Some Aspects of Reproductive Biology of the Crab *Chionoecetes bairdi*: Final Project Report

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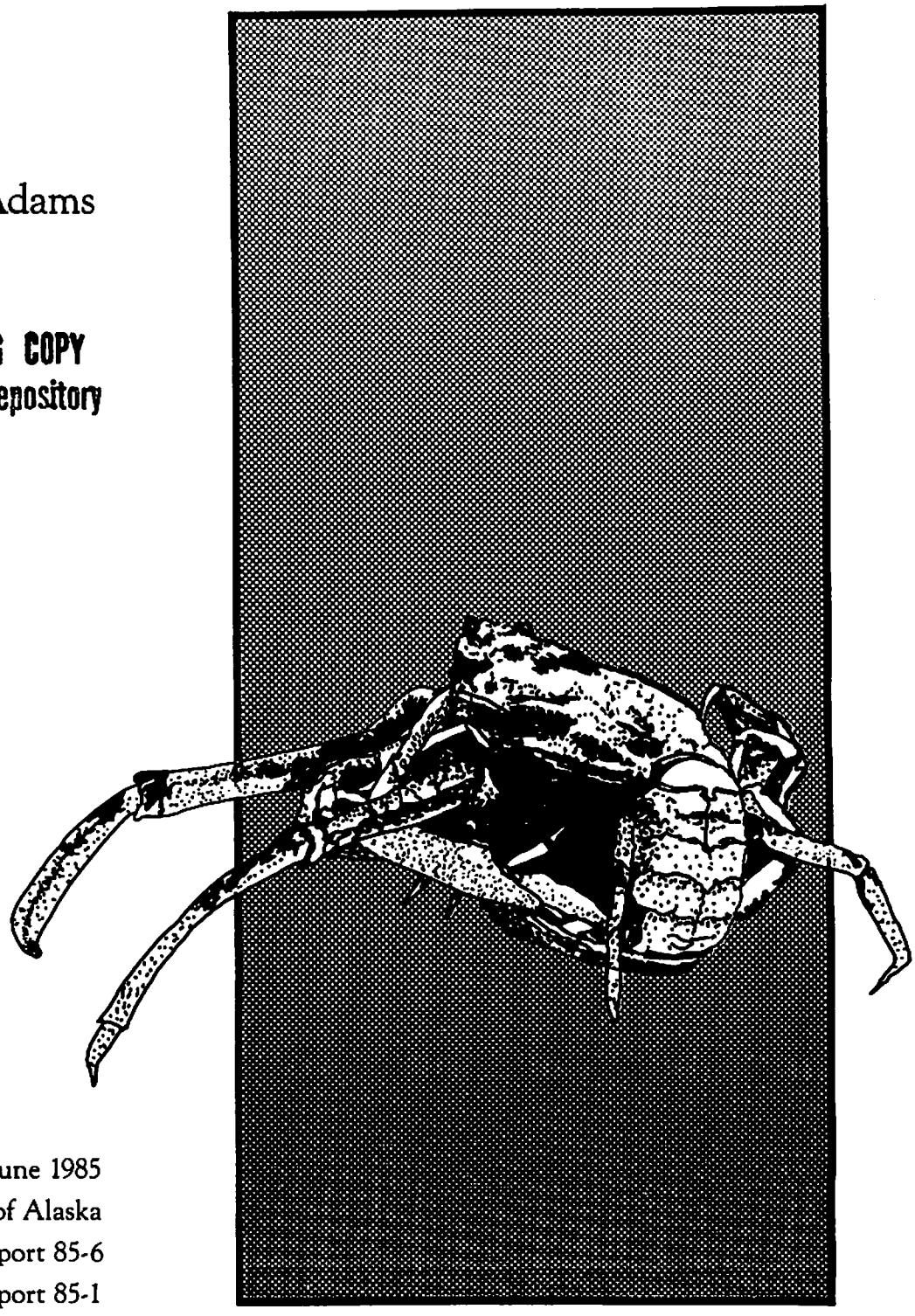

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Some Aspects of the Reproductive Biology of the Crab Chionoecetes bairdi: Final Project Report

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INTRODUCTION

The objective of this project was to obtain information on the reproductive biology of the economically important snow or tanner crab, Chionoecetes bairdi. Such information was recognized as essential to provide some guidance to the two agencies, the Alaska Department of Fish and Game and the North Pacific Fishery Management Council, that manage the fishery.

Alaska Sea Grant Report 83-1, February 1983 summarized findings of this project. In that report, the following six topics were addressed:

1. the length of time prior to and following molting that a female will attract breeding males;

2. the relationships between male parent size, the amount of sperm in the female's spermatheca shortly after egg extrusion, and the number of eggs produced by the female;

3. the frequency of mating and the size of male mates for old-shell females;

4. the number of eggs and viable egg clutches extruded by a female relying on stored sperm as compared with recently mated females;

5. the percentage of barren females that occur in a random sample of old-shell females; and

6. whether males mate more than once in a breeding season.

Other relevant information was obtained during the course of the project. Some of this material has been published in the Lowell Wakefield Fisheries Symposia Series (Adams 1982; Paul 1982), in other journals (Adams and Paul 1983), or is presently in preparation or review for publication. This report summarizes additional work conducted between November 1982 and June 1983.

The American fishery for C. bairdi harvests only male crabs, so it was crucial to determine the minimum size at maturity in males. Only then could even an educated guess be made about the possible effect of the catch size limit (in terms of carapace width restrictions) on crab stocks. Either underprotection or overprotection of C. bairdi could have negative effects on the fishery and the market. If underprotected, male crabs could be harvested before reaching maturity and the reproductive potential of the species could be severely reduced. If overprotected, the crab stocks would not be stressed by fishing pressure, but fishermen could initially realize a much lower catch of legal-sized crabs per unit effort.

In assessing minimum size at maturity in male C. bairdi, these four criteria were given primary consideration:

1. whether mating yielded viable zygotes;

2. whether the distal ends of the male's first pleopods (the intromittent organ) could spread apart to a sufficient

degree for proper insertion into the female's gonopores;

3. whether fully formed spermatozoa were produced and encapsulated within spermatophores; and

4. whether all accessory reproductive gland secretions were present in the reproductive tract.

METHODS

Large male and all female crabs were captured in otter trawl tows or by scuba at Womens Bay and Chiniak Bay, Kodiak by biologists William Donaldson (in 1979 - 1980), and William Colgate and David Hicks (1981 and 1982) of the Alaska Department of Fish and Game. Captured crabs were shipped from Kodiak either in the hold of the R/V Resolution or by air charter to the University of Alaska's Marine Science Center in Seward. Small male crabs were captured in Resurrection Bay, Seward by beam trawl from a chartered fishing vessel.

LABORATORY PROVISIONS AND OBSERVATIONS

Holding facilities at the Seward Marine Science Center included four cylindrical 1,895 liter capacity plastic tanks and three cylindrical 1,620 liter capacity plastic tanks (all manufactured by Frigid Units; Toledo, Ohio). Tanks received a continuous supply of sea water (salinity 30 to 33 ppt and temperature 4° to 6°C during the study) that was pumped into the laboratory from a depth of 60 m in Resurrection Bay.

Most mating experiments were conducted in a 168 liter aquarium with integral temperature control unit. A 120 liter capacity wooden tank with gravel substrate was used for approximately ten of the matings. Still other experiments were conducted in the 1,895 liter tanks. All detailed observations were made at night when external distractions could be limited.

Crabs were usually fed herring twice each week; however, pollock, shrimp, rock sole, and sablefish were substituted as food whenever possible.

HISTOLOGICAL TECHNIQUES

Live crabs were killed immediately prior to removal of their reproductive organs and the latter were placed directly into fixative. Various fixatives (including 10 percent neutral buffered formalin, Zenker's fluid (Gray 1954), Helly's fixative (Gray 1954), Bouin's fixative (Gray 1954), and calcium acetate - formalin (Clark 1973) were utilized. Fixed tissues were embedded in paraffin following a normal dehydration procedure of graded concentrations of ethyl alcohol (30, 45, 60, 75, 90, and 100 percent) followed by xylene, and lastly xylene:paraffin. Embedded tissues were serially sectioned at 5 µm to 10 µm thicknesses on a Jung microtome and the resulting sections were mounted on glass slides with Mayer's albumen adhesive (Gray 1954).

Histochemical stains employed in this study included: Mallory's triple stain for connective tissues (Mallory 1938); Mowry's combined Alcian Blue/Periodic Acid-Schiff method for carbohydrates (Putt 1972); Phifer's Alcian

Blue/Periodic Acid-Schiff/Orange G stain (Phifer 1973); and Himes and Moriber triple stain (Himes and Moriber 1956).

Sample size was 90 individuals for each method employed. Stained thin sections were examined under a Zeiss standard light microscope.

SPERM COUNTS

A spermatheca was excised from the female, dissected longitudinally, and any enclosed sperm and secretions were flushed from it with 4°C filtered sea water. The solidified matrix was macerated in 4°C filtered sea water and the settled volume of sperm and secretions was diluted by a factor of 10^3:1 or 10^4:1 with cold filtered sea water. Immediately after stirring, two subsamples were drawn and placed in a hemacytometer (with Fuchs-Rosenthal counting grid) and spermatozoa were allowed to settle for five minutes. Counts of intact sperm (those with brightly transmissive acrosomal vesicles) were made at 250X under a light microscope. A quantitative estimate of the number of sperm in one spermatheca was calculated with the following formula:

Original homogenate volume	X	Dilution factor	X	Mean sperm count in the two hemacytometer chambers	=	Total number of sperm

RESULTS AND DISCUSSION

MATING AND PRODUCTION OF VIABLE ZYGOTES

In the laboratory, 118 primiparous and 15 multiparous C. bairdi matings were observed. No male smaller than 110 mm mated with a multiparous female. However, males smaller than that size proved capable, in 49 instances, of successfully mating with primiparous females. In fact, the smallest male C. bairdi that mated with a female which subsequently produced viable zygotes measured only 55 mm in carapace width. Although males smaller than 55 mm carapace width also performed some actions that are unique to mating behavior, they were unable to copulate with any females. All males larger than 60 mm that mated with primiparous females did so successfully, with the single restriction that the mating had to occur within 28 days of the female's maturity molt. As shown in Table 1, ten out of fourteen (71 percent) matings that involved males smaller than 60 mm carapace width culminated in the extrusion of fertilized eggs by primiparous females. Development of the eggs was not followed beyond early gastrulation; however, cleavage of the fertilized eggs did proceed to that point.

Sperm production did not appear to be the factor limiting the reproductive success of males with carapace widths of 50 to 59 mm. The number of sperm remaining in spermathecae of primiparous females that had mated with these small males was not significantly different from that noted for females which mated with larger males (Table 2). Furthermore, testes from males of carapace widths 50 to 59 mm contained fully-formed spermatozoa (as determined by microscopic examination of histologically stained sections).

Since the reproductive tracts of small males contained mature sperm and some individuals even mated successfully, it seemed possible that the physical

Table 1. Early development of eggs extruded by primiparous Chionoecetes bairdi females that mated with small males

Male carapace width (mm)	Viable zygotes produced	Female number
55	Yes	288
55	Yes	273
55	Yes	231
55	No	561
55	Yes	598
56	No	287
57	Yes	275
57	Yes	237
57	No	261
59	Yes	221
59	Yes	224
59	Yes	252
59	No	256
59	Yes	261

Table 2. Number of spermatozoa remaining in single spermatheca following egg extrusion by primiparous Chionoecetes bairdi females mated to males of known carapace widths*

Male carapace width (mm)	Sperm count in one spermatheca			
	Mean	Standard-deviation	Range	Sample size
50 - 59	465,600	586,500	48,000 - 2,010,000	11
65 - 75	580,290	421,357	140,000 - 1,471,000	8
76 - 85	921,500	724,350	40,000 - 2,110,000	5
86 - 95	554,400	424,487	105,000 - 1,730,000	11
96 - 105	1,174,166	436,902	540,000 - 1,680,000	6
106 - 115	1,645,000	1,514,000	250,000 - 4,900,000	10
116 - 125	904,785	812,167	98,000 - 2,820,000	14
126 - 135	576,000	371,646	103,000 - 1,087,000	7
136 - 140	595,000	-	-	1

*All values for size groups 65 - 140 mm were reported in Table III of the 1982 final project report (Paul et al., 1983).

process of intromission was beyond the capability of some individuals. A closer examination of morphological and behavioral factors was obviously in order.

FEMALE INTER-GONOPORE DISTANCE VERSUS SPREAD OF THE MALE FIRST PLEOPODS

C. bairdi, like other brachyruan decapods, is noted for true intromission. That is, the male inserts his intromittent organs into the female's gonopores in order to internally transfer gametes to her reproductive tract. Therefore, the tips of the male's intromittent organs (the modified first pair of pleopods) must spread far enough apart to properly insert into the female's gonopores if the male is to couple with her. This distance had a maximum value of 23.7 mm in primiparous females (sample size = 89) and 26.3 mm in multiparous females. Male measurements indicated that 33 percent of those with carapace widths 50 to 59 mm (sample size = 15) could not physically couple with the largest primiparous female measured. However, all males in this size group could have mated with the smallest primiparous female measured. The smallest males which could spread their pleopods adequately (14.5 mm) for intromission with the smallest primiparous female measured 29 mm carapace width.

More noticeably, only 10 percent of the 50 to 59 mm wide males could have physically performed intromission with the largest multiparous female. Again, all males in this size group could spread their pleopods sufficiently to copulate with the smallest multiparous female. During the three seasons of behavioral observations, no males smaller than 110 mm carapace width mated with a multiparous female. As previously observed (Adams 1982), multiparous females are quite adept at escaping from the grasps of males that do not exceed their own size. This is significant in reproduction of the species because it means multiparous female C. bairdi are capable of thwarting the mating attempts of small but mature males. If this applies outside the laboratory then the crab populations must contain males larger than 110 mm carapace width to assure continued reproductive contributions of multiparous females.

Although primiparous females are initially (during the first hour after their molt to maturity) unable to offer much resistance to male mating attempts, these females also become increasingly evasive and resistive as the elapsed time since their maturity molt increases (Table 3).

HISTOLOGICAL CHARACTERISTICS OF MALE REPRODUCTIVE TRACT SECRETIONS

The reproductive tracts of C. bairdi males have two functions in addition to production of functional gametes. Cells of the middle and posterior vas deferens produce secretions that stain differentially in compound stains (such as those employed in this study). The second auxiliary function of the male reproductive tract, which entails transferring male gametes and secretions directly into the female reproductive tract, is performed by the intromittent organ complex. The latter group of organs includes the first and second pairs of pleopods and the pair of penes which insert into the base of the first pleopods.

Table 3. Summary of grasps, matings, and escapes involving Chionoecetes bairdi primiparous females

Type of outcome	Male carapace width (mm)	Elapsed time (in hrs) between female's puberty molt and observed behavior: -1440 to -25	-24 to -0.1	+0.1 to +24	+25 to +120	+121 to +240	+241 to +720	+721 to +8000
Mating	30 - 49	0[a]	0	0	0	0	0	0
		0[b]	0	0	0	0	0	0
	50 - 79	0	0	31	9	0	0	0
		0	0	40	36	0	0	0
	80 - 179	0	0	19	5	3	36	14
		0	0	86	63	37	42	18
Escape	30 - 49	0	0	6	3	0	0	0
		0	0	60	50	0	0	0
	50 - 79	0	4	12	11	0	5	2
		0	67	16	44	0	71	29
	80 - 179	0	0	0	0	0	14	9
		0	0	0	0	0	16	11
Female escapes during male vs. male combat	30 - 49	0	0	1	1	0	0	0
		0	0	10	17	0	0	0
	50 - 79	0	0	5	2	1	0	0
		0	0	6	8	100	0	0
	80 - 179	0	0	1	0	0	4	0
		0	0	5	0	0	5	0
Simple releases	30 - 49	0	3	3	2	0	0	1
		0	100	30	33	0	0	100
	50 - 79	1	2	29	3	0	2	5
		100	33	38	12	0	29	71
	80 - 179	50	11	2	3	5	31	56
		98	100	9	37	63	36	71
Total grasps	30 - 49	0	3	10	6	0	0	1
	50 - 79	1	6	77	25	1	7	7
	80 - 179	51	11	22	8	8	85	79

[a] Absolute frequency for noted event during the time span indicated.

[b] Percent of all grasps ended in noted event for appropriate male size group and during time indicated.

The results obtained from histology of the reproductive tracts (Table 4) and mating behavior observations suggest a correlation between complete functioning of the reproductive tract (production of all portions of the secretory matrix material including constituents of the spermatophore wall) and successful mating potential. All male C. bairdi that mated successfully were producing spermatozoa encapsulated by spermatophores. Furthermore, all such males contained a full complement (three distinctly staining components) of accessory reproductive gland secretions in their reproductive tracts. Larger males (up to 179 mm carapace width) contained no more than three major secretory products.

In Mallory's triple stain, the spermatophore wall became colored orange to red on its inner surface and blue on its outer surface. The outer spermatophore surface stained red to magenta in Mowry's AB/PAS and pink in Phifer's AB/PAS/OG while the inner portion of the spermatophore wall stained blue in both of these stains. The staining reactions are indicative of complex carbohydrates, acidophilic and basophilic compounds, and neutral mucosubstances. Himes and Moriber triple stain demonstrated that proteins exist throughout the spermatophore wall.

The distinct constituents of matrix fluid in the tract lumen stained red (acidophilic), gold (acidophilic), and blue (basophilic) in Mallory's triple. Red, blue, and gold staining occurred with Mowry's AB/PAS and Phifer's AB/PAS/OG, again indicating the presence of complex carbohydrates, neutral mucosubstances, and acidophilic compounds. Polysaccharides and proteins were demonstrated by Himes and Moriber triple staining in the reproductive tract secretions.

A description of the spermathecal sperm plug (a hardened mass of spermatophores plus matrix material), which is produced during intromission, was given in the October 1982 final report (Paul et al. 1983). As noted by several investigators of invertebrate reproduction (Spalding 1942, Knudsen 1960, Johnson 1980), the secretions that form the plug certainly play a role in lubrication of the reproductive tract and in assimilation and transportation of spermatophores. A possible nutritive role has also been suggested (Nishioka 1959) in instances where the spermatophores are stored in viable condition over a long time period. We (Paul et al. 1983) observed that female C. bairdi can retain viable spermatozoa in their spermathecae for as long as two years.

SUMMARY

All results from this study indicate that the minimum legal catch size of 140 mm carapace width allows for the protection of C. bairdi males which have developed full reproductive potential. Such males have mated with both primiparous and multiparous females in the laboratory, and viable zygotes have been produced from their mating.

As the size (carapace width) of the male approaches that of the female, both the male's size and elapsed time since the female's molt to maturity become limiting factors for reproductive success (Adams 1982). Multiparous females were capable of evading and resisting mating attempts by males of their own size (80 to 110 mm carapace width) or less. Therefore, it is possible that severe declines (during the late spring breeding season) in the abundance of

Table 4. Spermatophore wall formation and production of reproductive tract secretions by small male Chionoecetes bairdi

Male carapace width (mm)	Sample size	Spermatophore walls present (percent of sample)	Percent occurrence of number of moieties in reproductive tract secretions							
			Mallory's triple				Mowry's AB/PAS and Phifer's AB/PAS/OB			
			0	1	2	3	0	1	2	3
21 - 25	13	0	54	46	0	0	54	46	0	0
26 - 30	6	0	33	33	33	0	33	33	33	0
31 - 35	6	0	17	50	33	0	17	50	33	0
36 - 40	3	0	0	0	67	33	0	0	33	67
41 - 45	3	33	0	0	33	67	0	0	33	67
46 - 50	5	80	0	0	20	80	0	0	20	80
51 - 55	5	100	0	0	20	80				100
56 - 60	9	100	0	0	11	89				100
61 - 65	20	100				100				100
66 - 70	20	100				100				100

males larger than 110 mm carapace width could negatively effect the reproductive output of multiparous females.

If a male mates with a primiparous female more than 28 days after her maturity molt then it is unlikely that viable offspring will develop (Paul et al. 1983). Also, as the elapsed time since a primiparous female's maturity molt increases, it becomes more difficult for a male that is smaller than the female to mate with her.

The lower size limit for reproductively active males is determined by two characteristics: (1) the ability of the male to physically copulate with a female; and (2) the complete functioning of the male reproductive tract (production of all accessory reproductive gland secretions and enclosure of spermatozoa in spermatophores). There can be no reproduction without development of both of these traits. The smallest male C. bairdi that mated successfully measured 55 mm carapace width.

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