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ASPECTS OF EARLY DEVELOPMENT AND ATTACHMENT OF FERTILIZED KING CRAB EGGS

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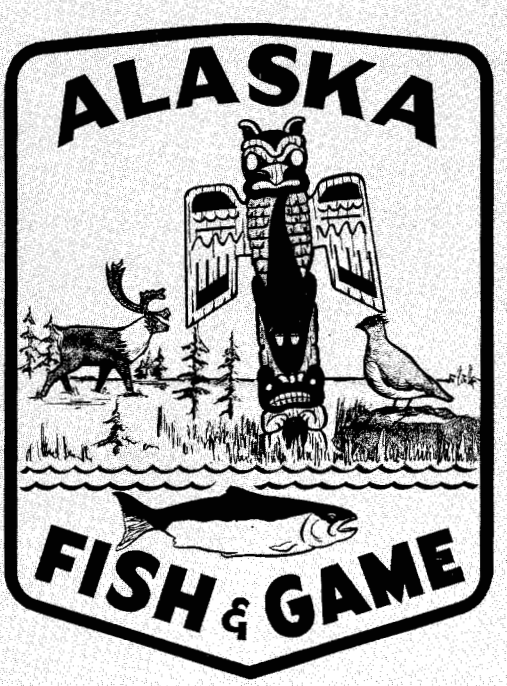

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ASPECTS OF EARLY DEVELOPMENT AND ATTACHMENT OF FERTILIZED KING CRAB EGGS[1/]

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INTRODUCTION

An early method of determining mating success of Alaskan king crabs, Paralithodes camtschatica, was limited to a gross examination of female king crabs to determine if they were ovigerous following the mating season. However, the presence of new eggs attached to a post-molt female king crab may not indicate that mating and fertilization have occurred (McMullen and Yoshihara, 1969). If king crab mating success is studied, eggs of a predetermined number of king crabs should be examined each brood year to determine if all ovigerous females are carrying fertilized eggs.

Egg development after fertilization can be determined by microscopic examination of unsectioned material. By this method, Marukawa (1933) presented drawings and listed representative days after fertilization at which each external cleavage stage might be observed. He did not, however, define the intervals of time associated with each cleavage stage.

This study is intended to expand upon Marukawa's work by presentation of the externally apparent cleavage stages as photographs, while more closely following egg development through the cleavage stages. Also, I wish to comment on the attachment of eggs to the female's pleopods.

METHODS AND MATERIALS

Collection of egg samples

Pre-molt female king crabs were captured at Kodiak Island during April, 1967. A group of 15 were held in floating screen boxes at Kodiak and immediately after molting were allowed to mate with a group of similarly captured males.

Dates of egg extrusion after mating were carefully observed and recorded. Groups of eggs were then removed from each female each day for 20 days beginning one day after extrusion. Removed eggs were fixed in Bouin's solution and stored in 70% alcohol after being passed through 30 and 50% solutions.

Water temperature was recorded at the king crab holding boxes, each day eggs were removed, from April 14 to May 9, 1969.

Determination of egg cleavage stages

About 100 eggs from each removal were examined for cleavage, using a low power microscope. Cleavage stages were recorded as present or absent for each group of eggs, but numerical composition of cleavage stages in each group was not determined. All groups of eggs were examined twice, and controversies between the first and second examinations were resolved with a third viewing.

Representative examples of each cleavage stage were photographically documented after all egg collections were examined.

Photomicrographs were taken with an American Optical unit attached to a binocular microscope. A 35mm camera back served as film holder. Eggs were photographed at a magnification of 60 and 80X at the film plane using Panotomic-X film. Two 100-watt bulbs furnished side lighting, and exposure time was 2 seconds. Individual eggs were photographed against a black background, while immersed in alcohol from their storage containers.

Egg attachment

Physical attachment of eggs to females' pleopods was studied by prying egg clutches apart and viewing them under the microscope. A single egg attachment was photographed in the manner previously described.

RESULTS AND DISCUSSION

Environment temperatures of developing eggs

Water temperatures within the crab holding boxes rose only 3^{o} F during the duration of this study (Figure 1). The water temperature was 37^{o} F when eggs were first extruded after mating. At the termination of the field program

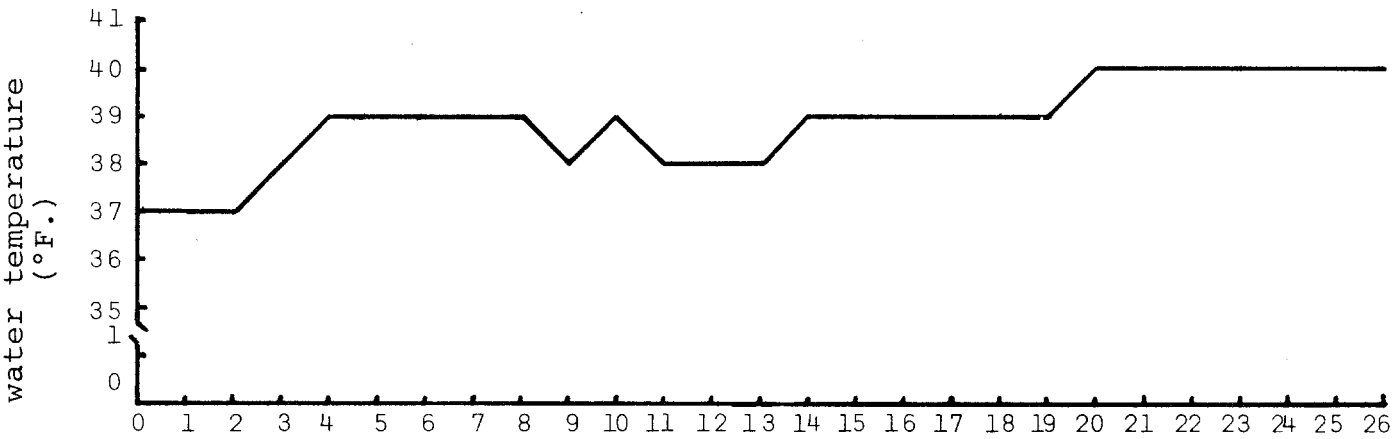

Figure 1. Temperatures of Kodiak harbor at location female king crab were being held as their eggs advanced from zygotes through 20 days of cleavage.

the water temperature had been stablilzed at 40° F for seven days.

Identification of cleavage stages

King crab eggs undergo superficial cleavage as described by Marukawa (1933), Barnes (1963) and Balinsky (1968). The first two divisions are of the nuclei and are not seen externally. Following the nuclear divisions, four nuclei migrate to the periphery of the egg and cleave to form eight identifiable blastomeres (Figure 2a). Eggs examined in this study displayed this cleavage stage 5-7 days after fertilization. The 16, 32 and 64 blastomere stages (Figures 2b, 2c and 2d) were achieved in rapid succession. By the twelfth day all eggs examined exhibited only the Morula stage (Figure 2e). The Blastula (final) cleavage stage (Figure 2f) was first observed at 15 days after fertilization. On that day the Blastula stage was present in 14 of the 15 egg collections. The Morula stage was last observed in one egg collection 18 days after fertilization, and only the Blastula stage was present in the 19 and 20 day collections.

Chronology of early egg development

Advancement of cleavage appeared to vary among eggs of individual females and between eggs of different females. Two or three cleavage stages were often seen among one group of eggs from a single female. However, the observed cleavage stages could be logically grouped (Table 1), although the possibility of several other arrangements of the data is not disputed. The first of the cleavage groups appearing in Table 1, appeared to exist to 5 days past fertilization, and was characterized by internal cleavage of one cell nucleus to four nuclei. Cleavage then appeared to advance rapidly from 6 to 9 days after fertilization. A different cleavage group was evident for the eggs removed on each of those days. Eggs removed at 6 days past fertilization generally exhibited 8 or 16 blastomere cleavage. Egg development advanced to the ninth day after fertilization at which time the 32 and 64 blastomere stages prevailed. The 64 blastomere and Morula stages characterized the eggs removed at 10 and 11 days after fertilization. Only the Morula stage was observed in 12 to 14 day-old eggs, then the Morula and Blastula existed simultaneously for three days. Finally, 18 to 20 day-old eggs were at the Blastula stage. No eggs were removed after 20 days, so the duration of the Blastula stage was not determined.

Development of a single king crab egg

Total days each cleavage stage was observed among the eggs removed

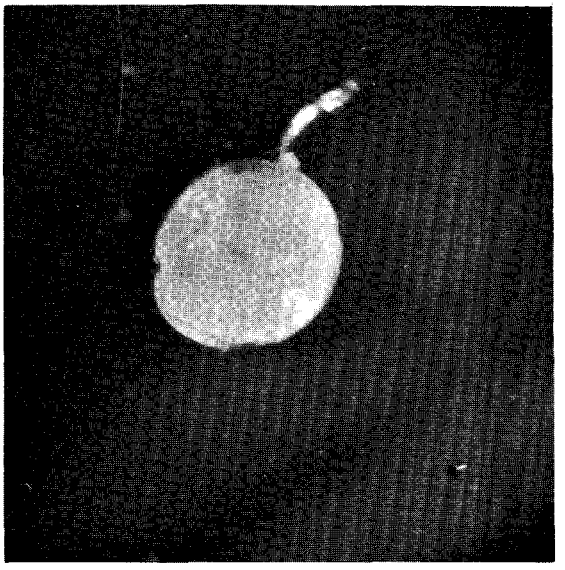

Figure 2a. King crab egg, 8 blastomere cleavage. 60X

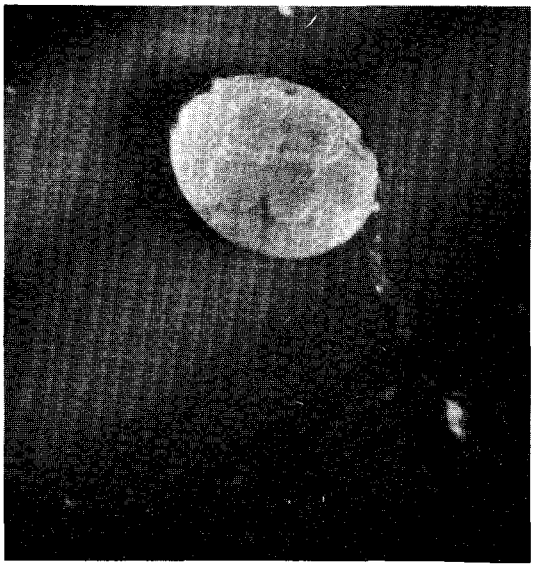

Figure 2b. King crab egg, 16 blastomere cleavage. 60X

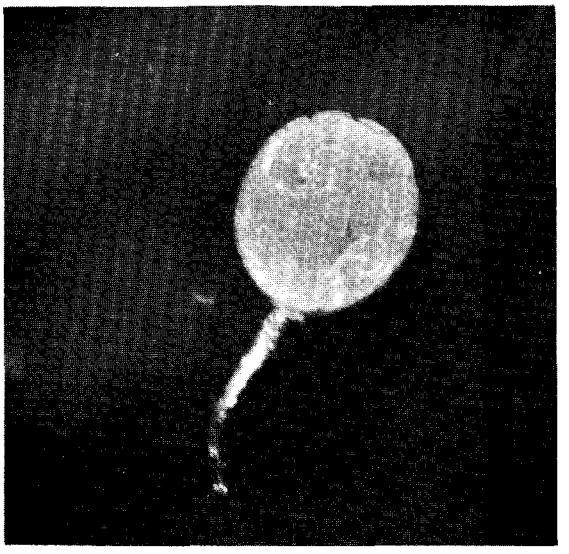

Figure 2c. King crab egg, 32 blastomere cleavage. 60X

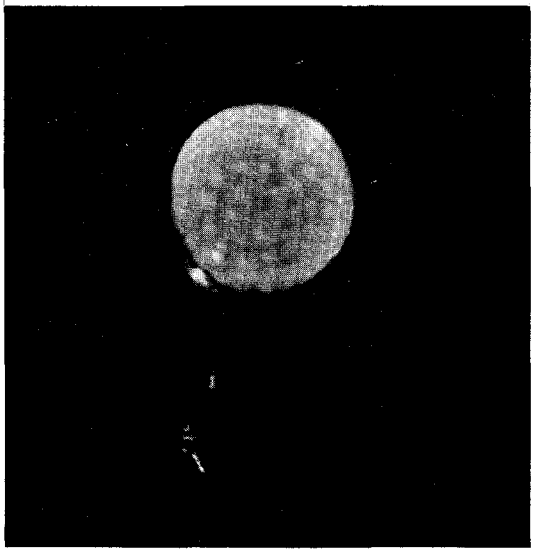

Figure 2d. King crab egg, 64 blastomere cleavage. 60X

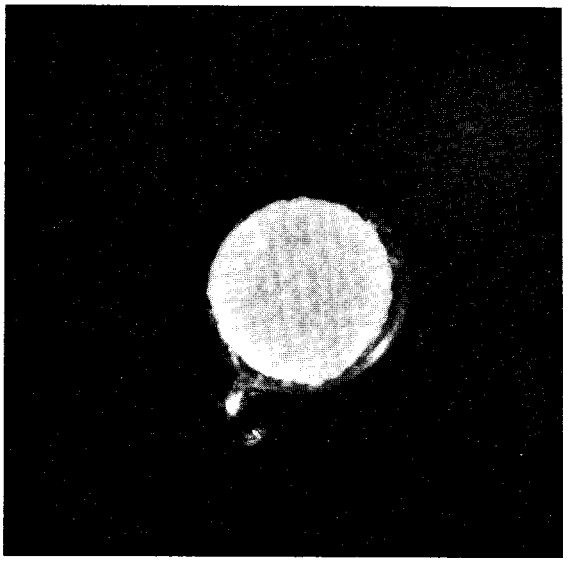

Figure 2e. King crab egg, Morula stage cleavage. 60X

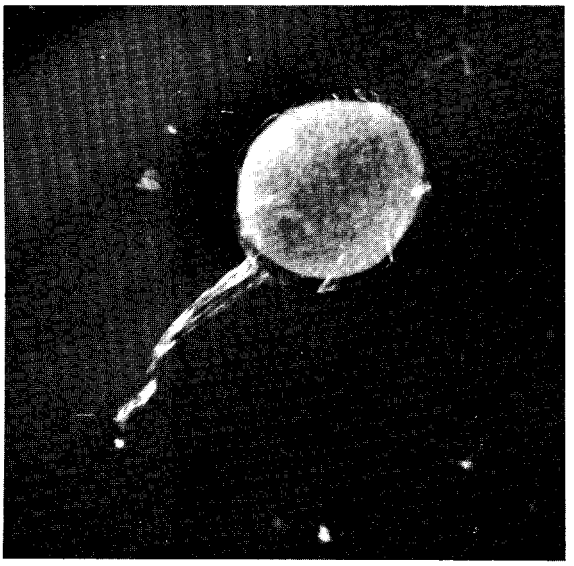

Figure 2f. King crab egg, Blastula stage cleavage. 60X

Table 1. Progression of king crab egg cleavage from 5-20 days following fertilization. The 8, 16, 32 and 64 blastomere, morula and blastula stages can be microscopically identified. The first two cleavage stages are nuclear, internal and not readily seen.

Specimen	Cleavage stages present each day																	
	Day 5	Day 6	Day 7	Day 8	Day 9	Days 10	11	Days 12	13	14	Days 15	16	17	Days 18	19	20
1	-	8	16	16	32	64	64	M	M	M	M	M				
			32	32	64	M	M				B	B	B	B	B	B
2	-	-	32	32	32	64	64	M	M	M	M	M	M	M		
			64	64	64	M	M					B	B	B	B	B
3	-	-	8	16	32	32	64	M	M	M	M	M	M			
			16	32	64	64	M				B	B	B	B	B	B
4	-	8	8	16	16	64	64									
		16	16	32	32	M	M	M	M	M	M	M	M			
		32	32	64	64						B	B	B	B	B	B
5	-	8	8	16	32	64	64									
		16	16	32	64	M	M	M	M	M	M	M	M			
			32	64	M						B	B	B	B	B	B
6	-	-	8	16	32	64	64									
			16	32	64	M	M	M	M	M	M	M	M			
				64							B	B	B	B	B	B
7	-	-	8	16	32	64	M	M	M	M	M	M	M			
			16	32	64	M					B	B	B	B	B	B
8	-	-	8	16	32	64	M	M	M	M	M	M	M			
			16	32	64	M					B	B	B	B	B	B
9	-	16	32	64	64	64										
		32	64	M	M	M	M	M	M	M	M	M	M			
		64									B	B	B	B	B	B
10	-	8	8	16	32	64										
		16	16	32	64	M	M	M	M	M	M	M	M			
			32	64	M						B	B	B	B	B	B
11	-	8	16	32	32	M	M	M	M	M	M	M	M			
		16	32		64						B	B	B	B	B	B
12	-	8	8	16	32	64	M									
		16	16	32	64	M	M	M	M	M	M	M	M			
			32	64							B	B	B	B	B	B
13	8	8	16	32												
	16	16	32	64	M	M	M	M	M	M	M					
			64	M							B	B	B	B	B	B
14	-	8	16	32	32	64	64	M	M	M	M					
		16	32	64	64	M	M				B	B	B	B	B	B
15	-	-	-	16	32	32										
				32	64	64	M	M	M	M	M	M	M			
				64		M					B	B	B	B	B	B

from the 15 female king crabs are included in Table 2. The totals for all cleavage stages are added to arrive at a grand total. Individual totals are then divided by the grand total to determine the relative duration (expressed as a percentage) of each cleavage stage. Each percentage is then multiplied by 16, the actual days eggs were removed from females. Resulting products represent the number of days that may be required to advance king crab egg cleavage through the general stages at 37^{o} to 40^{o} F water temperature. Eight blastomere cleavage occupies 4.9% of the 16-day period (see Table 2), or .78 days (Table 3). Time periods occupied by the next three stages increase from 1.42 to 2.26 days. The duration of the Morula is 5.58 days, and the 4.12 Blastula-days is open ended because egg development data was not available beyond 20 days after fertilization.

Physical attachment of king crab eggs

Attempts to define the method of king crab egg attachment to the female's pleopods have yielded only preliminary results to date. A single egg membrane includes a strand which becomes entangled with the strands of other eggs, forming groups of eggs. Egg groups become attached to pleopod hairs by the curling action of the egg strands (Figure 3). The existence of an adhesive is unknown at this time.

Use of study results

Studies concerning the reproduction of king crabs will be originated and continued until the importance of brood stock size, sex ratios, and the relationship between parent stocks and recruitment have been established. These studies will be conducted during the spring months when molting and mating of female king crabs occur. Then ability to identify cleavage in fertilized eggs will prevent the error of naming all egg bearing females as productive. Knowledge of rates of early egg development may also provide a time index for researchers studying calcification of new exoskeletons or rate of movement from known mating grounds.

Table 2. Duration of presence of the principal external cleavage stages of king crab eggs observed from 5-20 days following their fertilization.

Specimen	Number of days present					
	blastomere stage					
	8	16	32	64	Morula	Blastula
1	1	2	3	3	7	6
2	0	0	3	5	9	5
3	1	2	3	3	8	6
4	2	3	3	4	8	6
5	2	3	3	4	9	6
6	1	2	2	4	8	6
7	1	2	2	2	8	6
8	1	2	2	2	8	6
9	0	1	2	5	10	6
10	2	3	3	3	9	6
11	1	2	3	2	8	6
12	2	3	3	3	8	6
13	2	3	2	2	7	6
14	1	2	3	4	6	6
15	0	1	3	3	8	6
Totals	17	31	40	49	121	89[1/]
Grand total days			347			
Percent total	4.9	8.9	11.5	14.1	34.9	25.7

1/ Blastula days open-ended because egg collection terminated 20 days following their fertilization

Table 3. Possible duration of time required for a king crab egg to pass through the six external cleavage stages between 5-20 days following fertilization.[1/] Expected values are derived from observations of 300 egg collections from 15 females. During study, observed temperature of egg environment ranged from 37-40°F.

	Cleavage stages					
	8 blasto-mere	16 blasto-mere	32 blasto-mere	64 blasto-mere	Morula	Blastula
Days present	.78	1.42	1.84	2.26	5.58	4.12 +
Cumulative days	5.78	7.20	9.04	11.30	16.88	21.00[2/]

1/ External cleavage first seen at 5 days after fertilization. Earlier cleavage nuclear only.

2/ Cumulative days extend through 20 days.

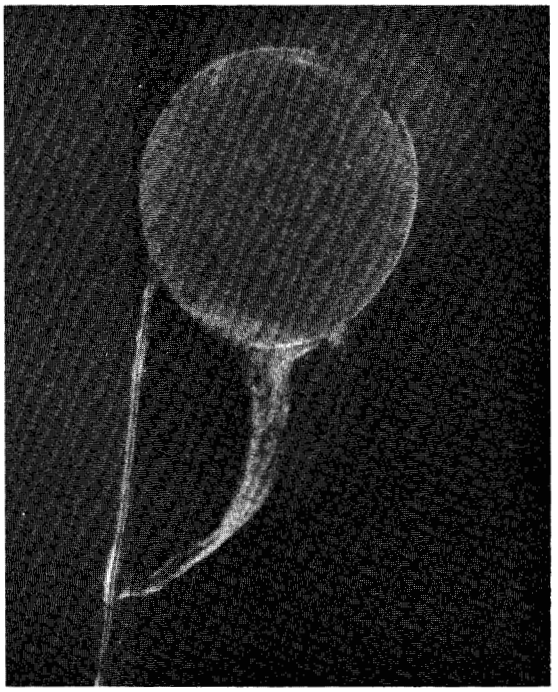

Figure 3. Attachment of single king crab egg to a pleopod hair. 80X

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