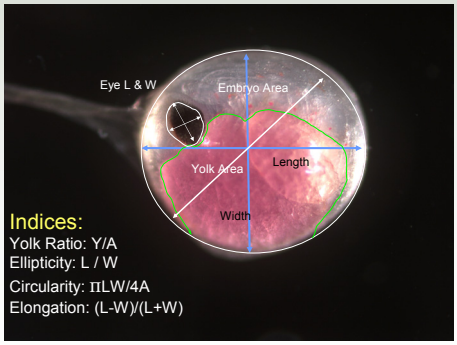
Embryonic Development of Pribilof Island blue king crab, *Paralithodes platypus*, studied using image analysis and clustering procedures

Bradley G. Stevens, NMFS/NOAA, Kodiak Fisheries Research Center, Kodiak, AK

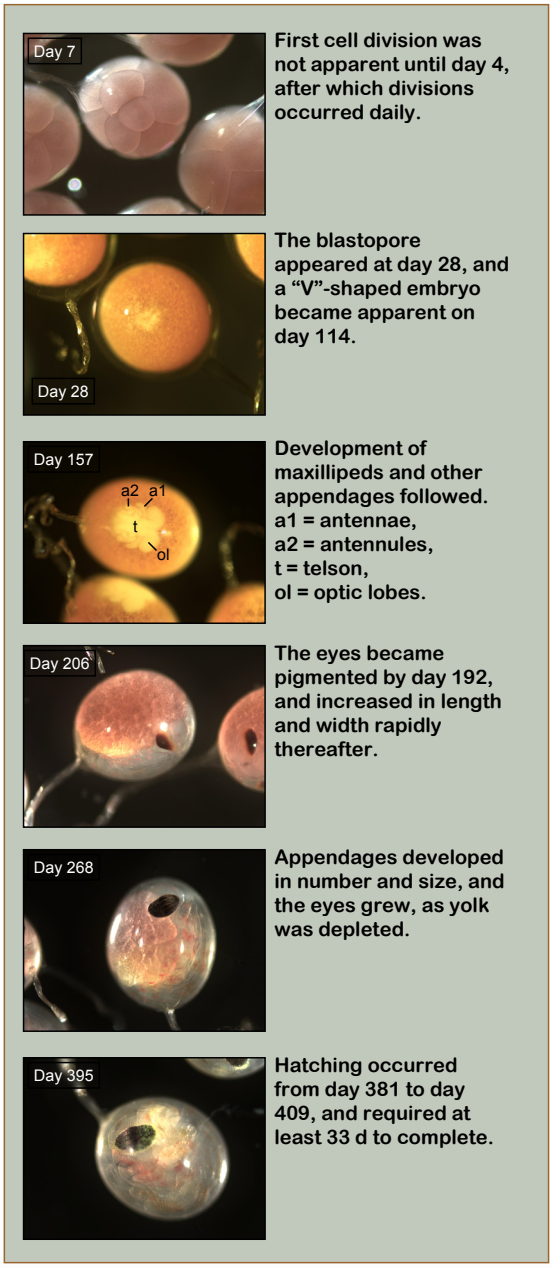
INTRODUCTION

Blue king crab *Paralithodes platypus* populations in the Pribilof Islands, Alaska, have declined 92% since 1995. Understanding the causes of such fluctuations requires knowledge about crab reproduction, its timing, and effects of climate change. Embryonic development of Alaskan king crabs has not previously been studied. Traditional studies of crab embryo development have relied on visual techniques that are subjective and highly variable and rarely applicable to other species. There is no standardized method for defining developmental stages for king crab or any other large, coldwater crab. I used image analysis techniques to study the development of blue king crab embryos during cultivation in the laboratory, and to standardize definitions of developmental stages using visual and morphometric characteristics. Crabs are particularly suitable research subjects because of their external and easily accessible embryos.



METHODS

Developing embryos were removed from a female crab at various intervals, digitally photographed under a compound microscope, and measured with Image-Pro Plus. Seven morphometric parameters were directly measured, including total area, yolk area, embryo length and width, average diameter, eye length and eye width. Four indices were calculated, including percent yolk, ellipticity, elongation, and circularity.



First cell division was not apparent until day 4, after which divisions occurred daily.

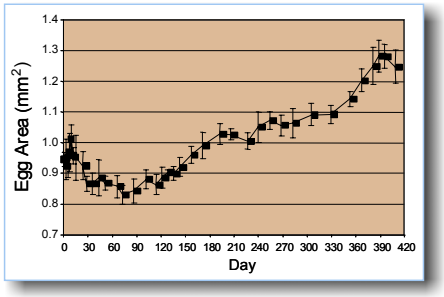
The blastopore appeared at day 28, and a "V"-shaped embryo became apparent on day 114.

Development of maxillipeds and other appendages followed. a1 = antennae, a2 = antennules, t = telson, ol = optic lobes.

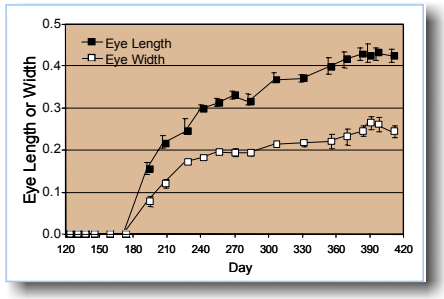
The eyes became pigmented by day 192, and increased in length and width rapidly thereafter.

Appendages developed in number and size, and the eyes grew, as yolk was depleted.

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 mm² on day 1 to 0.83 mm² on day 72, and then increased to 1.28 mm² on day 388. Growth reached a plateau between days 240 and 353, and then increased rapidly until day 395.



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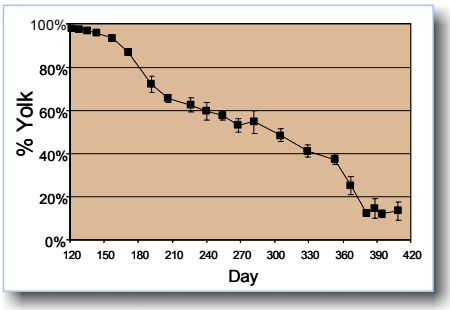
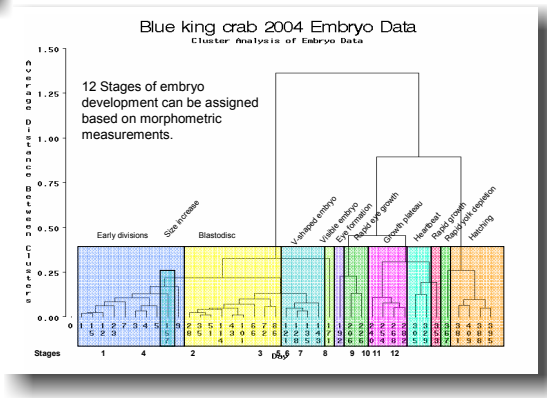


The embryo became apparent at day 114, and increased in size steadily, as yolk area decreased. Percent yolk (the ratio of embryo area to yolk area) declined steadily, with a rapid drop to about 12% just before hatching.

Twelve developmental stages were defined using cluster analysis of the morphometric data. Most stages were clustered in chronological order. Visual methods were better at defining early changes, but morphometrics were better for defining middle and later stages. This research demonstrates that multivariate analysis of embryo morphometrics can provide objective, repeatable classification of embryo stages.



Morphometric analysis techniques may lead to improved understanding of crustacean embryogenesis, allow standardization of staging, and enable studies of environmental effects. These techniques also have application in the aquaculture industry, for selection and classification of broodstock.

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