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# Egg and Yolk Sac Larval Development of Pacific Halibut (*Hippoglossus stenolepis*)

by

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## ABSTRACT

Pacific halibut (*Hippoglossus stenolepis*) were reared from the egg stage through full yolk sac absorption. Fertilization success and egg and larval development were monitored. Forty percent fertilization was achieved and hatching success ranged from 15 to 40%. Eggs hatched between 312 and 434 hours at temperatures ranging from 5 to 7°C. At 6°C, the yolk sac was fully absorbed by day 55 from hatch. Salinity of neutral buoyancy was measured and estimates of the location of developing eggs and larvae in the natural environment were made.

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## INTRODUCTION

Pacific halibut (*Hippoglossus stenolepis*) range from southern California, across the continental shelf in the Bering Sea and down the Asian coast as far as Hokkaido, Japan (Bell and St-Pierre 1970). It is one of the most important commercially utilized species of groundfish in the northeast Pacific ocean, with an estimated commercial harvest of 33,754 mt and a landed value of $94.9 million U.S. in 1988. The largest concentrations occur from northern Vancouver Island, British Columbia to Unimak Pass, Alaska. Halibut spawn from late November through March (St-Pierre 1984), with peak spawning occurring from late December to late January. Spawning takes place along the whole coast over the continental slope at depths of approximately 200 to 500 m, based on catches of eggs. Eggs hatch within 2 to 3 weeks depending on temperature. Field collections suggest that early yolk sac larvae ascend in the water column, although the timing and rate of this upward movement is unknown (Parker 1989).

In recent years the pleuronectiformes have become increasingly important in the field of mariculture. Cultivation of the Japanese flounder (*Paralichthys olivaceus*), in Japan; and the turbot (*Scophthalmus maximus*), and Dover sole (*Solea solea*), in Europe, is now well established (Hiramoto and Kobayashi 1979, Jones et al. 1981, Person-LeRuyet 1986). Norway, Iceland, Great Britain and Canada all have ongoing mariculture programs for the Atlantic halibut, (*H. hippoglossus*). However, research on the Pacific halibut has concentrated mainly on documenting the ecology and life history of this species (Thompson 1914, Thompson and Van Cleve 1936, Van Cleve and Seymour 1953, St-Pierre 1984, 1989; Best and St-Pierre 1986), and on various methods of population assessment (Quinn et al. 1985). One laboratory study done in the early 1930's, unpublished, involved artificial spawning of captive halibut and the subsequent fertilization and development of the eggs for a number of days. Another study reported development through to hatching (Forrester and Alderdice 1973). Subsequent work has been directed at culturing Pacific halibut since those studies. A cooperative culture project between the International Pacific Halibut Commission and the U.S. Fish and Wildlife Service was initiated in 1986.

Recent culture studies on the sablefish, (*Anoplopoma fimbria*), have focused on rearing and documenting the early larval stages of this bathypelagic species (Alderdice et al. 1988; McFarlane and Nagata 1988). In view of the many similarities in the ontogeny and ecology of the early stages of sablefish and halibut, an experiment was undertaken at the Pacific Biological Station, Nanaimo, to examine the feasibility of halibut mariculture.

This paper describes the results of that study and presents information on gamete collection, fertilization, salinity of neutral buoyancy, the influence of temperature on egg development, and the developmental stages of larvae through to full yolk sac absorption. In addition, this information is used to estimate the depth of developing

eggs and larvae in the natural environment.

## MATERIALS AND METHODS

In July 1988, halibut were captured by longline off the west coast of Vancouver Island, aboard a vessel chartered by the International Pacific Halibut Commission. A total of 12 fish were transported aboard the vessel to the Pacific Biological Station on July 31, 1988. The fish were held in a 62,000 L tank with flowing sea water for a period of 5 months. They were fed herring, squid and walleye pollock to satiation, twice a week. Seven fish were selected for the experiment and transferred to two 7,000 L tanks equipped with flowing sea water.

To induce ovulation, these fish were injected with two doses of Luteinizing Hormone Releasing Hormone (LHRH) analog, according to the method developed for sablefish (Solar et al. 1987), the first at a concentration of 0.10 mg/kg and the second 9 days later at a concentration of 0.05 mg/kg. The injections were made into the peritoneal cavity. Prior to injection, the fish were anesthetized with MS222 (Tricaine methane sulfonate). Fish were inspected daily for visual signs of ripeness. Gametes were obtained from ripe fish by gentle abdominal pressure and collected into 2 L plastic containers (ova), or 200 mL sealable plastic bags (sperm).

Milt was diluted with 35°/oo sea water at a dilution ratio of 1/200, based on fertilization methods developed for sablefish (Alderdice et al. 1988). This mixture was immediately added to the eggs, followed by gentle mixing with a glass stirring rod. At one-min intervals, two more batches of activated sperm were added to the eggs. The final egg to fluid (i.e. seawater and milt) ratio was about 1/3. Floating eggs were carefully removed with a fine meshed strainer and serially rinsed in clean 35°/oo seawater. These eggs were placed in the incubator described below.

Fertilized eggs were incubated in an experimental incubator, designed specifically for marine eggs and larvae (Figure 1). The shape and dimensions were designed to produce a gentle, non-turbulent upwelling flow for the developing eggs and larvae. A cone, constructed of molded 3.2 mm transparent PVC, with a small (3°) angle was chosen as a means of presenting eggs and larvae with constant upwelling flow. The cone diameter ranged from 5.08 to 15.24 cm over a height of 120 cm. These dimensions were chosen to yield an upwelling velocity of 0.3 cm/s midway in the cone with a flow rate of 1.5 Lpm.

Water was introduced via an inflow ring with 3.2 mm holes, into a mixing chamber constructed of 3.2 mm thick PVC and measuring 25 cm in diameter. Nitex screen (Swiss Silk Bolting Cloth Mfg. Co. Ltd., CH9425, Thal, (St. Gall) Switzerland) (300 micron mesh) was cemented to the bottom of the cone to prevent eggs or larvae from getting into the mixing chamber if the flow was stopped. A removable Nitex screen covered the top of the cone.

A small subgroup of eggs was divided into three samples of 50 eggs each and fertilized using the same procedure as described above. These eggs were placed in 100 mL crystalizing dishes in a water bath at 6°C for 7 h. At that time, development was determined and 20 normally developing eggs were placed in each of four modified salmonid incubators, at four incubation temperatures (4 to 7°C). These incubators maintained the eggs in a layer in perfusing water (see Alderdice et al. 1988, Figure 1). The incubators were housed in 40 L constant temperature (± 0.1°C) tanks. Salinities averaged 29.6°/oo.

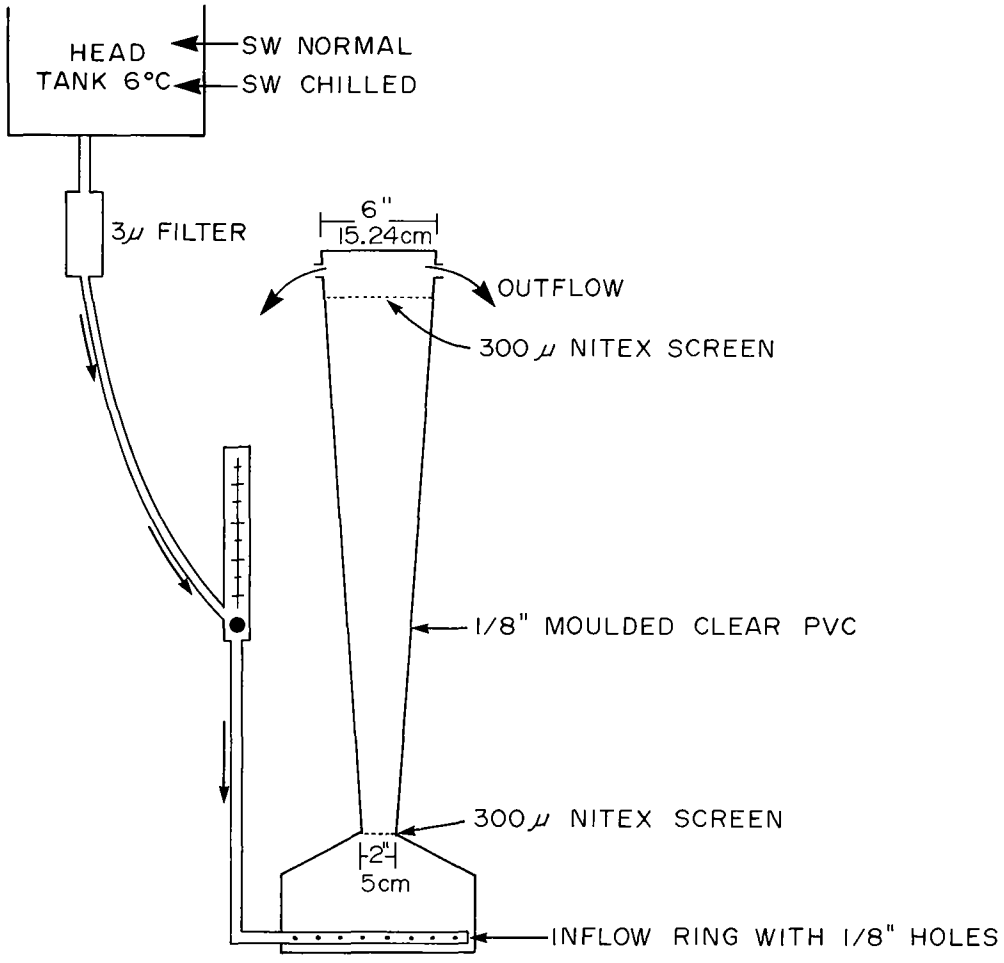



**Figure 1. Conical incubator designed for culture of pelagic marine fish eggs.**

## RESULTS

Of the seven halibut selected for the experiment, three were females and four males. All males spermiated, however eggs were obtained from only one of the females. A total of 5,376 eggs was obtained and fertilization success of 39.6% was achieved (Table 1).

**Table 1. Test fertilization results of Pacific halibut (at 7.1 h after fertilization).**

| Rep# | Total eggs | Non-developed | 2-cell | 4-cell | % development |
|---|---|---|---|---|---|
| 1 | 67 | 36 | 14 | 17 | 46.3 |
| 2 | 73 | 41 | 16 | 16 | 43.8 |
| 3 | 54 | 37 | 7 | 10 | 31.5 |
| | | | | | Mean fertilization = 39.6% |

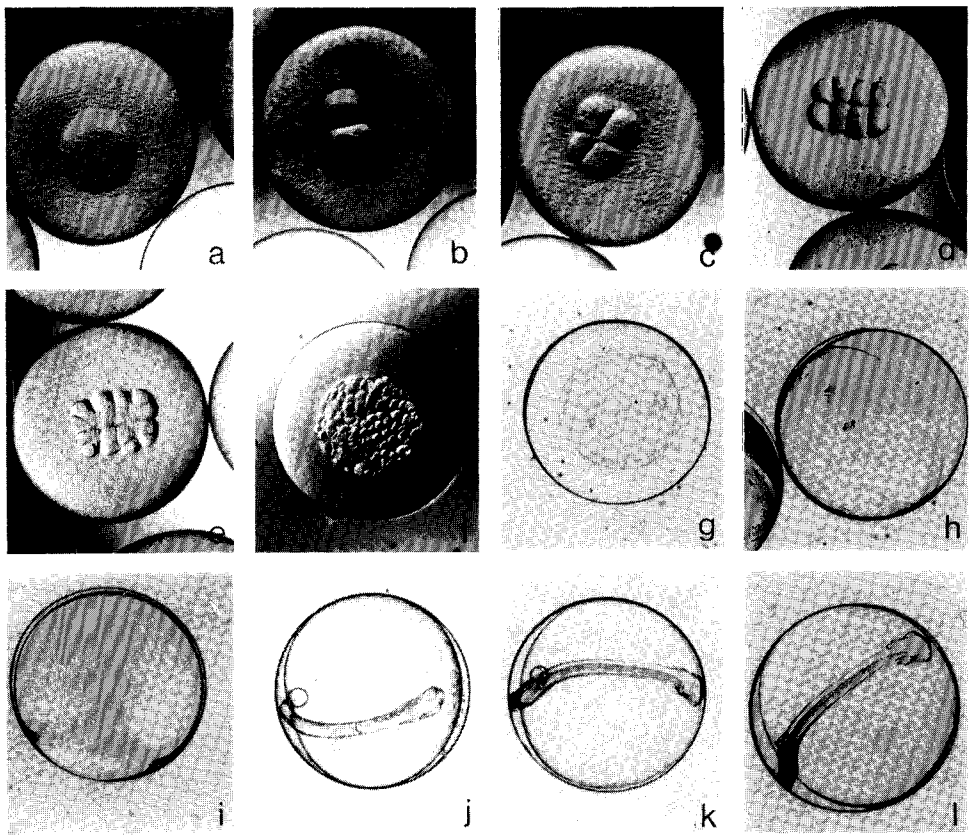



**Figure 2. Stages of embryonic development - (a) blastodisc, (b) 2- cell, (c) 4-cell, (d) 8-cell, (e) 16-cell, (f) 64-cell, (g) germ ring (bottom view), (h) 1/2 epiboly, (i) 2/3 epiboly, (j) yolk plug near closure, Kupffer's vesicle, (k+l) advanced embryonic stages, (m) tail bud lifting off egg.**

## Egg Development

Developmental stages of eggs (Figure 2) were similar to those reported by Forrester and Alderdice (1973) for Pacific halibut and by Rollefsen (1935) for Atlantic halibut. The egg is non-adhesive, transparent and extremely fragile (Forrester and Alderdice 1973). The mean diameter of fertilized, water-hardened eggs was 3.4 ± 0.9 mm. Egg development was monitored at 4 to 7°C (Table 2). Hatching occurred at 5, 6, and 7°C; at 4°C all eggs stopped developing and died by half epiboly stage. Time to hatch was temperature dependent, ranging from 312 to 434 h (Table 2; Figure 3) and was slightly less than that reported by Forrester and Alderdice (1973).

Survival to hatching in the incubators at 5 to 7°C ranged from 30 to 40%, higher than that reported by Forrester and Alderdice (1973). We attribute this higher rate to the selection of only normally developing eggs for the experiment. Survival was greatest at 6°C (40%) and lowest at 7°C (30%). However, due to the small number

**Table 2. Estimates of incubation time (h) to various development stages at temperatures ranging from 4 to 8°C of Pacific halibut (data from current studies (1989) at the Pacific Biological Station and from Forrester and Alderdice (1973)).**

| Development stage | Temperature (°C) and Year | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 4° | | 5° | | 6° | | 7° | | 8° |
| | 1973 | 1989 | 1973 | 1989 | 1973 | 1989 | 1973 | 1989 | 1973 |
| 2-cell | - | - | - | - | - | 7 | - | - | - |
| 4-cell | - | - | - | - | - | 10 | - | - | - |
| 16-cell | - | 22 | - | - | - | - | - | - | - |
| 32-cell | - | - | - | 22 | - | - | - | - | - |
| 64-cell | - | 42 | - | - | - | 22 | - | 22 | 24 |
| Early morula | 72 | 70 | - | 37 | - | - | - | 30 | - |
| Late morula | 96 | 94 | - | 58 | - | 46 | - | 46 | 48 |
| Germ ring formed | 120 | 119 | - | 94 | - | 70 | - | 70 | 72 |
| Germ ring overgrowth begins | - | 143 | - | - | - | 94 | 96 | - | 96 |
| 1/4 to 1/2 epiboly | 168 | 165 | - | 131 | 149 | - | 101 | 94 | 120 |
| 1/2 to 3/4 epiboly | 240 | - | - | - | - | 119 | - | - | - |
| Near yolk plug closure | - | - | - | - | - | 165 | - | 119 | - |
| Yolk plug closed | - | - | - | - | 192 | - | 163 | - | 144 |
| 1-15 somites, Kupffer's vesicle | - | - | - | - | - | - | - | 143 | - |
| 15-25 somites | - | - | - | - | - | - | - | 165 | - |
| >25 somites, optic cups develop | - | - | 252 | - | 221 | 216 | - | - | 168 |
| Tailbud lifted off yolk | - | - | - | - | 300 | 240 | - | 216 | 216 |
| Heart beating | - | - | - | 335 | - | 288 | - | 240 | - |
| Hatching | - | - | 487 | 434 | 396 | 360 | 336 | 312 | 300 |

of eggs available for this experiment, no replicates were possible.

The relationship between the rate of egg development, temperature and hatching time in the natural environment was modelled using data (Table 3) from this study and that of Forrester and Alderdice (1973). A general curve fitting model developed by Schnute (1981) was employed:

$$y = (y_1^b + ((y_2^b - y_1^b) \cdot (1 - \exp(-a(x - x_1))/1 - \exp(-a(x_2 - x_1))))) ^{1/b}$$

where

$y$ = time to 50% hatch, h
$x$ = actual temperature at depth, °C
$y_1$, $y_2$ = upper, lower limits of y, given $x_1$, $x_2$
$x_1$, $x_2$ = minimum and maximum temperatures used, °C
$a$, $b$ = constants

Parameter estimates determined from the data of Table 3 were:

$y_1 = 568.47$ $\quad$ $y_2 = 300.00$
$x_1 = 4.0$ $\quad$ $x_2 = 8.0$
$a = 1.596$ $\quad$ $b = 7.454$

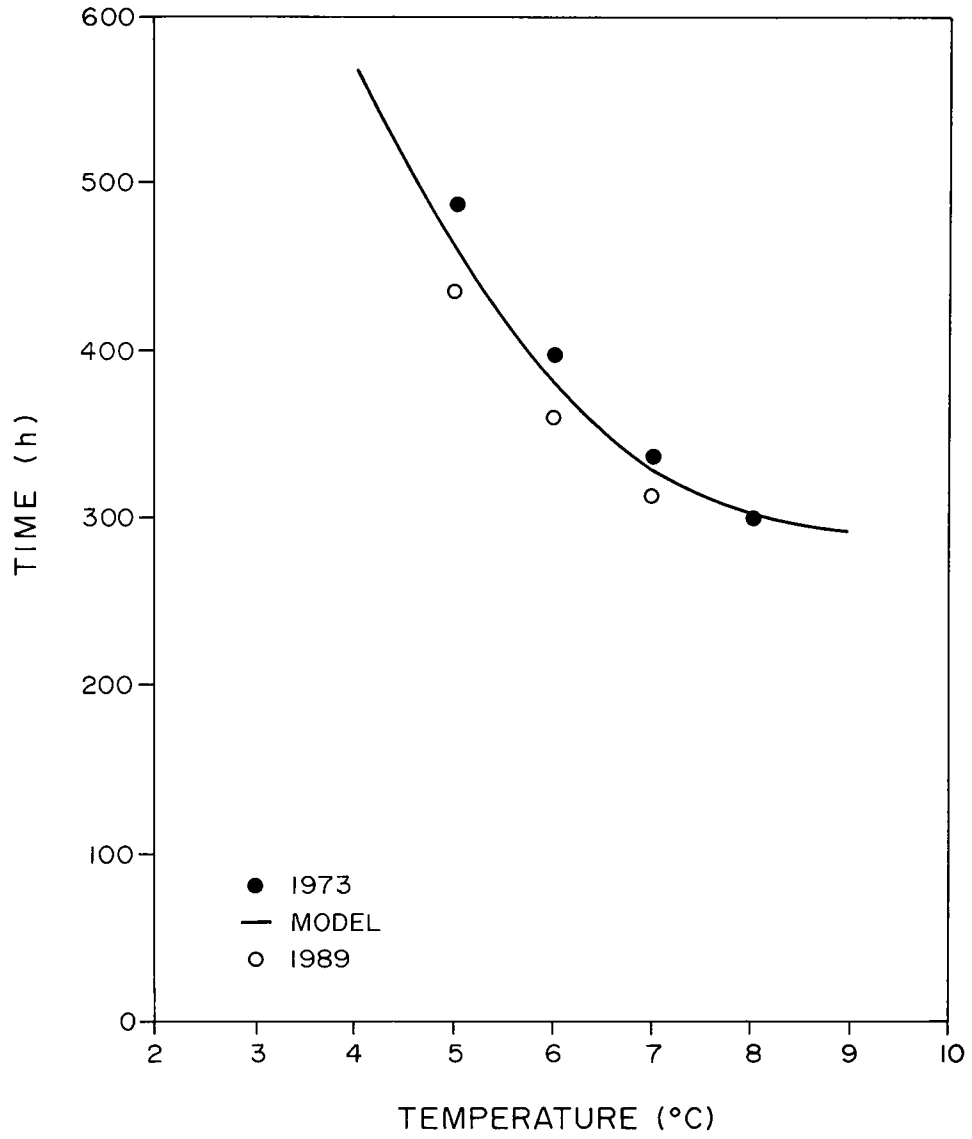



**Figure 3. Incubation time to 50% hatch in relation to temperature.**

Salinity of neutral buoyancy (SNB) was measured for developing halibut eggs at 5.3°C in a salinity gradient column. These estimates were combined with SNB data reported by Forrester and Alderdice (1973) (Table 4) and a series of salinity and temperature profiles to 1000 m (Table 5) compiled from salinity temperature depth (STD) data collected in February and March 1986 and March 1987, off the west coast of Vancouver Island (McFarlane, unpublished data). Calculations of vertical egg movement in a typical ocean water column were then possible by determining the differences in egg density and that of the surrounding water. The rate of ascent was calculated using Stokes' equation (Alderdice and Forrester 1971):

$$-W = \frac{2}{9} g \frac{(p1 - p2)}{\mu} r^2$$

**Table 3. Mean temperatures for the incubation period, survival to hatch (%), and the incubation time to 50% hatch (observed and predicted) of Pacific halibut.**

| Year | X Incubation temp (°C) | Survival to hatch (%) | Incubation time (h) | |
|---|---|---|---|---|
| | | | Observed | Predicted |
| 1973 | 5.0 | | 497.00 | 460.56 |
| 1989 | 5.0 | 35.0 | 434.00 | 460.56 |
| 1973 | 6.0 | | 396.00 | 377.92 |
| 1989 | 6.0 | 40.0 | 360.00 | 377.92 |
| 1973 | 7.0 | | 336.00 | 323.98 |
| 1989 | 7.0 | 30.0 | 312.00 | 323.98 |
| 1973 | 8.0 | | 300.00 | 300.00 |

**Table 4. Observed and interpolated salinity of neutral buoyancy (SNB) values used in calculating Pacific halibut egg depths over time. The interpolated SNB values shown at 24 h intervals were obtained by interpolation of the observed values shown in column two (SNB, Pacific Biological Station 1989). For elapsed times beyond 270 h, data from Forrester and Alderdice (1973) were used.**

| Elapsed time (h) | SNB PBS 1989 (ppt) | PBS 1989 + Forrester and Alderdice 1973 | |
|---|---|---|---|
| | | Elapsed time (h) (at 24 h intervals) | SNB values interpolated (ppt) |
| 0 | 34.00 | 0 | 34.00 |
| 12 | - | 12 | 33.95 |
| 48 | 33.80 | 60 | 33.70 |
| 72 | 33.70 | 84 | 33.55 |
| 96 | 33.60 | 108 | 33.40 |
| 108 | 33.50 | 132 | 33.25 |
| 120 | 33.40 | 156 | 33.25 |
| 125 | 33.30 | 180 | 33.10 |
| 131 | 33.25 | 204 | 32.90 |
| 144 | - | 228 | 32.85 |
| 146 | 33.25 | 252 | 32.85 |
| 150 | - | 276 | 32.85 |
| 168 | - | 300 | 32.85 |
| 173.5 | 33.15 | 324 | 32.70 |
| 175 | - | 348 | 32.55 |
| 195.5 | 32.90 | 372 | 32.70 |
| 200 | - | 396 | 32.70 |
| 223 | 32.90 | 420 | 32.80 |
| 225 | - | 444 | 32.70 |
| 239.5 | 32.80 | 468 | 32.80 |
| 250 | - | 492 | 32.70 |
| 270 | 32.90 | 516 | 32.70 |

**Table 5. Salinity, temperature, and depth data (combined February and March 1986 and March 1987, west coast of Vancouver Island, British Columbia) used to model salinity and temperature as a function of depth. Additional depth intervals* were added and corresponding temperatures and salinities were calculated to provide a complete depth profile of predicted values.**

| Depth (m) | Temperature(°C) | | Salinity (ppt) | |
|---|---|---|---|---|
| | Observed | Predicted | Observed | Predicted |
| 0 | 8.70 | 9.37 | 31.25 | 31.38 |
| 0 | 9.40 | 9.37 | 31.50 | 31.38 |
| 0 | 9.15 | 9.37 | 31.25 | 31.38 |
| 10 | 8.70 | 9.27 | 32.25 | 31.58 |
| 10 | 9.40 | 9.27 | 31.50 | 31.58 |
| 10 | 9.15 | 9.27 | 31.25 | 31.58 |
| 20 | 8.70 | 9.18 | 32.20 | 31.76 |
| 20 | 9.40 | 9.18 | 31.75 | 31.76 |
| 20 | 9.10 | 9.18 | 31.50 | 31.76 |
| 50 | 8.80 | 8.90 | - | 32.25 |
| 50 | 9.40 | 8.90 | 32.20 | 32.25 |
| 50 | 9.15 | 8.90 | 31.90 | 32.25 |
| 100 | 8.80 | 8.46 | 32.90 | 32.86 |
| 100 | 9.40 | 8.46 | 32.70 | 32.86 |
| 100 | 8.90 | 8.46 | 32.80 | 32.86 |
| 200 | 7.80 | 7.63 | 33.70 | 33.60 |
| 200 | 8.00 | 7.63 | 33.75 | 33.60 |
| 200 | 7.60 | 7.63 | 33.85 | 33.60 |
| 300 | 6.60 | 6.88 | 33.90 | 33.96 |
| 300 | 6.75 | 6.88 | 33.95 | 33.96 |
| 300 | 6.60 | 6.88 | 33.96 | 33.96 |
| 400* | - | 6.20 | - | 34.13 |
| 500 | 5.20 | 5.58 | 34.04 | 34.21 |
| 500 | 5.20 | 5.58 | 34.07 | 34.21 |
| 600* | - | 5.02 | - | 34.25 |
| 700* | - | 4.52 | - | 34.27 |
| 800* | - | 4.06 | - | 34.28 |
| 900* | - | 3.65 | - | 34.29 |
| 1000 | 3.50 | 3.28 | 34.36 | 34.29 |
| 1000 | 3.40 | 3.28 | 34.37 | 34.29 |

where W = vertical egg velocity (cm/s); g = 980.621 cm/s$^2$; p1 = egg density (g/cm$^3$); p2 = density of the surrounding water (g/cm$^3$); $\mu$ = 0.0150 (dynamic viscosity of sea water estimated at 35°/oo and averaged for 5 to 10°C) (Sverdrup et al. 1946); r = radius of the egg (cm).

Schnute's (1981) general growth model was used to obtain empirical models to (1) estimate changes in egg density in relation to ambient temperature and (2) to describe changes in salinity and temperature in relation to depth. This allowed calculation of water density (Millero and Poisson 1981), in the ocean water column to depths of 1000 m.

To calculate the location of eggs in the water column, these relationships were incorporated into an interactive model that, given an initial spawning depth, calculated egg density (dependent on temperature and time from fertilization). This density

value was input into the Stokes' equation to determine the vertical egg velocity at a given time and depth. The model then calculated, first at 12 h followed by 24 h intervals, the egg's new location in the water column. At this new location the water temperature and salinity were re-calculated, based on the modelled STD data.

Then, the mean temperature exposure was determined and the corresponding egg development rate was calculated so that the SNB of the egg could be determined. The new SNB was again input into the Stokes' equation to calculate a new vertical egg velocity. This process was repeated until 510 h past hatching. A series of estimated vertical locations of eggs and larvae, with corresponding percentage development to hatching, was generated, based on spawning depths ranging from 200 to 600 m (Figure 4). Depending on spawning depth, the fertilized egg rose to between 100 and 200 m during development. Time to hatch (temperature-dependent) ranges from 300 to 425 h (12.5 to 18 days).

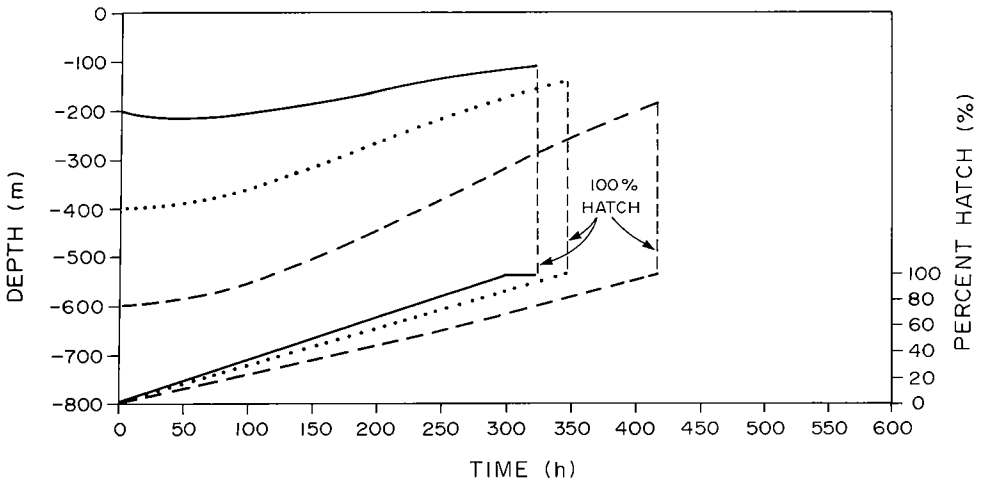



**Figure 4. Modeled location of halibut eggs and time to 100% hatch at spawning depth of 200, 400, and 600 m. Temperature and salinity data used in model are presented in Table 5.**

## Yolk Sac Larval Development

Pacific halibut larvae were 7.5 ± .21 mm (total length) at hatch. Newly hatched larvae were primitive, with no pigmentation, no mouth, and the head still attached to the yolk sac (Figure 5). In the incubators, larvae floated passively in the water column with the head pointing downward.

Table 6 lists the major developmental features of halibut larvae in a temporal sequence from hatch until full yolk sac utilization at 6°C, while Figure 5 shows a photographic record of these developmental events. Time from 50% hatch until full yolk sac utilization was 55 days, similar to that reported for Atlantic halibut (Pittman et al. 1988). By day 6, and a length of approx 9.6 mm, the eyes were pigmented. This was followed over the next 10 to 20 days by the development of the internal organs. By day 16 the liver had developed from a group of cells on the anterior surface of the yolk sac, and the mouth had formed, as a small opening. By day 24 (larval

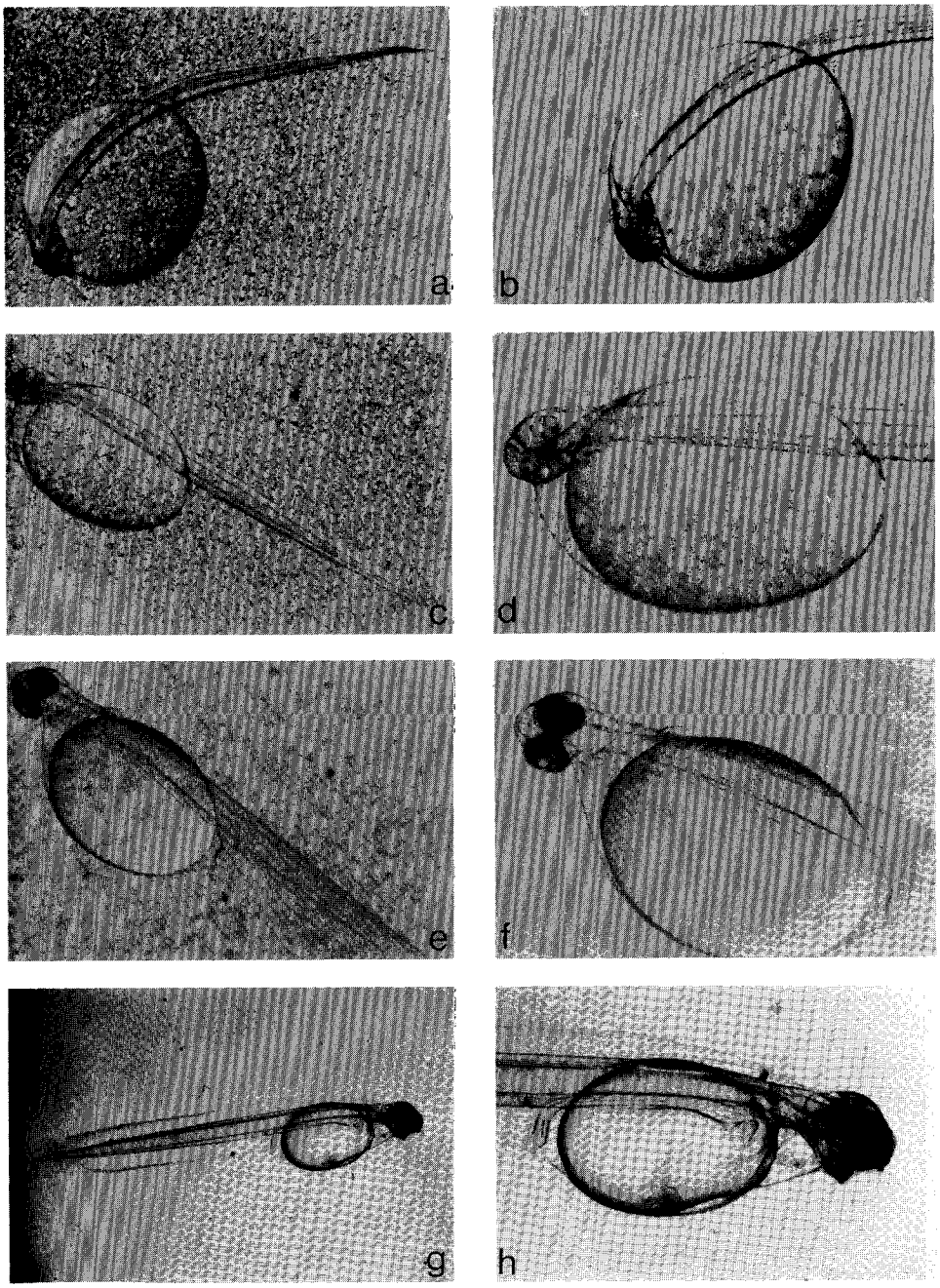
length 11 mm), pigmentation was present along the alimentary canal and on the ventral surface of the larvae. At this time the intestine changed from a straight tube and formed a single loop in the anterior portion, as the yolk was utilized. Pigmentation extended over the yolk sac by day 32, and by day 40 the mouth was functional with fully formed jawbones. During the remaining 15 days of the yolk sac stage the intestine differentiated, with the appearance of sphincter muscles separating the intestine from the colon (day 44), the pectoral fin and lower jaw became pigmented (day 51), and yolk was fully utilized (day 55). At full yolk absorption the larvae had not yet initiated feeding. Ten larvae reached the full yolk sac absorption stage. Seven died within the following two weeks (day 70), with the remaining three lasting to days 81, 85 and 102.

Growth in length of larvae was rapid for the first 10 to 15 days after hatch (Figure 6). After this time, growth was slightly reduced and linear. During the latter part of the yolk sac stage, growth rate was further reduced, presumably as the larva began to put energy into growth in myotome height as opposed to length.

**Table 6. Chronological sequence of major developmental stages of yolk-sac larvae of Pacific halibut at 6.0°C**

| Date | Days from fertilization | Days from hatch | Event | Size (mm) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | TL | NL | SA | YL | YW |
| Feb 10 | 0 | | Fertilization | - | - | - | - | - |
| Feb 25 | 15 | 0 | 50% hatch | 7.58 | 7.33 | 3.58 | 3.33 | 2.70 |
| Feb 26 | 16 | 1 | 100% hatch | - | - | - | - | - |
| Mar 3 | 21 | 6 | Eye pigmentation | 9.58 | 9.33 | 4.00 | 3.08 | 2.42 |
| Mar 13 | 31 | 16 | Liver development and mouth open | 11.00 | 10.80 | 4.30 | 2.50 | 2.00 |
| Mar 21 | 39 | 24 | Alimentary and ventral pigmentation, beginning of foregut coil | 11.10 | 10.80 | 4.20 | 2.40 | 1.83 |
| Mar 29 | 47 | 32 | Pigmentation extending ventrally over yolk-sac | 12.00 | - | 4.17 | 2.08 | 1.92 |
| Apr 6 | 55 | 40 | Mouth functional | 13.00 | 12.40 | 4.80 | 1.70 | 1.80 |
| Apr 10 | 59 | 44 | Gut musculature contracting, intestine fully differentiated | 13.20 | - | 4.80 | 1.70 | 1.70 |
| Apr 17 | 66 | 51 | Pectoral fin and lower jaw pigmentation | 13.40 | - | 5.00 | 1.20 | 0.90 |
| Apr 21 | 70 | 55 | 100% yolk utilization | 13.50 | - | 5.20 | - | - |

TL = total length; NL = notochord length; SA = snout to anus length; YL = yolk sac length; YW = yolk sac width.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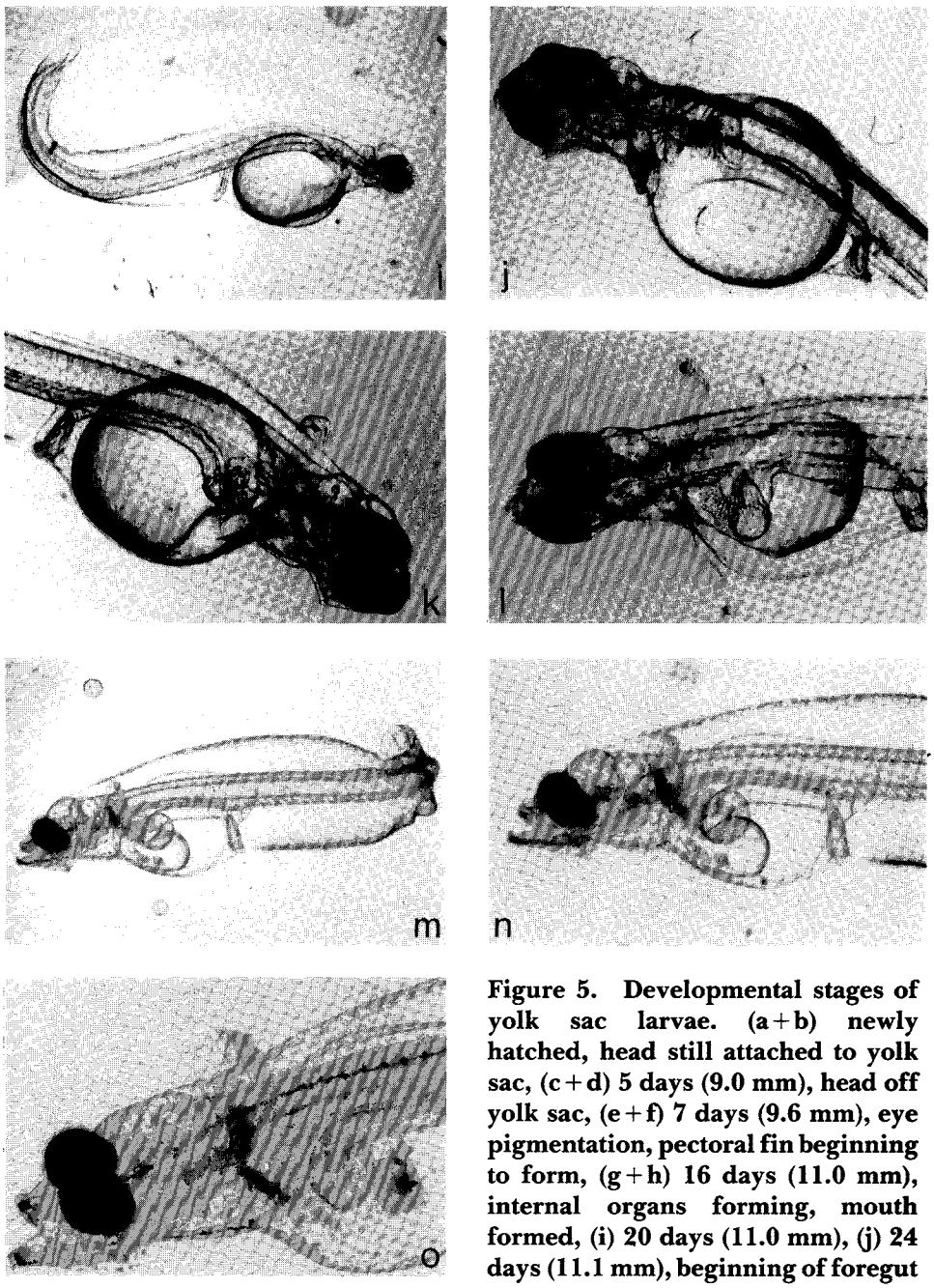


**Figure 5. Developmental stages of yolk sac larvae. (a+b) newly hatched, head still attached to yolk sac, (c+d) 5 days (9.0 mm), head off yolk sac, (e+f) 7 days (9.6 mm), eye pigmentation, pectoral fin beginning to form, (g+h) 16 days (11.0 mm), internal organs forming, mouth formed, (i) 20 days (11.0 mm), (j) 24 days (11.1 mm), beginning of foregut coil, (k) 32 days (12.0 mm), (l) 44 days (13.0 mm ), (m+n) 51 days (13.2 mm), (o) 56 days (13.4mm) 100% yolk sac absorption.**

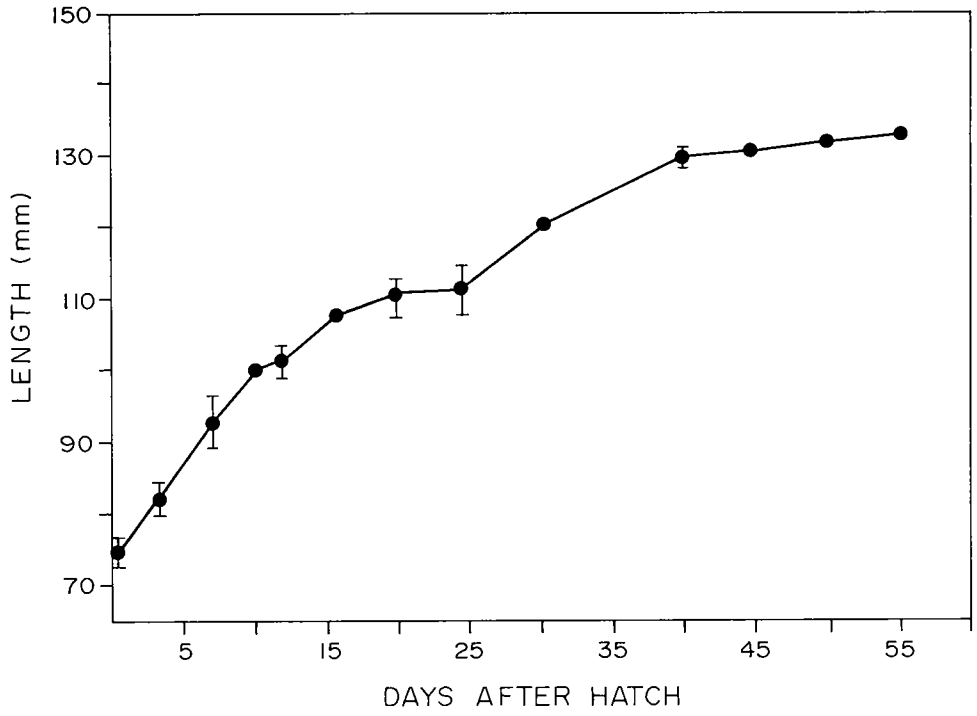


**Figure 6. Growth of yolk sac larvae at 6°C.**

## DISCUSSION

On the west coast of North America most aquaculture effort has focused on salmonids. The success of these efforts has stimulated interest in other fish species. In particular, the feasibility of culturing Pacific halibut is now being considered. It was anticipated that, as with Atlantic halibut and sablefish, development of successful techniques for egg incubation and larval rearing would be the greatest obstacles to the establishment of this potentially valuable industry. In this paper we have presented preliminary results of our work in this area. To our knowledge, this is the first reported success in rearing Pacific halibut from fertilized egg to full yolk sac absorption. Previous investigators (Forrester and Alderdice 1973) reported success in egg incubation studies for this species, although all yolk sac larvae died within 10 days of hatching. More recently, investigators at the University of Washington have reared yolk sac larvae for a maximum period of 20 days after hatching (Han Wu Liu, University of Washington, Seattle, pers. comm.).

Pacific and Atlantic halibut exhibit many similarities in their early life history stages. In particular, egg and larval development rates are similar, and growth rate of Pacific halibut larvae (at 6°C) was identical to that reported for Atlantic halibut reared at the same temperature (Pittman et al. 1988). Developmental abnormalities of larvae have been reported in the literature. Pittman et al. (1988) describe two problems which cause significant larval mortality in Atlantic halibut. The first, gaping mouth syndrome (lockjaw), is a condition where the larvae is unable to close its mouth. Although the cause is unknown, it appears to be associated with high rearing

temperatures (>7°C). The second abnormality, edema, is the buildup of fluids in the intestinal spaces. In Atlantic halibut this appears as a swelling of the peritoneal and/or pericardial cavities causing larvae to float to the surface. Although it appears that mortalities are a result of a failure to feed, it is probable that the underlying cause of the problem arises in the early yolk sac stage.

These two developmental abnormalities have also been identified for sablefish larvae (McFarlane 1988), but were not observed in our study. We believe that this is a result of our experimental upwelling incubators. These cone incubators provided a gentle nonturbulent upwelling flow of high quality water for the larvae. The controlled flow rate compensated for changes in SNB of the eggs and larvae thereby more closely simulating the natural environment.

Temperature and pressure fluctuations in our water supply on day 19 resulted in greater than 90% larval mortality. Therefore, only 10 larvae reached the full yolk sac absorption stage. Nevertheless, we believe that our results indicate the biological feasibility of Pacific halibut mariculture. Success has been achieved in developing fertilization and incubation techniques for induced gametes, and determining optimal physical conditions for incubation of eggs and yolk sac larvae. Although biological constraints remain, we anticipate they will be resolved within the next few years. In particular, the similarities between Atlantic and Pacific halibut suggest that solutions to problems in one species will be directly applicable to the other.

St-Pierre (1984) reported that, based on observations at sea, Pacific halibut spawn at depths between 180 and 550 m along the continental shelf edge. Most eggs (68%) were found at depths between 85 and 212 m with the majority of the remainder found down to 680 m. Estimates of water temperature at spawning depth ranged from 2.3 to 3.5°C off the Asian coast, 3.5 to 5.5°C in the Bering Sea and from 4.7 to 9.7°C off the west coast of British Columbia. Hatching time was estimated to be from 30 to 45 days off the Asian coast and from 11 to 23 days off the west coast of North America. Developmental rates at depth of eggs and yolk sac larvae are unknown.

It was not previously possible to determine these rates in the laboratory because we lacked the ability to keep larvae alive through to yolk sac absorption. Using the incubation system described in this paper we believe detailed studies can now be conducted. From the information obtained in this study, we have attempted to predict the vertical movement and development of halibut eggs and larvae in the natural environment. From our study and that of Forrester and Alderdice (1973), it is apparent that for successful development, eggs should be released in waters ranging in temperature from 5 to 7°C since egg development ceased at the 1/2 epiboly stage at 4°C and hatching success was reduced at 8°C in Forrester and Alderdice's (1973) study. However, further research is required to determine optimum temperatures for development, particularly for eggs collected in more northerly environments where eggs have been found in waters 4°C.

We have applied the laboratory results to the natural environment, to predict vertical movement of eggs off the west coast of Canada at depths ranging from approximately 200 to 600 m. After fertilization, the egg will rise in the water column to a depth of between 100 and 200 m, depending on spawning depth. Estimated time to hatch will range from 12.5 days (if spawned at 200 m) to 18 days (if spawned at 600 m), based on the temperature profiles used in this paper. Although it was difficult to make accurate SNB measurements on larval fish, based on visual observations of early yolk sac larvae in the incubator it was possible to continue monitoring larval positions in the water column. After hatch, larvae remained at hatching depth for a period of 5 to 10 days. By day 6, eye pigmentation occurred and the larvae became

extremely active. For the next 20 days larvae increased their swimming activity and were able to maintain themselves anywhere in the water column of the incubator. In the ocean they would be capable of remaining in the upper portion of the water column and positioning themselves in the upper 100 m, to be transported by currents. This study confirms the observations summarized by St-Pierre (1984) from field collections. It also provides additional developmental information necessary for a more thorough analysis of egg and larval drift models over time and area in the northeast Pacific Ocean.

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