AKCRRAB Draft Study Plan 2011

Hatchery culture and release strategies for red king crab (*Paralithodes camtschaticus*)

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ABSTRACT
The Alaska King Crab Research Rehabilitation and Biology (AKCRRAB) program was formed in 2006 with the goal of investigating the feasibility of hatchery rearing of Alaskan king crab species for the purpose of population rehabilitation. Large-scale larval culture conducted at the Alutiiq Pride Shellfish Hatchery from 2007 to 2010 using red king crab (*Paralithodes camtschaticus*) has been increasingly successful. In 2007, experiments using Kodiak broodstock yielded overall survival to the first juvenile stage of less than 1%. Bristol Bay broodstock were used in for hatchery experiments in 2008, 2009, and 2010. Experiments in 2008 yielded an average of 31% survival to the glaucothoe stage and 11% to the first juvenile stage. Research in 2008 elucidated possible causes of mortality in the previous year, allowing for adjustments in larval rearing techniques that improved larval survival. Research in 2009 and 2010 further refined rearing techniques increasing survival to glaucothoe to over 50% and survival to C1 to 21%. Each year improvements in culture technology are made with the understanding that fine tuning larval and juvenile husbandry techniques are an ongoing process. With further refinement of rearing techniques, our ultimate goal is to achieve 50% survival to the first juvenile stage on a large scale. It is unclear if alternate stocks will have similar success using improved rearing techniques. Proposed experiments in 2011 will compare larval viability between broodstock collected in Bristol Bay and Juneau and continue to fine tune variables that yielded highest larval, glaucothoe, and juvenile survival in previous years. We are optimistic that we will see similar success using Juneau stocks and improve overall survival in 2011. Additionally, we plan to examine survival of hatchery-raised crabs in the field near Juneau, Alaska in 2011 using a small-scale tethering experiment to determine optimal sizes and timing for release. This research will give further insight into the feasibility of mass culture of king crab in terms of methodology, crab survival, and costs incurred which are critical to assess the feasibility of rehabilitation.

INTRODUCTION
Hatchery rearing has the potential to be an effective tool for rehabilitation of depleted stocks and for fishery management and is currently in progress for crab and lobster species in the US and worldwide (Secor, 2002; Stevens, 2006c). However, before implementation, research is needed to assess the feasibility, effectiveness, and possible consequences of a rehabilitation 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 begin the necessary research to assess the feasibility of rehabilitation for king crabs in Alaska. Commercial harvest of Alaskan king crab was for decades active and lucrative. However, many stocks declined drastically over 20 years ago and have not rebounded, even in the absence of fishing. We propose to study the early life history of red king crab to develop methods and determine feasibility of hatchery rearing. This study plan addresses methods for culture of larvae and juveniles in the Alutiiq Pride Shellfish Hatchery in
Seward, Alaska and investigates survival of juveniles in the field based on size and season in a small-scale tethering experiment.

Laboratory culture of *Paralithodes* sp. to the C1 stage has been extensively investigated in Japan (reviewed by Stevens 2006d), Russia (Kovatcheva et al., 2006), and Alaska (Paul et al., 1989; Shirley and Shirley, 1989; Persselin 2006a; Stevens et al., 2008; Daly et al., 2009). Red king crab larvae are especially vulnerable to stress, and relatively high mortality has been observed throughout larval development, especially during molting (Stevens 2006d, Kovatcheva 2006). Although methods for large-scale rearing of 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, Stevens et al., 2008). In an experiment that examined the effect of diet, temperature, and larval density, blue king crab larval survival to C1 varied from 27% to 91% dependent on treatment (Stevens et al., 2008).

Several other species of crab are cultured at the hatchery scale. Successful techniques for rearing larval crabs at the hatchery 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 rehabilitation may be possible for this species (Davis et al., 2005b).

**2007 - 2010 RESULTS**

Studies on hatchery-scale larval culture of red king crabs were conducted in 2007, 2008, 2009, and 2010 at the Alutiiq Pride Shellfish Hatchery to investigate optimal culturing techniques. The first hatchery-scale experiments in 2007 investigated effects of stocking density and varying diet on overall larval survival and had limited success, likely because of fluctuations in water supply, suboptimal temperature, and nutrition. Survival decreased dramatically throughout the zoeal and glaucothoe stages to less than 1% by the first juvenile instar (C1) in all treatments. Due to the low survival in hatchery-scale culture, juvenile studies were not possible in 2007.

Red king crab hatchery-scale culture in 2008 proved successful. Production scale experiments in nine 1200 L tanks investigated effects of diet and stocking density and yielded overall survival of approximately 31% from Z1 to glaucothoe in 2008. Diets consisted of enriched San Francisco Bay (SFB) *Artemia*, and some tanks were also fed *Chaetoceros* sp. algae. Highest survival in a single production scale tank to glaucothoe was 68% which yielded 40,800 glaucothoe. The tank was stocked at 50 larvae L$^{-1}$, fed enriched SFB *Artemia* and *Chaetoceros* sp. algae at 50,000 cells ml$^{-1}$, and treated with EDTA once daily. Production in 1200L tanks yielded over 120,000
healthy glaucothoe. From these, approximately 35,000 first stage juveniles were produced, which were used in juvenile rearing experiments.

Red king crab hatchery-scale culture in 2009 and 2010 proved increasingly successful (Fig. 1). Hatchery survival of red king crab achieved during 2009 and 2010 was substantially higher than that obtained during the first two years of the AKCRRAB project. During 2009, approximately 250,000 glaucothoe were produced in eight 1200 L tanks with a mean survival from stocking of Z1 to the glaucothoe stage of 53.3% and a mean survival of Z1 to C1 of 21%. The greatest number of glaucothoe produced in a single tank was 40,000. Approximately 100,000 C1’s were produced and subsequently used in nursery rearing experiments and ecological studies. During 2010, approximately 300,000 glaucothoe were produced in eight 1200 L and twelve 190 L tanks with a mean survival from stocking of Z1 to the glaucothoe stage of 50% and a mean survival of Z1 to C1 of 20%. Approximately 108,000 C1’s were produced and subsequently used in nursery rearing experiments and ecological studies.

Hatchery survival of red king crab achieved during the 2009 and 2010 trials was significantly higher than that obtained during the first two years of the AKCRRAB project. Improved survival was due to further refinements of larval rearing protocols and hatchery infrastructure, including installing immersion heaters in the seawater supply reservoirs which delivered a constant adequate supply of 8°C and 12°C seawater. It is unclear if similar survival rates can be achieved using broodstock from alternate locations.

**Potential ecosystem interactions of hatchery-raised crab**
Predation likely plays an important role in the demography of early benthic phase juvenile red king crab and may create a population bottleneck. Cannibalism is a potential source of predation because it occurs frequently in the lab (Stevens and Swiney, 2005), yet whether it occurs in the field is unknown. Little is known about predator-prey interactions of juvenile red king crabs in
the field, however gut contents analysis suggest that fish eat juvenile red king crabs (Livingston, 1989, 1991; Livingston et al., 1993). Fish co-occur with juveniles and are abundant in settling habitats (Dean et al., 2000). Stoner (2009) demonstrated that recently settled juvenile red king crabs are capable of detecting fish predators in the lab and adjust their behavior by seeking habitats with increased structural complexity. Further, early juvenile red king crabs exhibit behavioral shifts in habitat selectivity, which may be due to shifting foraging demands, shelter requirements, or anti-predation behavior (Pirtle and Stoner, 2010).

Another important question in evaluating the feasibility of stock enhancement of red king crabs is determining the size and season at which individuals are to be released in the wild. Body size is widely recognized as an important factor for post-release survival of hatchery-reared individuals (Wahle and Steneck, 1992; Leber, 1995; Willis et al., 1995; Johnson et al., 2008). For example, vulnerability to predation of tethered juvenile American lobsters (*Homarus americanus*) decreases with increasing body size (Wahle and Steneck, 1992), which may be due to ontogenetic shifts in behavior of the juveniles to predators (Wahle, 1992). Additionally, effects of season are important to survival (Glazer and Jones, 1997; Leber et al., 1997; van der Meeren, 2000) and often have interactions with body size (Johnson et al., 2008). For example, survival of hatchery-reared juvenile blue crabs increases with size; however, optimal size varies depending on season (Johnson et al., 2008).

Juvenile red king crabs have ontogenetic shifts in behavioral responses to predators (Stoner, 2009). In the laboratory, recently settled juveniles are highly cryptic with little movement within the substrate. Later stage juveniles become increasingly active in food searches and antagonistic behavior with conspecifics increases, thus crypsis decreases with crab size or age (Stoner, 2009). With increasing activity, larger crabs may demonstrate anti-predator behaviors such as refuge searches or aggressive displays. It is unclear whether the cryptic behavior of recently settled juveniles or the greater level of activity (refuge searches, aggressive displays) of later juveniles is a more effective anti-predator mechanism.

Hatchery-cultured red king crab juveniles vary in size as they grow. The first juvenile instars (C1s) are relatively uniform in size; however, size variability develops through successive molts. Mechanisms for this size variability are unknown, but theories include genetic variability and rearing artifacts such as varying feeding and cannibalism rates, artificial rearing conditions, and diet. Regardless, a specific size may be optimal to maximize survival in the natural environment for a release. If larger animals survive better, then there is a tradeoff between increased costs of hatchery culture and potential improvements in post-release survival with increased size. Therefore, our second proposed experiment investigates the comparative survival of different size classes of juvenile red king crabs during different seasons.

**OBJECTIVES AND METHODS:**

Experiments in 2011 will investigate effects of broodstock origin on large-scale production using alternate stocks. Additionally, survival of juvenile crabs at different sizes and seasons will be investigated.

The objectives of the hatchery-scale culture and field experiments in 2011 are the following:

- Determine larval and post-larval viability using alternate stocks for large-scale rearing
• Determine effect of size and timing on survival of hatchery-cultured red king crab juveniles in the field.

**BROODSTOCK ACQUISITION AND HATCHING**
We will collect 20 ovigerous female red king crab from Juneau, Alaska during the ADF&G fall crab survey and 20 from Bristol Bay, Alaska during the commercial fishery (pending permits). Crabs will be shipped to the Alutiiq Pride Shellfish Hatchery in Seward.

**Part 1: RED KING CRAB HATCHERY PRODUCTION:**
**Effect of broodstock origin on production success**

**SPRING 2011**

The following experiment will compare larval viability of Bristol Bay and Juneau broodstock when reared on a large scale. All previous hatchery rearing that was successful used Bristol Bay broodstock. We would like to investigate whether conditions that are ideal for rearing larval and juvenile crabs are universal and apply to more than one stock. We propose to rear red king larvae from both Juneau and Bristol Bay using identical conditions to determine if broodstock origin influences success of hatchery production.

We plan to examine the effects of broodstock origin on larval survival using 1200 L larval rearing tanks at the Alutiiq Pride Shellfish Hatchery.

*Hypothesis*

**H10:** There will be no difference in survival or intermolt duration of king crab larvae from Bristol Bay and Juneau broodstock.  
**H1a:** Survival and intermolt duration differs between different stocks.

**METHODS**
Larvae will be collected from at least three females from each stock and mixed randomly. Larvae will be stocked at densities of 50 larvae L\(^{-1}\) in eight 1200 L tanks. Four tanks will contain Bristol Bay larvae and four tanks will contain Juneau larvae. All tanks will be fed SFB *Artemia* enriched with DC DHA Selco (Inve Aquaculture). Larvae will be placed in circular, conical bottomed, polyethylene 1200 L tanks at the Alutiiq Pride Shellfish Hatchery’s Mariculture Technical Center. Water will be maintained at 12°C. Incoming water filtration will include particle filtration to 5 \(\mu\)m, a carbon filter, and an ultraviolet light sterilizer. Water flow will be maintained so that water is exchanged within each tank on a daily basis. Seawater will enter the tanks near the surface and exit through a banjo filter near the surface fitted with 105 \(\mu\)m screens to retain larvae and food in the tanks. Each morning, the 105 \(\mu\)m screens will be replaced with 500\(\mu\)m screens to allow *Artemia* from the previous day to flush out of the tanks. When the tanks are flushed of *Artemia*, the 105 \(\mu\)m screens will be replaced and all larvae will be fed enriched *Artemia* nauplii grown for 24 hrs and enriched for an additional 24 hrs. Larvae will be feed *Artemia* at a density of 2-4 ml\(^{-1}\). This density falls into suggested feeding ranges based on daily feeding rates of *P. camtschaticus* in laboratory conditions (Epelbaum and Kovatcheva, 2005). Each tank will receive a daily dose of EDTA, a synthetic chelator commonly used in larval
rearing of marine shrimp and other shellfish. EDTA may have an inhibitory effect on some pathogenic bacteria and also may protect larvae from toxic heavy metals. Feeding will be terminated when all zoeae in the tank molt to the glaucothoe stage. Percent survival will be compared at each zoae and glaucothoe stage between the two treatments using contingency table analysis.

LARVAL HEALTH ASSESSMENTS
Larval health will be monitored during each larval stage. Lipid content, gut content, and bacteria load will be examined via microscopy to determine nutritional status and overall health of the larvae. Number of lipids and maximum diameter of the lipids will be measured. Bacteria will be quantified by estimating % body coverage on the larvae. Sampling will occur during the late intermolt as filamentous bacterial infection is typically most severe shortly prior to molting.

QUARANTINE PROCEDURES
For each of the previously described hatchery experiments, quarantine precautions will include disinfection of transport containers with 100 ppm iodophore or 200 ppm bleach, control of aerosolization from the broodstock holding containers either by placement in a separate room or placing lids over the tanks, controlled access to the tanks either by separate room or visqueen barrier, use of disinfectant footbath in the controlled access corridor, use of separate utensils for the king crabs, and disinfection of the effluent with ozone and/or chlorine.

ADDITIONAL PROJECTS
Mass culture of king crab larvae provides the opportunity to investigate other features of early life history with hatchery-produced larvae and juveniles. Larvae and juveniles for additional studies will be provided by the larval culture described here. Additional projects may include genetic analyses, behavioral experiments to determine fitness of hatchery-reared individuals, and studies of molting, growth and habitat selection. These projects will require that hatchery-produced individuals be sent to research labs of collaborators to include Ginny Eckert (UAF Juneau), Al Stoner (NOAA Newport), Pam Jensen (NOAA Seattle), David Tallmon (UAS Juneau), and Sherry Tamone (UAS Juneau). Transport permits will be requested as needed for these projects.

Part 2: OUTPLANTING SIZE AND TIMING:
Effect of out-planting size and timing on survival of hatchery-cultured red king crabs in the field
The mission of AKCRRAB is to understand the large-scale culturing needs of wild king crab stocks, and to perfect strategies for hatching and rearing king crab to a stage where they can be released into the wild and contribute to reversing low wild stock abundance in Alaska. Acquiring this knowledge base will aid policymakers in making informed decisions about whether to one day pursue active rehabilitation of depressed wild king crab stocks through hatchery culture.

AKCRRAB has made great strides and has successfully produced over 100,000 red king crab juveniles in both 2009 and 2010 at the Alutiiq Pride Shellfish Hatchery. The next research step in AKCRRAB is to determine how and when to outplant juveniles and to determine ecosystem interactions of hatchery-reared juveniles in the wild. The outcome will be an informed strategy
for outplanting hatchery-cultured red king crabs and recommendations for hatchery production regarding size and season of juvenile crabs. This component is a vital part of the feasibility study as the size and timing that is needed to produce hatchery crabs has a very large impact on the economic feasibility of production.

SUMMER 2011

Hypotheses

$H_{10}$: In situ survival rates of juvenile king crabs are similar regardless of size.

$H_{1A}$: Larger crabs have higher in situ survival than smaller crabs.

$H_{20}$: In situ survival rates of juvenile king crabs are similar regardless of seasonality.

$H_{2A}$: In situ survival rates of juvenile king crabs have temporal variation

METHODS

Study site

Field experiments will be conducted in summer/fall 2011 near Juneau, Alaska, in the southern Lynn Canal, at Yankee Cove (134° 54.366’ W, 58° 35.431’ N). The site is composed of shallow subtidal (0-8 m depth) rocky reefs that host dense stands of the kelps *Saccharina subsimplex*, *Laminaria yezoensis*, and *Agarum clathratum*, several species of prostrate red algae, encrusting algae, as well as benthic invertebrates. These reefs transition into flat, sandy substrate at approximately 6-8 m depth. Annual surveys of community composition, including fish predators have been conducted since 2007 by Eckert and her colleagues and will be continued through the duration of this project. This study site was used in Fall 2009 for predation experiments examining the role of habitat on predation of age-0 and age-1 juvenile red king crabs (Pirtle et al., in prep.).

Producing hatchery crabs

Twenty ovigerous females will be obtained with pots in Juneau, Alaska during fall 2010 during the Alaska Department of Fish and Game fall Southeast Alaska crab survey and shipped to the Alutiiq Pride Shellfish Hatchery in Seward, Alaska, USA. Crabs will be placed in tanks containing flow through ambient seawater and fed 20 g chopped herring and squid per crab twice per week. Once hatching begins (anticipated winter/spring 2011), females will be placed in separate bins to isolate larvae. Larvae from each female will be mixed and raised in 1200 L cylindrical tanks until the first juvenile instar (C1) stage. Larvae will be fed enriched San Francisco Bay strain *Artemia* nauplii daily. After a 2 month nursery rearing period, hatchery-cultured juvenile red king crabs will be sorted into three size classes (2-4 mm CW, 5-8 mm CW, 9-12 mm CW). Crabs will be shipped back to the Juneau Center of the School of Fisheries and Ocean Sciences and held in running seawater aquaria, following established methods of rearing juvenile king crabs (Daly et al., 2009) until crabs are needed for field experiments.

Experimental design

Tethering platforms will be constructed of cement and PVC (Fig. 2). The tether platform will be 60 x 60 x 2 cm PVC sheets secured 50 cm off the bottom. Field experiments in Juneau have revealed predation by the sea star, *Pycnopodia helianthoides*, on tethered crabs is significant (Pirtle, pers. comm.), and thus the raised platform is needed to prevent *P. helianthoides* from
consuming the tethered crabs. Untethered juvenile red king crabs avoid *P. helianthoides* by actively moving away (Pirtle, pers. comm.). Therefore, predation by *P. helianthoides* may be an artifact of tethering. We expect that demersal fish will be the primary predators of juvenile red king crabs (Livingston, 1989, 1991; Livingston et al., 1993; Tyler and Kruse, 1996) and that effects of the raised tethering platform on predation rates by fish are likely minimal. Modifications will be made to the tether platform design, as needed for stability or to evade *P. helianthoides*.

Natural shell hash substrate will be placed on the platforms. Crab will be tethered with a 25 cm monofilament fishing line and placed on the experimental platforms. Substrate type will be constant for all replicates.

![Tether Platform Diagram](image)

Figure 2. Conceptual plan for the tether platform

Experimental trials will consist of three platforms so that one crab of each size class is tethered per platform. A fourth platform will be surrounded with 1 cm mesh and will contain a tethered crab as a control to assess handling mortality. Survival will be assessed after 24 h. Trials will be repeated on five consecutive days for temporal replication. Crabs remaining at the end of the trials will be returned to the laboratory but will not be reused. Underwater color HD video cameras (Well Vu) will be installed above each platform and recordings will be made to identify predators, calculate attack rates, and observe crab behavior. These cameras are cabled to shore for power and connection to a digital video recorder. This setup was used successfully for predation experiments examining the role of habitat on juvenile king crab predation in Fall 2009 (Pirtle et al., in prep.) All crabs were successfully recovered or observed to have been predated, and none escaped. Experiments will be conducted during both July and September to determine seasonality in size effects on survival. Survival will be analyzed comparing seasons and sizes with two-way analysis of variance (ANOVA).

**LITERATURE CITED**


Persselin, S. 2006b. Enriched Artemia nauplii as diet for red (Paralithodes camtschaticus) and blue (P. platypus) king crab larvae in the laboratory. J. Shell. Res. 25, 762.


