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EMBRYO DEVELOPMENT AND MORPHOMETRY IN THE BLUE KING CRAB *PARALITHODES PLATYPUS* STUDIED BY USING IMAGE AND CLUSTER ANALYSIS

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ABSTRACT In this paper the embryonic development of laboratory-reared blue king crab, *Paralithodes platypus,* from the Pribilof Islands in the eastern Bering Sea is described. Developing embryos were removed from a female crab at various intervals, digitally photographed under a compound microscope and analyzed using Image-Pro Plus. Nine morphometric parameters were used, including seven measurements (total area, yolk area, embryo length and width, average diameter, eye length and width) and two calculated indices (percent yolk and elongation). First cell division was not apparent until day 4, after which divisions occurred daily until the blastopore appeared at day 28. A "V"-shaped embryo became apparent on day 114, followed by rapid appendage development. The eyes became pigmented by day 192. Hatching occurred from day 381 to day 409, and required at least 33 d to complete. Embryo area declined from an initial value of 0.95 mm^2 on day 1–0.83 mm^2 on day 72 and then increased to 1.28 mm^2 on day 388. Growth of all characters reached a plateau between days 240 and 353, and then increased rapidly until the middle of hatching (day 390). Visual examination was better at defining early changes, but cluster analysis of morphometric measurements was a better technique for defining middle and later stages. Both techniques resulted in an optimum selection of 12 developmental stages. Embryonic development has been described for few decapod crustaceans, and no standard exists for defining developmental stages. Multivariate analysis of morphometric measurements may lead to improved understanding of crustacean embryogenesis, allow standardization of staging and enable studies of environmental influence on development. The technique also has applications in the aquaculture industry.

KEY WORDS: king crab, development, hatching, incubation, embryo, morphometry, image analysis

INTRODUCTION

Studies of crustacean population fluctuations must include techniques for assessing environmental impacts such as climate change on reproduction, including embryonic development rates, diapause and irregular embryogenesis. Previous studies of embryo development in decapods have relied on traditional visual techniques to define developmental stages (Moriyasu & Lanteigne 1998, Yamaguchi 2001). However, the subjective nature of these methods leads to high variability because of the lack of standardized techniques or equipment, and such studies can rarely be applied to other species. Recent developments in digital imaging equipment and software have made it possible to improve the quality and reliability of morphological assessments in human and veterinary medicine. Image-analysis techniques have been used successfully to assess sperm morphology and quality in humans (Verstegen et al. 2002), horses (Hidalgo et al. 2005), fish (Gage et al. 2002), and marine mammals (Kita et al. 2001), and these techniques are easily adapted to studies of embryos as well. Crustaceans are particularly suitable research subjects because of their external and easily accessible embryos. Morphometry of embryos has been used to describe developmental stages in the freshwater prawn *Macrobrachium borellii* (Lavarias et al. 2002).

King crabs are large anomurans that are commercially exploited in many parts of the world. Commercially valuable species include red and blue king crab *Paralithodes camtschaticus* (Tilesius, 1815) and *P. platypus* Brandt, 1850, respectively, golden king crab *Lithodes aequispinus* Benedict, 1894, scarlet king crab *L. couesi* Benedict, 1894, European king crab *L. maja*, southern king crab *L. santolla* and others. Despite their value, embryonic development has only been studied for red king crab in Japan (Nakanishi 1987). There is no standardized scheme for describing developmental stages for king crab or any other large decapod crustacean.

Blue king crab (BKC) have historically supported lucrative fisheries in the eastern Bering Sea (EBS) at St. Matthew Island and near the Pribilof Islands (St. Paul and St. George). In 1999, both populations declined drastically, and their fisheries were closed, leading to renewed interest in research on their biology. Blue king crab have a 2-y reproductive cycle; in the first year of this cycle (the spawning year), female crabs molt, extrude eggs, mate and carry the developing fertilized embryos for approximately 1 y (Somerton & MacIntosh 1983, Jensen et al. 1985, Somerton & MacIntosh 1985, Jensen & Armstrong 1989). During the second (or hatching) year, larvae are released, but the crabs do not molt or mate again (Stevens in press), unlike female red king crabs that hatch, molt, mate and extrude annually (Stevens & Swiney, in press).

This research was undertaken as part of a larger study on the early life history of BKC, including development of cultivation techniques to ensure a supply of small crab for future research (Stevens et al., in press). This article describes the embryonic development of BKC during cultivation in the laboratory and methods to standardize definitions of developmental stages using visual as well as morphometric characteristics.

MATERIALS AND METHODS

Blue king crabs were captured by trawl about 20 miles northeast of St. Paul Island, in the eastern Bering Sea, during October 2003 (for dates and locations see Stevens in press). Crabs were kept in recirculating seawater aboard ship for several days until returning to Dutch Harbor, Alaska. They were then packed in insulated coolers between layers of wet burlap, kept chilled with frozen ice packs and shipped by air to Kodiak, Alaska. Upon arriving in Kodiak, crabs were placed in a 2500-L tank containing chilled (4°C) seawater. Most female crabs were new-shelled and ovigerous, but two female crabs had old shells and empty egg cases, evidence that they had released larvae the previous spring.

When one of the old-shell crabs subsequently molted, it was

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Reference to trade names does not constitute endorsement by the National Marine Fisheries Service.

placed in a tank with a male crab and observed daily during grasping until new clutches of eggs were extruded and the female was released. Examination of embryos began 1 d after fertilization (day 1). A small cluster of 50–100 eggs was removed every other day for the first 3 wk and at 2–3 wk intervals thereafter. Half of the eggs from each sample were examined live, and the remaining eggs were stained for 5–15 min in Bouin's solution prior to examination. Eggs were placed on a glass slide in 1 ml of filtered seawater and examined under a compound microscope at ×50-magnification using reflected light (darkfield background) from a fiber-optic source. Digital photographs of live embryos were taken with a 2-megapixel digital camera (Diagnostic Instruments Spot Insight camera) and analyzed using Image-Pro Plus, version 4.5. From days 3–12, individual photographs were taken of 3–5 embryos, but after day 12, 10–12 embryos were photographed on each sampling date. Embryos were only photographed if they were rotated at 90° to the sagittal plane. The image analysis system was calibrated using digital photographs of a stage micrometer set to the height of the midplane of crab embryos; the mean value (pixels · mm^{-1}) from three digitized images was used. Measurements were made by first outlining the embryo on the computer screen using the computer mouse; if the inner and outer embryo membrane were clearly separated (as was often the case during the first 90 d of development), then the inner membrane was outlined. If the embryo outline was clearly defined and free of background clutter, then the automatic tracing option was used. The outline was then captured using a smoothing value of 5 (on a scale of 1–9). For the first 3 mo, digital measurements collected for each embryo included area (A), maximum diameter (L), minimum diameter (W) and mean diameter (calculated from 180 measurements taken at 2° intervals around the perimeter). After the embryo became apparent at day 114, the area of the yolk mass was determined, and the percentage of apparent cross-sectional area occupied by yolk (PAY) was calculated. After day 192, 10 additional embryos were photographed where the eyespot was rotated to the top central position, and L and W of the pigmented eyespots were measured. In addition to the measured parameters, three metrics of embryo shape were calculated, including ellipticity (L/W), elongation ([L – W]/[L + W]), and circularity (πLW/4A) (Hidalgo et al. 2005). Measurements were output directly to an Excel spreadsheet for analysis.

When hatching began, the female crab was placed into a 70-L plastic tub fitted with a bulkhead fitting and drain on the lowest portion of the sidewall. Tubs received flowing sand-filtered seawater at a rate of 4–5 L · min^{-1} at ambient temperature (4.8 ± 0.4°C) during the hatching period. Larvae exiting the drain passed up through an exterior standpipe and into a fine mesh net. The net was removed daily and larval volume measured to the nearest 0.5 mL in a graduated cylinder. Mean hatching date was determined as the weighted average of larval production over time, that is, by multiplying the daily volume of hatched larvae by day-of-the-year, summing the products over time and dividing by total volume of larvae released.

Stage Descriptions

Stages of embryonic development were defined using two different methods. One was the traditional method using developmental changes that are visually observable in the stained or unstained embryos. However, no standard criteria exist by which to define stage endpoints, so stage definitions tend to be subjective. As a general guide, reference was made to descriptions of embryonic development in snow crabs *Chionoecetes opilio* (Moriyasu & Lanteigne 1998) and red king crab (Nakanishi 1987).

In the attempt to develop a more objective morphometrically-based method of classifying embryonic stages, cluster analysis of embryo morphometry was used as a second technique (Ludwig & Reynolds 1988) using SAS PROC CLUSTER. By grouping together samples (dates) with similar characteristics, the clustering technique should identify groups of dates (which may represent periods of development if they are sequential) during which the embryo metrics are most similar to each other and thus represent stages of development with little change. Different stages of development should be grouped into different clusters. Sampling units were defined as dates (with individual embryos as replicates), and the method used was average Euclidean distance. However, the actual sample dates were not used as input data because they would have influenced the resulting order of clusters, whereas the goal of this analysis is to determine the stage of development in randomly sampled (wild) crabs whose fertilization dates are unknown. All 11 measured and calculated metric parameters were used in the initial cluster analysis except yolk area (which was only used for calculating percent area of yolk, PAY). Subsequent analyses were made by removing calculated indices until the clusters were aligned in best chronological order. The number of clusters defined is somewhat arbitrary, with a maximum up to the total number of samples. However, selection of an appropriate number can be guided by looking for peaks in the pseudo-F statistic, and valleys in the pseudo-t^2 statistic (SAS 2004). Values of morphometric parameters are given in the text as Mean ±1 SD.

The utility of this method for classifying the stages of eggs from wild crabs with unknown developmental histories depends on the conditions under which it is applied. As a test, sampled data from three different crabs with embryos of different known ages were included and classified along with data from crab #1. Each test sample consisted of measurements from 10 embryos, and each crab had been held at different temperatures, as follows: Crab #2, 2°C, 167 d; Crab #3, 4°C, 192 d; Crab #4, 6°C, 223 d.

RESULTS

Embryo Development

Female crab #1 molted on January 17, 2004, was grasped and mated by the male the next day (18 January) and was observed to have extruded eggs on the next day, 19 January 2004, which was designated as day 0. Water temperature increased gradually from 4°C to 6°C during the study; mean temperature was 5.2°C ± 1.2°C during the incubation period (January 19, 2004 to March 3, 2005) (Fig. 1). The following description of development is organized according to the 12 stages defined by morphometric cluster analysis (see later).

Stage 1 (Cleavage)

During this period dividing cells were easily distinguishable prior to blastodisc formation, and little change in morphometry occurs. Eggs were initially lavender colored; first divisions occurred on day 4, but true 2-cell stages were not observed. By day 7, all embryos were multicellular, including 4-cell stage and 8-cell stages, and a few with 16 cells (Fig. 2A, a). On day 9, most embryos were at the 32-cell stage and one was 64-cells. Nuclei were apparent as diffuse light-colored spots in the center of each

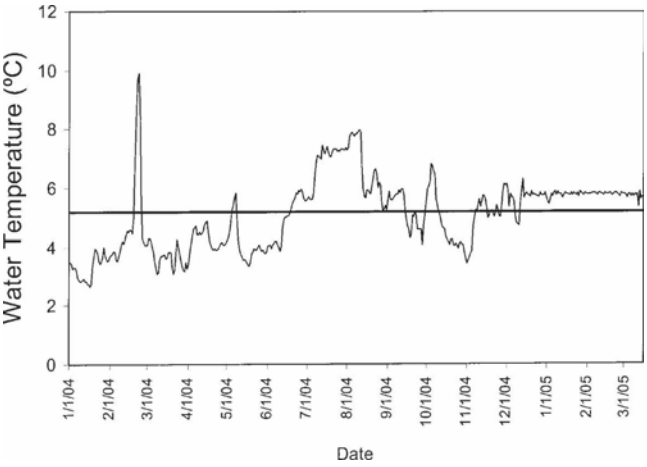

Figure 1. Water temperature during embryonic development of blue king crab (*Paralithodes platypus*). Mean temperature (heavy line) was 5.2 ± 1.2°C.

cell. Embryos were separated from the outer egg membrane, which had increased slightly in size. Cell numbers continued to double at 2-d intervals, to 64 on day 10, 128 on day 12, 256 on day 14, etc. By day 19, yolk had broken up into small irregular globules and cell borders were no longer distinct, although nuclei were apparent on eggs preserved in Bouin's. This period corresponds to visual stages V1-V2 in Table 1.

Stage 2 (Blastula-Gastrula)

Individual cells and structures are not visible. The blastodic became apparent on day 28 and the blastopore was visible on some eggs (Fig. 2B, b). Embryos continued to decrease in size until day 72, but developmental changes were not discernible. Corresponds to V3-V5.

Stage 3 (V-embryo to Nauplius)

During this period embryonic lobes are becoming visible and are increasing in size. On day 114, the V-shaped embryo became distinct from the yolk in stained eggs; it is now clear in profile and can be measured (Fig. 2C, c). By day 121, most embryos had distinct lobes that would become the antennules, antennae and mandibles. Optic lobes are diffuse and indistinct, and the abdomen is a diffuse round lobe at the base of the "V". By day 128, most embryos have distinct mandibles, and by day 143, the optic lobes are clearly defined. By the end of this stage, the antennules and antennae are elongated, and the abdomen is distinct. This stage corresponds to V-6, and is similar to stage 31 (metanauplius) of Nakanishi (1987) (abbreviated as N-31), or to stage 6 (prenauplius) of Moriyasu and Lanteigne (1998) (abbreviated as ML-6).

Stage 4 (Prenauplius)

Defined by a single observation on day 157 (Fig. 2D, d). The optic lobes are large and rounded. Rudiments of the antennules and biramous antennae are clearly defined, the latter with a medial epipodite. The mandible is forming medial to the antennae. The abdomen is folded over the embryo for about half of its length. Size and shape of the embryo is identical to that of Stage 1, equivalent to stage V-7 and similar to N-33 or ML-8.

Stage 5 (Metanauplius)

Observed on day 171 only. Optic lobes extend lateral to the rest of the embryo. The tail is about two-thirds the length of the embryo. The telson is forked, but setae are not apparent. Maxilliped rudiments are barely visible lateral to the tail. Embryo area and diameter surpass the starting values. Included in V-7, similar to N-38 or ML-9.

Stage 6 (Eye Formation)

The eyes are large, lightly pigmented, and extend almost to the edges of the egg (Fig. 2E, e). The telson has 6 or 7 spines (or setae) and reaches the anterior margin of the optic lobes. Lateral appendages have setae. Up to four chromatophores can be seen. Similar to N-42 (day 201) or ML-10.

Stage 7 (Chromatophore Formation)

This is a period of rapid eye growth and formation. The eyes changed from strongly pigmented crescents (Fig. 2F, f), to being oval-shaped (Fig. 2G, g). Six to eight chromatophores are visible on each side. Maxillipeds are elongated with rudimentary setae. In side view, the embryo takes up one-third of the egg. The telson extends past the optic lobes. Similar to N-44, this stage and the next are included in stage V-8.

Stage 8 (Diapause)

Yolk is divided down dorsal midline into left and right halves, as well as distinct anterior (pinkish) and posterior (orange) lobes (Fig. 2H, h). The embryo is crescent-shaped and wraps three quarters of the way around the yolk, covering the entire surface in ventral view. Embryo area and diameter reach a "plateau," and do not increase further until after day 329. Growth rate of eye length slows down, and eye width levels off. Heartbeat becomes distinct.

Stage 9 (Eye Enlargement)

Embryo takes up >50% of egg in side view (Fig. 2I, i). Posterior lobe of yolk is visibly reduced, relative to anterior lobe. Yolk lobes are clearly separated in dorsal view (Fig. 2J, j). This stage is equivalent to V-9.

Stage 10 (Rapid Growth Phase)

Area, length and width of embryo increase rapidly. Dorsal edge of yolk is separated from the perimeter of the embryo case. All measured dimensions start to increase. Equal to V-10.

Stage 11 (Yolk Depletion)

Area of yolk decreases rapidly, as other dimensions increase during this period of rapid growth. Maxillipeds are well defined and pigmented (Fig. 2K, k). Equal to V-11.

Stage 12 (Hatching)

Hatching starts. Embryo length and width, and eye length and width reach maximum values. Ommatidia develop a greenish-yellow fringe, producing a "halo" effect around eye. PAY reaches lowest value. Anterior and posterior regions of yolk become distinct, and the latter is reduced to individual lipid globules. The presence of hemocyanin gives the embryo a bluish tinge. Day 395 (Fig. 2L, (l) is midpoint of hatching period. Equal to V-12.

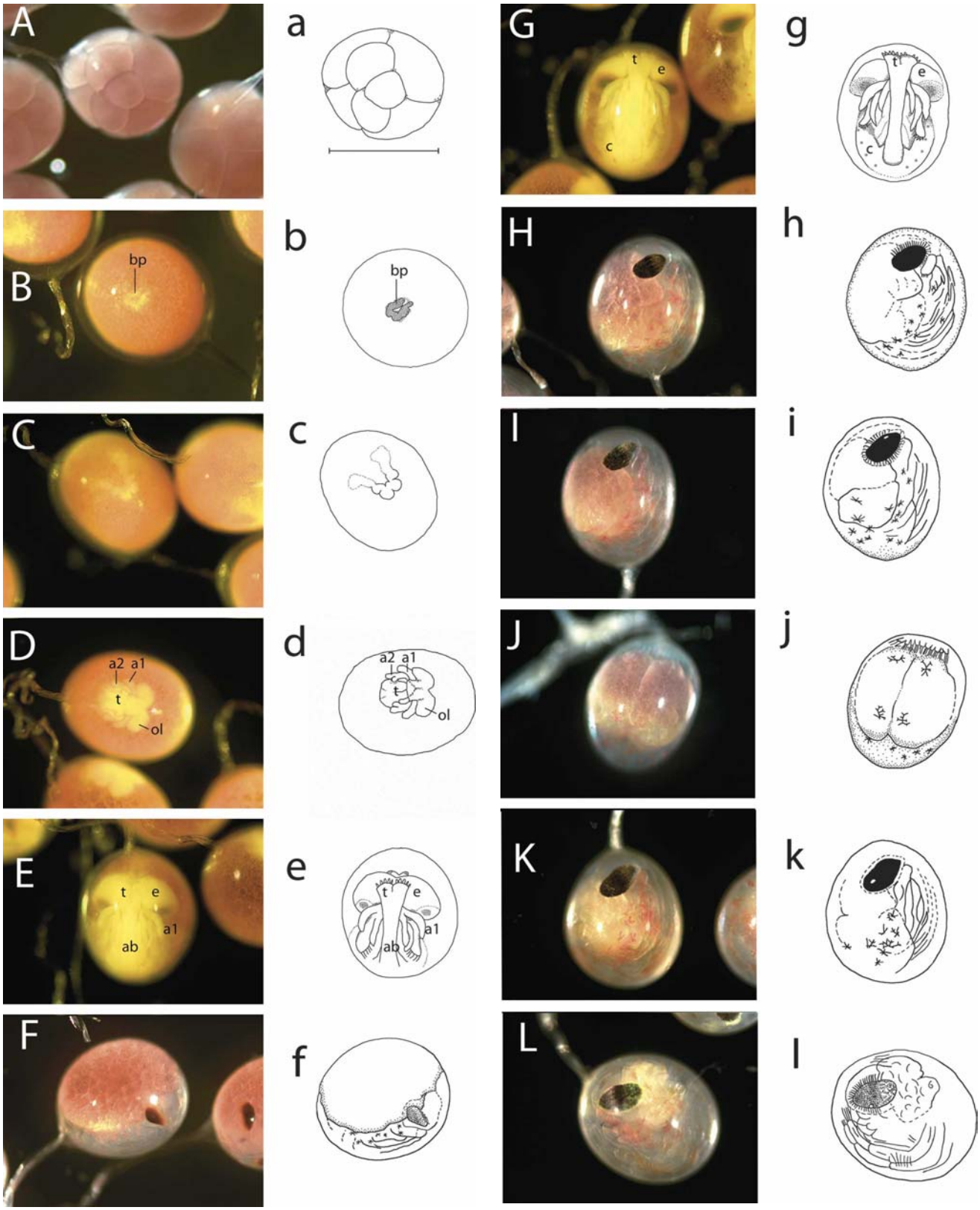

Figure 2. Blue king crab embryo development based on morphometric staging: A, (a) Stage 1, day 7, 16 cells (scale bar = 1.0 mm); B, (b) Stage 2, day 28, blastodisc, stained; C, (c) Stage 3, day 114, "V"-embryo, stained; D, (d) Stage 4, day 157, stained; E, (e) Stage 6, day 192, stained (note setae on maxillipeds and telson); F, (f) Stage 7, day 206; G, (g) Stage 7, day 206, stained; H, (h) Stage 8, day 268; I, (i) Stage 9, day 305; J, (j) Stage 9, day 305, dorsal view showing divided yolk; K, (k) Stage 11, day 367; L, (l) Stage 12, day 395, hatching. Abbreviations: a1, antennule; a2, antennae; ab, abdomen; bp, blastopore; c, carapace; e, eye; ol, optic lobe; t, telson.

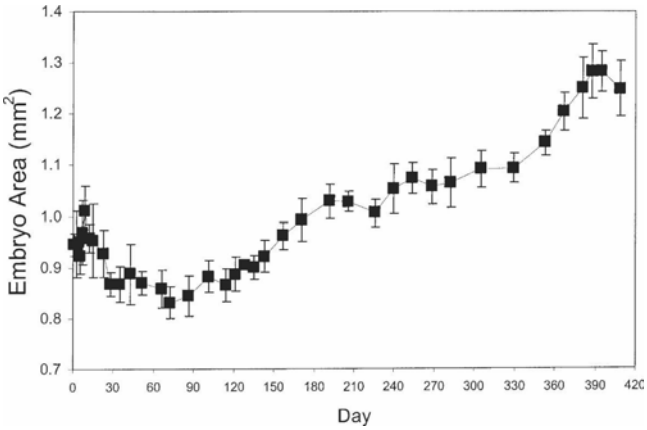

Figure 3. Mean ±1 SD of area for blue king crab (*Paralithodes platypus*) embryos, from fertilization to hatching.

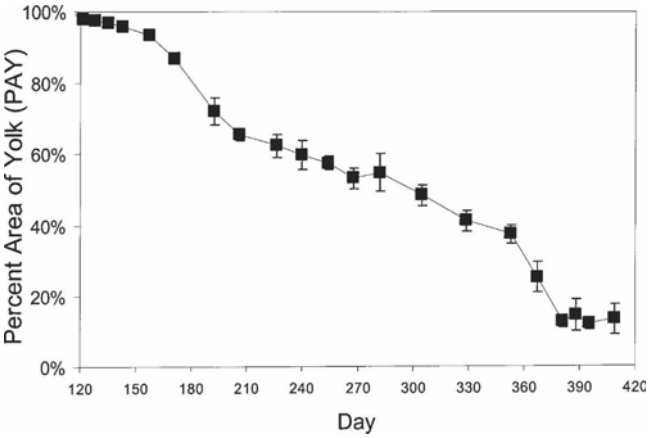

Figure 5. Mean ±1 SD of percent yolk visible in side view of blue king crab (*Paralithodes platypus*) embryos.

Morphometric Changes

When first extruded, the mean area of eggs was 0.95 ± 0.02 mm^2 (Fig. 3), and length and width were 1.17 ± 0.05 mm and 1.03 ± 0.03 mm, respectively (Fig. 4). Mean area gradually declined to a minimum of 0.83 ± 0.03 mm^2 on day 72 then began increasing steadily. Length and width followed similar trends. Embryo area, length and width reached a plateau between days 240 and 329 and then increased rapidly from day 353 to day 381, when hatching started. Maximum values were reached for embryo length (1.37 ± 0.02 mm) on day 381, for embryo area (1.28 ± 0.05 mm^2) on day 388, and for embryo width (1.19 ± 0.03 mm) on day 395. In profile view, yolk occupied 100% of the area of the egg until day 121, when the embryo first became apparent, and PAY was 97.8% (Fig. 5). Yolk area declined as the embryo grew, with a steep decline between days 157 and 206. By day 305, PAY was <50% of total profile area. From day 353 to day 381, during the last month before hatching started, PAY declined most rapidly, from 37% to 12.5%. PAY leveled off during hatching, but reached its lowest value of 12% on day 395. Eye pigment was first observed on day 192, when length and width of the pigmented area were 0.155 ± 0.017 mm and 0.077 ± 0.011 mm, respectively (Fig. 6). Eye length and width both increased steadily thereafter, with the rate of increase leveling off after day 305. Eye length and width increased rapidly after day 353, reaching maximal values on days 395 (0.431 ± 0.012 mm) and 388 (0.265 ± 0.016 mm) for length and width, respectively.

Stage Classification

A total of 39 samples (dates of observation) were used for the analysis. Table 1 defines the stages, starting and endpoint dates (as day number), duration of each stage in days, and percent of total development, as defined by both the traditional (visual) method and the morphometric (clustering) method. The best results were obtained after eliminating the circularity and ellipticity metrics, which showed no linear trend over time. Similar results were obtained regardless of whether calculations were made using PAY as raw data, or after angular or log transformation. Clusters were selected in more-or-less chronological order, even though day number was not used as a variable. Twelve stages were defined by both methods, those stages found to be similar by both methods appear on the same line. In the cluster analysis, a sharp change occurred in both statistical guidelines (the pseudo-F and pseudo-t^2 values) after 11 clusters. In the dendrogram (Fig. 7), a horizontal line drawn at an average Euclidean distance of about 0.15 (on the vertical axis) would cut across 11 vertical branches, each of which defines a cluster. Two clusters (days 9 and 157) were misplaced, so the former was combined with Cluster 1 and the latter removed

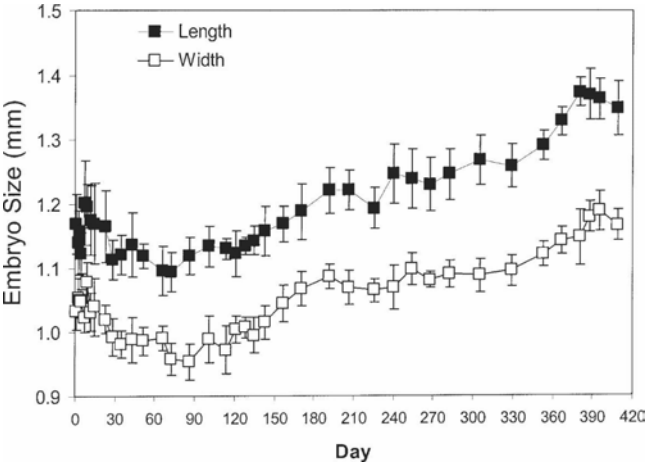

Figure 4. Mean ±1 SD of minimum (width) and maximum (length) diameters for blue king crab (*Paralithodes platypus*) embryos.

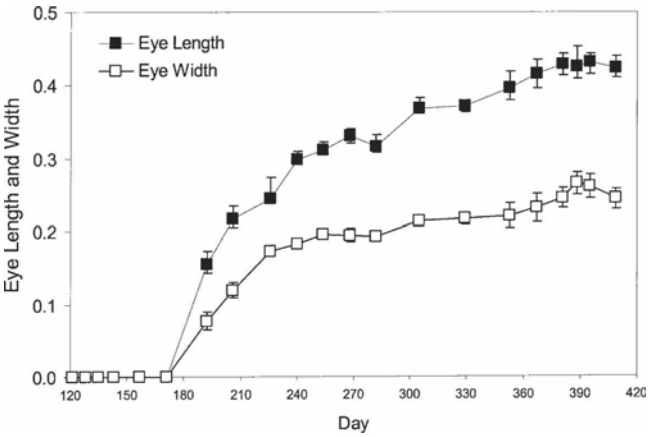

Figure 6. Mean ±1 SD of length and width for the pigmented eye of blue king crab (*Paralithodes platypus*) embryos.

TABLE 1.

Developmental stages of blue king crab. Stages were defined either by the traditional visual method, or by analysis of morphometrics. Begin and End are midpoint days between observations and are numbered from fertilization; Days is duration of stage; Percent is duration relative to complete development. Similar stages occur on same lines. Some stages were represented by a single sampling date.

Stages Assigned by Morphometrics		Values at Beginning of Stage								Stages Assigned by Visual Method				
Stage	Description	Begin	End	Days	Percent	Egg Area	Egg Length	Eye Length	Pct Yolk	Stage	Begin	End	Days	Percent
1	Cleavage	0	25	25	6.1%	0.945	1.170			1	0	4	4	1.0%
										2	4	26	22	5.4%
2	Blastula-Gastrula	25	117	92	22.4%	0.867	1.114			3	26	39	13	3.2%
										4	39	108	69	16.8%
										5	108	125	17	4.1%
3	V-embryo	117	150	33	8.0%	0.886	1.122		0.980	6	125	139	14	3.4%
4	Pre-nauplius	150	164	14	3.4%	0.961	1.168		0.935	7	139	182	43	10.5%
5	Meta-nauplius	164	182	18	4.4%	0.992	1.188	0.000	0.870					
6	Eye formation	182	199	17	4.1%	1.028	1.220	0.155	0.721					
7	Chromatophore formation	199	233	34	8.3%	1.028	1.221	0.216	0.655	8	182	233	51	14.4%
8	Diapause	233	294	61	14.9%	1.051	1.247	0.298	0.597					
9	Eye enlargement	294	340	46	11.2%	1.091	1.267	0.368	0.484	9	233	294	61	14.9%
10	Rapid growth	340	360	20	4.9%	1.142	1.291	0.397	0.374	10	294	341	47	11.4%
11	Yolk depletion	360	374	14	3.4%	1.203	1.328	0.415	0.252	11	341	374	33	8.0%
12	Hatching	374	410	36	8.8%	1.249	1.374	0.428	0.126	12	374	410	36	8.8%
					100.0%									100.0%

from it. One additional cluster was further split posthoc into Clusters 2 and 3, leaving 12 useful clusters as stages.

Embryos from three test crabs were classified into three different stages relative to those of Crab #1. Embryos from Crab #2, incubated at 2°C (167 d old) were developmentally delayed because of colder temperatures and were classified as stage 3 (between days 128 and 135 for Crab #1). Embryos from Crab #3, raised at 4°C (192 d old), were classified as a distinct cluster between stage 5 (171 d) and stage 6 (192 d). Embryos from Crab #4 (6°C, 223 d) were more advanced because of warmer temperatures and were classified as a distinct cluster between stages 8 (282 d) and 9 (305 d).

Hatching

Hatching of larvae was first observed on February 2, 2005, but the female crab was not placed into the isolation tub until February 4, because of space limitations, and larvae were first collected the next day. Therefore, it is likely that several days of larval hatching were missed. On the first night of larval capture 35 mL of larvae were collected (Fig. 8), a much larger volume than on subsequent days. Whereas unusual, such spikes occasionally occur when hatching is delayed because of disturbance or disruption of light cycles. Measurable numbers of larvae were collected for 31 d, and the mean date of hatching was February 12 (day 390). If the 2 d prior to the beginning of larval collections (after female isolation) are included, hatching lasted for a total of at least 33 d.

DISCUSSION

During early stages, BKC embryos developed at almost the same rate as described for red king crab by Nakanishi (1987), who reported that cleavage was first seen on day 4, a distinct 2-cell stage was not observed, the 4-cell stage appeared on day 5, 8-cells on day 8, and cell numbers doubled daily thereafter. Development of BKC embryos slowed between days 240 and 330 (mid September to mid December) and then increased rapidly until hatching. The total length of development was longer (390 d to the mean hatch date) than that for 12 primiparous red king crabs (365 d) or for 19 multiparous red king crab (326 d) that were held at an average temperature of 6°C (Stevens & Swiney, in press). Length of hatching, although possibly underestimated at 33 d, was slightly longer than the mean of 28 days determined for 23 BKC in 2004 (Stevens, in press), yet similar to that for red king crabs, which averaged 31 d regardless of parity (Stevens & Swiney, in press). Snow crabs in the Gulf of St. Lawrence also have a 2-y spawning cycle like BKC, but embryos require 2 y to develop (Moriyasu & Lanteigne 1998), versus 13 mo for BKC. Moriyasu and Lanteigne (1998) described 14 developmental stages that roughly correspond to the 12 identified for BKC, plus two earlier stages prior to, and during, funiculus formation; analysis of morphometry did not distinguish such stages in BKC embryos. Nakanishi (1987) examined red king crab eggs at more-or-less weekly intervals, and subsequently described 53 stages.

Crustacean growth and development rates increase with temperature, as has been shown for larval stages of both red (Nakanishi 1981) and southern king crab (Anger et al. 2004), and snow crab (Kogane et al. 2005) and embryos of northern shrimp *Pandalus borealis* (Brillon et al. 2005). Crustaceans from warmer water environments typically have shorter embryonic development on the order of days to weeks. With short developmental periods, observations made at daily intervals are often different enough to be characterized as individual stages. For example, embryos of the fiddler crab *Uca lactea* require an average of 15.4 d to develop, reach the 32-cell stage within 24 h and can be categorized into 15 distinct stages (Yamaguchi 2001). Embryonic development of the redclaw crayfish, *Cherax quadricarinatus*, requires 42 d at 26.0°C, and was categorized into 10 prehatching and 3 posthatching stages (Garcia-Guerrero et al. 2003). Unlike crabs in the families Lithodidae and Paguridae, *Aegla platensis*, a riverine anomuran in Brazil, develops through the zoea and decapodid stages (equivalent to the megalops or glaucothoe) inside the egg and hatches after 35 d (Lizardo-Daudt and Bond-Buckup 2003).

Because of the great disparity in development time, there is no

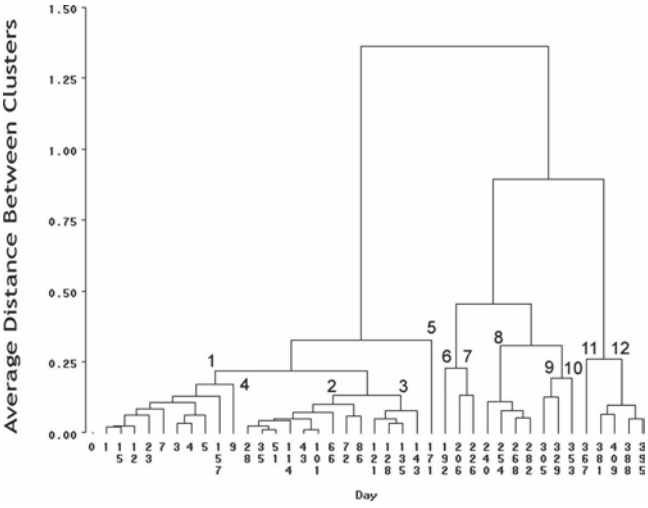

Figure 7. Dendrogram of blue king crab (*Paralithodes platypus*) embryo stages as defined by cluster analysis. 12 clusters are identified, one of which (#4, day 157) was misclassified.

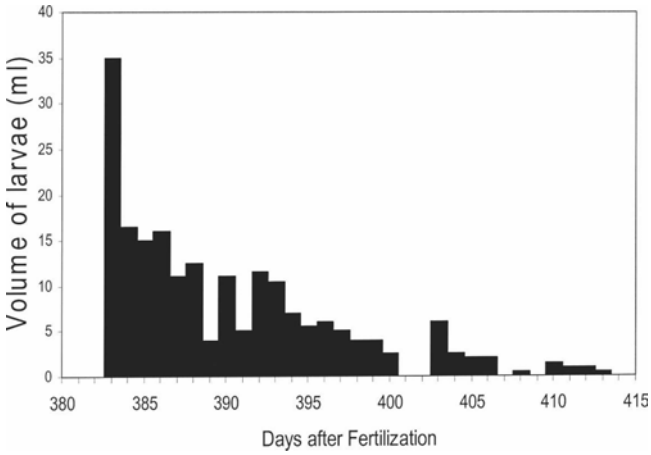

Figure 8. Total volume of larvae hatched each day by the single blue king crab (*Paralithodes platypus*) used in this study.

standardized scheme for characterizing developmental stages of crabs or any other decapod crustacean. The number of described stages ranges from 10–15, depending on the length of development and the utility of each stage in describing changes observable by eye. The use of embryo morphometrics is a more quantitative method, and may be a useful approach for comparing development between different populations, environmental conditions or species. Lavarias et al. (2002) used embryo morphometry to describe development in the freshwater prawn *Macrobrachium borellii* and found that predetermined stages could be identified using four metrics, but they did not use a multivariate approach to classify stages based on their similarity. In order for this approach to be useful, however, it requires a larger number of samples than the number of expected stages. Therefore, for species with short development periods, multiple samples per day would be required. However, stages defined by multivariate analysis, whereas relatively easy to construct using morphometric measurements, are not a substitute for examining the embryo by eye to determine the relative development of various appendages, chromatophores and other organs, and some samples (e.g., day 157) could not be accurately classified without visual observations. Examination of embryos from the three extra crabs, whereas not definitive because of the different holding temperatures, does indicate that unknown embryo samples can be classified on a relative scale of development. A more definitive system could be developed by employing classification and regression tree (CART) analysis to define discrete developmental stages based on specific criteria.

The techniques of morphometric analysis used in this study are partly adapted from those used in the medical and veterinary sciences for classifying the "quality" of sperm cells (Verstegen et al. 2002). Automated techniques such as computer-assisted sperm analysis (CASA) (Verstegen et al. 2002) and automated sperm morphometry analysis (ASMA) (Hidalgo et al. 2005) could provide new insight into the study of crustacean embryo development, revealing differences too subtle to be detected by the naked eye that may result from environmental change, anthropogenic disturbance or pollution. Morphometric classification of gametes and embryos may also be useful for identifying and selecting high-quality brood stock for the aquaculture industry or for preservation of endangered species.

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