Effect of Temperature and Rearing Density in Golden King Crab, *Lithodes aequispinus*, Larval Cultivation

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INTRODUCTION

Golden king crab (GKC) *Lithodes aequispinus* are a commercially valuable species in Alaska waters in the Bering Sea, Pribilof Islands, Gulf of Alaska, and the Aleutian Islands (Fig. 1a). Along the Aleutian Islands, bottom temperatures range from 2.0 to 6.5ºC (mean = 4.0ºC), and GKC are found in depths > 800 m (Fig. 1b). GKC became commercially important in Alaska waters as other king crab species declined in the early 1980's. The commercial fishery remains relatively high yet little information exists for the early life history of this species. They are described as aseasonal and asynchronous in their reproductive cycle (Paul and Paul 2001, Sloan 1985). Their lecithotrophic development (Shirley and Zhou 1997), narrow deep depth range of juveniles and adults (Sloan 1985), and absence from extensive plankton tows down to 50 m (Shirley and Shirley 1989, Somerton and Otto 1986), suggest that GKC larvae are demersal. GKC females are known to migrate at deeper depths prior to larval release (Sloan 1985) and the juveniles have been found deeper than the adults (Blau et al. 1995, B. Stevens Pers. Comm., UMASS).

OBJECTIVES

The goal of this study is to successfully cultivate GKC larvae to obtain an adequate number of glaucothoe to conduct experiments on settlement behavior and habitat preference of juveniles. This will include monitoring ovigerous females, hatching eggs, and cultivation of larvae based on previous work on blue king crab *Paralithodes platypus* (Stevens 2006b, Stevens et al. 2008) and GKC (Shirley and Zhou, 1997).

MATERIALS AND METHODS

During September 2005, 11 ovigerous female GKC with eyed eggs were captured from commercial crab pots along the Aleutian Islands and collected by fishery observers. GKC were shipped live in coolers by ADF&G staff from Dutch Harbor to the AFSC Kodiak Laboratory. At the Kodiak sea water lab the crabs were tagged, weighed (g), and measured (CL mm). Crabs were maintained in a flow-through filtered seawater system in a 2500 L tank chilled at 3-4°C.

Monitoring Embryo Development and Hatch Timing

Beginning in October, developing embryos were monitored for hatching by sampling eggs monthly and photographing embryos. Ten eggs from each female were digitally photographed at 50X magnification with a 2 megapixel digital camera under a compound microscope. Eggs were measured using Image-Pro Plus, version 4.5, after calibration with a stage micrometer (Fig. 2). Digital measurements were made of egg area (A), maximum diameter (L), minimum diameter (W), mean diameter (MD), and areas of yolk mass were measured to calculate the percentage of yolk (PAY). Also L and W of the pigmented eyespots were measured. Measurements were plotted to determine changes in egg morphometrics for the late stage eggs.

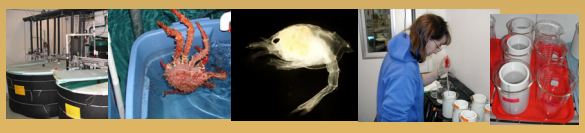
Experimental Design

Experiment 1 (Survival at 2 temperatures and 3 densities using controlled static conditions)

Since GKC are lecithotrophic, larvae were not fed until the crab stage (CI). The experiments were conducted with newly hatched larvae in controlled cold rooms set at 4 and 6°C (4.1, 6.6°C ± 0.3). Larvae were placed in 7.5 cm D x 16.5 cm H PVC tubes with a 625 µm mesh bottom and inserted into a 1 L beaker filled to 80 ml sterile UV treated seawater. Larvae were counted and dead larvae and exuviae were removed daily, and water was changed on alternate days. For each temperature there were three densities of 10 (A), 20 (B), and 40 (C) larvae per beaker (larvae/L) and four replicates of each (Table 1).

Experiment 2 (Compare survival of experiment 1 using a low flow-through system)

The same design using larvae that hatched the same day from the same female was used with the PVC inserts immersed in a low flow-through, filtered and UV treated, tank (190 cm L x 101 cm W x 30 cm H) at ambient temperature (7°C ± 0.5). Each tube received a slow drip inflow from the top except another treatment (Cont. flow) had a continuous, low, but steady water inflow with a medium density of 20 larvae/L.



Chilled tanks for holding ovigerous females. Hatching female in hatchery tub. Newly hatched zoea (ZI). Removing dead zoea and exuviae from beakers in cold room. Beakers with PVC inserts.

Table 1. Experimental design for cultivation study of golden king crab.

Temp °C	Cultivation System	Legend	Density (#Larvae/L)	#Larvae/ Treatment Total Larvae
Experiment 1				
4	Cold room 1L beakers in PVC tubes	A	10	10 x 4 =40
4	Cold room 1L beakers in PVC tubes	B	20	20 x 4 = 80
4	Cold room 1L beakers in PVC tubes	C	40	40 x 4 = 160
6	Cold room 1L beakers in PVC tubes	A	10	10 x 4 =40
6	Cold room 1L beakers in PVC tubes	B	20	20 x 4 = 80
6	Cold room 1L beakers in PVC tubes	C	40	40 x 4 = 160
Experiment 2				
7	Low flow-through system in 1L PVC tubes with drip inflow	A	10	10 x 4 =40
7	Low flow-through system in 1L PVC tubes with drip inflow	B	20	20 x 4 = 80
7	Low flow-through system in 1L PVC tubes with drip inflow	C	40	40 x 4 = 160
7	Low flow-through system in 1L PVC tubes with continuous flow inflow	Cont.	20	20 x 4 = 80

LITERATURE CITED

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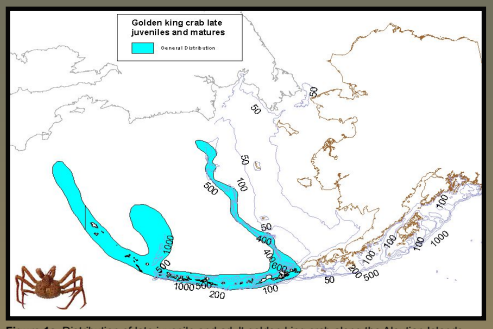


Figure 1a. Distribution of late juvenile and adult golden king crab along the Aleutian Islands and eastern Bering Sea (AFSC).

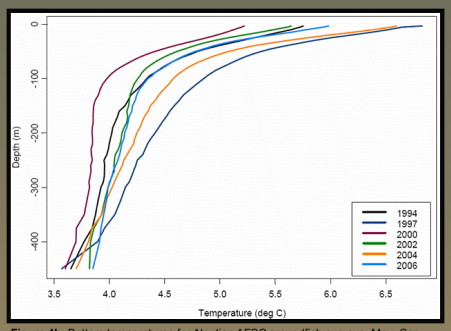
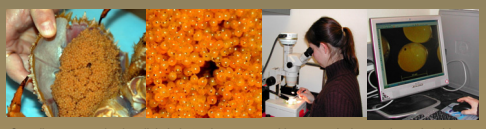


Figure 1b. Bottom temperatures for Aleutian AFSC groundfish surveys. May- Sep., 1994-2006.



Sampling eggs and using digital photomicroscopy and image analysis to estimate hatching for golden king crab embryos.

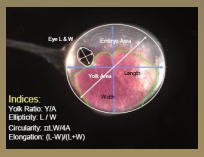
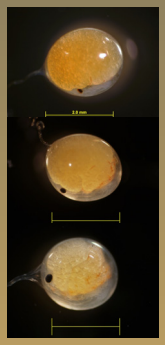


Figure 2. Egg measurement indices used to estimate hatching in golden king crab (Stevens 2006a).



Golden king crab embryos of known age, showing change in yolk and eye area from eye formation (est. day 200) to hatching (est. day 430).

RESULTS

Monitoring egg development and hatching

The embryos hatched with large quantities of yolk, comprising >50% PAY (Fig. 3). Females #694, 688, 690, 693, and 689 all hatched with 50.7 - 58.1% yolk from November through January. The rest (#691, 684, 686, 687, 692, and 685) hatched from February through March and the percent yolk measured during January was 61.4 - 73.2%. This large quantity of yolk in hatching and developing lecithotrophic golden king crab larvae is in sharp contrast to the almost complete yolk absorption seen in planktotrophic blue king crab, *Paralithodes platypus*, with 12% (Stevens 2006a).

RESULTS

Larval cultivation (Fig. 4)

Experiment 1

A Two-factor ANOVA was conducted with temperature and density as factors. Survival of GKC from hatching to 50% glaucothoe (G50) varied between temperatures ($p<<0.001$) but not among rearing densities ($p=0.64$). Proportions of survival were 59.2 and 54% at 6.6 and 4.1ºC, respectively. Furthermore, there was no interaction between factors ($p=0.71$). Time from hatching to G50 differed significantly between both temperatures (test-T $p<<0.001$). Larvae raised at 4.1 and 6.6 ºC required 47.4 ± 1.0 days and 33.5 ± 0.8 days, respectively to reach G50. As in similar studies (Shirley and Zhou 1997), larvae raised at 4.1, 6.6, and 7ºC skipped either the 3rd or 4th larval instar.

Experiment 2 (Fig. 5)

Larvae cultivated in the flow-through system with a slow drip inflow into the PVC tubes had similar results to the cold room treatment at 6.6 ºC. However larvae with a continuous but low inflow into the PVC tubes showed the highest survival of 67% to G50.

These results suggest that temperature delays molting and development of GKC larvae, and reduces survival to G50. Cultivation techniques described in this study are useful for future research on settlement behavior and habitat preference which are dependent on the production of an adequate number of glaucothoe.

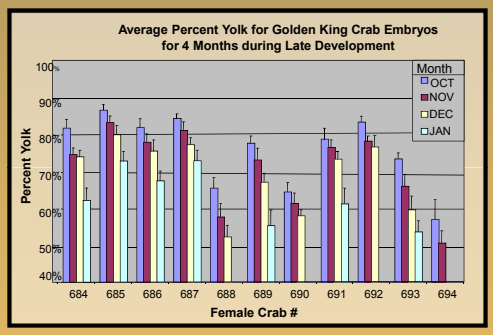


Figure 3. Average percent yolk area (PAY) for 11 late stage golden king crab embryos over a four month period and hatching at different times.

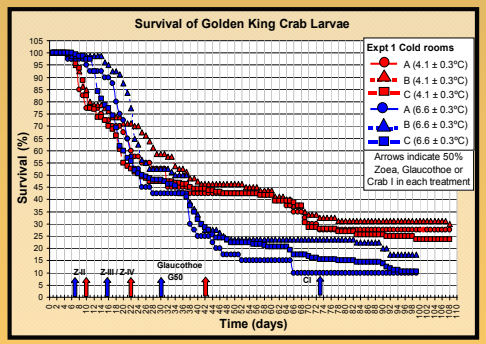


Figure 4. Survival results for larval cultivation of golden king crab in cold rooms at 4ºC (red) and 6 ºC (blue).

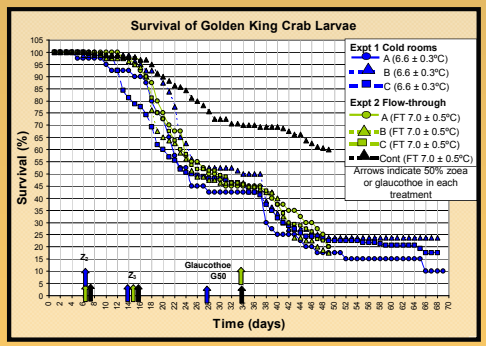


Figure 5. Survival results for larval cultivation of golden king crab in a cold room at 6.6ºC (blue) and in an experimental flow-through system at 7ºC, with a slow drip inflow (green) and a continuous inflow (Cont.) (black).

CONCLUSIONS

- There was no difference in survival for rearing densities of 10, 20, and 40 larvae/L
- Development time from hatching (ZI) to G50 was 13.9 ± 1 days longer for 4.1 versus 6.6 ºC.
- Survival of GKC to G50 was greater at 6.6 versus 4.1 ºC.
- However survival from G50 to crab stage (CI) is lower for 6.6 ºC.
- There was no difference in survival to G50 for the 7ºC slow drip inflow flow-through system and the 6.6ºC treatment in the cold room.
- However, the continuous low flow flow-through treatment appears promising with survival of 67% to G50 and has led to testing other technology for cultivating greater numbers.
- Other experiments are underway to test variation in survival from early, mid, and late hatch as well as maternal effects.
- Also studies to compare survival to CI at different temperatures and energetic costs are of interest.

ZI (zoea I, larval stage I)

Glaucothoe

CI (crab stage I)

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