99664. Pers. commun. 17 March 1992.

opment of walleye pollock embryos incubated at 6.0–7.0°C (Yusa, 1954) for comparison with morphological characteristics of eggs reared at 5.7°C in this study. We used hallmarks of each stage (e.g. number of cells, germ ring advancement, number of myomeres, tail growth around yolk) to distribute Yusa's (1954) descriptions into 21 stages. Yusa's (1954) descriptions were similar to ours up to stage 11. Beginning with stage 11, Yusa (1954) described the development of some structures occurring one or more stages earlier than this study: myomeres and nares were sighted one stage earlier; brain differentiation and eye lenses, two stages earlier; the heart, three stages earlier; and the otic capsules, five stages earlier (Table 5). Otoliths sighted by Yusa (1954) were not visible in our specimens. Conversely, eye pigment was observed in our study one stage earlier than that observed by Yusa (1954). Other structures appeared at the same stage in each study: optic vesicles, Kupffer's vesicle, liver, gut, and pectoral-fin anlagen. Also, after stage 13, similar numbers of myomeres were visible at like stages in both studies as was the beating of the heart.

Differences between the two studies may be the result of egg condition when examined: Yusa (1954) described live eggs, whereas most of our descriptions were of preserved eggs. Formalin preservation may obscure myomeres or destroy structures such as embryonic otoliths (McMahon and Tash, 1979). Stockard's solution darkens embryonic tissue and obscures fine details. Also, morphological development may differ between western and eastern North Pacific walleye pollock, further emphasizing the need to restrict data collection to specific areas of interest to increase accuracy of interpretation.

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Table 5

Descriptions of morphological development of *Theragra chalcogramma* embryos at comparable stages by Yusa (1954) and this study.

Stage	Yusa (6.0–7.0°C)	This study (5.7°C)
11	medullary plate and optic vesicles visible	optic vesicles visible
12	5–7 myomeres; 3 sections of brain visible	myomeres begin to differentiate
13	9–13 myomeres; heart, otic capsules, otoliths, eye lenses, and Kupffer's vesicle visible	7–12 myomeres; Kupffer's vesicle visible
14	16–17 myomeres; nares and pigment along dorsum visible	14–16 myomeres; pigment along dorsum visible; mid- and hindbrain differentiation
15	18–30 myomeres; liver, gut, and pectoral anlagen visible; 3 sections of brain formed	20–25 myomeres; eye lens, nares, pectoral anlagen, liver, and gut visible
16	35 myomeres	24–36 myomeres; heart visible; 3 sections of brain formed
17	37 myomeres; heart beating	27–36 myomeres; heart beating
18	40 myomeres	36–41 myomeres; otic capsules visible
19		44–48 myomeres
20		48–49 myomeres; eye pigment appears
21	eye pigment appears	

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