Techniques for larval culture of red king crab *Paralithodes camtschaticus*, 2008

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ABSTRACT
The Alaska King Crab Research, and Rehabilitation and Biology (AKCRRAB) program was formed in 2006 as a partnership between local and statewide agencies with the goal of investigating the feasibility of large scale Alaska king crab culture for the purpose of population rehabilitation through ocean ranching. Following red king crab (*Paralithodes camtschaticus*) through their planktonic larval and settling glaucothoe stages, this study will address the effect of dietary enrichment with algae species on larval growth and survival. Also under examination are the potential influences of location and scale of rearing on larval *P. camtschaticus* survival. This research will build on rearing techniques for crab species around the world and findings of red and blue king crab rearing experiments in 2007 as the AKCRRAB program continues to investigate the feasibility of mass larval culture of Alaskan king crab species on a hatchery scale.

INRODUCTION
Commercial harvest of Kodiak red king crab (*Paralithodes camtschaticus*) was for decades an active, lucrative fishery growing rapidly until the 1969 earthquake, which damaged many of the region's fish processing plants (Kruse 2006). The fishery moderately rebounded over the next five years followed by a swift decline in catch from the mid-1970s to 1983, the last year the fishery was active. Annual trawl surveys conducted by the Alaska Department of Fish and Game (ADFG) indicate that the population remains at historically low levels and the fishery must remain closed until the threshold of 5.5 million breeding females is met (Ruccio et al. 2001). Definitive reasons for the lack of natural population rehabilitation are unknown but are likely due to low survival through the early juvenile life stages caused in part by highly variable predation effects (Blau 1986), drift of planktonic larvae from suitable habitat (Shirley and Shirley, 1989a), food availability, and temperature (A. Paul, pers. comm.).

Stock enhancement has the potential to be an effective tool for rehabilitation of Alaska red king crab and blue king crab (*Paralithodes platypus*) populations and is currently in under way for crab and lobster species in the United States and worldwide (Secor 2002, Stevens 2006c). Before implementation in Alaska, research is needed to assess the feasibility, effectiveness, and possible consequences of a stock enhancement program (Leber 1999, 2002). The Alaska King Crab Research, and Rehabilitation and Biology (AKCRRAB) program was created in 2006 as a partnership between the University of Alaska Fairbanks, Alaska Sea Grant, the Alutiiq Pride Shellfish Hatchery (APSH), NOAA Fisheries, and several community-based groups to answer those questions through scientifically sound research.

Female red king crab reproduce annually and hold their extruded fertilized eggs for 11 months, releasing planktotrophic larvae over a period of 2-3 weeks between January and March, depending on environmental and physiological factors (Stevens 2006b; Stevens and Swiney 2007). For approximately 40 days, larvae molt from prezoea

through four zoeal stages (ZI-ZIV) followed by a non-feeding glaucothoe (G) stage that lasts 30-40 days. Glaucothoe seek structurally complex habitat then metamorphose into a first-instar juvenile crab (C1) (Stevens and Kittaka 1998, Stevens 2003) where they begin life as benthic oriented scavengers. Both red and blue king crab species mature at approximately 5-7 years of age and recruit to the fishery at 7-9 years of age (Jensen and Armstrong 1989, Zhou et al. 1998, Loher et al. 2001, Stevens et al. 2008). The relationship between larval abundance and recruitment to the breeding population is unknown and complex (Shirley and Shirley, 1989a, 1989b).

Laboratory culture of *Paralithodes* sp. to the C1 stage has been extensively investigated in Japan (reviewed by Stevens 2006d), Russia (Epelbaum and Kovatcheva 2005, Epelbaum et al. 2006, Kovatcheva et al. 2006), and Alaska (Shirley and Shirley 1989b, Persselin 2006a, Stevens et al. 2008). Red king crab larvae are vulnerable to stress and cannibalism, and relatively high mortality has been observed throughout larval development, especially during molting (Stevens 2006d, Kovatcheva et al., 2006). Although methods for large-scale rearing of red king crab were developed several decades ago in Japan (Nakanishi and Naryu 1981), hatchery production was highly variable from year to year, and from 1982 to 1996, production of Hanasaki king crab (*P. brevipes*) ranged from 0 to 800,000 C1 per year, with an average survival of about 42% (Stevens 2006d). Survival to the C1 stage for red and blue king crab in small-scale culture experiments in Kodiak has also been highly variable (Persselin 2006a, 2006b). Survival in a recent Kodiak-based experiment examining the effects of diet, temperature, and larval density for blue king crab to the C1 stage varied from 27% to 91% (Stevens et al. 2008).

Several other species of crab are cultured at the hatchery scale. Successful techniques for rearing larval crabs on a large scale were developed 30 years ago for swimming crab (*Portunus trituberculatus*) in Japan, more recently for Chinese mitten crab (*Eriocheir sinensis*) in China (Zhang et al. 1998, Li et al. 2001), and blue crab (*Callinectes sapidus*) in Chesapeake Bay (Secor et al. 2002, Zmora et al. 2005). During four culturing cycles from February through September 2002, the hatchery in Chesapeake Bay produced 40,000 juvenile blue crabs, of which 25,000 were released in the wild (Zmora et al. 2005). Researchers working on blue crab have developed tagging techniques (Davis et al. 2004a), investigated fitness of hatchery-raised individuals (Davis et al. 2004b), and developed techniques for morphological conditioning to improve juvenile fitness (Davis et al. 2005a). Estimated survival to maturity of hatchery-raised blue crab released into the wild was 5 to 20% during initial investigations, indicating that stock enhancement may be possible for this species (Davis et al. 2005b).

Red king crab larvae have been cultured successfully using *Artemia* sp. enriched with *Thalassiosira nordenskioeldii* (Persselin, 2006a). *T. nordenskioeldii* (T-NORD) is a coldwater, chain forming algae that grows best at 3°C and achieves relatively low densities of 50,000-200,000 cells/mL (S. Persselin, pers. comm.) compared to other species cultured at APSH that reach 4-5 million cells/mL. Due to the cold water requirement and low density yield, large-scale culture of this species would be costly. However, if a comparably high survival rate as demonstrated in laboratory scale rearing (75.9%) could be duplicated on the hatchery scale it may be worthwhile. The increase in survival when king crab zoea are fed enriched *Artemia* sp. versus unenriched *Artemia* sp. or starvation has been established; feeding unenriched *Artemia* sp. to larvae resulted in

survival of 43.1% in small scale culture of *P. camtschaticus* (Kittaka et al., 2002) and will be used as the control for this study. *Isochrysis galbana* Tahitian strain (T-ISO) is an algal species grown at APSH that may serve as a cost-effective alternative enrichment species to T-NORD. T-ISO is widely used in the aquaculture industry and has a higher proximate composition of lipid and protein than T-NORD despite its considerably smaller size (Table 1). There is little data available on the nutritional value of T-NORD as it is not used in aquaculture, but given the high survival rates when used in the NMFS Kodiak lab it is expected to do well.

Table 1. Chlorophyll *a*, protein, carbohydrate, and lipid values for algae species of interest.

Species	Dry Weight (pg./cell)	Cell Size (µm)***	Chl *a*	Protein	Carbohydrate	Lipid
			Proximate composition (%) of dry weight			
Thalassiosira nordenskioeldii	--	L: 10-22 W: 10-22	--	10.6**	--	9.5**
Isochrysis galbana	30.5*	L: 4-8 W: 0-0	0.98*	29*	12.9*	23*
Pavlova lutheri	102.3*	L: 5-8 W: 3-5	0.84*	29*	9.0*	12*

**Data from Brown, 1991; **Data from Kittaka et al., 2002; ***Data from CCMP: http://ccmp.bigelow.org*

Preliminary studies of hatchery-scale larval culture were conducted in 2007 at the Alutiiq Pride Shellfish Hatchery to investigate optimal feeding and handling methods in the culture of early life stage red and blue king crab. The necessity of finding a cost effective food with high survival for red king crab aquaculture was noted by Kovatcheva et al. in 2006. Red king crab broodstock were collected in 2006 from Alitak Bay on Kodiak Island during ADFG annual trawl surveys (Permit No. CF-06-032), and blue king crab broodstock were collected by F/V *Aleutian Beauty* near St. Paul Island (Permit No. CF-06-085) then transported to the Seward Marine Center and upon hatching in 2007 moved to APSH. Larvae from 4-5 broodstock were combined and stocked into experimental tanks to reduce the effect of genetic influence of a single parent's offspring on our results.

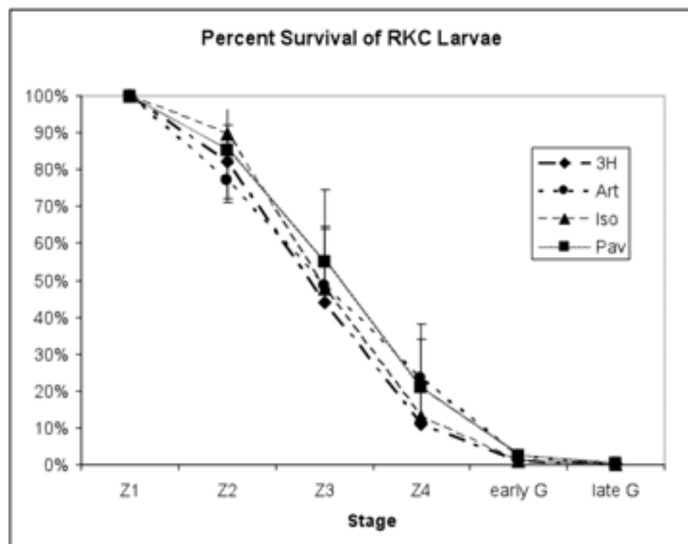

Fig. 1: Percent survival of red king crab larvae in 2007 diet experiments from ZI to the late glaucothoe stage.

Red king crab larvae were cultured from February to April 2007 in sixteen 190 L tanks and fed newly hatched *Artemia* sp. at a density of 2/mL/day and one of three algal species, *Thalassiosira pseudonana* (3H), *Isochrysis galbana* Tahitian strain (T-ISO), or *Pavlova lutheri* (PAV) at a density of 50,000 cells/mL/day. A control treatment of larvae fed *Artemia* sp. only (ART) was also included.

Crabs were stocked at a density of 15/L and survival was assessed at each zoeal stage by draining tanks and sub-sampling. Survival decreased throughout larval development and continued through the glaucothoe stage (Fig. 1).

In response to these results and our observations a study plan was developed for blue king crab larval culture. Blue king crab larvae were cultured from May to July 2007 under similar tank conditions with two handling and two diet treatments. Preliminary observations of T-ISO concentrate fed to red king crab larvae in hatchery trials indicate that larvae are able to ingest these cells and they do not adhere to the crabs' shell (J. Swingle and C. Leroux, per. obs.) as was seen in observations with algal pastes (S. Persselin) that use crushed cells versus the whole, unfrozen cells found in algae concentrate. As a potentially cost-effective alternative to culturing live algae, T-ISO concentrate along with live T-ISO were selected as diet treatments. In reaction to the consistent and dramatic drop in red king crab larval survival over time, the possible effect of handling stress on survival was also investigated. For half of the tanks on each diet treatment, survival at each stage of development was assessed by draining the tank through a mesh screen and counting all live zoea. This also allowed us to clean rearing tanks with fresh water and remove dead bodies as they may be a source of bacterial buildup. Larvae in the remaining tanks were not handled during zoeal development, counted only when initially stocked and as glaucothoe. Survival for both handling and diet treatments was not significantly different (Figs. 2 and 3).

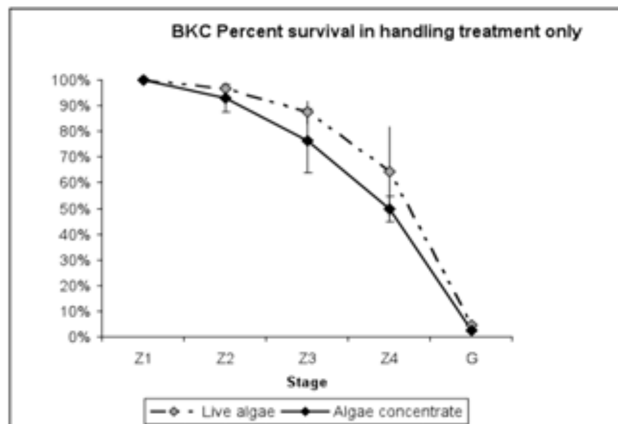

Fig. 2: BKC survival in handling treatment tanks.

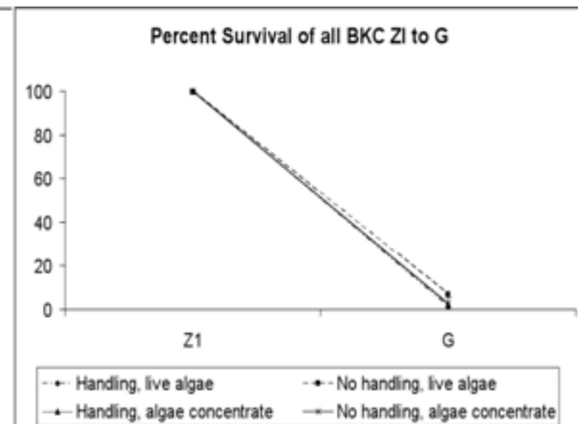

Fig. 3.: BKC survival for all treatments.

Although there was not a significant difference in survival for red or blue king crab zoea fed any diet treatments tested, the findings of Stevens et al. in 2007 lead us to believe algae may still benefit the culture that was masked by zoeal death due to causes as yet unidentified but under active investigation by the AKCRRAB science team. We can conclude that the practiced handling methods do not significantly impact the survival

of king crab larvae, and as the handling process allows for tank cleaning it decreases biotic buildup and may reduce the incidence and proliferation of disease. The results of the 2007 rearing experiments have instigated the AKCRRAB science team to critically examine all potential factors contributing to tank conditions and optimize these for survival in 2008 larval rearing experiments. This will incorporate changes to tank setup, water treatment, tank flushing and more. Also under investigation are the two most fundamental changes between small scale rearing in the NMFS Kodiak lab where high survival has been repeatedly demonstrated and large scale rearing at APSH, namely location and scale. This will be examined by conducting concurrent small scale experiments in Kodiak and Seward that parallel the treatments of sixteen large scale tanks.

HYPOTHESES

($H1_0$) There will be no difference in survival of *Paralithodes camtschaticus* throughout zoeal development when fed *Thalassiosira nordenskioeldii, Isochrysis galbana* live or as a concentrate in addition to *Artemia* sp. or if fed *Artemia* sp only.

> **($H1_a$)** Survival of *P. camtschaticus* will differ significantly between diet treatments.

($H2_0$) There will be no difference in growth of *P. camtschaticus* as determined by dry weight and/or spine length measurements from the first zoeal stage to the glaucothoe stage in any tank, regardless of diet treatment.

> **($H2_a$)** Growth of *P. camtschaticus* zoea as determined by dry weight and/or spine length measurements per tank will differ significantly between diet treatments.

($H3_0$) Results of **H1** for large scale rearing will also apply to small scale experiments conducted in Seward, Alaska.

> **($H3_a$)** Results of **H1** for large scale rearing will differ significantly from results in small scale experiments conducted in Seward, Alaska.

($H4_0$) Results of small scale experiments conducted in Seward, Alaska will also apply to small scale experiments conducted using the same methods in Kodiak, Alaska

> **($H4_a$)** Results of small scale experiments conducted in Seward, Alaska will differ significantly from identical experiments conducted in Kodiak, Alaska

OBJECTIVES

- Compare survivorship of *P. camtschaticus* through zoeal development when fed four different diets.
- Compare survivorship of *P. camtschaticus* through zoeal development when reared on a small scale versus a large scale in Seward, AK.
- Compare survivorship of *P. camtschaticus* through zoeal development when reared on a small scale in Seward, AK versus a small scale in Kodiak, AK.

METHODS

Ovigerous female red king crabs will be collected (permit process pending) and transported in chilled coolers to the Seward Marine Center where they will be maintained in 1500 L tanks of unfiltered seawater chilled to 7-9ºC as needed. Broodstock will be fed

a mixed diet of herring, squid, capelin, razor clams, silversides, etc. At the start of larval release, individual females will be transported to APSH and isolated in 5.3 L containers with a 150µm mesh bottom in flowing ambient seawater. Broodstock for Kodiak-based experiments will be cared for under similar conditions. Hatching females will be release larvae over an 8-10 hour period (overnight) at which point newly hatched zoea will be collected. *Paralithodes camtschaticus* zoea tend to gravitate toward the top and sides of the container, therefore healthy larvae will be selectively collected by scooping them out with a beaker from these areas as is done in the NMFS Kodiak lab. ZI stage larvae will be collected from at least three females and mixed randomly for stocking into all experimental tanks and beakers. All treatments will be maintained on a 12:12 L:D cycle using indirect fluorescent lighting. Temperatures will recorded by electronic data loggers placed in similar beakers and all large scale tanks.

As with the 2007 larval rearing experiments, all tanks will be fed newly hatched *Artemia* sp. of the Great Salt Lake strain at a density of 2/mL daily. This is similar to the feeding density used in the NMFS Kodiak lab (Stevens et al., 2007) and availability of food is well above daily intake requirements for zoeal *P. camtschaticus* of all stages (Kovatcheva et al. 2006). Each larval tank/beaker will also be enriched with algae at a density of 50,000 cells/mL daily for tanks fed T-ISO Live or T-ISO Concentrate and 10,000 cells/mL daily for T-NORD. Due to the larger cell size of T-NORD this reduced cell density produces a comparable green water effect (S. Persselin, pers. obs.). *T. nordenskioeldii* will be obtained from the Center for Culture of Marine Phytoplankton (CCMP) at Bigelow Laboratory for Ocean Sciences (West Boothbay Harbor, ME, USA) and cultured in the Mariculture Technical Center portion of APSH in a cold room. Although at the National Marine Fisheries Service (NMFS) Kodiak lab this species is grown at 3ºC, it will be grown near 6ºC at APSH, a temperature within the species tolerance as listed on the starter culture website (CCMP Bigelow Labs). Attempts at *T. nordenskioeldii* culture at APSH in 2006 and 2007 have not produced sufficient growth for this experiment to be conducted as planned; if culture in 2008 proves insufficient for use as a feeding treatment those tanks will be fed *Pavlova lutheri* (data in Table 1) at a density of 50,000 cells/mL daily. *I. galbana, P. lutheri*, and *T. pseudonana* cultures will be grown in the main algae rearing room of APSH at 20ºC using filtered seawater to 1µm. The control treatment will be larvae fed *Artemia* sp. without algae as the survival rate using this treatment on a small scale (43.1%) is published (Kittaka et al. 2002). In case of failure in *Artemia* sp. culture, tanks will be fed hatched, frozen *Artemia* sp. at the same density. In the unlikely event that all cultures of an algae species at APSH simultaneously fail, all tanks will not be fed algae on that/those day(s).

LARGE SCALE REARING

2,850 larvae will be counted and stocked into each 190 L ChemTainer™ conical bottom experimental tank to give a starting density of 15/L. Four tanks (one of each treatment) will be stocked per day over a period of four days to total 16 experimental tanks. Seawater will be sourced from depth so the incoming temperature will be 4-6ºC as is typical this time of year in Resurrection Bay and within salinity tolerance of *P. camtschaticus* larvae. A new water filtration system will be installed to filter incoming seawater to 1µm through sand and cartridge filters, other filters may be added as necessary. Temperature in all tanks will be recorded at 30 minute intervals using HOBO Temperature loggers and salinity will be monitored once daily in all tanks. Seawater will

enter near the base of the tank through a submerged PVC tube and exit through a banjo filter fitted with 500 µm Nitex™ screen to prevent larvae from exiting the tanks but allowing uneaten algae and *Artemia* sp. to be filtered out. Water exchange rate will be sufficient to regulate temperature, remove uneaten food and waste daily, and effectively mix contents of the tank. In place of air stones used in 2007 rearing experiments, food and larvae will be suspended via water upwelling through the submerged incoming water line (Calado et al. 2003) as excessive mixing and interaction with bubbles from air stones may unduly stress or damage these delicate organisms (A. Epelbaum, pers. comm.). To ensure random mixing of environmental conditions, feeding treatment and start date have been randomly assigned (Table 2).

Survival of larvae will be determined once per zoeal stage. Each tank will be emptied through a 400µm drop screen and carefully rinsed into 2 L of seawater. All larvae will then be counted using a 5 mL glass pipette fitted backwards to a 5 mL bulb. This pipette setup will allow larvae to pass into the pipette and be counted without damaging their fragile structure. The final sampling event will establish a survival of larvae through the ZIV molt to the glaucothoe stage. Glaucothoe actively settle on artificial seaweed (C. Leroux and B. Daly, pers. obs.) therefore a set length of this material will be placed in each tank at the end of the ZIV stage and left in tanks until all have molted to glaucothoe. Artificial seaweed "collectors" will be removed daily, glaucothoe shaken off and counted. and the empty collector replaced. It is expected that all glaucothoe will eventually settle on collectors as no alternative substrate will be present and, by being replaced daily, they will not be saturated. Daily monitoring of glaucothoe settlement will provide additional information on molting time with different diet treatments. When all visible crabs have molted to glaucothoe the tank will be emptied and any remaining live glaucothoe collected in a drop screen, counted and pooled with other surviving glaucothoe for that tank. At this point glaucothoe may be sampled for fatty acid methyl ester (FAME) analysis, growth rate and dry weight measurements, or they may be used in juvenile rearing experiments.

Table 2. Treatments and start dates for RKC larval rearing tanks.

Tank	Enrichment Sp.	Start Day
1	T-ISO Live	2
2	T-ISO Con. Drip	4
3	T-ISO Con. Pulse	3
4	T-ISO Con. Pulse	2
5	T-ISO Live	3
6	T-ISO Con. Drip	2
7	T-ISO Con. Drip	3
8	NONE	2
9	T-ISO Con. Drip	1
10	T-ISO Live	1
11	T-ISO Con. Pulse	4
12	NONE	4
13	T-ISO Live	4
14	NONE	1
15	T-ISO Con. Pulse	1
16	NONE	3

From each tank at each stage from ZI to G, 20 crabs will be collected for growth rate measurements. 10 crabs will be preserved in 5% buffered formalin and 10 will be frozen. The crabs preserved in formalin will be used for spine length measurements and the frozen samples will be freeze-dried to obtain dry weight data. This will give additional information on growth rate for each treatment. These samples are collected by scooping larvae out of tanks with a 3 L beaker and collecting them in a small screen.

SMALL SCALE REARING

Small and large scale diet studies will be conducted concurrently at APSH and the NMFS Laboratories in Kodiak, AK. Larvae will be collected from at least three females and stocked at a density of 15/L in sixteen 1L beakers filled with 800 mL of seawater. Experiments will be conducted by placing larvae at a density of 15/L inside a 150mm length of 75 mm diameter PVC tube, with 675 µm polyethylene netting glued to the bottom. Each tube is set into a 1 L glass beaker filled with 800 ml of seawater that had been filtered to 5 µm and UV-sterilized. Larvae will be transferred by gently lifting the PVC container into beakers with fresh food and seawater daily. Both small scale experiments will be conducted in a cold room kept at 6°C. Feeding will be terminated and final survival assessed at each zoeal stage and when all zoea in the beaker molt to the glaucothoe stage. Small scale experiments will not be subject to sampling for dry weight or spine lengths.

Survival from ZI to G will be compared using two-way repeated measures ANOVA with density and scale as factors. Post-hoc comparisons of each treatment will be conducted with Tukey's HSD test. Values of $p < 0.05$ will be considered significant. Multifactorial design repeated measures ANOVA will be used to test for significance between location and diets over time.*

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