

## North Pacific Research Board: Final Report

Project #: R0316

Title: EFH for Blue King Crab *Paralithodes platypus*: Development of larval cultivation techniques

Principal Investigator(s) and Recipient Organization(s):

Dr. Bradley G. Stevens [Bradley.g.stevens@noaa.gov](mailto:Bradley.g.stevens@noaa.gov)  
And Ms. Sara Persselin  
NMFS, Kodiak Fisheries Research Center  
301 Research Ct., Kodiak, AK 99615 (907) 481-1726

Contract Period and Amount of Funding: 1 July 2003 to 1 July 2004, \$85,561

Report Period: Jan 16 to July 15, 2004

Report Date: 14 January, 2005

Lead Author of Report: Brad Stevens

Co-Author: Sara Persselin

Project Summary: The goal of this project was to understand the relationship between Essential Fish Habitat (EFH) for “overfished” Pribilof Islands blue king crab (BKC) and survival in the first year of life. The first year’s goals were to develop techniques for cultivation of BKC larvae, verify our ability to raise them in the laboratory, and determine the optimum conditions for cultivation.

### Progress Summary

Larvae of the blue king crab (*Paralithodes platypus*) were cultivated in a non-factorial experiment to test the effects of diet, temperature, and rearing density. Diets tested included no feeding (UNFED), *Artemia* nauplii enriched by feeding with diatoms *Thalassiosira nordenskioeldii* (THAL), unenriched *Artemia* fed in addition to *Thalassiosira* (A+THAL), and a control diet of *Artemia* enriched by feeding with frozen *Isochrysis* paste (ISO). All diets were tested at 6 C, and a density of 10 zoea·l<sup>-1</sup>, with 6 replicates per treatment. The ISO diet was also tested at 3 C (ISO 3) and 9 C (ISO 9), and at densities of 20 (ISO 20) and 40 (ISO 40) zoea·l<sup>-1</sup>. Survival on the A+THAL diet (91.7%) was significantly higher than all others, whereas UNFED larvae died within two weeks. Survival decreased slightly with increasing temperature, but not significantly. Density had no significant effect on survival, but final mean density (16 zoea·l<sup>-1</sup>) was similar in the ISO 20 and ISO 40 treatments suggesting that a maximum carrying capacity for these conditions had been reached. Length of development to the first juvenile crab stage (C1) was significantly longer (109 d) at 3 C than at 6 C (70 d), but did not decrease further at 9 C. Half of the replicates in the ISO 20 and ISO 40 treatments were fed continuously during the postlarval (glaucothoe) stage (all other treatments were not); survival of continuously fed larvae was higher in the latter treatment but not the former. We concluded from this research that blue king crab larvae can be cultivated with high survival using the proper diet, that larvae are not

lecithotrophic (i.e., they need to feed), and that glaucothoe do not feed. These results can be used to produce larger numbers of juvenile crab for laboratory research, and could be modified for use in stock enhancement.

**Introduction:** The blue king crab (*Paralithodes platypus*) is a commercially valuable crustacean in the Bering Sea and Gulf of Alaska, with landings of 5.2 million lbs, worth \$12.0 million in 1997. Abundance of blue king crab in the St. Matthew Island and Pribilof Islands populations declined precipitously in the late 1990s, and those areas have been closed to fishing since 1999 and 2002, respectively. Adult female blue king crab have a biennial spawning cycle (Jensen et al., 1985). First-year spawners produce new eggs in late winter that develop for a year and hatch the following spring (Somerton and MacIntosh, 1985). One year later, they subsequently molt, mate, and extrude new eggs. Like red king crab, blue king crab develop through four zoeal and one postlarval stage (called a glaucothoe, equivalent to the megalops) before metamorphosis to the first juvenile crab (C1) stage (Sato, 1958; Hoffman, 1968). Juvenile (1-3 year old) blue king crab have been found among shell hash in the Pribilof Islands that is associated with fouling organisms (Armstrong et al., 1985). Efforts to study the settlement behavior and habitat preferences of these crabs are dependent on the need to develop cultivation methods to produce adequate numbers of small crab for laboratory research. Larvae of both species have been cultivated in the laboratory (Abrunhosa and Kittaka, 1997; Stevens and Kittaka, 1998; Stevens, 2003) on a diet of *Artemia* with or without diatoms (Kittaka et al., 2002), but success with either species has been inconsistent. In the wild, survival of newly hatched red king crab zoeas depends on the availability of diatoms (*Thalassiosira sp.*) (Paul et al., 1989; Paul and Paul, 1990). The present experiment was conducted as part of a research program to improve knowledge of the reproduction and early life history of blue king crab, from fertilization through the first year of benthic life. In order to ensure an adequate supply of young crab for research purposes, we investigated the best conditions for cultivation of the larvae from hatching to stage C1.

## Materials and Methods

Female blue king crabs with fertilized, eyed eggs were collected from the Pribilof Islands in July and September 2003 and shipped to Kodiak in chilled coolers, where they were held in a chilled recirculating water system at 4 C. Hatching began in February 2004, and on 13 April 2004, 240 newly-hatched larvae were collected from each of three female crabs that had been releasing larvae concurrently for several days. Larvae were mixed randomly, for a total of 720 larvae. The chain-forming diatom *Thalassiosira nordenskiöldii* was cultured in filtered (5  $\Phi$ m), UV-sterilized seawater enriched with f/2 medium plus sodium metasilicate, and grown under a 16:8 L:D cycle in a temperature-controlled room at 3 C. *Artemia* cysts were hatched in 2-L plastic cones in aerated seawater sterilized as above. For the A+THAL diet, newly hatched (<24 hours old) *Artemia* nauplii were collected, rinsed in freshwater and fed to the larvae. For the THAL diet, the nauplii were first collected 30 hours after hydrating the cysts, rinsed with freshwater, and returned to a hatching cone filled with *T. nordenskiöldii* culture. Nauplii were collected again 18 hours later, rinsed with freshwater and fed to the zoeas. Enrichment of *Artemia* for the ISO treatments followed the same enrichment procedure as for *T. nordenskiöldii*, but after the initial collection, the nauplii were returned to a hatching cone with *Isochrysis* paste added to sterilized seawater at 0.5 ml/liter.

## Cultivation of Crab Larvae

The experiment consisted of eight treatments grouped by diet, temperature, and density (Table 1). Treatments 1-4 consisted of larvae fed four different diets at  $6.3 \pm 0.1$  C : 1) larvae receiving no food (UNFED); 2) larvae receiving *Artemia* nauplii enriched with *Isochrysis* paste (ISO 6), considered the “standard” diet; 3) larvae receiving *Artemia* nauplii enriched with *Thalassiosira nordenskiöldii*, (THAL); 4) larvae receiving unenriched *Artemia* nauplii plus live *T. nordenskiöldii* directly in the beaker (A+THAL). Each treatment comprised six replicates with 10 zoeas per beaker. All beakers were fed daily with approximately 1750 *Artemia* nauplii/beaker (i.e. 2.2 nauplii/ml). Treatments 5 and 6 were also conducted using 10 zoeas per beaker on the ISO diet, but beakers were held at  $3.1 \pm 0.1$  C (ISO 3) and  $9.0 \pm 0.4$  C (ISO 9). Treatments 7 and 8 were fed the ISO diet at 6 C, but consisted of beakers with 20 (ISO 20) or 40 zoeas·l<sup>-1</sup> (ISO 40). All treatments were maintained on a 12:12 L:D cycle at approximately 70 lux using indirect fluorescent lighting. Temperatures were recorded by electronic data loggers placed in adjacent beakers. All experiments at 3 and 6 C were held in separate constant-temperature rooms, whereas the 9 C treatment occurred in two small incubators. Experiments were conducted by placing larvae inside a 150-mm length of 75 mm diameter PVC tube, with 675 µm polyethylene netting glued to the bottom. Each tube was set into a 1-l glass beaker filled with 800 ml of seawater that had been filtered to 5 µm and UV-sterilized. Larvae (in tubes) were transferred to clean beakers with fresh seawater daily prior to feeding. Feeding was terminated when all zoeas in the beaker had molted to the glaucothoe stage, except for three replicates in each of the ISO 20 and ISO 40 treatments, which were fed throughout the glaucothoe stage for comparison. Surviving larvae were counted once each week in all beakers, after which beakers were rinsed in fresh and distilled water prior to reuse. Molts and mortalities were removed daily to determine the start and end time of molting to glaucothoe or first crab (C1) stage. Mid-G and Mid-C were defined as the number of days to the middle of the molting period for the transition to glaucothoe or C1 stages. Final survival for each beaker was determined when all surviving crabs had molted to stage C1.

## Data Analysis

Proportional survival data were subjected to angular transformation. Homogeneity of variances was tested using Levene’s test prior to use of Anova. Survival to stage C1 was compared between treatments by Anova, and post-hoc multiple comparisons of each treatment versus the ISO 6 diet were conducted with Dunnett’s test. A two-sample t-test was used to compare survival between fed and unfed glaucothoe in the ISO 20 and ISO 40 treatments (after angular transformation). Anova was also used to compare raw numbers of surviving crab in each of the three density treatments that used the same diet and temperature (ISO 6, ISO 20, and ISO 40). However, because raw numbers were discrete counts, they were converted to  $\log_{10}(x+1)$  prior to analysis, and post-hoc multiple comparisons were made using Tukey’s HSD test. Mean days to Mid-G and Mid-C were compared directly by Anova without transformation, and mean values were compared to the ISO 6 diet with Dunnett’s test. Values of  $P < 0.05$  were considered significant. Mean values  $\pm 1$  standard deviation (SD) are given where appropriate.

## **Results**

Survival of larvae from hatching to to stage C1 (Fig. 1) varied significantly ( $F=7.21$ ,  $P<0.000$ ), and variances were homogeneous (Levene  $F = 2.11$ ,  $P = 0.0647$ ). Best survival (91.7  $\pm$  9.8%) was obtained with the A+THAL diet (at 6 C, 10/beaker), and was significantly greater

than in any other treatment (Table 2). Survival in the UNFED treatment was significantly lower than the control (ISO 6) diet; all larvae died within 21 days (Fig. 1; Table 2). Survival in all other treatments was not significantly different from the control diet. Temperature had little effect, as mean survival was similar between the ISO 3 ( $57.9 \pm 30.9\%$ ), ISO 6 ( $48.3 \pm 41.2\%$ ), and ISO 9 ( $41.7 \pm 34.3\%$ ) treatments (Table 2). There was no difference in survival to stage C1 between crabs that were fed or not during the glaucothoe period in the ISO 20 treatment ( $t = 0.316$ ,  $P = 0.768$ ), but fed crabs had significantly higher survival ( $t = 2.812$ ,  $P = 0.048$ ) in the ISO 40 treatment; however, much of the difference in survival occurred during the zoeal stages, prior to the glaucothoe phase.

Proportional survival among the three density treatments was not significantly different, though it was greater in the ISO 20 treatment ( $64.2 \pm 25.4\%$ ) than in the ISO 6 or ISO 40 treatments ( $27.5 \pm 17.0\%$ ). However, the actual numbers of surviving larvae in the ISO 20 and ISO 40 treatments (77 and 66, respectively) were similar (Fig. 2, Table 2). Anova of raw (log transformed) data showed that mean numbers of surviving C1 crab were significantly different ( $F = 3.90$ ,  $P = 0.433$ ) among the three density treatments, but post-hoc tests showed they were similar between the ISO 20 ( $12.8 \pm 5.1$ ) and ISO 40 ( $11.0 \pm 6.8$ ) treatments, and between the latter and the ISO 6 treatment ( $4.8 \pm 4.1$ , Tukey's test). These results imply that the experimental conditions of diet, *Artemia* concentration, and temperature used could support an upper limit of about 13 zoeas per 800 ml beaker, or about  $16.2 \text{ zoeas} \cdot \text{l}^{-1}$ .

Time to the midpoint of molting to glaucothoe (Mid-G) differed significantly between treatments (Anova,  $F = 238.6$ ,  $P < 0.0001$ ) and variances were homogeneous (Levene  $F = 2.16$ ,  $P = 0.072$ ). Larvae raised at 3 C (ISO 3) required significantly more time (mean  $71.8 \pm 2.2$  d) to reach Mid-G than did the control (ISO 6) treatment ( $44.7 \pm 2.0$  d; Table 3), whereas all other treatments were similar to the control, requiring an average of  $44.9 \pm 1.7$  d (Fig. 3). Time to Mid-G for larvae raised at 9 C (ISO 9;  $44.9 \pm 1.7$  d) was not significantly different from that for those raised at 6 C (ISO 6) or the overall mean. Time to Mid-C also differed significantly between treatments (Anova,  $F = 692.7$ ,  $P < 0.0001$ ) and variances were homogeneous (Levene  $F = 1.79$ ,  $P = 0.1408$ ). Larvae in three treatments required significantly more time to reach the midpoint of molting to stage C1 than did the control (ISO 6,  $69.9 \pm 0.6$  d); these were ISO 3 ( $108.8 \pm 2.3$  d), ISO 20 ( $72.7 \pm 0.8$  d) and ISO 40 ( $73.4 \pm 0.9$  d) (Table 3).

## Discussion

In our experiment, extremely high survival (92%) of blue king crab larvae from hatching to the first juvenile crab stage was obtained on a diet of unenriched *Artemia* and culture water supplemented with *Thalassiosira*, at a temperature of 6 C. Much lower survival occurred when *Artemia* were enriched with *Thalassiosira* or *Isochrysis* sp. prior to feeding them to crab zoeas. UNFED zoeas died within a few weeks, demonstrating that BKC zoeas are not lecithotrophic like those of the golden king crab *Lithodes aequispinus*. Based on these results, we infer that crab larvae may obtain additional nutrients directly from the diatoms that they do not obtain when the diatoms are first consumed by *Artemia*. The presence of diatoms in the culture water may also provide other benefits such as removal of nitrogenous wastes, the addition of oxygen and helping to balance pH. Survival on the standard diet (*Artemia* enriched with frozen *Isochrysis* paste) decreased with increasing temperature, from 3 C to 9 C, though not by a significant amount. However, development time from hatching to stage C1 was identical (mean 74 days) at 6 C and 9 C, yet it was lengthened considerably (105 d) at 3 C. Thus, slightly increased survival at 3 C is offset by much longer development time, with associated labor costs

and higher risk of failure. Comparison of fed vs. unfed glaucothoe was equivocal, with greater survival in one group, and lower in another; the latter was partially due to mortality prior to the glaucothoe phase. We conclude from these data that blue king crab do not need to be fed during the glaucothoe phase, and probably do not eat, similar to red king crab glaucothoe.

Our experimental conditions were adequate to produce numbers of larvae (several hundred) useful for laboratory experimentation, and could be repeated at this level. However, cultivation in larger volumes will be necessary to produce more larvae for future research, and will require different laboratory apparatus, including a flow-through water system with built in filtration, temperature control, and a different feeding regime. Ultimately, these techniques could be adapted for use to produce larger quantities of small crab for enhancement of natural stocks. Japanese fishing cooperatives have conducted a large scale enhancement project with *P. brevipes* for over a decade, releasing 200,000-500,000 early stage crabs annually<sup>1</sup>. The success rate of this endeavor is unknown, however, due to lack of monitoring efforts.

Table 1. Summary of culture conditions (treatments) for blue king crab larvae.

Treatment	Temperature	Food	Density	Beakers	Total Larvae
1	6 C	Unfed	10	6	60
2	6 C	ISO	10	6	60
3	6 C	THAL	10	6	60
4	6 C	A+THAL	10	6	60
5	6 C	ISO	20	6	120
6	6 C	ISO	40	6	240
7	3 C	ISO	10	6	60
8	9 C	ISO	10	6	60
<b>Total</b>				<b>48</b>	<b>720</b>

Table 2. Survival of BKC larvae from hatching through 22 June. Best survival was on the A+Thal diet with 10 larvae/liter at 6C. Groups that were not significantly different are designated by capital letters.

Beaker	Unfed	Iso 6C	Thal	A+Thal	Dens 20	Dens 40	Iso 3C	Iso 9C
1	0.0	100.0	80.0	80.0	60.0	15.0	100.0	50.0
2	0.0	20.0	0.0	100.0	25.0	25.0	80.0	80.0
3	0.0	90.0	40.0	100.0	75.0	7.5	80.0	20.0
4	0.0	70.0	80.0	90.0	60.0	32.5	40.0	100.0
5	0.0	0.0	50.0	100.0	95.0	32.5	80.0	20.0
6	0.0	20.0	70.0	80.0	80.0	55.0	40.0	0.0
<b>Mean</b>	<b>0.0</b>	<b>50.0</b>	<b>53.3</b>	<b>91.7</b>	<b>65.8</b>	<b>27.9</b>	<b>70.0</b>	<b>45.0</b>
		<b>A</b>	<b>A</b>	<b>A</b>	<b>A</b>		<b>A</b>	<b>A</b>
<b>Similar</b>		<b>B</b>	<b>B</b>		<b>B</b>	<b>B</b>	<b>B</b>	<b>B</b>
<b>Groups</b>	<b>C</b>	<b>C</b>	<b>C</b>		<b>C</b>	<b>C</b>	<b>C</b>	<b>C</b>

<sup>1</sup> J. Kittaka, Nemuro City Fisheries Research Institute, Onnemoto 168, Nemuro, Hokkaido, 087-0166, JAPAN, pers. commun.

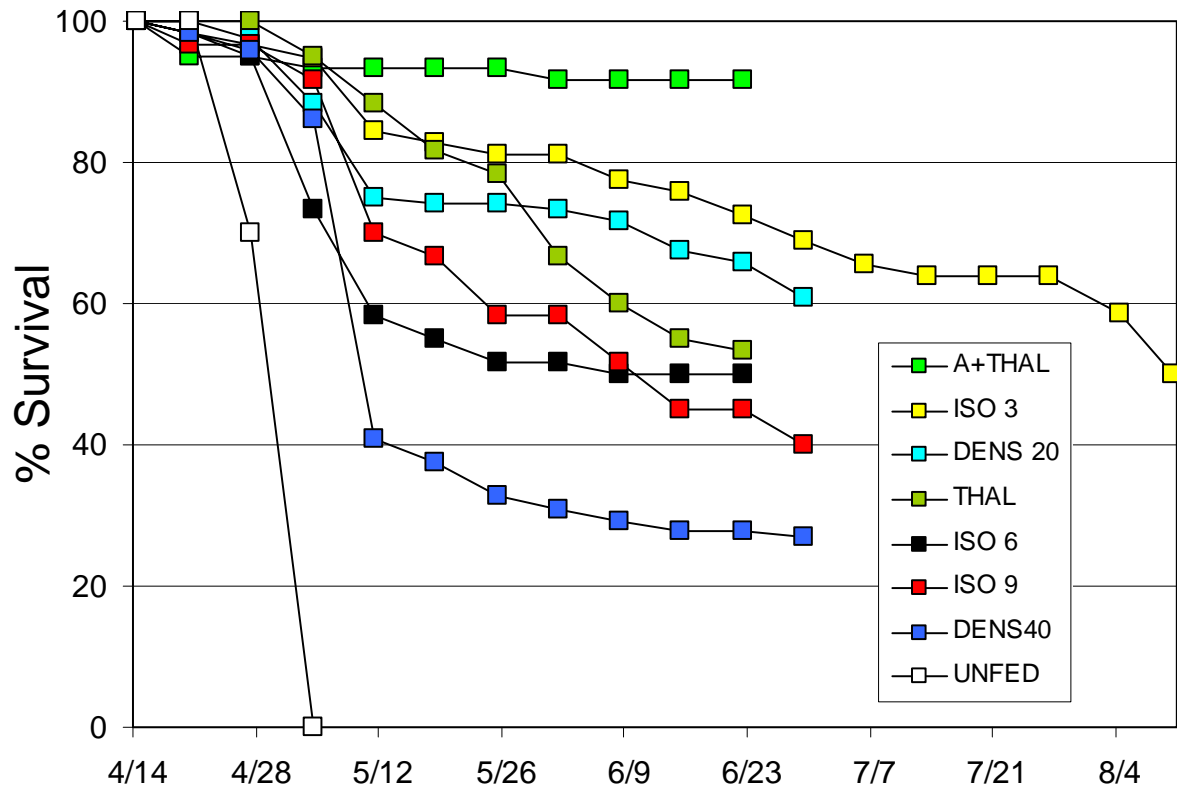


Figure 1. Percent survival of blue king crab (*P. platypus*) larvae at weekly intervals, from hatching to stage C1, under different culture conditions. All points are means of 6 replicates. See text for description of treatments.

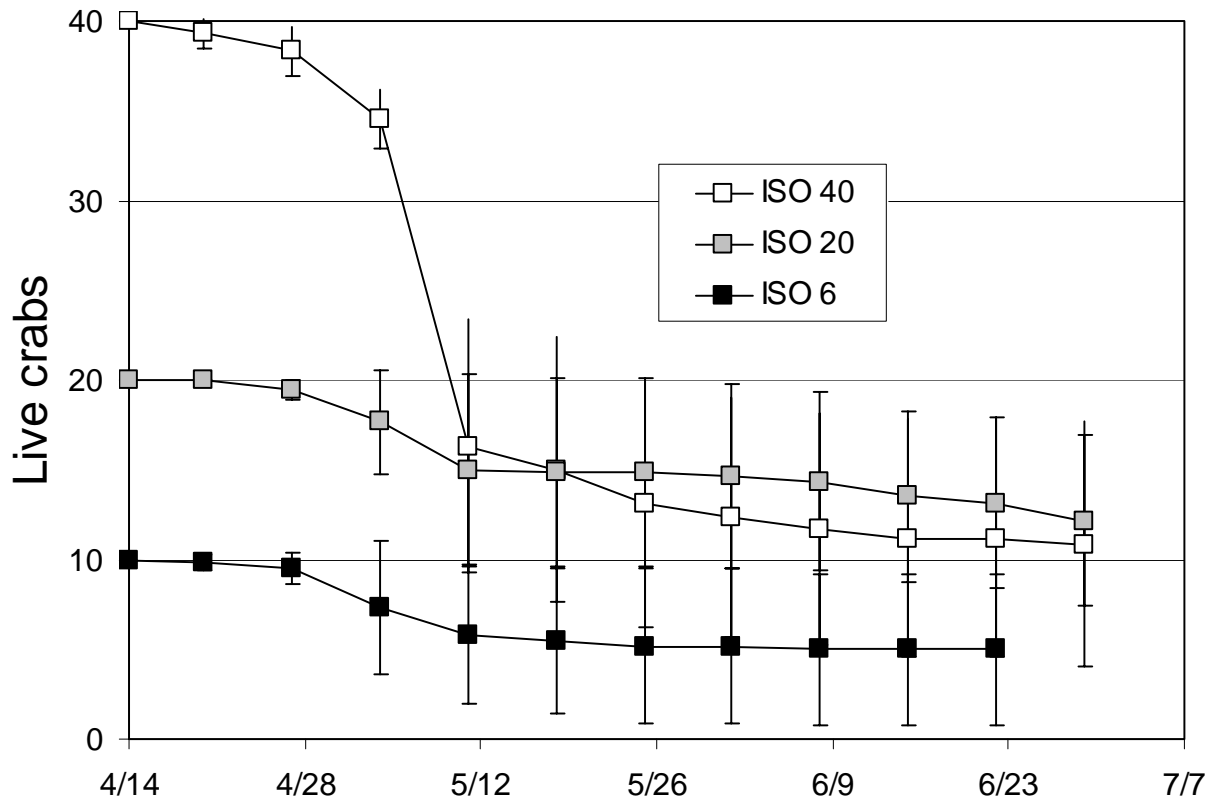


Figure 2. Mean number of surviving blue king crab (*P. platypus*) larvae at weekly intervals, from hatching to stage C1 in the three density treatments. All were fed the ISO diet at 6C. ISO 6 treatment started with 10 larvae per beaker, whereas the ISO 20 and ISO 40 started with 20 or 40 larvae per beaker, respectively.

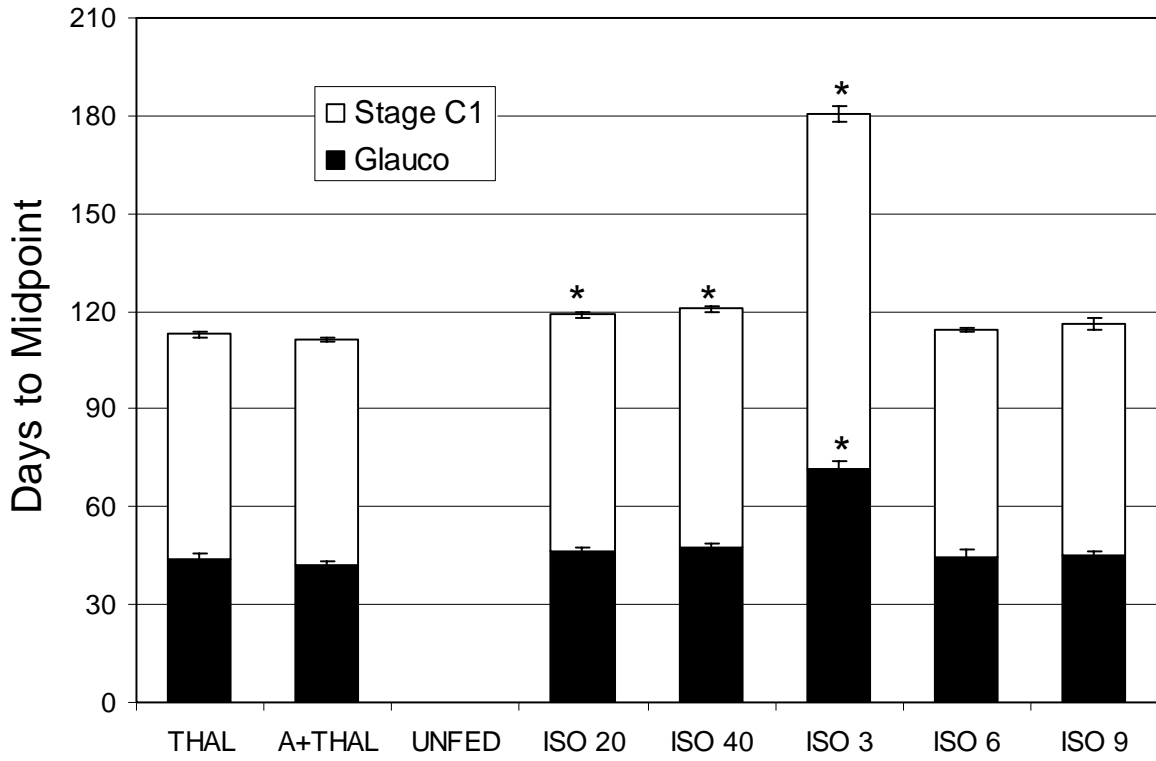


Figure 3. Mean number of days from hatching to the midpoint of molting to glaucothoe (black) and stage C1 (white) for larvae of blue king crab (*P. platypus*). \* above bar indicate those that were significantly different from the control (ISO6) treatment. Error bars represent 1 SD.

## Literature Cited

- Abrunhosa, F. A., and J. Kittaka. 1997. Functional morphology of mouthparts and foregut of the last zoea, glaucothoe and first juvenile of the king crabs *Paralithodes camtschaticus*, *P. brevipes* and *P. platypus*. Fish. Sci. 63:923-930.
- Armstrong, D. A., R. Palacios, G. Williams, G. C. Jensen, and W. Pearson. 1985. Early life history of juvenile blue king crab, *Paralithodes platypus*, around the Pribilof Islands. In B. Melteff (ed.) Proceedings of the International King Crab Symposium. Anchorage, AK. January 22-24, 1985. University of Alaska Sea Grant. Rep. No. 85-12, p. 211-230.
- Hoffman, E. G. 1968. Description of laboratory-reared larvae of *Paralithodes platypus* (Decapoda, Anomura, Lithodidae). J. Fish. Res. Bd. Can. 25:439-455.
- Jensen, G. C., D. A. Armstrong, and G. Williams. 1985. Reproductive biology of the blue king crab, *Paralithodes platypus*, in the Pribilof Islands. In B. Melteff (ed.) Proceedings of the International King Crab Symposium. Anchorage, AK. January 22-24, 1985. University of Alaska Sea Grant. Rep. No. 85-12, p. 109-121.
- Kittaka, J., B. G. Stevens, S. Teshima, and M. Ishikawa. 2002. Larval culture of the king crabs *Paralithodes camtschaticus* and *P. brevipes*. In Crabs in cold water regions: Biology, Management, and Economics. Anchorage, AK. 2002. Alaska Sea Grant Program. Rep. No. AK-SG-02-01, p. 189-209.
- Paul, A. J., and J. M. Paul. 1990. Growth of stage I king crab larvae of *Paralithodes camtschatica* (Tilesius) (Decapoda:Lithodidae) in natural communities. J. Crust. Biol. 10:175-183.
- Paul, A. J., J. M. Paul, and K. O. Coyle. 1989. Energy sources for first feeding zoeae of king crab *Paralithodes camtschatica* (Tilesius)(Decapoda, Lithodidae). J. Exp. Mar. Biol. Ecol. 130:55-69.
- Sato, S. 1958. Studies on larval development and fishery biology of king crab, *Paralithodes camtschatica* (Tilesius). Bull. Hok. Reg. Fish. Res. Lab. 17.
- Somerton, D. A., and R. A. MacIntosh. 1985. Reproductive biology of the female blue king crab *Paralithodes platypus* near the Pribilof Islands, Alaska. J. Crust. Biol. 5:365-376.
- Stevens, B. G. 2003. Settlement, substrate preference, and survival of red king crab *Paralithodes camtschaticus* (Tilesius, 1815) glaucothoe on natural substrata in the laboratory. J. Exp. Mar. Biol. Ecol. 283:63-78.
- Stevens, B. G., and J. Kittaka. 1998. Postlarval settling behavior, substrate preference, and time to metamorphosis for red king crab *Paralithodes camtschaticus*. Mar. Ecol. Prog. Ser. 167:197-206.