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Laboratory Observations on Early Development of the Pacific Halibut

by

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PREFACE

The International Pacific Halibut Commission was established in 1923 by the Convention between Canada and the United States for the preservation of the halibut fishery of the North Pacific. The Convention was the first international agreement providing for joint management of a marine fishery. The Conventions of 1930, 1937, and 1953 extended the Commission's authority and specified that the halibut stocks be developed and maintained at levels consistent with the maximum sustained yield.

Three Commissioners are appointed by the Governor General of Canada and three by the President of the United States. The Commissioners appoint the Director of Investigations who supervises the scientific and administrative staff. The scientific staff collects and analyzes statistical and biological data to manage the halibut fishery. The headquarters and laboratory are located at the University of Washington in Seattle, Washington. Each country provides one-half of the Commission's annual appropriation.

The Commissioners meet annually to review the regulatory proposals made by the scientific staff and consider advice from the Conference Board that represents vessel owners and fishermen. The regulatory measures are submitted to the two governments for adoption, and the fishermen of both nations are required to observe these regulations.

CONTENTS

ABSTRACT

Eggs of the Pacific halibut *(Hippoglossus stenolepis)* were artificially fertilized and incubated in water of 33 $^{0}/_{00}$ salinity at temperatures of 2 to 12°C. Hatching occurred at temperatures of 5, 6, 7 and 8°C. Time to 50% hatching ranged from 12.5 days (8°C) to 20 days (5°C). Mean larval size at hatching ranged from 6.03 to 7.24 mm; the largest larvae were hatched at 6°C. At hatching the length of yolk sac was approximately 50% of the total larval length. The observations complement information provided through earlier field studies by the International Pacific Halibut Commission.

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by
C. R. Forrester* and D. F. Alderdice*

INTRODUCTION

Information on the early development of the Pacific halibut *(Hippoglossus stenolepis)* has been gained largely from examination of eggs and larvae taken in plankton tows (Thompson and Van Cleve, 1936; Van Cleve and Seymour, 1953; Pertseva-Ostroumova, 1961). Production of ripe eggs and milt by specimens held in captivity at Vancouver, British Columbia, during the winters of 1971-72 and 1972-73, afforded the opportunity of examining the effects of temperature on the rate of development of the Pacific halibut egg. The initial objective of the tests was to study the effects of salinity and temperature, in a closed system under controlled conditions, on aspects of early egg and larval development. When it became apparent that eggs were highly susceptible to mechanical injury, the study was expanded to consider a means of improving survival during the incubation period. This report summarizes observations made during these studies and complements those obtained earlier by International Pacific Halibut Commission field studies.

METHODS

Live halibut were obtained in May 1970 in Queen Charlotte Sound, British Columbia. The adult fish were transported to and held at the West Vancouver Laboratory of the Pacific Environment Institute (Tomlinson and Baker, 1973). The specimens matured sexually and released eggs and milt in the holding tank during the winters of 1971-72 and 1972-73. Eggs were stripped from the live fish (February 17, 1972; February 13, 1973) and fertilized in sea water of either 26.8 $^{0}/_{00}$ or 33.0 $^{0}/_{00}$ S (salinity). The eggs were transported in refrigerated containers (5.5-7.8°C) to the Pacific Biological Station, Nanaimo, and placed in constant salinity-temperature conditions 6 hours after fertilization.

1972 Experiments

Eggs were dispensed volumetrically into seven incubators, each standard incubator (Alderdice and Velsen, 1968) holding four subsamples of eggs (mean, 228; range, 164-363 eggs). Each incubator was held at different constant salinity-temperature conditions: 33 $^{0}/_{00}$ S at 2, 4, 6, 8, 10 and 12°C; and 29 $^{0}/_{00}$ S at 6°C. The salinity of 33 $^{0}/_{00}$ was considered to be representative of conditions at depths near the bottom of the upper layer zone off British Columbia where eggs incubate in nature (Thompson and Van Cleve, 1936; Van Cleve and Seymour, 1953). The salinity of 29 $^{0}/_{00}$ was

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an approximation of coastal surface water salinity. Temperatures from 3.5-9°C were selected to conform with those at the bottom of the upper zone in the northeastern Pacific Ocean (Gulf of Alaska) during the winter period (Dodimead et al., 1963).

1973 Experiments

Eggs were incubated at 33 °/₀₀ S at 5, 6 and 7°C, conditions which produced the best results in 1972. The same incubators were used as in 1972. However, subsample size was reduced to approximately 50 eggs because of a suspected oxygen deficiency in the 1972 trials. Perfusion velocity of the incubation medium past the eggs was also raised from 350 cm/hr in 1972 to approximately 580 cm/hr in 1973.

The low hatching success in 1972 was attributed to egg fragility and susceptibility to mechanical shock. Attempts were made to alleviate mechanical shock by incubating eggs in other types of containers: (a) incubators providing a slow, toroidal circulation; (b) submerged plastic-nylon mesh cylinders providing a slow internal circulation; and (c) open 1-litre glass jars with slow circulation provided by gentle aeration (Table 1).

Antibiotics were used in the 1973 tests to inhibit bacterial growth; those used were sodium penicillin G (50 I.U./ml), and streptomycin sulphate (0.05 mg/ml) (Shelborne, 1963).

Table 1. Percent hatch and numbers of Pacific halibut eggs held in three types of incubator at 33 °/₀₀ S (1973).

Incubator Type	Temperature (°C)	No. of eggs					Percent hatch				
		Subsample					Subsample				
		1	2	3	4	Total	1	2	3	4	Mean
Standard	5	62	44	55	52	213	8.1	6.8	7.3	13.5	8.9
	6	62	58	55	54	229	11.3	10.3	10.9	14.8	11.8
	7	50	55	56	48	209	24.0	30.9	28.6	22.9	26.6
Toroidal circulation	5					241					6.6
	6					208					19.2
	7					238					24.0
Cylinder	7					161					39.1

RESULTS

Development of Eggs

The unfertilized halibut egg is plastic, nonadhesive and transparent, with the surface showing a network of discontinuous striations (Figure 1a). Mean diameter of unfertilized eggs (within 15 minutes of extrusion) was 3.14 mm (range, 2.87-3.36 mm). Mean diameter of fertilized eggs was 3.2 and 3.34 mm in 1972 and 1973 (range, 2.95-3.52 mm). Full diameter was reached 3-4 hours following fertilization. Average egg diameter cannot be directly compared with measurements of preserved eggs made by Thompson and Van Cleve (1936) who noted variations in egg size with time and locality.

Development appeared to be normal in most eggs, and proceeded with formation of a small perivitelline space, concentration of protoplasm into the blastodisc, and subsequent meroblastic cleavage (Figure 1 b-k). Development appeared identical to that for eggs of the Atlantic halibut (Rollefsen, 1935). Development was slowest at 2°C and did not proceed beyond germ ring formation, which occurred 7 days after fer-

tilization. At 4°C development did not proceed beyond formation of the neural keel, which occurred 10 days after fertilization.

In 1972, early mortality of eggs was extensive at temperatures of 6°C or greater. At 8 and 10°C, surviving eggs proceeded to early blastodermal cap formation 24 hours after fertilization. At 10 and 12°C, there was no further development after 3 and 2 days, respectively; at 12°C, development ceased at the 8-cell stage.

In 1973, increased egg survival provided the opportunity for more extended observations of development. Near the time of blastopore closure, between the 9- and 19-somite stages of development, 1 to 35 Kupffer's vesicles appeared. The greatest concentration of vesicles was in the usual location, ventral and anterior to the tip of the tail; others occurred anteriorly as far as the location of the first somites (Figure 1 1-m). The appearance of multiple Kupffer's vesicles also has been observed for some other species (Rosenthal and Fonds, in press).

Daily estimates of egg density were obtained during the course of development. Eggs fertilized and incubated in water of 33 $^{0}/_{00}$ S increased from an initial density of approximately 1.025, and stabilized at 1.026 between 200 and 300 hours after fertilization (Figure 2). Density determinations indicated that eggs and newly hatched larvae would be buoyant in salinities greater than about 31.5 $^{0}/_{00}$.The estimates of egg density generally correspond with observations by investigators of the International Pacific Halibut Commission; incubating eggs have been found in subsurface layers where water density is about 1.026-1.027 (Thompson and Van Cleve, 1936).

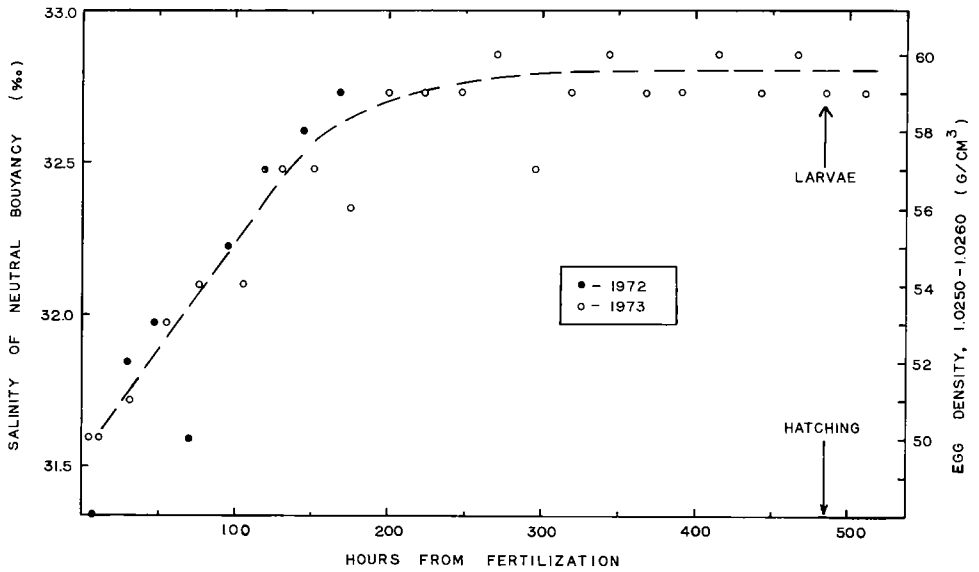
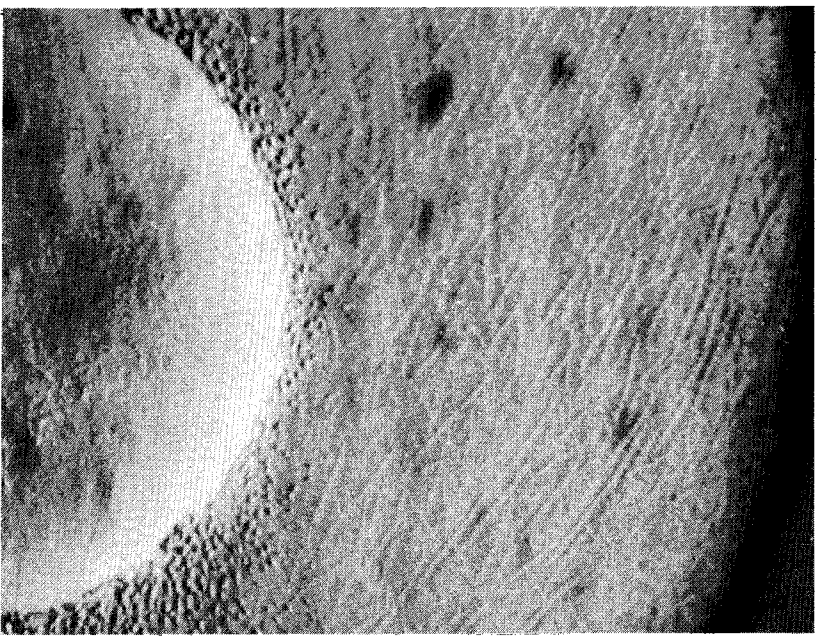
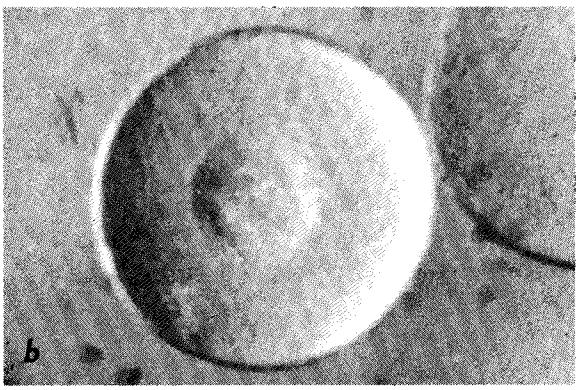


Fig. 2. Salinity of neutral buoyancy of Pacific halibut eggs fertilized and incubated in 33 $^{0}/_{00}$ S sea water, and corresponding egg and larval densities calculated at 5°C (U.S. Navy Hydrographic Office 1952). Incubation temperatures: 1972, 5.5°C; 1973, 5.0°C.

Hatching Efficiency

Twenty larvae hatched in 1972, and at least 594 in 1973 (278 from incubators, the balance from supplementary containers). Hatching success in 1973 varied between 7 and 40% in the various types of incubator employed (Table 1); it was lowest at 5°C and highest at 7°C. The standard incubators were not opened until after the

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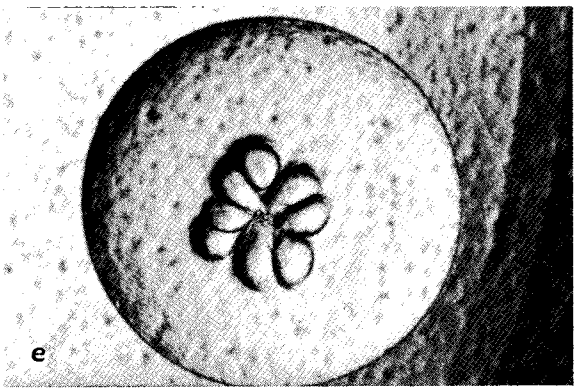
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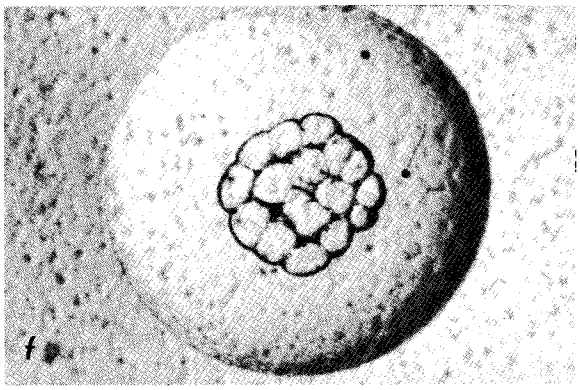
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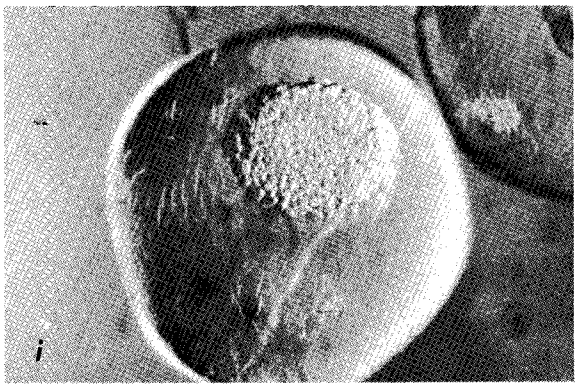
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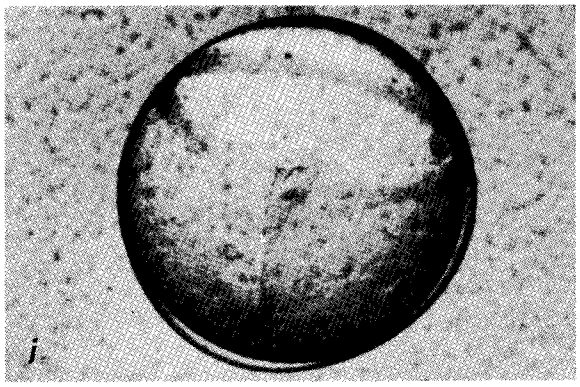
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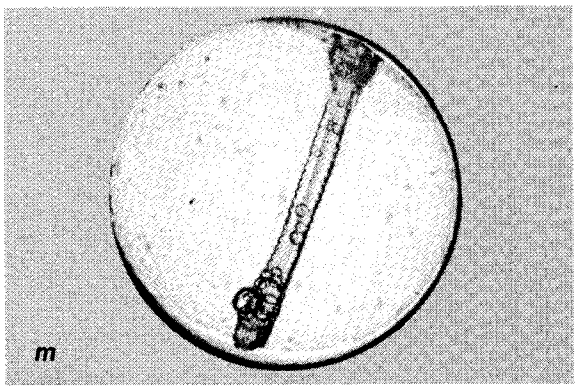
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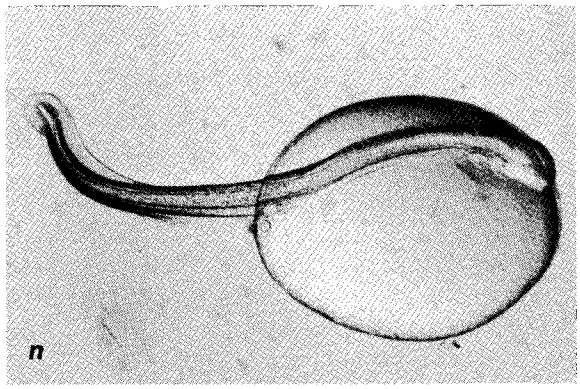
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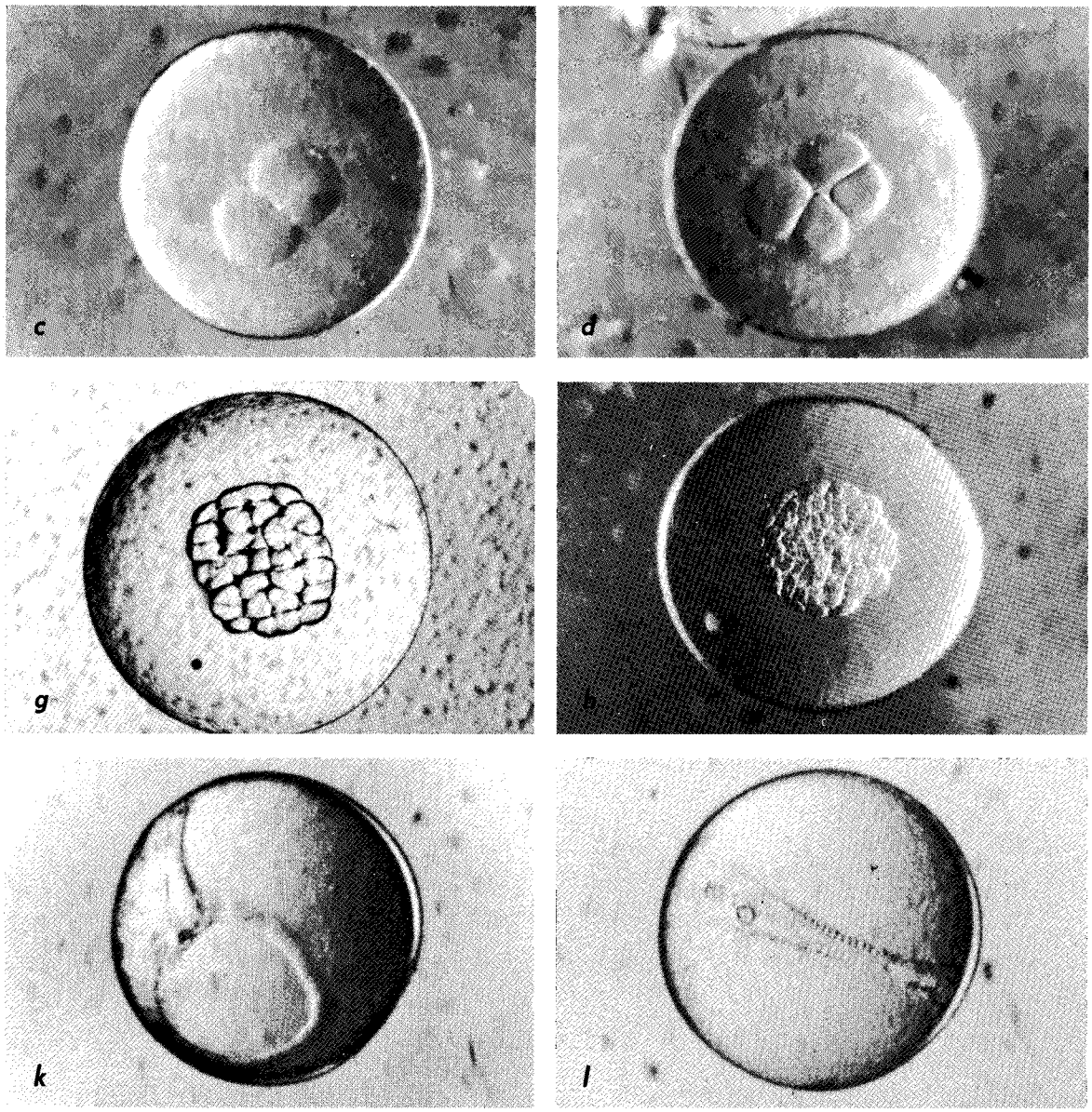

Fig. 1. Stages in development of the Pacific halibut egg: a) blastodisc prior to first cell division. Note the network of discontinuous striations on the chorion; b) blastodisc; c) 2-cell; d) 4-cell; e) 8-cell; f) 16-cell; g) 32-cell; h) 64-cell; i) early blastodermal cap; j) migration of germ ring, formation of neural keel; k) blastopore nearing closure; l) 14-somite embryo, multiple Kupffer's vesicles; m) early embryo, about 27 somites. Note cluster of vesicles near tail bud; two smaller clusters are also evident, one near the midbody region and another near the anterior somites; n, o) newly hatched larvae, total length about 6 mm.

time of blastopore closure. Extensive mortality apparently had occurred at an early stage of development. Observations on egg samples from open jars indicated that early mortality probably had occurred shortly after formation of the blastodermal cap.

Hatching occurred at temperatures of 5, 6, 7 and 8°C; it did not occur at 2, 4, 10 or 12°C. Incubation time to different developmental stages, for eggs held at various temperatures, is shown in Figure 3. Time to hatching ranged from 12.5 days (8°C) to 20 days (5°C) (Figure 4). At the time of hatching, embryos encircled less than the full diameter of the yolk. Five abnormal larvae, characterized by abnormal spinal curvature, were observed among 278 larvae removed from incubators.

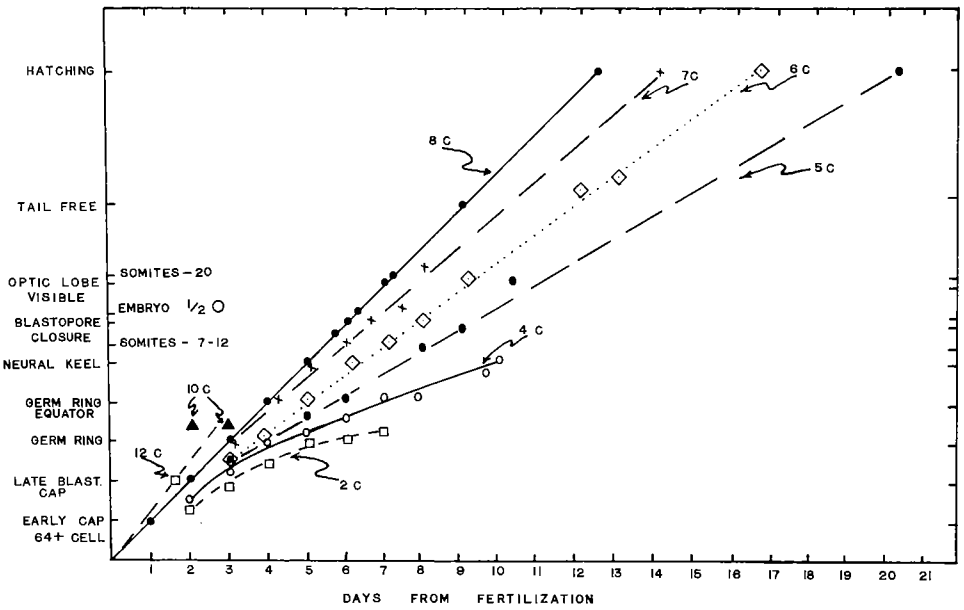

Fig. 3. Time to various stages of development in Pacific halibut eggs incubated at temperatures from 2° to 12°C and 33 °/₀₀ S. Stages of development for eggs held at 8°C were arbitrarily placed on a straight line.

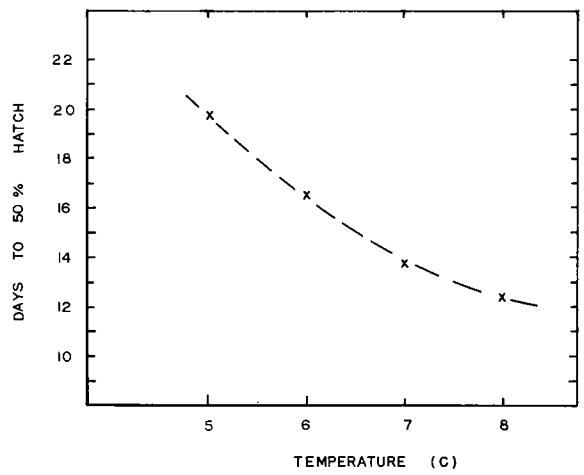

Fig. 4. Estimated time to 50% hatch of Pacific halibut eggs incubated at various temperatures in 33 °/₀₀ S sea water.

Posthatching Development

Newly hatched larvae lacked pigmentation, and were virtually transparent (Figure 1 n-o). They ranged in size from 5.33-7.79 mm total length, and were generally larger when incubated at 6°C (Table 2). Newly hatched larvae appeared similar to those marine pelagic larvae described by Budd (1940) and Shelbourne (1956). They closely resembled larvae of the Atlantic halibut (Rollefsen, 1935) and the smaller larvae of the petrale sole (Alderdice and Forrester, 1971). The anteroposterior length of the yolk sac of those larvae hatching initially at each temperature was greater than 50% of the total length of the larvae. The ratio of yolk length to total length decreased in larvae hatching on subsequent days (Table 2).

Table 2. Total length and anteroposterior yolk sac length in newly hatched Pacific halibut larvae. Incubation: 5°, 6°, or 7°C at 33 ‰ S.

Date	Temp. (°C)	No.	Average total length (mm)	Range (mm)	Average yolk sac length (mm)	Range (mm)	Ratio: yolk length/ larval length
March 5	5	5	6.69	6.39-6.80	3.51	3.36-3.77	0.52
6		14	7.18	6.15-7.79	3.29	2.63-3.77	0.46
March 2	6	10	6.95	6.72-7.38	3.61	3.44-3.93	0.52
3		20	7.24	6.72-7.71	3.39	2.87-3.69	0.47
Feb. 26	7	19	6.03	5.33-6.48	3.48	3.36-3.61	0.58
27		13	6.41	6.07-6.80	3.37	2.62-3.69	0.53
28		16	6.53	6.07-7.13	3.36	3.20-3.61	0.51
March 1		10	7.03	6.39-7.62	3.32	3.03-3.52	0.47

Larvae survived a maximum of 10 days after hatching. No attempt was made to feed larvae. They increased in length for 3-4 days after hatching and then regressed in size until death (Table 3). Regression in size appeared to be anomalous, and was

Table 3. Posthatching growth of Pacific halibut larvae at 5°, 6°, 7°C, 33 ‰ S.

Hours after hatching	Temp. (°C)	No.	Larval length (mm)		Yolk sac length (mm)		Ratio: yolk length/ larval length
			Mean	Range	Mean	Range	
12	5	14	7.18	6.15-7.79	3.29	2.63-3.77	0.46
36		6	7.20	6.56-8.03	2.92	2.62-3.28	0.41
60		9	7.31	6.97-7.71	3.01	2.79-3.28	0.41
84		7	7.60	7.38-7.87	3.08	2.87-3.28	0.41
108		7	7.48	6.89-8.61	2.94	2.71-3.28	0.39
156		3	7.38	6.56-8.03	2.95	2.87-3.11	0.40
12	6	20	7.24	6.72-7.71	3.39	2.87-3.69	0.47
36		5	7.46	7.21-7.87	3.28	3.03-3.36	0.44
60		10	7.31	7.05-7.62	3.08	2.95-3.36	0.42
84		17	7.45	7.21-8.20	3.05	2.79-3.20	0.41
108		10	7.79	7.38-8.20	3.08	2.87-3.52	0.40
132		10	7.63	7.38-7.87	3.00	2.79-3.28	0.39
156		8	7.55	6.89-8.12	3.06	2.95-3.28	0.41
180		7	7.47	6.56-8.12	2.80	2.62-3.03	0.37
204		4	7.46	6.80-8.20	2.66	2.62-2.71	0.36
24	7	26	6.72	6.07-7.62	3.34	3.03-3.61	0.50
48		7	7.61	7.30-8.12	3.24	3.11-3.28	0.43
72		7	8.00	7.71-8.36	3.13	2.87-3.28	0.39
96		10	7.98	7.54-8.52	3.11	2.95-3.36	0.39
120		11	7.97	7.38-8.61	3.11	2.95-3.28	0.39
144		8	7.49	6.56-8.36	2.90	2.79-3.03	0.39
168		4	7.64	7.21-8.03	2.83	2.62-2.95	0.37
192		3	7.76	7.71-8.12	2.90	2.71-3.03	0.37

associated with a deterioration of the trunk and tail posterior to the vent. Prior to death larvae were beginning to resemble the Stage 1 halibut larvae described by Thompson and Van Cleve (1936) as having average length of 11.2 mm and a yolk sac constituting 31% of the total larval length. In this experiment largest larval length attained was 8.6 mm and the lowest yolk sac/total length factor was 0.33. In addition the mouth was not clearly defined as in the Stage 1 larvae, being rudimentary, not easily visible and located ventral and posterior to the eyes and just above the anterior junction of the yolk sac.

Areas of pigmentation began to appear 2-4 days after hatching. Melanophores appeared first around the outer margin of the eye and intensified with continued development. They also appeared at the anterior insertion of the pectoral fin buds, and subsequently on the body slightly posterior to the pectoral fins.

Egg Fragility

During microscopic examination of eggs undergoing the first few cell divisions, very slight movements of the mechanical stage produced nonsynchronous oscillations of individual blastomeres. These observations lead to the conclusion that minor mechanical shocks could disrupt the process of early cell division. Anomalies during early development presumably associated with mechanical shock were observed particularly: 1) at the very early blastodermal cap stage; and 2) just prior to blastopore closure. In the former, there was a separation of cells in the cap and cessation of normal development. In the latter, ectodermal overgrowth of yolk was interrupted and the ectoderm shrank away from the location of the presumptive blastopore.

We assume that a measure of insulation from mechanical shock is provided to the developing egg by the perivitelline space. In the halibut egg the perivitelline space is very small, suggesting that in its natural environment the egg usually is not subjected to sudden accelerative or decelerative forces, which, if imparted to the chorion, would be transmitted directly to the developing embryo. Velocities to which eggs were subjected intermittently or continually in some of the incubators ranged between about 0.2-1 cm/second. Similar difficulties related to egg fragility and mechanical damage were experienced in a study of egg development in the flathead sole *(Hippoglossoides elassodon)* (Forrester and Alderdice, MS 1968). In that species, however, survival to hatching was high in almost all salinities tested. This could be attributed to insulation from mechanical shock produced by the very large perivitelline space in the flathead sole egg.

Other workers have reported that marine, pelagic eggs are fragile and difficult to incubate either in open or closed incubation systems: Atlantic cod, *Gadus morhua* (Earll, 1880); Atlantic halibut, *Hippoglossus hippoglossus* (Rollefsen, 1935); and petrale sole, *Eopsetta jordani* (Alderdice and Forrester, 1971). Experimental studies assessing the effects of environmental factors on egg development in controlled environments would benefit from an examination of the hydraulics of incubator design. In closed incubation systems the use of antibiotics is also recommended. The use decreases the need for water changes and the attendant risks of egg damage.

Much of the available information on development of Pacific halibut eggs has come from field studies which necessarily drew inferences from available field samples. The complementary information provided here may assist in defining the needs for further developmental studies in the laboratory.

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