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BIOCHEMICAL COMPOSITION OF EMBRYONIC BLUE CRABS
CALLINECTES SAPIDUS RATHBUN 1896
(CRUSTACEA: DECAPODA) FROM THE GULF OF MEXICO

Janet R. Jacobs, Patricia M. Biesiot, Harriet M. Perry and Christine Trigg

A B S T R A C T

Blue crab *Callinectes sapidus* embryos from the Mississippi Sound were sampled in spring and in late summer to determine patterns of biochemical composition and of yolk utilization during embryogenesis and to ascertain potential seasonal differences in biochemical composition. The diameter of spring embryos was ~6% greater than summer embryos but this significant size difference was due to increased water content, not to increased organic content. The general trend in initial biochemical composition was similar in both seasons; protein was the primary component at ~50% of initial dry weight followed by lipid (~30%), ash (~8%) and carbohydrate (6%). The general trend for utilization of organic reserves during embryogenesis was also similar seasonally. Lipid was the primary component metabolized during embryogenesis (44–48% of initial stores were utilized) followed by protein (13–16% utilized) and carbohydrate (~13% utilized). Calculated on a dry weight basis, spring embryos had significantly lower lipid but significantly higher ash than summer embryos; there were no significant seasonal differences in protein or carbohydrate. Caloric expenditure on a dry weight basis was significantly different seasonally. There appear to be geographic differences among blue crabs; our results differ from those of a previous study of blue crab embryos from North Carolina waters.

The blue crab *Callinectes sapidus* Rathbun, 1896 is a portunid species that ranges from Nova Scotia to northern Argentina; it is an abundant and economically important member of estuarine and coastal communities in temperate, tropical and subtropical waters (Williams, 1974). The blue crab is an important commercial and recreational fisheries species in both the Atlantic Ocean (Milliken and Williams, 1984) and the Gulf of Mexico (Perry, 1975; Perry et al., 1984; Steele and Perry, 1990). United States commercial landings in 1995 amounted to 214.7 million pounds, of which the Gulf contributed 54.0 million pounds (NMFS, 1996). According to FAO statistics (1992), the blue crab fishery is the largest single-species crab fishery in the world.

Many aspects of blue crab biology have been well-studied, including life history (Churchill, 1919; Darnell, 1959; Tagatz, 1968; Williams, 1974, 1984; Millikin and Williams, 1984; Pattillo et al., 1997) and recruitment. Recruitment studies include megalopal peak abundances in the plankton (Stuck and Perry, 1981; McConaugha et al., 1983) and settlement of the megalopae in nearshore waters (Olmi et al., 1990; Metcalf and Lipcius, 1992; Olmi, 1994; Perry et al., 1995; Rabalais et al., 1995; van Montfrans et al., 1995). Other studies have focused on blue crab physiology including the effect of temperature on the developmental rate of embryos (Amsler and George, 1984a) and juveniles (Leffler, 1972), larval survival rates at various salinities and temperatures (Sandoz and Rogers, 1944), larval diet (Sulkin, 1975, 1978), and adult diet (Laughlin, 1982; Stoner and Buchaan, 1990; Hsueh et al., 1992).

Relatively few studies have been performed on the biochemical composition of *Callinectes sapidus* and, prior to the present study, none have dealt with blue crabs from the Gulf of Mexico. Whitney (1969) reported selected lipid classes and fatty acids of embryos, ovaries and midgut gland of North Carolina blue

crabs. Tsai et al. (1984) analyzed lipid and cholesterol content of the midgut gland, gonads and muscle of blue crabs from a Maryland estuary. Amsler and George (1984b) analyzed the biochemical composition of blue crab embryos from North Carolina waters and found seasonal differences in embryo composition. Previous work on blue crab larvae demonstrated seasonal differences in the size of zoeae, megalopae and first crab from the Gulf of Mexico (Stuck and Perry, 1982) as well as geographic differences in size between Atlantic coast (North Carolina waters) and Gulf of Mexico (Mississippi waters) populations (Costlow and Book-hout, 1959; Stuck and Perry, 1982). Results from these studies suggest that there might also be seasonal and geographic differences in size and in biochemical composition of blue crab embryos.

Development and metamorphosis of planktotrophic larvae, including decapod crustaceans, depends to a great extent on nutrition. Both exogenous nutrients, obtained by feeding, and endogenous nutrients, from the yolk reserves, can be important during early postembryonic development (Sasaki et al., 1986). There are a few studies which quantify the relationships among stored energy reserves (lipid, protein and carbohydrate) during embryogenesis of brachyuran crabs (Amsler and George, 1984a; Babu, 1987; Sanchez-Rubio, 1992; Biesiot and Perry, 1995). These maternally derived endogenous nutrients are catabolized throughout embryogenesis and also during the transition to exogenous feeding by the first zoeal stage. The relationships among the primary biochemical components of invertebrate eggs and the potential consequences of this variability are often considered in various models relating to the evolution and maintenance of specific life history patterns (Jaeckle, 1995).

The objectives of the present study were to 1) determine the pattern of biochemical composition, as proxies for yolk storage and for yolk utilization, during development of blue crab *Callinectes sapidus* embryos from the Gulf of Mexico; 2) determine potential seasonal differences in biochemical composition of embryos sampled during the two peak periods of spawning in the Gulf (late spring and late summer/early fall); and 3) compare the results with those for shallow-water and deep-sea crustacea. For example, we noted in a previous study (Biesiot and Perry, 1995) that embryos from different female deep-sea red crab *Chaceon quinquedens* showed different patterns of biochemical composition and subsequent metabolism of stored components during embryogenesis. There was a range of lipid to protein ratios among the separate broods examined, indicating plasticity in the relative proportions of lipid and protein in the yolk of that species. It is not known, however, whether these are general results that also apply to shallow-water species, such as the blue crab.

MATERIALS AND METHODS

Ovigerous female blue crabs *Callinectes sapidus* were collected from the Mississippi Sound near Ocean Springs, Mississippi, U.S.A. during May and again during late August/early September, hereafter termed spring and summer, respectively. The spring samples were taken just prior to the earliest annual peak of zoeae in the Gulf of Mexico whereas the summer samples were timed for the last zoeal peak (Perry, 1975). The egg masses (broods) were all bright orange in color, indicating that the embryos were from one to seven days old (Bland and Amerson, 1974).

The crabs were captured by a local fisherman in standard blue crab traps from waters with a surface salinity of 4 to 14 ppt. Bottom salinities were not measured, but historically they have been reported as greater than 20 ppt, ranging from 20.1 to 29.4 ppt, in the area where the crabs were collected (Christmas and Eleuterius, 1973). Mean water temperature in the field was 25°C in May and 28°C in August/September.

Immediately after capture, the crabs were brought to the Gulf Coast Research Laboratory, Ocean Springs, Mississippi. Only primiparous crabs, those bearing their first brood, were used in this study. Each ovigerous female had a brightly colored, unfouled carapace and was carefully examined to

Table 1. Morphometric characteristics (mean ± SD) of ovigerous blue crab *Callinectes sapidus*. Means were not significantly different between seasons (*t*-test; $P = 0.218$ for carapace length, $P = 0.070$ for carapace width; $P = 0.327$ for wet weight).

Season	Carapace length (mm)	Carapace width (mm)	Wet weight (g)
Spring	64 ± 5	164 ± 13	173 ± 48
Summer	61 ± 4	153 ± 10	164 ± 24

confirm the absence of egg remnants on the pleopods. Carapace length (CL), from the groove between the rostral teeth to the posterior edge of the carapace, and carapace width (CW), between the tips of the lateral spines at the widest part of the body, were measured to the nearest 1 mm using Max Cal® electronic calipers which were calibrated before use. Crabs were blotted dry with paper towels, and total wet weight (WW) was determined to the nearest gram using a Sartorius® Model MP9 analytical balance. There were no significant seasonal differences in size of the ovigerous female crabs as measured by CL, CW or WW (Table 1) (*t*-tests; $P = 0.218$ for CL, $P = 0.070$ for CW, $P = 0.327$ for WW).

Crabs were held individually in 30-gallon aquaria equipped with biological filters and maintained at 25 ppt salinity until the embryos hatched. Sandoz and Rogers (1944) reported survival of blue crab zoeae to be highest in the salinity range 23–28 ppt. Water temperature in the laboratory was 23 ± 2°C in the spring and 25 ± 1°C in the summer. Although mean water temperatures were slightly lower in the laboratory than in the field, the differential was similar in both seasons.

Beginning on the initial day of capture and every other day thereafter, an aliquot of approximately 700 mg (wet weight) was taken of the embryos from each ovigerous female until the embryos hatched, at which time the zoeae were sampled. Small clumps of embryos were snipped from random locations in each brood using sharp scissors. Each aliquot was stored separately at −80°C in labeled 1.5 ml polypropylene microcentrifuge tubes until the biochemical analyses were performed. Eleven spring broods and ten summer broods were analyzed.

Although we planned to calculate the biochemical composition on a per embryo basis, it proved impractical to do so. Because the embryos were small (ranging in diameter from 255–300 μm, depending on season and stage of development), it was necessary to pool them for this study. It was not possible to clip the attachment material from individual embryos without tedious dissection and it was difficult to maintain an accurate count of the embryos that were so separated. Hence, the entire sample, including the attachment materials, was homogenized. The biochemical composition is reported as μg of component per mg of sample dry weight whereas water content is reported as % of sample wet weight.

Frozen embryos were thawed on ice and approximately half of each sample was homogenized in distilled water using a VirtiShear® tissue homogenizer. Frozen zoeae were treated similarly, but homogenized using a hand-held ground-glass micro-homogenizer (total capacity 2 ml). The remaining portion of each sample was refrozen at −80°C and stored for later embryo diameter analysis (see below). After homogenization, both the embryo and zoea samples were treated with a Fisher® sonic cell disrupter. Aliquots of each homogenate were used in the following biochemical analyses. Protein was measured using a modified Lowry method (Hartree, 1972) with bovine serum albumin as the standard. The carbohydrate assay was according to Dubois et al. (1956) with a glucose standard. Total lipid was analyzed gravimetrically according to Sasaki and Capuzzo (1984) which is a combination of Folch et al. (1957) and Bligh and Dyer (1959). Dry weight and ash weight were determined according to Paine (1971). Samples were dried to constant weight at 80°C in a laboratory oven and weighed. The dried samples were then combusted overnight at 500°C in a Blue M® muffle furnace to determine ash weight. Weights were measured using a Cahn® C-31 microbalance. All analyses were conducted in duplicate.

Embryo diameters were measured using a Stinger® video overlay/capture card (VideoLabs, Inc., Minneapolis, Minnesota) with a Gateway 2000 P75® computer running Windows 95®. A microscope was used to enlarge the image of the embryos and a video camera relayed the image to the computer. Development of the compound eye and the relative amount of yolk present were also documented using video microscopy to monitor potential differences in developmental rates between embryos from spring and summer.

Statistical analyses were performed using SPSS for Windows (Version 10.0). *T*-tests were used to determine whether size of the ovigerous crabs differed between seasons. Repeated measures analysis of variance (ANOVA) General Linear Model was used to test the effect of season (independent variable) on embryo diameter and on biochemical composition during the course of development; days before hatching was the dependent variable. Because embryo samples were taken on the day of
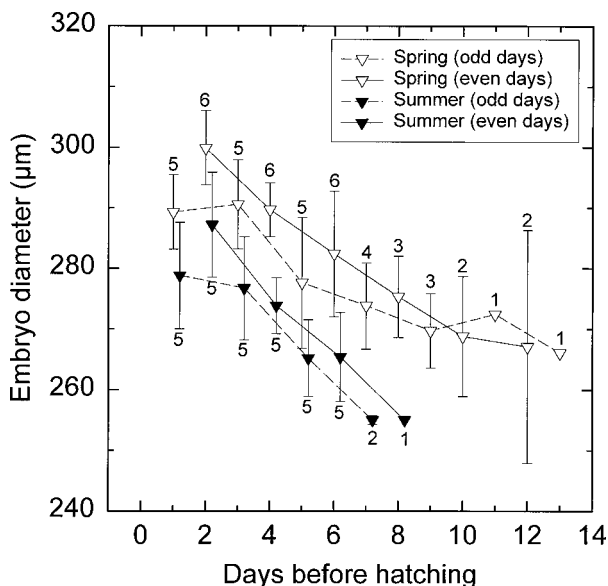
Figure 1. Diameter (mean ± SD) of blue crab *Callinectes sapidus* embryos during embryogenesis. Open symbols, spring; closed symbols, summer. Solid lines represent samples collected on even numbered days before hatching; dashed lines represent samples collected on odd numbered days before hatching. Sample sizes for each time point are given above and below the data for spring and summer, respectively. The data for each season were collected on the same relative day, but summer data points are offset slightly to the right so they are not obliterated by spring data points.

initial capture and every other day thereafter until hatching (day 0), there are data within each season for even-numbered days and day 0 for some broods (n = 5 broods in both spring and summer) and for odd-numbered days and day 0 for others (n = 6 broods in spring, n = 5 broods in summer). Data for the ANOVA were collapsed by accounting for sampling on odd versus even days within each season; *t*-tests using the Bonferroni correction were performed for each variable (embryo diameter, protein, lipid, carbohydrate, ash and water) in each season. The null hypotheses that mean values for each variable on successive pairs of days within a season were equal (i.e., day 1 = day 2; day 3 = day 4; day 5 = day 6) were accepted (*t*-tests; $P > 0.05$ for each comparison), which permitted collapsing of the data. Only data for the later portion of embryonic development (from six days before hatch to day of hatch) were included in the ANOVA because sample sizes were too small from 13 to seven days before hatching and each season was not adequately represented prior to six days before hatching.

RESULTS

The time spent in the laboratory (from the day of initial capture to the day of hatching) ranged from five to 13 days in spring and from five to eight days in summer (cf. Fig. 1), with 75% of the embryos from each season spending six to eight days in the laboratory before hatching. The observed difference in length of embryonic development between seasons was probably a reflection of the degree of embryonic development at the time of initial capture rather than a true reflection of development time in the wild. Although only ovigerous crabs with a bright orange sponge, indicating recent extrusion of the brood, were used in this study, the broods were probably extruded on different relative days and thus were at different stages of embryonic development when first brought into the laboratory. The timing of compound eye development, which was similar for both spring and summer embryos, supports this hypothesis. Eighty percent of spring embryos and 20% of summer embryos developed eye slits five days prior to

hatching and 100% of embryos from both seasons had eye slits by four days before hatching.

Spring embryos were significantly larger in diameter than summer embryos (ANOVA, $F_{1,19}$ = 38.177, P < 0.001) by ~6% (Fig. 1). Although absolute embryo sizes were different in the two seasons, the embryo diameter within each season increased by a similar proportion as hatching approached; between day 8 and day 0, there was a 8.4% increase in diameter among spring embryos and a 9.4% increase for summer embryos.

The general pattern of biochemical composition was similar for embryos from each season (Fig. 2A). Protein was the primary constituent of blue crab embryos at ~500 µg per mg of the initial dry weight (DW) (50% of the initial DW in both seasons) and showed a more or less steady decline until about four days before hatching. Protein remained relatively constant until two days before hatching and then decreased rapidly. Lipid initially contributed ~300 µg per mg DW (30% and 29% of the initial DW in spring and summer, respectively) of the embryos. Lipid decreased only slightly from 13 to six days before hatching, and then showed a steeper rate of decline until hatching. Carbohydrate was a minor constituent of blue crab embryos at ~60 µg per mg DW (6% of initial DW in both seasons). It remained fairly constant throughout embryogenesis until just prior to hatching when it dropped slightly. Ash (inorganic salts) was also a minor constituent (~8% of initial DW) but as the organic composition of the embryos declined, the ash increased. Ash increased dramatically at the time of hatching and was related to the uptake of seawater during the embryonic molt to the first zoeal stage. The percentages of lipid, protein and carbohydrate were lower for newly-hatched zoeae than for the developing embryos.

The general pattern of yolk utilization was also similar in each season (cf. Fig. 2A); only the eight days prior to hatching are considered here because there are no earlier data for summer embryos. About 60 µg of protein per mg DW (~13% of the protein stores) were metabolized during this time period in spring compared to ~80 µg per mg DW (~16% of the protein stores) in summer. Lipid contributed more toward the energy utilized during embryogenesis than did protein. About 120 µg of lipid per mg DW (~44% of the lipid stores) were metabolized during embryogenesis in spring compared to ~140 µg per mg DW metabolized during summer (~48% of the lipid stores). Carbohydrate did not contribute much to the organic reserves that were metabolized during embryogenesis; about 8 µg carbohydrate per mg DW (~13% of the carbohydrate stores) were metabolized in each season.

Although the general patterns of biochemical composition (protein > lipid > carbohydrate) and of the utilization of stored reserves during embryogenesis (lipid > protein > carbohydrate) were similar for embryos from each season, there were some significant differences in absolute biochemical composition with respect to season. Lipid was greater in summer than in spring, although at a marginal level of significance ($F_{1,18}$ = 4.474, P = 0.049), and ash was significantly greater in spring than in summer ($F_{1,18}$ = 34.831, P < 0.001). However, protein was not significantly different between seasons ($F_{1,18}$ = 2.023, P = 0.172) nor was carbohydrate ($F_{1,18}$ = 2.636, P = 0.122). There were significant interaction effects between season and day of sampling for lipid ($F_{3,16}$ = 5.124, P = 0.011), carbohydrate ($F_{3,16}$ = 7.562, P = 0.002) and ash ($F_{3,16}$ = 6.005, P = 0.006) but not for protein ($F_{3,16}$ = 3.082, P = 0.057).

Water content as % wet weight of the sample (Fig. 2B) was significantly greater in spring than in summer ($F_{1,18}$ = 18.385, P < 0.001) and there was a significant interaction effect ($F_{3,16}$ = 7.658, P = 0.002). At eight days prior to hatching,
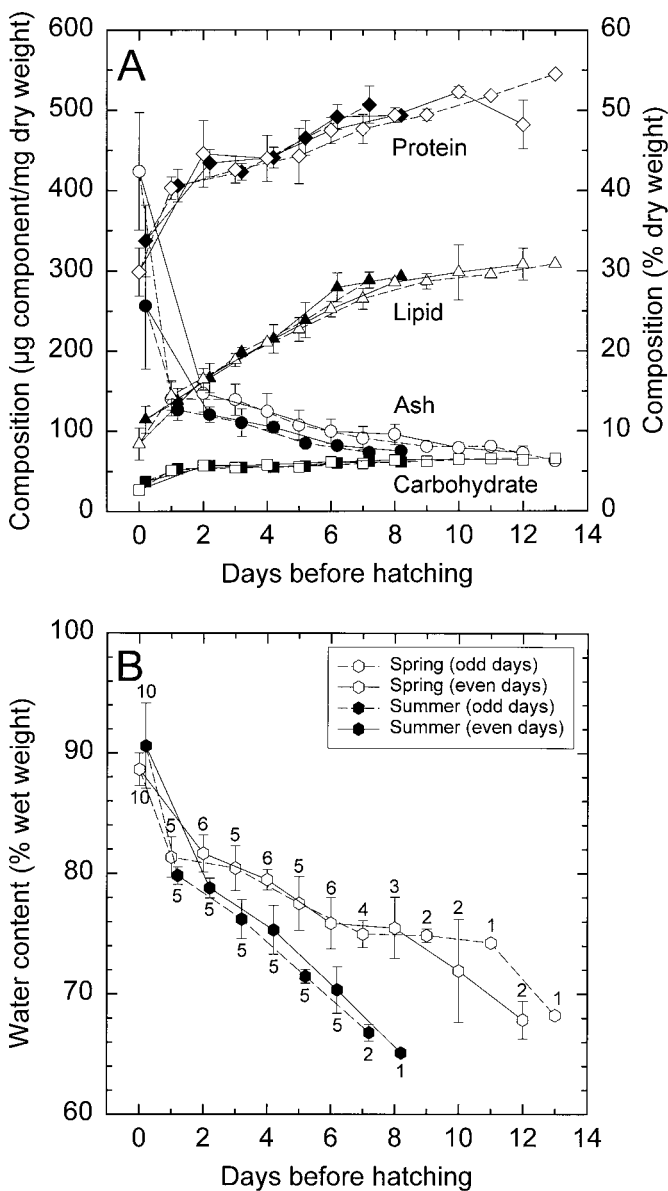
Figure 2.   Composition (mean ± SD) of blue crab *Callinectes sapidus* embryos during embryogenesis; day 0 data are for newly-hatched zoeae. (A) Biochemical composition as μg of component per mg dry weight (DW) and as % DW. Diamonds, protein; triangles, lipid; squares, carbohydrate; circles, ash. (B) Water content as % wet weight. Open symbols, spring; closed symbols, summer. Solid lines represent samples collected on even numbered days before hatching; dashed lines represent samples collected on odd numbered days before hatching. The data for each season were collected on the same relative day, but summer data points are offset slightly to the right so they are not obliterated by spring data points. Embryo sample sizes for each time point are the same as in Figure 1; sample size for zoeae is 10 for each season.

Table 2. Calories per mg dry weight (mean ± SD) expended during the last six days of embryogenesis in the blue crab *Callinectes sapidus*.

	Protein	Lipid*	Carbohydrate	Sum*
Season				
Spring	216 ± 198	828 ± 136	20 ± 16	1,064 ± 256
Summer	332 ± 116	1,017 ± 149	13 ± 8	1,362 ± 217
Statistics				
$F_{1,19}$	2.626	9.256	1.241	8.233
P value	0.122	0.007	0.279	0.010

*, significant difference between seasons.

spring embryos were ~76% water whereas summer embryos were ~65% water. The difference in water content became less pronounced as hatching approached. By one day prior to hatch, embryos from both seasons were 80% water; the newly-hatched zoeae were 90% water.

The amount of energy used by spring and summer embryos (on a dry weight basis) during the last five to six days of embryogenesis (the only time interval with sufficient sample size for the calculations) is shown in Table 2. These values were calculated by converting the biochemical composition (mean protein, lipid and carbohydrate) of the embryos to their caloric equivalents by using heat of combustion values of 5.7 cal/mg for protein, 9.5 cal/mg for lipid and 4.0 cal/mg for carbohydrate (Crisp, 1984). Values for the caloric equivalents of day 1 and day 2 embryos were subtracted from those of day 5 and day 6 embryos, respectively. The total number of calories used during this time period was greater for summer embryos than for spring embryos because of the significantly greater expenditure of lipid calories ($F_{1,19} = 9.256$, $P = 0.007$) by the summer embryos.

DISCUSSION

There are statistically significant seasonal size differences in blue crab embryos from the Gulf of Mexico. Mean diameter of spring embryos was ~270 μm at the earliest stage sampled and it increased to ~300 μm by one to two days before hatching whereas summer embryos were ~255 μm and ~280 μm at the same respective times. The pattern in proximate composition of blue crab embryos is similar during both seasons for all components except ash. Protein was the primary component of blue crab embryos followed by less, although still substantial, lipid and minimal carbohydrate. The absolute amount of both protein and carbohydrate was not significantly different between seasons. Although lipid was significantly greater in summer than in spring, the level of significance was marginal ($P = 0.049$). Ash was significantly higher in spring than in summer and was paralleled by the significantly greater water content of spring embryos. The pattern of yolk utilization during embryogenesis is also similar seasonally. Based on changes in the absolute amount of each organic substrate, lipid was the primary constituent metabolized followed by protein with minor contribution by carbohydrate. During the latter part of embryogenesis, there are seasonal differences in the total number of calories expended and in the calories contributed by specific biochemical substrates; summer embryos utilized more lipid calories (and more total calories) than spring embryos.

The biochemical composition of blue crab embryos from the present study is reported in Table 3 along with comparable data from Amsler and George (1984b) in order to consider intraspecific geographic and seasonal variation. The general

Table 3. Composition (protein, lipid, carbohydrate, ash) of blue crab *Callinectes sapidus* embryos as percent dry weight; data for embryos from two deep-sea red crabs *Chaceon quinquedens* are shown for comparison. Data reported for the present study are for eight days prior to hatch (earliest sampling date with data for both seasons) and for the initial reported composition in the two other studies. The components do not sum to 100% because ash was not reported in all cases and because crustacean embryos contain some unidentified materials (the so-called ''remainder fraction'') which are not estimated by the analytical methods used. MS, embryos from Mississippi waters; NC, embryos from North Carolina waters.

Species	% Dry weight					Reference
	Prot.	Lipid	Carb.	Ash	Sum	
Callinectes sapidus (MS, spring)	47.7	26.5	5.9	9.1	89.2	present study
C. sapidus (MS, summer)	50.7	28.8	6.3	7.3	93.1	present study
C. sapidus (NC, spring)	58.9	26.4	1.8	—	87.1	Amsler and George, 1984b
C. sapidus (NC, summer)	58.7	21.5	2.0	6.7	88.9	Amsler and George, 1984b
Chaceon quinquedens (crab #7)	56	33	7	—	96	Biesiot and Perry, 1995
C. quinquedens (crab #10)	43	47	7	—	97	Biesiot and Perry, 1995

pattern of composition is similar for blue crab embryos in both studies; protein was the major constituent of blue crab embryos, followed by lipid whereas carbohydrate was much less. This pattern is typical for crustacea (Barnes, 1965; Pandian, 1970; Archituv and Barnes, 1976; Holland, 1978; Sasaki et al., 1986). The biochemical composition of blue crab embryos does vary geographically. Mississippi embryos were initially ~50% protein whereas North Carolina embryos were ~60% protein. The lipid of spring embryos from both regions was similar but that of summer embryos was ~34% greater among Mississippi embryos compared to North Carolina embryos. Carbohydrate, although low in both regions, was about three times greater for the Mississippi embryos. Ash of summer embryos was similar in both regions. In the present study, there were no seasonal differences in the protein and carbohydrate of blue crab embryos but lipid was slightly higher in summer and ash was higher in spring. Amsler and George (1984b) also demonstrated seasonal differences in composition of blue crab embryos and also only for lipid. There was ~20% more lipid in spring North Carolina embryos but equivalent amounts of protein and carbohydrate in both seasons. Ash values were reported only for summer embryos in their study, so seasonal comparisons were not possible.

That the organic content of blue crabs in the present study was similar in both seasons was somewhat surprising, considering that spring embryos were ~6% larger in diameter than summer embryos; the calculated volume differential just prior to hatching is ~19% greater. Large invertebrate eggs often have greater organic content than small eggs (Herring, 1974; Clarke et al., 1991; Clarke and Gore, 1992; Clarke, 1993) but egg size is not always an accurate predictor of organic content in decapods (Shakuntala and Reddy, 1982) or in starfish (McEdward and Carson, 1987; McEdward and Coulter, 1987). It appears that the larger size of spring blue crab embryos is due for the most part to increased water, and the concomitant increase in inorganic salts (ash) commonly seen in crustacean embryos (Pandian, 1970; Green, 1971), and not to increased organic content. At eight days before hatch, spring embryos had ~75% water compared to ~65% for summer embryos but as embryogenesis progressed the percent water converged to concentrations that were not significantly different between seasons. By one day prior to hatch, embryos from both seasons were 80% water whereas the newly-hatched zoeae were 90% water. Amsler and George (1984b) also demon-

strated an increase in water content during blue crab embryonic development, from ~58% in the gastrula stage to ~72% prior to hatching, but there was no seasonal difference.

Data for embryos of the deep-sea red crab *Chaceon quinquedens* (Biesiot and Perry, 1995) are also included in Table 3 in order to compare *Callinectes sapidus,* a shallow-water crab, with a deep-sea species. *Chaceon quinquedens* from the Gulf of Mexico typically spawn in early summer (May–June), brood the embryos for ~9 mo and have a peak of hatching in spring (February–March) (Erdman et al., 1991). Biesiot and Perry (1995) collected 18 ovigerous deep-sea red crab in August, maintained them in the laboratory and performed biweekly proximate analysis of embryos from each crab. Although embryos from most of the clutches were stripped off by the females after six to eight weeks in the laboratory, embryos from two females (crabs #7 and #10) hatched after ~150 days in lab. The most obvious difference between *C. sapidus* and *C. quinquedens* is that embryos of the former show only one pattern of biochemical composition (protein > lipid > carbohydrate) regardless of season and locale whereas embryos of the latter demonstrate diverse patterns, with protein dominating in embryos of crab #7 and lipid dominating in embryos of crab #10. It was suggested that the differences in biochemical composition among the different clutches of red crab embryos might relate to the state of maternal nutrition because the deep sea is food limited. In the present study, blue crab females almost certainly encountered different feeding regimes. Those females that produced spring broods most likely mated the previous fall and overwintered before extruding their broods whereas the females that produced summer broods most likely mated in spring. Unlike blue crabs along the Atlantic coast, however, blue crabs that overwinter in the Gulf of Mexico typically continue to feed during the winter (Harriet Perry, personal observation).

It is not altogether surprising that embryos of Gulf of Mexico blue crab demonstrate seasonal size differences. Stuck and Perry (1982) previously documented that older life history stages of blue crab from the Gulf, including zoeae, megalopae and first crabs, were larger in spring than later in the year. The differences in size between their laboratory-reared spring (April) and summer (August) blue crab larvae persisted until about the fifth zoeal stage, but in wild-captured megalopae the size difference persisted through development of the first crab. The authors suggested that seasonal variations in size of megalopa and first crab in the wild could be related to seasonal trends in food availability which would not have affected the laboratory-reared larvae since they were fed *ad libitum.* Amsler and George (1984b) did not report a seasonal size difference in their study of blue crab embryos from North Carolina; however, their data were not collected in a manner that would allow statistical comparison of embryo size between seasons (Margaret Amsler, University of Alabama, personal communication).

The present study suggests that there are geographic size differences in blue crab embryos. The diameter of blue crab embryos from North Carolina waters was 225 μm at the blastula stage and 275 μm just prior to hatch (Amsler and George, 1984b), which is less than the size of blue crab embryos from the Mississippi Sound at similar developmental stages (270 and 300 μm, respectively, in spring and 255 and 280 μm, respectively, in summer). The observed geographic difference in embryo size may be related to some extent to differences in water content. In the present study, spring embryos had ~75% water at eight days prior to hatch compared to ~65% for summer embryos at the same stage but as embryogenesis progressed, the percent water converged to concentrations that were not significantly different between seasons. By one day prior to hatch, embryos from both seasons were ~80% water; the newly-hatched zoeae were ~90% water.

Amsler and George (1984b) also noted an increase in water content during blue crab embryonic development, from ~58% in the gastrula stage to ~72% prior to hatching, which was lower than in the present study; furthermore, there was no seasonal difference in their study.

Although the mechanism for seasonal variation in the size of blue crab embryos from the Gulf may be explained by an increase in water content rather than an increase in organic content, an obvious question remains. Why are there seasonal, and geographic, size differences in blue crab embryos? For some decapod species, embryo size increases with increasing female size (Attard and Hudon, 1987; Gardner, 1997). This was not a factor in the present study because size of the ovigerous females was not significantly different between seasons. Embryo size is known to decrease with successive broods over the spawning season for some species (Clark et al., 1985; Sainte-Marie, 1993). Blue crabs are capable of spawning successive broods, but because only primiparous females were used in the present study, this would not have contributed to the observed seasonal variation.

Other studies (Pandian and Schumann, 1967; Boddeke, 1982; Skadsheim, 1984; Clarke et al., 1985) have noted seasonal variation in embryo size, with larger embryos produced in winter/spring and smaller embryos in spring/summer. Temperature, rather than season alone, can influence embryo size such that larger embryos are produced at lower temperatures and smaller embryos at higher temperatures (Crisp, 1959; Patel and Crisp, 1960). There is a general trend for larger embryos at higher latitudes (Clarke et al., 1985), which is the reverse for the observed geographical difference among blue crab embryos from Mississippi (present study) and North Carolina (Amsler and George, 1984b). However, as Wehrtmann and Kattner (1998) pointed out, this trend can be modulated by differences in physical and chemical conditions including temperature, salinity and feeding. Temperature may not explain the variation in blue crab embryo size because temperature conditions were similar for both seasons in the present study and for the summer embryos from North Carolina (Amsler and George, 1984b). Salinity also may not be a factor because this parameter was maintained at 25 ppt in the laboratory during both seasons in the present study although it was 30 ppt for the North Carolina embryos (Amsler and George, 1984a).

Seasonal differences in crustacean embryo size have also been correlated with predictable changes in seasonal food supply (Cooney and Gehrs, 1980; Willows, 1987) or seasonal predators (Kerfoot, 1974; Brambilla, 1982) that impact the newly-hatched larvae. Therefore, larval size at the time of hatching, rather than embryo size per se, may be a critical factor influencing the observed seasonal size differences among Gulf blue crab embryos.

Variation in crustacean egg size can be controlled genetically (Hancock et al., 1998) or it can represent phenotypic plasticity (Walsh, 1993; Odinetz Collart and Rabelo, 1996; Hancock, 1998). McMillen-Jackson et al. (1994) performed a geographically broad genetic survey of *Callinectes sapidus* and found no geographic pattern in the clustering of populations in nearshore waters from New York to Texas. The species has high potential for dispersal and there appears to be substantial gene flow over its range. Despite the high level of gene flow, however, these authors did note geographic differentiation related to genetic patchiness. Six allozymes exhibited genetic patchiness on local and range-wide geographic scales whereas *EST-2* varied temporally. Furthermore, there was a cline in the *EST-2* locus in estuaries along the Atlantic coast but no apparent cline in the Gulf of Mexico. The latter results were presumed to be due to a combination of low adult long-distance migration and a high retention of locally-spawned larvae along the

Atlantic coast compared to potential long-distance migration of females in some regions of the Gulf or masking by genetic patchiness.

The explanation for variable embryo size in the blue crab is unknown. There may be a genetic component to the size differences between Mississippi and North Carolina populations, but local selection for size is a valid alternative hypothesis. Seasonal differences in Mississippi blue crab embryos may be due to phenotypic plasticity which suggests either a seasonal trend in food availability or a seasonal predator that favors larger size at hatching or at metamorphosis in the spring. However, the nature of the putative cause (predator or prey) is not known at this time.

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