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Biochemistry of Red King Crab (*Paralithodes camtschaticus*) from Different Locations in Alaskan Waters

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Executive Summary

Samples of female red king crabs (*Paralithodes camtschaticus*) from Bristol Bay and the Pribilof Islands, Kodiak Island, and Petrel Bank were taken in June, September, and November 2006, respectively. Whole crab weight and the usual biometric parameters were recorded as well as weight of the ovary, hepatopancreas, and eggs. Moisture, ash, total lipid, and protein content of each organ were determined to investigate whether differences in biochemistry existed among crab from different areas and to initiate annual examination of these values to determine the extent of year-to-year variability.

Relative gonadosomatic index values of crab from the different areas were consistent with the time interval between date of capture and typical mating season in their respective areas. Therefore, gonadosomatic index values were used as the estimator of the stage of annual reproductive cycle, as it was not possible to determine the stage of embryonic development. Crabs from Kodiak Island and Petrel Bank were further along in the reproductive cycle than crabs from Bristol Bay or the Pribilof Islands. The weight of the hepatopancreas relative to whole weight was the same in samples from the four areas.

Crabs from Kodiak Island and Petrel Bank had higher lipid content in the hepatopancreas and ovary than crabs from Bristol Bay and the Pribilof Islands on a dry weight basis. Eggs of crabs from Kodiak Island had higher lipid content than crab from other areas. This may indicate that crabs from Kodiak Island had higher lipid content at fertilization.

The results of this preliminary study indicate that a more extensive experiment, in which crab would be taken from the same area throughout the yearly cycle, would greatly benefit our understanding of the biochemical changes associated with reproduction. Determination of the fatty acid profiles and lipid classes of the lipid from such samples would also provide valuable information on the nutritional status of this species.

Acknowledgments

For providing samples and collected biometric information on crabs from Bristol Bay and the Pribilof Islands, I thank personnel from the Resource Assessment and Conservation Engineering Division of the Alaska Fisheries Science Center. For providing samples and collecting biometric information on crabs from Kodiak Island and Petrel Bank, I thank Kally Spalinger and other personnel of the Kodiak office of the Alaska Department of Fish and Game Shellfish Division, and Ryan Burt of the Dutch Harbor office of the Shellfish Division, respectively.

Introduction

The decline of the red king crab (*Paralithodes camtschaticus*) population and its failure to recover despite two decades of restricted fishing is an enduring and perplexing problem for those tasked with the management of biological resources in the Bering Sea and the Gulf of Alaska. Commercial catch peaked in 1980 at 130 million lb but dropped quickly thereafter and has remained low (NPFMC 2008). The population has averaged 12.9 million legal-sized males over the last 5 years in the Bristol Bay and Pribilof Islands areas (Chilton et al. 2008).

While there is no widely accepted consensus as to what caused the collapse of the king crab fishery or why it has failed to recover, one possibility is climatic change. Anderson and Piatt (1999) noted the occurrence of an ocean climate regime shift from cold to warm in the Gulf of Alaska in 1977 that resulted in a decrease in fatty forage species. Conners et al. (2002) reported a similar climate change in the southeastern Bering Sea in the early 1980s. Crabs may not be obtaining optimal levels of certain nutrients such as essential fatty acids that are necessary for reproduction.

The role of nutrition of female crustaceans on reproductive success has been studied, especially for species associated with commercial aquaculture (Harrison 1990, Wouters et al. 2001). Ovarian development in crustaceans during the annual reproductive cycle is not only marked by an increase in mass, but also changes in biochemical composition (Teshima et al. 1988, Harrison 1990, Smith et al. 2004).

It is believed that dietary lipids accumulate in the hepatopancreas of decapods and are then transferred to the ovary during the annual reproductive cycle (Teshima et al. 1988, Harrison 1990). A diet high in polyunsaturated fatty acids resulted in an increase in ovary weight, fecundity, and egg hatch rate in Chinese mitten crab (*Eriocheir sinensis*) (Wen et al. 2002). Fecundity was correlated with eicosapentaenoic acid (EPA), C20:5(n-3), content and hatch rate with docosahexaenoic acid (DHA), C22:6(n-3), content of the eggs. Both fecundity and hatch rate were affected by n-3/n-6 fatty acid ratio (Wen et al. 2002). Xu et al. (1994) reported that diets low in the omega-3 fatty acids EPA and DHA resulted in reduced fecundity and hatchability in Chinese prawn (*Fenneropenaeus chinensis*). Larvae from spiny rock lobster (*Jasus edwardsii*) fed a beef-based diet were smaller, had a lower survival rate, and were less active than those from wild broodstock (Smith et al. 2004). Rosa et al. (2007) reported that the fatty acid profiles of decapod embryos reflected differences in diet and ecological niche.

It may be possible to use total lipid content, fatty acid profile, and lipid profile as an indicator of the nutritional status of king crab. Differences in nutritional status among crab from different locations or from one year to the next may add an important metric in addition to the usual biometric data in assaying the health of various crab stocks. For this study, samples of red king crab for biochemical analysis could be obtained during the annual survey of crab population by the National Marine Fisheries Service (NMFS) and the Alaska Department of Fish and Game

(ADF&G). The primary objective was to compare the biochemical profile of hepatopancreas, ovaries, and embryos of red king crab taken from different locations in Alaskan waters and to establish a baseline to determine whether these profiles change from year to year or vary due to location.

Methodology

Collection of Crab and Preparation of Samples

Crabs from Bristol Bay (BB) and the Pribilof Islands (PI) were obtained during the annual NMFS survey in June 2006, conducted by the Resource Assessment and Conservation Engineering Division of the Alaska Fisheries Science Center. Crabs from Uyak Bay, Kodiak Island (KI), were obtained during survey work of the Shellfish Division of the ADF&G Kodiak office in September 2006. Crab from the Petrel Bank (PB) (lat 57.42°N, long 153.82°W), were obtained during survey work of the Shellfish Division of the ADF&G Dutch Harbor office in November 2006. Biometric information recorded included carapace length (CL), shell and egg clutch condition, and whole weight. For crab caught by NMFS, the standard codes for biometric information were used as follows:

	Shell condition
0	Pre-molt
1	Soft and pliable
2	Thin and clean
3	Hard shell, slightly worn
4	Hard shell, worn
5	Hard shell, very worn

	Clutch size
0	Immature
1	Mature, no eggs
2	Trace to ⅛ full
3	¼ full
4	½ full
5	¾ full
6	Full

	Egg color
0	No eggs
1	[Not used]
2	Purple
3	Brown
4	Orange
5	Purple-brown
6	Pink

For crab caught by ADF&G, biometric codes were converted to those used by NMFS for ease of comparison. During the NMFS survey, it was possible to accurately weigh whole crab at sea; the crab were then sacrificed to obtain the egg clutch, ovary, and hepatopancreas. The egg clutch was removed by snipping the pleopods with a pair of scissors and transferred to a resealable plastic bag. The ovary and hepatopancreas were similarly removed and transferred to separate, resealable plastic bags. Excess air was expelled prior to closing, then the specimens were frozen and kept frozen until further analysis. These samples were landed at Dutch Harbor and shipped by air to Kodiak. For KI samples, all four crabs were frozen whole at sea, as there was no access to a motion compensated balance needed to obtain whole weights if the seas were not calm. For the PB samples, only three crabs could be processed at sea while eight were frozen whole. PB samples were landed at Dutch Harbor and shipped by air to Kodiak. For whole specimens frozen at sea, crab were tempered overnight at 4°C in a cool room prior to being processed to obtain biometric data. Since the crab were not completely thawed, it was possible to obtain whole weights without compensating for thaw drip. Specimens were then thawed enough to obtain tissue samples.

Eggs were stripped from pleopods with forceps and the egg clutch weight recorded. While it was possible to process the egg clutches from crab frozen whole, freezing and thawing sometimes had a detrimental effect on the hepatopancreas, so the weight of the hepatopancreas was not recorded from those specimens. The ovary tissue from crab frozen whole was not as

detrimentally affected as the hepatopancreas tissue, but it was not possible to recover the entire ovary in every case. For those specimens whose organs were intact enough for reliable measurements, the gonadosomatic index (GSI) was calculated as follows.

$$GSI = \text{ovary weight} \times 100/\text{whole weight}$$

Indexes for egg clutch weight (ratio of egg weight to body weight, aka eggsomatic index [ESI]) and hepatopancreas (hepatosomatic index [HSI]) were similarly calculated.

Biochemistry

For tissue samples from crab processed at sea, the samples were partially thawed at 4°C and weight was recorded. For egg clutches, eggs were stripped from the pleopods using forceps, and the weight of eggs recorded. Hepatopancreas and ovary tissue was homogenized using a PowerGen Model 125 homogenizer (Fisher Scientific, Pittsburgh, Pennsylvania) equipped with a 10 mm generator prior to being assayed for proximate analysis. Material not needed for proximate analysis was placed either in amber glass vials or plastic containers, flushed with nitrogen, and stored at –80°C. Tissue samples from crab frozen at sea were similarly treated. Determination of moisture, ash, protein, and total lipid content was performed in duplicate using the methods of Reppond et al. (2008). Ash, lipid, and protein values were converted to a dry weight basis to compensate for differences in moisture content among samples. Protein content was not determined on all samples. In some cases, there was insufficient material left once samples were taken for other analyses. In other cases, the instrument used for protein analysis was under repair and was unavailable until after the experiment had to be terminated due to closure of the lab.

Statistical Analysis

Ten crabs were obtained from BB, while 2, 4, and 11 crabs came from PI, KI, and PB, respectively. These are referred to as location replicates. Biometric data were subjected to a one-way analysis of variance (ANOVA) to determine the effect of location. Moisture, ash, and lipid data were subjected to a nested ANOVA where experimental results for location replicates were nested within location using SAS 6.2 statistical programming (SAS Inc., Cary, North Carolina). Protein values were not subjected to a nested ANOVA because data was not available for all samples. To make comparison among locations easier, the mean moisture, ash, lipid, and protein values of each location replicate were calculated and used in a least significant difference determination using Statistical Package for Social Sciences 6.1 (SPSS Inc., Chicago, Illinois). Correlation coefficients among mean experimental values were determined using the same programming.

Results

Biometrics

Crabs from BB and KI were all shell code 2, while both crabs from the PI area were classified as shell code 1. Eight of the PB crabs were shell code 2, and three were shell code 3. Egg clutch size codes were either 5 (3/4 full) or 6 (full) for crabs from each location. Of the crabs from BB, eight had clutch size code 5 and two had clutch size code 6. All the crabs from PI had clutch size code 6 and all the crabs from KI had clutch size code 5. Eight of the crabs from PB had clutch size code 5, and three had clutch code size 6. Eggs of crabs from BB, PI, and KI were all uneyed. Eight of the PB crabs had uneyed eggs, while three were eyed. Egg color was either purple or purple-brown in all samples with only a few classified as brown as follows:

	Purple	Brown	Purple-brown
Bristol Bay	3	1	6
Pribilof Islands	2	–	–
Kodiak Island	1	1	2
Petrel Bank	5	1	2

Crabs from BB and PI had recently mated, and it was not always possible to locate and remove the ovary from the other organs due to its small size. Therefore, some crabs from BB lacked ovary data but had a sufficiently sized egg clutch and hepatopancreas for those organs to be analyzed. Unfortunately, only two crabs were available from PI, but their ovaries were large enough for sampling. Difficulties with obtaining ovary and hepatopancreas samples from PB crabs are described in the Methodology section.

Crabs from BB were smaller than crabs from the other locations whether measured by CL or whole weight (Table 1). Crabs from KI were heavier than those from BB or PB but similar to those from PI. By weight, crabs from BB had the smallest amount of eggs as might be expected by the relative size of the crabs. PI and KI crabs had the highest egg weight with PB crabs somewhat lower. Since larger crab would be expected to have a larger egg mass, a more useful measurement would be the ESI. PI crabs had the highest ESI and there was no significant difference among crabs from the other locations (Table 1). The small sample size of the PI crabs made comparison with crabs from other locations problematic.

The weight of the hepatopancreas followed the same pattern as noted with whole weight, smallest for crabs from BB and largest in crabs from KI (Table 1). Accordingly, no significant differences were seen in the HSI values among the crab from different locations. The weight of ovaries was smallest for the crabs from BB and largest for crabs from KI and PB (Table 1). Crabs from PI had ovaries with weights between these two groups. GSI values followed the same pattern.

Table 1. Mean and SD of CL, whole weight, egg clutch weight, ESI, hepatopancreas weight, HSI, ovary weight, and GSI of red king crab from different locations. N = number of crab from each location. The superscript letters ($^{A, B, C}$) indicate means sharing a common letter were not significantly different from each other, $p > 0.05$.

Location	Date	Value	CL (mm)	Whole weight (g)	Clutch weight (g)	ESI	Hepato-pancreas weight (g)	HSI	Ovary weight (g)	GSI
Bristol	June	Mean	106.9 ^{A}	914 ^{A}	71.3 ^{A}	7.8 ^{A}	48.5 ^{A}	5.3 ^{A}	7.5 ^{A}	0.8 ^{A}
Bay	2006	SD	±6.2	±157	±14.3	±1.0	±15.5	±1.5	±2.0	±0.2
		N	10	10	10	10	10	10	10	10
Pribilof	June	Mean	154.5 ^{B}	1,960 $^{B, C}$	226.5 ^{C}	12.3 ^{B}	85.1 $^{B, C}$	4.4 ^{A}	27.9 ^{B}	1.5 ^{B}
Islands	2006	SD	±3.5	±665	±7.8	±4.6	±18.0	±0.6	±1.2	±0.6
		N	2	2	2	2	2	2	2	2
Kodiak	Sept.	Mean	141.0 ^{B}	2,137 ^{C}	180.0 ^{C}	8.4 ^{A}	108.2 ^{C}	5.0 ^{A}	59.1 ^{C}	2.8 ^{C}
Island	2006	SD	±9.4	±256	±26.0	±0.4	±29.8	±1.0	±13.7	±0.5
		N	4	4	4	4	4	4	4	4
Petrel	Nov.	Mean	142.8 ^{B}	1,711 ^{B}	148.2 ^{B}	8.6 ^{A}	82.2 ^{A}	4.9 ^{A}	55.7 ^{C}	3.3 ^{C}
Bank	2006	SD	±10.7	±291	±34.3	±1.1	±25.1	±0.5	±19.9	±0.5
		N	11	11	11	11	3	3	3	3

To determine whether there was any relationship among biometric parameters, correlation coefficients were determined. CL and whole weight were positively correlated with weights of organs and weights of organs were correlated with each other (Table 2). Of the three indexes, only GSI had a significant correlation with CL, whole weight, hepatopancreas weight, and ovary weight. None of the three indexes were correlated with each other.

Hepatopancreas Biochemistry

A nested ANOVA treatment of the hepatopancreas moisture data indicated significant differences existed between locations and among replicates within the same location (Table 3). For ash content, the variation among crab within locations was significant but there were no significant differences among locations. The lipids results were similar to that noted for moisture content, as both location and replicates within location were a significant source of variance. The moisture content of the hepatopancreas of crabs from KI was lower than that seen in crabs from BB and PI but was not statistically different in the PB crabs (Table 4). As

Table 2. Number of samples (N) and probability (*p*) of correlation coefficient (R) of CL, whole weight, egg weight, hepatopancreas weight, ovary weight, ESI, GSI, and HSI of red king crab.

		Whole weight	**Egg weight**	**Hepato-pancreas weight**	**Ovary weight**	**ESI**	**GSI**	**HSI**
CL	R	0.895	0.920	0.579	0.829	0.457	0.723	–0.465
	N	27	27	27	25	27	25	27
	p	>0.001	>0.001	0.002	>0.001	0.016	>0.001	0.014
Whole weight	R		0.894	0.791	0.848	0.208	0.663	–0.307
	N		27	27	25	27	25	27
	p		>0.001	>0.001	>0.001	0.297	>0.001	0.120
Egg weight	R			0.694	0.745	0.619	0.591	–0.296
	N			27	25	27	25	27
	p			>0.001	>0.001	0.001	0.002	0.134
Hepato-pancreas weight	R				0.725	0.134	0.533	0.303
	N				25	27	25	27
	p				>0.001	0.506	0.006	0.124
Ovary weight	R					0.162	0.939	–0.250
	N					25	25	25
	p					0.440	>0.001	0.229
ESI	R						0.152	–0.113
	N						25	27
	p						0.467	0.573
GSI	R							–0.263
	N							25
	p							0.204

Table 3. Nested ANOVA of red king crab hepatopancreas composition where location replicates (Rep) are nested within location.

	Source	df[a]	SS[b]	MS[c]	F[d]	*p*
Moisture	Location	3	758.23	252.740	7.19	<0.050
	Rep (location)	15	527.10	35.140	237.88	<0.001
	Error	19	2.81	0.148		
Ash	Location	3	68.51	22.840	1.25	NS[e]
	Rep (location)	15	273.32	18.220	7.54	<0.001
	Error	19	45.95	2.418		
Lipid	Location	3	1,639.40	546.470	7.88	<0.050
	Rep (location)	15	1,040.10	63.940	157.45	<0.001
	Error	19	8.37	0.440		

[a] df = degrees of freedom.
[b] SS = sum of squares.
[c] MS = mean square.
[d] F = F-distribution value.
[e] NS = not significant, $p > 0.05$.

indicated by the nested ANOVA results, the variation of the hepatopancreas ash values due to location was not significant. Lipid contents of the hepatopancreas of crabs from BB and PI were significantly lower than that found in crabs from KI and PB. Hepatopancreas protein content was lower in crabs from KI than crabs from BB and PI. Moisture content of the hepatopancreas had a significant positive correlation with ash content and a significant negative correlation with lipid content (Table 5). Hepatopancreas moisture content was also negatively correlated with whole weight, hepatopancreas weight, ovary weight, and GSI. Ash content of the hepatopancreas was negatively correlated with lipid content, hepatopancreas weight, and HSI. Lipid content of the hepatopancreas had a positive correlation with hepatopancreas weight, ovary weight, and GSI.

Ovary Biochemistry

For ovary samples, variation due to location and replicate crab within location were significant for moisture and for lipid content (Table 6). For the ash content of the ovary samples, only variation due to location was statistically significant. The ovaries of crabs from PI had a higher moisture and ash content than seen in crabs from the other locations (Table 4). The ovaries of crabs from KI and PB had lower moisture and ash content than crabs from BB. Lipid content of ovaries was lower in the crabs from BB and PI than in crabs from KI and PB. Protein content of ovaries from PI crabs was greater than that in crabs from KI.

As seen in the hepatopancreas results, ovarian moisture content was positively correlated with ash content but negatively correlated with lipid content (Table 7). Ovarian moisture content was not correlated with CL and whole-weight measurements of the size of the crab. Ovarian moisture content was negatively correlated with hepatopancreas weight, ovarian weight, and GSI. Ovarian ash content was negatively correlated with lipid content, ovary weight, and GSI. Lipid content of the ovary was positively correlated with hepatopancreas weight, ovary weight, and GSI.

Table 4. Mean and SD of proximate composition of hepatopancreas and ovary tissue of red king crab from different locations. Ash, lipid, and protein values were adjusted to a dry basis; N = number of replicates from each location. The superscript letters ($^{A, B, C}$) indicate means sharing a common letter were not significantly different from each other, $p > 0.05$.

Location	Date	Value	Moisture	Ash	Lipid	Protein
Hepatopancreas						
Bristol Bay	June 2006	Mean	78.2 ^{A}	10.0 ^{A}	20.8 ^{A}	53.1 ^{A}
		SD	±5.2	±3.8	±7.2	±6.0
		N	10	10	10	10
Pribilof Islands	June 2006	Mean	81.0 ^{A}	11.4 ^{A}	16.4 ^{A}	58.7 ^{A}
		SD	±2.7	±0.0	±5.3	±4.0
		N	2	2	2	2
Kodiak Island	Sept. 2006	Mean	67.9 ^{B}	7.7 ^{A}	34.7 ^{B}	40.2 ^{B}
		SD	±1.4	±1.1	±2.3	±2.1
		N	4	4	4	4
Petrel Bank	Nov. 2006	Mean	73.0 $^{A, B}$	7.3 ^{A}	31.4 ^{B}	—
		SD	±1.2	±0.2	±1.8	
		N	3	3	3	
Ovary						
Bristol Bay	June 2006	Mean	77.8 ^{B}	8.1 ^{B}	11.9 ^{A}	—
		SD	±1.9	±1.4	±4.7	
		N	5	5	5	
Pribilof Islands	June 2006	Mean	82.0 ^{C}	13.0 ^{C}	13.0 ^{A}	76.2 ^{A}
		SD	±0.3	±0.7	±4.3	±2.1
		N	2	2	2	2
Kodiak Island	Sept. 2006	Mean	57.0 ^{A}	3.3 ^{A}	27.2 ^{B}	63.6 ^{B}
		SD	±2.5	±1.2	±1.0	±1.3
		N	4	4	4	4
Petrel Bank	Nov. 2006	Mean	56.5 ^{A}	4.0 ^{A}	28.3 ^{B}	—
		SD	±2.3	±0.5	±0.9	
		N	3	3	3	
Eggs						
Bristol Bay	June 2006	Mean	58.7 ^{A}	5.5 ^{A}	30.8 ^{B}	60.2 ^{A}
		SD	±2.0	±1.2	±0.9	±2.9
		N	10	10	10	10
Pribilof Islands	June 2006	Mean	61.2 ^{A}	3.9 ^{A}	30.2 $^{A, B}$	62.3 $^{A, B}$
		SD	±0.1	±0.1	±0.1	±0.1
		N	2	2	2	2
Kodiak Island	Sept. 2006	Mean	66.9 ^{B}	5.2 ^{A}	33.7 ^{C}	66.0 ^{B}
		SD	±1.2	±0.3	±2.4	±2.0
		N	4	4	4	4
Petrel Bank	Nov. 2006	Mean	68.8 ^{B}	5.5 ^{A}	28.9 ^{A}	—
		SD	±1.6	±0.6	±1.6	
		N	11	11	11	

Table 5. Correlation coefficient (R) and associated probability (*p*) of hepatopancreas moisture, ash, and lipid content with CL, whole weight, egg weight, hepatopancreas weight, ovary weight, ESI, GSI, and HSI. Number of replicates was 19.

	Moisture		Ash		Lipid	
	R	***p***	**R**	***p***	**R**	***p***
Ash	0.804	<0.001				
Lipid	–0.763	<0.001	–0.558	0.013		
CL	–0.342	0.151	–0.189	0.439	0.268	0.267
Whole weight	–0.458	0.049	–0.257	0.288	0.374	0.115
Egg weight	–0.314	0.191	–0.135	0.583	0.236	0.332
Hepatopancreas weight	–0.591	0.008	–0.469	0.043	0.527	0.021
Ovary weight	–0.607	0.006	–0.390	0.099	0.617	0.005
ESI	0.084	0.731	0.139	0.57	–0.145	0.553
GSI	–0.612	0.005	–0.414	0.078	0.667	0.002
HSI	–0.308	0.200	–0.515	0.024	0.302	0.209

Table 6. Nested ANOVA of red king crab ovary composition where location replicates (Rep) are nested within location.

	Source	**df**[a]	**SS**[a]	**MS**[a]	**F**[a]	***p***
Moisture	Location	3	2,379.12	793.04	72.93	<0.001
	Rep (location)	9	97.87	10.87	95.00	<0.001
	Error	13	1.49	0.11		
Ash	Location	3	275.71	91.90	47.70	<0.001
	Rep (location)	8	15.41	1.93	1.04	NS[b]
	Error	11	20.43	1.86		
Lipid	Location	3	1,534.26	511.42	25.38	<0.001
	Rep (location)	9	181.32	20.15	4.09	<0.050
	Error	13	64.02	4.92		

[a] See Table 3 for definition of abbreviations.
[b] NS = not significant, $p > 0.05$.

Eggs Biochemistry

Variation among different locations and variation among replicate crab within location were both significant for moisture and lipid content of red king crab eggs (Table 8). For the ash content of the egg samples, only the variation among replicates within location was statistically significant. Moisture content of eggs of BB and PI crabs was lower than that noted for crabs from KI and PB (Table 4). As noted in the hepatopancreas samples, ash content was not affected by location. The eggs of crabs from KI had a higher lipid content than the eggs of crabs from the other locations. The eggs of crabs from BB and PI had similar lipid content. Eggs from PB crabs had less lipid than crabs from BB, but there was no significant difference in the egg lipid content between crabs from PI and PB.

Table 7. Correlation coefficient (R) and associated probability (*p*) of red king crab ovary moisture, ash, and lipid content with CL, whole weight, egg weight, hepatopancreas weight, ovary weight, ESI, GSI, and HSI.

	Moisture			Ash			Lipid		
	R	**N***	***p***	**R**	**N**	***p***	**R**	**N**	***p***
Ash	0.902	13	>0.001						
Lipid	–0.941	13	>0.001	–0.771	12	0.003			
CL	–0.299	14	0.299	0.017	13	0.957	0.335	13	0.263
Whole weight	–0.489	14	0.076	–0.225	13	0.459	0.580	13	0.038
Egg weight	–0.214	14	0.463	0.103	13	0.738	0.254	13	0.402
Hepatopancreas weight	–0.538	14	0.047	–0.313	13	0.297	0.637	13	0.019
Ovary weight	–0.817	14	>0.001	–0.595	13	0.032	0.805	13	0.001
ESI	0.335	14	0.241	0.533	13	0.061	–0.438	13	0.134
GSI	–0.881	14	>0.001	–0.657	13	0.015	0.824	13	0.001
HSI	–0.091	14	0.756	–0.230	13	0.449	0.1739	13	0.570

*N = number of samples for each correlation.

Table 8. Nested ANOVA of red king crab egg composition where location replicates (Rep) are nested within location.

	Source	**df**[a]	**SS**[a]	**MS**[a]	**F**[a]	***p***
Moisture	Location	3	1,155.35	385.12	67.73	<0.001
	Rep (location)	23	130.78	5.69	46.37	<0.001
	Error	26	3.19	0.12		
Ash	Location	3	10.15	3.38	2.38	NS[b]
	Rep (location)	23	32.72	1.42	4.60	<0.001
	Error	26	8.05	0.31		
Lipid	Location	3	137.63	45.88	10.65	<0.001
	Rep (location)	23	99.11	4.31	5.01	<0.001
	Error	27	23.23	0.86		

[a] See Table 3 for definition of abbreviations.
[b] NS = not significant, $p > 0.05$.

Unlike the hepatopancreas and ovarian tissue, the moisture content of red king crab eggs was not significantly correlated with either ash or lipid content (Table 9). Moisture content was positively correlated with CL and whole weight, indicating that the eggs from larger crabs were higher in moisture content. Moisture content was also positively correlated with egg weight, hepatopancreas weight, ovary weight, and GSI. Moisture content of the eggs was negatively correlated with HSI. Ash content was correlated only with ESI and that correlation coefficient was negative.

Table 9. Correlation coefficient (R) and associated probability (*p*) of red king crab egg moisture, ash, and lipid content with CL, whole weight, egg weight, hepatopancreas weight, ovary weight, ESI, GSI, and HSI.

	Moisture			**Ash**			**Lipid**		
	R	**N***	***p***	**R**	**N**	***p***	**R**	**N**	***p***
Ash	0.069	27	0.733						
Lipid	–0.254	27	0.201	–0.143	27	0.478			
CL	0.724	27	<0.001	–0.258	27	0.194	–0.192	27	0.337
Whole weight	0.669	27	<0.001	–0.205	27	0.306	0.063	27	0.755
Egg weight	0.582	27	0.001	–0.345	27	0.078	0.040	27	0.841
Hepatopancreas weight	0.406	27	0.036	–0.164	27	0.414	0.244	27	0.219
Ovary weight	0.884	25	<0.001	–0.042	25	0.842	–0.080	25	0.704
ESI	0.110	27	0.586	–0.463	27	0.015	–0.025	27	0.904
GSI	0.913	25	<0.001	–0.015	25	0.943	–0.212	25	0.308
HSI	–0.391	27	0.044	0.075	27	0.711	0.235	27	0.239

*N = number of samples for each correlation.

Discussion

While it would have been preferable to have all crab with the same shell condition and clutch size and to be able to determine the stage of egg development, some interesting comparisons can still be made. The stage in the reproductive cycle of crab from each area relative to the others can be reasonably estimated using the GSI values as it typically increases throughout the seasonal reproductive cycle of crustaceans. Somerton and MacIntosh (1985) reported that mean GSI values increased from about 1.0 to 8.0 during the biannual reproduction cycle of blue king crab (*Paralithodes platypus*) from PI. The range of GSI values was greater in their study as their samples were taken over a greater portion of the reproductive cycle than the limited time range of this study. Accordingly, crabs from BB and PI were earlier in their yearly reproduction cycle than crabs from KI and PB based on the GSI values. This would be consistent with observations of others based on the dates the samples were taken and the typical times for mating for the crab in the different areas of this study.

Mating for red king crab in Bristol Bay can occur anytime from January to June (Otto et al. 1990, Stevens and Swiney 2007). Mating for red king crabs in Kodiak waters usually occurs primarily in April and May (Powell et al. 2002). The relative ranking of the reproductive stage of the crab in this study based on GSI values is also similar to that based on shell codes. Female king crab molt before mating; therefore, specimens with low shell codes have likely molted more recently than ones with higher shell codes. Although the crabs from PI had a higher mean ESI value than crab from other areas, it would be unwarranted to draw any conclusions based on such a small sample size of crabs from PI. A crab with a higher ESI value could mean it was further along in the reproductive cycle.

The lack of any significant differences in HSI values among crab from different locations could be interpreted as indicating the crab were equally recovered from the hatching, molting, and mating process, but this may be unwarranted based on the limited number of crabs from the PI and KI areas. Turner et al. (2003) noted that the caloric content of hepatopancreas and reproductive organs of blue crab (*Callinectes sapidus*) increases linearly with time after molting once muscle caloric content has reached a threshold value. The lack of sampling throughout the reproductive cycle for crab in any of the areas of this study precluded the sort of analysis used by Turner et al. (2003), but the expectation is that a similar process is likely to occur in red king crab. The positive correlations among CL, whole weight, and weight of organs were what would be expected, as larger individuals should have larger organs. The lack of any significant correlation among ESI, HSI, and GSI may indicate that the change in weight of these tissues relative to whole weight occurred independently of each other at least during the limited portion of the reproductive cycle sampled in this study.

In most cases, differences in the biochemical composition, especially lipid content, of the hepatopancreas and ovary of crab from different locations followed the pattern that would be expected given the likely differences in time between typical mating season and capture date. The higher lipid content in the hepatopancreas and ovary of crabs from KI and PB was likely due

to having more time to recover from molting and mating. Decreasing moisture content and increasing lipid content of ovaries during the reproductive cycle have been noted with other crustaceans (Teshima and Kanazawa 1983, Lautier and Lagarrigue 1988, Mourente et al. 1994, Wouters et al. 2001, Reppond et al. 2009). The lower ovarian ash content of crabs from KI and PB may be due to the dilution effect of increasing lipid content. Some of the difference among samples could be due to location alone, however.

Increasing moisture content and decreasing lipid content during embryonic development have been reported for several crab species (Petersen and Anger 1997, Gardner 2001, Reppond et al. 2008). The higher moisture content in eggs of crabs from KI and PB is consistent with the likelihood that crabs from these locations were further along in the seasonal sexual development than crabs from BB or PI. Differences among the lipid values were not consistent with the likely differences in time since mating, however, as KI crab eggs had the highest lipid content. A lower lipid value in the eggs would be expected in more mature eggs if lipids were the main energy source of developing red king crab embryos. While it was not possible to determine the stage of embryonic development for these samples, as the samples were frozen at sea, it is unlikely that eggs from crabs from Kodiak were less mature than eggs of crabs from other locations given the GSI and egg moisture values. Therefore, the higher lipid content of KI crab eggs is likely the result of more lipid being deposited in the developing oocytes prior to mating.

The negative correlation between the moisture content of the hepatopancreas with hepatopancreas weight and GSI indicates that the moisture content of the hepatopancreas decreased as the crab proceeded through the reproductive cycle. Similarly, the positive correlation of the lipid content of the hepatopancreas with GSI indicates that a net increase in lipid was occurring as the crab progressed during the reproductive cycle. The negative correlation of ovarian moisture and ash content with GSI indicates that the ovary was decreasing in moisture and ash as the crab proceeded through the reproductive cycle. The positive correlation of ovarian lipid content with GSI indicates that lipid was being deposited as the ovaries matured. The positive correlation of egg moisture values with GSI indicates that moisture should increase during seasonal development.

The positive correlation of egg moisture content with both hepatopancreas weight and ovarian weight can be explained by the fact that both organs are likely to increase in size during the reproductive cycle during which time the moisture content of the eggs is also increasing. Somewhat surprising was the lack of a significant negative correlation between egg lipid content and GSI or any other parameters studied. This could be the result of the limited embryonic development time range between the samples. In fact, the range in egg lipid values among the samples was much smaller than the ranges in hepatopancreas or ovary lipid values. In other words, change in egg lipid values was less than changes in hepatopancreas and ovary lipid values over the developmental time covered by the samples in this study. These results could change if more samples were taken later in the seasonal development.

The results of this preliminary study indicate that a more extensive experiment, in which crab would be taken from the same area throughout the yearly cycle, would greatly benefit our understanding of the biochemical changes associated with reproduction. It would also prove beneficial in the future to take samples from the same regions at the same time of year in order to see if there is a year-to-year variation in the nutritional status of red king crab.

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