2012-2013 Study Plan

Nutrition and condition of red king crab larvae: enhancement of king crabs to improve sustainability of Alaskan coastal communities

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Abstract

King crab enhancement has the potential to restore king crab stocks, which would economically benefit the fishing industry and coastal communities throughout Alaska. The recent success of king crab culture in Alaska suggests that enhancement with hatchery animals is possible, yet mortality during the last larval rearing stage is a bottleneck in hatchery production of juveniles. A larval nutritional deficiency has been proposed to explain the source of the bottleneck, and we propose to test this hypothesis. We aim to improve the production of healthy juvenile red king crab (Paralithodes camtschaticus) and juvenile blue king crab (Paralithodes platypus) through a multidisciplinary approach to understanding their bioenergetics and optimize their condition. We propose biochemical, visual, and stress tests that will provide industry with a better understanding of the nutritional factors that affect quality during the larval stages of crab production. The outcome of this project will be the potential to design aquaculture live-food enrichments that are customized to Alaska king crab. This project falls within Alaska Sea Grant’s funding theme through the development of aquaculture technology that will lead to improvements in the economic and sociocultural sustainability of Alaskan coastal communities, and king crab enhancement is a strategy for coastal communities to adapt to change.
I. DESCRIPTION AND NEED

Statement of Need
The growing global demand for seafood has increased pressure on fish stocks (Pauly et al., 1998). As such, approximately 80% of world fish stocks are currently fully exploited or overexploited (FAO, 2009). Almost all of the historical red king crab (Paralithodes camtschaticus) fisheries (six of the eight stocks) in Alaska crashed and are now closed; most have been closed since the early 1980s due to low population size. Red king crab fisheries in Kodiak, Cook Inlet, Prince William Sound, the Aleutians, and the Alaska Peninsula were closed in the 1980’s and remain closed today. The red king crab fishery in Southeast Alaska has been intermittently open and closed and is currently closed. The last bastion of the red king crab fishery is in Bristol Bay, where the maximum historical annual US retained catch was 59,000 t in 1980, but in 2009 was only 9,221 t (ADFG, 2010). Blue king crab (Paralithodes platypus) are in even worse shape, as the Pribilof Islands stock was declared overfished in 2002 and is currently undergoing a second rebuilding effort ten years later (NPFMC 2010). King crabs are iconic in Alaska and were historically and are still in many regions important for sport, subsistence, and community harvest.

The collapse of Alaskan crab fisheries coincided with decadal changes in climate (Zheng and Kruse 2000), and hypotheses linking climate and fishery declines include changes in larval advection and survival (Tyler and Kruse 1996, 1997) and increased disease and/or predation (Bechtol and Kruse 2009). Coherence in declines across larger geographic areas and across species support a link between climate and crab stocks (Orensanz et al. 1998). Shifts in climate may be causative factors for changes in community structure and trophic linkages. Shifts in climate and oceanography may be advantageous for predators that remove small juvenile crabs, preventing population recovery (Zheng and Kruse 2006). However, correlational studies of biological populations and climate can suggest linkages but are generally speculative, because specific mechanisms on scales relevant to biological populations are lacking (Sinclair and Frank 1995) and because other factors such as recruitment pulses or fishing-down stocks may have coincided with population changes (Orensanz et al. 1998). Climate may be the proximate cause for a shift from a crustacean-dominated to a flatfish- and gadid-dominated system; the ultimate cause is as yet unknown, but likely involves overfishing. The lack of recovery in stocks, such as Kodiak red king crab and the Pribilof Islands blue king crab, which have not recovered in the absence of fishing for decades, suggests that alternative approaches are needed to rebuild king crab stocks in Alaska. Stock enhancement is one alternative (Leber, 1999, 2002).

Relevance and Impacts
This project falls within Alaska Sea Grant’s funding theme through the development of aquaculture technology that will lead to improvements in the economic and sociocultural sustainability of Alaskan coastal communities, and king crab enhancement is a strategy for coastal communities to adapt to change. Alaska Sea Grant’s mission includes “research, education, and extension programs and partnerships to help sustain economic development, traditional cultural uses, and conservation of Alaska's marine and coastal watershed resources”.

Impacts

Economic (market and non-market) and societal benefits (jobs created and retained) derived from the discovery and/or application of new aquaculture production and management models or techniques that lead to increased sustainability and productivity

King crab enhancement has the potential to restore king crab stocks, which would have economic benefits for the fishing industry and coastal communities throughout Alaska. The knowledge gained through the proposed research will aid in the development of successful release strategies for hatchery-cultured king crabs. The ability to maintain a sustainable crab fishery by augmenting natural stocks would provide employment opportunities for fisherman and their children. Enhancing depressed stocks may also replenish depleted stocks to the degree necessary to reopen areas currently closed to fishing, further increasing job opportunities. Furthermore, impacts of this research can be applied to other crustacean stock enhancement programs, as many components of red king crab rearing may be applied to other species.

Adoption and implementation of economically and environmentally sustainable aquaculture development practices and policies as a result of Sea Grant activities

Stock enhancement practices can be adopted and implemented by coastal communities to increase the sustainability and productivity of local crab fisheries. The results from this research will customize larval food for king crab aquaculture. Stock enhancement outreach may foster a local interest in the development of king crab aquaculture practices in coastal communities.

Diverse and sustainable coastal communities, where residents have the knowledge and skills they need to adapt to natural and man-made changes in resource use and availability.

The proposed research will move towards developing sustainable king crab fisheries by augmenting the natural stocks with hatchery-cultured individuals. Understanding the feasibility of outplanting through outreach may foster a local interest in the development of economically and environmentally sustainable king crab aquaculture practices in coastal communities. Increasing hatchery culture of king crabs and implementing effective outplanting may eventually be adopted on a regional scale, which could help residents maintain economically viable coastal communities in incredibly remote regions of Alaska.

Background Information

The potential for stock enhancement

Stock enhancement 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 et al., 2002; Stevens, 2006). Globally, several species of crab are cultured at the hatchery scale. Successful techniques for rearing larval crabs 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 in Chesapeake Bay (Secor et al., 2002; Zmora et al., 2005). Larval rearing research on king crab has been conducted in Japan (Nakanishi, 1987), Russia (Kovatcheva et al., 2006), and Alaska (Shirley and Shirley, 1989; Persselin, 2006; Stevens et al., 2008).

King crabs are suitable candidates for stock enhancement, because they are some of the most commercially valuable crustaceans in the world, and recruitment limitation has been proposed to explain their lack of recovery in the absence of fishing (Blau, 1986). The Russian introduction of
red king crabs to the Barents Sea in the 1960s suggests that it is, in theory, possible to restore
king crabs to their historic range. The Alaska King Crab Research and Rehabilitation and
Biology (AKCRRAB) Program was created in 2006 to assess the feasibility of stock
enhancement for king crabs in Alaska. The AKCRRAB program is the first and only program to
conduct large-scale king crab aquaculture in the US. Since its inception in 2006, the AKCRRAB
program successfully demonstrated that king crabs can be cultured on a large-scale in a hatchery
setting. The ultimate goal of AKCRRAB is to increase survival of larvae and early juveniles in
the hatchery and release juveniles into the wild to rehabilitate depleted stocks.

During the last four years of research at the Alutiiq Pride Shellfish Hatchery in Seward, Alaska,
large-scale larval rearing experiments have successfully investigated rearing needs of red king
crab larvae and juveniles (Daly et al., 2009). Established techniques for larval rearing from
hatching to the glaucothoe stage have been refined and hatchery infrastructure improved
(Swingle et al., in prep). Red king crab larval survival has steadily improved from 2% in 2007 to
31% in 2008 and approximately 50% in 2009 and 2010. Over the first four years of the project,
the total number of red king crab first stage juveniles (C1s) produced increased from less than
1,000 in 2007 to approximately 108,000 in 2010 (Figure 1). In nature, survival from hatching to
the first juvenile stage is thought to be less than 0.1% (Marukawa, 1933, Kovatcheva et al.,
2006). In 2010, hatchery production yielded an average survival from hatching to the first
juvenile stage of 20%, a tremendous increase over natural survival rates.

**Fitness of hatchery-cultured crabs**

Despite recent production success, glaucothoe rearing is a bottleneck in hatchery culture.
Glaucothoe are non-feeding post-larvae that rely on nutritional reserves acquired during larval
rearing to reach the first juvenile stage (C1). The process of molting from larvae to glaucothoe to
first-feeding juveniles is a stressful period during red king crab culture, as the animals undergo
significant morphological and physiological changes (Marukawa, 1933). The bioenergetics and
nutritional requirements to successfully complete these transitions in body form are likely
significant and have not been documented. Increased glaucothoe mortality may be due to
inadequate nutrition stored during the feeding larval stages. Hatchery biologists have developed
a method for subjectively assessing larval and glaucothoe health through visual analysis of gut
content, number and size of lipid globules, and bacterial infection using a rank scale. However,
there is currently little understanding of the bioenergetics behind this variation in condition,
which is a critical step in developing technologies for culture of this species.

Lipids are vital to marine organisms as an energy source and as important structural components
of cell membranes (Sargent et al., 1989; Arts et al., 2001), where they maintain membrane
fluidity in environments with cold or variable temperatures (Cossins et al. 1997). Lipids are a
major source of energy in juvenile and larval crustaceans (Coutteau et al. 1996); moreover, they
are crucial to elevated growth and molting success (Wen et al. 2006). In crustaceans, cell
membrane structure depends largely on the combination of specific essential lipids and proteins.
Lipid droplets often visibly accumulate in specific tissues, such as the hepatopancreas, that serve
as the major site of energy storage (Yepiz-Plascencia et al., 2000). Certain lipids are essential to
larval crustaceans, as they are required preformed in the diet and are vital to normal growth,
development and survival. These dietary essential lipids are those that cannot be synthesized in
adequate amounts *de novo* within an animal from dietary precursors; however, they are often not
found in adequate levels in commercially available live-foods. The major lipid classes that impact condition in crustaceans are triacylglycerols (TAG), sterols (ST), and phospholipids (PL) (Ouellet & Taggart, 1992; Coutteau et al. 1996). Specifically, the ratio of different lipid classes within larvae (TAG/ST) has previously been used to indicate condition in a number of finfish and crustaceans (Fraser et al., 1989; Harding and Fraser, 1999; Copeman et al., 2002, 2008).

Fatty acids are the major constituent of both TAG and PL and the importance of polyunsaturated fatty acids (PUFAs) have been extensively investigated in marine larval nutrition (Wantanabe and Kiron, 1994; Sargent et al., 1999). Specifically, docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (AA, 20:4n-6) are essential fatty acids (EFAs) for many crustaceans (Merican and Shim, 1996; Holme et al., 2007), however live-foods commonly used for culturing larvae, including Artemia, are naturally low in these EFAs (Navarro et al., 1999). As such, enrichment of live foods with EFAs prior to feeding is often necessary (Sorgeloos et al. 2001). Levels of EFAs in crustaceans can be a good indicator of overall health, as seen in Pacific white shrimp which showed increased tolerance to handling stress when fed a diet enriched with EFAs (Mercier et al., 2009). The importance of dietary PUFAs to the culture of juvenile crustaceans has been investigated for several crab (Suprayudi et al., 2004; Zmora et al., 2005), lobster (Limbourn and Nichols, 2009), and shrimp (Lavens and Sorgeloos, 2000) species. Numerous studies have shown that the fatty acids DHA, EPA, and AA play an important role in the early growth and development of crustaceans such as Kuruma shrimp (Penaeus vannamei) (Lim et al., 1997), mud crab (Scylla paramamosain) (Takeuchi et al., 1999; Suprayudi et al., 2004), Chinese mitten crab (Sui et al., 2007), snow crab (Chionoecetes opilio) (Kogane et al., 2007), and swimming crab (Hamasaki et al., 1997).

EPA and DHA play differing roles in crab larvae, with EPA showing benefits to survival and DHA playing an important role in intermolt duration and carapace width (Takeuchi et al., 1999). EPA and DHA are both essential to marine larvae, however many species have a higher level of membrane specificity for DHA (Copeman et al., 2002; Rodriguez et al., 1997). DHA is naturally found at high levels in neural tissue and may impact neural membrane structure and function (Bell and Dick, 1991). Lower dietary DHA/EPA ratios may negatively impact neural function, growth and survival of larvae (Bell et al., 1995; Rodriguez et al., 1997). Further, insufficient DHA relative to EPA has previously been demonstrated to result in developmental retardation and/or metamorphosis failure, as seen in mud crab zoeae (Nghia et al., 2007). Specific Artemia enrichment mediums containing 30% n-3 highly unsaturated fatty acids (HUFA) with a minimum DHA/EPA ratio of 1:1 were recommended to improve mud crab survival and growth (Nghia et al., 2007). Membrane lipids, such as EPA and DHA play an important role in thermal adaptation of marine ectotherms and are often found at elevated levels in marine organisms living at cold temperatures (Dunstan et al., 1999; Hall et al., 2000, 2002). As such, optimal n-3 HUFA levels are likely to be higher for red king crab, than in better studied warm water species.

Essential fatty acids (EFAs) are likely a key component to red king crab development. “Nature Knows Best” has often been used as a starting point in the development of hatchery nutritional protocols for new culture species (Sargent, 1989, 1999); however, no explicit comparison has been conducted to investigate lipid composition between hatchery-cultured and wild red and blue king crabs. Hatchery-reared crabs are likely missing EFAs, therefore, an understanding these insufficiencies in glaucothoe will advance the development of proper live-food enrichment
protocols. A comparison of the hatchery health index with the biochemical composition of the glaucothoe will provide a “standard curve” to assess larval condition. This inexpensive method can be easily used in the hatchery setting to quickly assess larval condition. This information will also provide further understanding of the nutritional requirements of the early life history stages of king crab and thus improve enrichment protocols for live-foods used in the hatchery.

II. OBJECTIVES AND APPROACH

OBJECTIVES:
Our goals are to (1) gain information on the dietary requirements of larval and juvenile red and blue king crab and (2) develop a quality index for hatchery-cultured king crab larvae and juveniles for stock enhancement. To achieve this, our specific objectives are to (1) determine a lipid profile of hatchery-cultured and wild-collected red king crab glaucothoe and juveniles to compare their bioenergetics and nutritional profile, (2) determine a lipid profile of hatchery-cultured blue king crab glaucothoe and juveniles, (3) perform live-feed dietary experiments to determine the EFA requirements for red king crab during the larval stages along with hatchery health indices and salinity stress tests, and (4) inform interested industry, fishery managers, and other stakeholders of advancements in king crab aquaculture through outreach.

PROCEDURES
Conduct nutritional comparisons of hatchery and wild red king crab glaucothoe and juveniles
The transition from the post-larval stage to the first juvenile stage is thought to be energetically costly. Therefore, previously hatchery-cultured and wild crabs will be assessed for lipid classes and fatty acids both during the critical transitional glaucothoe stage and in the newly settled juvenile stage. We are currently collecting lipid samples from hatchery-raised red and blue king crab glaucothoe and juveniles during the 2011 culturing season in anticipation of this project. Wild red king crab glaucothoe will be collected for comparison using artificial collectors in Juneau, Alaska. The artificial collectors have an outer skin of tubular plastic netting stuffed with conditioned gillnet and have been successfully used to passively collect young-of-year red king crabs in Alaska (Blau and Byersdorfer, 1994). The collectors will be deployed in spring 2012 and the collectors will be monitored regularly to identify the settlement window with anticipated retrieval in July to obtain wild glaucothoe. Using this method we have successfully collected both glaucothoe and juvenile crabs from Juneau during the summers of 2008 to 2010, three years during which settlement timing was quite consistent (Pirtle 2010). We will freeze half of the wild glaucothoe and hold the other half in the laboratory and monitor them daily until they molt into juveniles, at which point they will also be frozen. Proximate composition and weights (see Proximate composition methodology below) will be compared between a) hatchery-cultured red and blue king crab glaucothoe, b) hatchery-cultured red and blue king crab juveniles, c) glaucothoe and juveniles for both species, d) hatchery-cultured and wild red king crab glaucothoe and e) hatchery-cultured and wild recently settled juvenile red king crab. Statistical comparisons will be made by comparing the levels of lipids, fatty acids, dry weights and organic weights using ANOVA and Principal Components Analysis (see Statistical analyses below) to test the following hypothesis.
**Hypothesis**

**H10:** There will be no difference in lipid profile among hatchery-cultured and wild red king crabs in the glaucothoe and recently settled juvenile stages.

**H20:** There will be no difference in lipid profile among hatchery-cultured red and blue king crabs in the glaucothoe and recently settled juvenile stages.

**Determine the lipid classes and fatty acids of hatchery-cultured red king crab glaucothoe in relation to hatchery health indices**

Ten ovigerous females will be obtained with pots in Juneau, Alaska in fall 2012 during the Alaska Department of Fish and Game fall Southeast Alaska crab survey and either shipped to the Alutiiq Pride Shellfish Hatchery in Seward, Alaska or maintained at the UAF School of Fisheries and Ocean Sciences Lena Point Facility. Larvae will be hatched and reared using established protocols used for larval culture (Figures 2-3, Swingle et al., in prep) with modifications to the larval food. To quantify the dietary DHA/EPA requirements of larval red king crab, we will compare glaucothoe reared using a commercially-available enrichment (DC DHA Selco®, INVE Aquaculture, UT, USA) to larvae fed *Artemia* enriched with three different experimental emulsions (ICES standardized enrichments, Table 1) as well as this control DC DHA enrichment. Based on previous experience, king crab larvae do not survive to the glaucothoe stage on unenriched *Artemia*, and therefore we will not use this treatment. The three experimental emulsions, which have been developed by researchers at the Laboratory of Aquaculture and Artemia Reference Center in Ghent, Belgium and the International Council for Exploration of the Sea (ICES) (hereafter called ICES emulsions), have a constant level of total lipid and proportion of n-3 PUFA. ICES emulsions are routinely provided for a small formulation and shipping fee to researchers conducting nutrition work with marine larvae (i.e., Martins et al. 2006, Stottrup and Attramadal, 2007). These ICES emulsions will be utilized to determine the optimal supplementation of DHA/EPA for larval red king crab. Use of controlled standardized ICES emulsions will allow comparisons of the dietary requirements of red king crab larvae and glaucothoe to those of other candidate aquaculture species (i.e., mud crab, Nghia et al., 2007). The use of experimental emulsions, rather than commercial emulsions, will provide an explicit test of the effects of the DHA/EPA ratio on larval crabs, while keeping all other nutritional components of the live-food constant (i.e. total lipids, proteins, lipid classes). *Artemia* cysts will be disinfected with chlorine, incubated and hatched daily following standard methods (Sorgeloos et al., 1986). Newly hatched *Artemia* will be enriched starting 12 h post-hatch for 24 h at a concentration of 200 individuals ml⁻¹. For all emulsions, an initial dose of 0.6 g L⁻¹ will be added to the enrichment tanks. Temperature and salinity of the *Artemia* culture will be kept at 30°C and 30 g L⁻¹, respectively (Nghia et al., 2007).

**Table 1.** Experimental emulsions used in determining dietary DHA/EPA requirements of juvenile red king crab (Garcia et al., 2008; Nghia et al., 2007; ICES reference emulsions).

<table>
<thead>
<tr>
<th>Lipid parameters</th>
<th>DC-DHA-selco</th>
<th>ICES Emulsion 30/0.6</th>
<th>ICES Emulsion 30/2.3</th>
<th>ICES Emulsion 30/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total n-3</td>
<td>~30%</td>
<td>30%</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>DHA/EPA</td>
<td>~3.5</td>
<td>0.6</td>
<td>2.3</td>
<td>4</td>
</tr>
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</table>
Larvae will be reared in experimental larval tanks, with three replicates of each of the four *Artemia* enrichment treatments. Larvae will be fed enriched *Artemia* nauplii (Table 1) at a density of 2-4 ml⁻¹. This feeding density falls into suggested feeding ranges based on daily feeding rates of *P. camtschaticus* in laboratory conditions (Epelbaum and Kovatcheva, 2005) and has yielded high survival in previous hatchery-scale experiments at the Alutiiq Pride Shellfish Hatchery (Swingle et al., in prep). Feeding will be terminated when all zoeae in the tank molt to the glaucothoe stage. Seawater and unconsumed *Artemia* will be flushed from the tanks each evening by replacing the 105 µm screens (that retain larvae and *Artemia*) with 500 µm screens (that retain only larvae). After flushing, the 105 um screens will be replaced. Larvae will be reared at 12°C to increase overall production by decreasing intermolt period and filamentous bacteria load. Incoming water filtration will include particle filtration to 5 µm, a carbon filter, and an ultraviolet light sterilizer. Water flow to each larval rearing tank will be maintained at a continuous rate of 1 L min⁻¹ to maintain stable temperature and excellent water quality in all experimental tanks. Survival will be quantified for each tank at each larval and post-larval stage. Visual health assessments will be conducted at each larval stage to qualitatively assess overall larval and post-larval health (see below).

**Hatchery health indices: visual inspection**

We have developed an inexpensive method for king crab larvae to determine relative nutritional status of larvae and post-larvae by visually inspecting lipid globules. Larvae and glaucothoe reared using the four different *Artemia* enrichments will be compared.

**Hypothesis**

**H₃ₒ:** There will be no difference in lipid score among larvae and glaucothoe reared with four different *Artemia* enrichment diets.

Visual analysis will be conducted during the larval and glaucothoe rearing periods for each tank to qualitatively assess overall larval and post-larval health. Specifically, twenty randomly selected larvae from each tank will be assessed at each zoeal and glaucothoe stage and given a relative condition score based accumulation of lipid globules. Glaucothoe collected in the field will be scored using this index as well. A score will be given to each individual based on the estimated number of lipid globules greater than 50 µm diameter. No lipids will result in “0”, 1-5 lipid globules will result in “1”, 6-15 lipid globules will result in “2”, and >15 lipid globules will result in “3”. Additionally, live and dead glaucothoe from the salinity stress test (described below) will be scored for lipid globules to provide a direct comparison of the visual hatchery health index to the differential survival of larvae exposed to a salinity stress challenge.

**Hatchery health indices: Stress test**

Health indices are important for evaluating the relative health of hatchery-cultured individuals. Stress tests are often used for assessing the health of hatchery-cultured post-larvae of commercially important crustacean species including Pacific white shrimp (*Penaeus vannamei*) (Villalon, 1991; Samocha et al., 1998), tiger prawns (*Penaeus monodon*) (Briggs, 1992), and freshwater crabs (*Eriocheir sinensis*) (Naihong et al., 1999). A salinity shock is the most common stressor used (Brock and Main, 1994), which involves assessing survival rates after short term exposure to an elevated or reduced salinity. Higher survival suggests better quality post-larvae, while lower survival suggests poorer quality post-larvae. Exposure of *P. vannamei*
post-larvae to a salinity of 5 ppt at 20°C for one hour with survival greater than 60% indicates good quality (Villalon, 1991). Additionally, a survival rate of greater than 50% after exposure of *P. vannamei* post-larvae to a salinity of 3 ppt at 26°C for two hours suggests good quality post-larvae (Samocha et al., 1998). For tiger prawns, exposure to a salinity of 10 ppt at 20°C for one hour is used to assess relative health (Briggs, 1992). A similar method to evaluate the health of freshwater crab post-larvae has been developed, with animals exposed to an elevated salinity of 60 ppt for two hours with cumulative mortality recorded at ten min intervals (Naihong et al., 1999). Hardier crab post-larvae survive up to two hours while weaker groups fed inferior diets succumbed within one hour (Naihong et al., 1999).

**Hypothesis**

**H40:** There is no difference in mortality among glaucothoe reared with four different *Artemia* enrichment diets when exposed to reduced salinity.

Red king crab larvae reared to the glaucothoe stage using diets containing four different *Artemia* enrichment emulsions (Table 1) will be subjected to a salinity stress test to measure their relative health. Twenty randomly selected glaucothoe from each tank will be placed in separate Petri dishes with seawater salinities of 5 and 32 ppt in a cold room at 12°C. The 32 ppt salinity is equal to the larval rearing salinity and will serve as a control. The 5 ppt salinity was selected because preliminary testing in 2010 resulted in 100% glaucothoe mortality after one hour with differential survivals at 10 min intervals (Swingle and Daly, unpublished data). Distilled water will be mixed with filtered seawater to produce the desired salinity. During the salinity stress tests, counts of surviving glaucothoe will be made at 10 min intervals over a 1 h period. Live glaucothoe will be defined as those showing any movement of their antennae, pereiopods, and/or pleopods, while dead glaucothoe will be defined as those showing no movement for at least 15 seconds after gentle agitation. Wild glaucothoe will be subjected to the stress test for comparison. We expect that glaucothoe fed the best diet will exhibit highest survival during the stress tests. The percent of live larvae at each time interval will be compared among diet treatments using repeated measures analysis of variance (ANOVA) with post-hoc comparisons to identify differences at individual time periods.

**Nutritional profile of hatchery-cultured glaucothoe**

Glaucothoe from the different diets, the stress test, and the visual health index will be biochemically analyzed for lipid classes and fatty acid content to determine which lipid parameters are associated with elevated growth, survival, and condition (Table 2). Within each dietary treatment, glaucothoe will be pooled by result of stress test (live vs. dead) and by visual health index (healthy vs. poor) to obtain sufficient material for detailed lipid class and fatty acid analysis (~10 individuals, >100 µg). This will allow direct correlation of the fitness of the glaucothoe with their essential fatty acid content and lipid class composition. Additionally, late stage larvae (mid Z4) in each of the dietary treatments will be collected and pooled to obtain sufficient material for a comparison of the EFA content and lipid class composition compared to the glaucothoe (Table 2). All emulsions, enriched *Artemia*, and early-stage zoeae will be sampled to provide a comparison of the lipid profiles of the food and the larvae (Table 2).
Table 2. Lipid samples collected for hatchery-culturing experiments.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Treatments</th>
<th>replicates</th>
<th>lipid samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICES emulsions &amp; DHA Selco</td>
<td>4 diets</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>enriched Artemia</td>
<td>4 diets</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>early Z1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>pooled at start</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>mid Z4&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4 diets</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>G&lt;sup&gt;3&lt;/sup&gt; - stress test</td>
<td>4 diets - live &amp; dead</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>G&lt;sup&gt;3&lt;/sup&gt; - visual insp.</td>
<td>4 diets – poor &amp; healthy</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>83</td>
</tr>
</tbody>
</table>

<sup>1</sup>Z1= newly hatched larvae, <sup>2</sup>Z4= late stage larvae, <sup>3</sup>G= glaucothoe

**Hypotheses**

**H5<sub>0</sub>:** There will be no difference in lipid profile among late-stage zoeae and glaucothoe reared with four different Artemia enrichment diets.

**H6<sub>0</sub>:** There will be no difference in lipid profile among glaucothoe that survived the salinity stress test compared to those that did not survive the stress test.

**H7<sub>0</sub>:** There will be no difference in lipid profile among glaucothoe that have a relatively “poor” lipid score compared to those with a relatively “healthy” lipid score using visual inspection.

**Proximate composition methodology**

Larval and juvenile samples will be frozen at the time of collection and shipped on dry ice to the Alaskan Fisheries NOAA Laboratory in Newport Oregon. Upon arrival in Newport, lipids will be extracted in chloroform/methanol according to Parrish (1987) using a modified Folch procedure (Folch et al., 1957). Within three months, frozen extracts will be shipped to Dr. Chris Parrish’s laboratory at the Ocean Sciences Centre, Memorial University in St. John’s, Newfoundland, Canada. Dr. Parrish’s laboratory was chosen due to a long standing highly productive relationship between Louise Copeman (Newport, NOAA) and Dr. Parrish’s lab (Copeman et al. 2002; Copeman and Parrish 2002, Copeman et al. 2008; Copeman et al. 2009; Copeman and Laurel 2010, Stoner et al. 2010, Copeman et al. 2010). Dr. Parrish is a world expert in lipid class and fatty acid analyses of marine samples (Parrish 1987, Parrish 1998). He leads one of the only laboratories in North America that is able to routinely perform both detailed lipid class and fatty acid analysis on small quantitative marine samples. Further, his laboratory provides expertise in the analyses of new culture species for cold water aquaculture (Garcia et al. 2008; Parrish et al. 1999, Parrish 1998).

Lipid classes will be determined using thin layer chromatography with flame ionisation detection (TLC/FID) with a MARK VI Iatroscan (Iatron Laboratories, Tokyo, Japan) as described by Parrish (1987). Extracts will be spotted on silica gel coated Chromarods and a three-stage development system will be used to separate lipid classes. The first separation consists of 20 min developments in 98.95:1:0.05 hexane:diethyl ether:formic acid. The second separation consists of a 40 min development in 79:20:1 treatment. The last separation consists of 15 min
developments in 100 % acetone followed by 10 min developments in 5:4:1 chloroform:methanol:water. Data peaks will be integrated using Peak Simple software (ver. 3.67, SRI Inc) and the signal detected in mvolts will be quantified using lipid standards (Sigma, St. Louis, MO, USA). Lipid classes will be expressed both in relative (mg g⁻¹ dry weight) and absolute amounts (µg animal⁻¹).

Total lipid will be analysed for fatty acid composition. Fatty acid methyl esters (FAME) will be prepared by transesterification with 14% BF₃ in methanol at 85°C for 90 min (Morrison and Smith, 1964; Budge, 1999). FAMEs will be analyzed on an HP 6890 GC FID equipped with a 7683 autosampler and a ZB wax+ GC column (Phenomenex, USA). The column will be 30 m in length, with an internal diameter of 0.25 µm. The column temperature starts at 65°C and is held at this temperature for 0.5 min. The temperature will be increased to 195°C (40°C min⁻¹), held for 15 min then increased again (2°C min⁻¹) to a final temperature of 220°C. Final temperature will be held for 3.25 min. The carrier gas will be hydrogen, flowing at a rate of 2 ml min⁻¹. The injector temperature will start at 150°C and increased (200°C min⁻¹) to a final temperature of 250°C. The detector temperature will be held constant at 260°C. Peaks will be identified using retention times based upon standards purchased from Supelco (37 component FAME, BAME, PUFA 1, PUFA 3). Chromatograms will be integrated using Galaxie Chromatography Data System (Ver. 1.9.3.2, Varian).

Dry weight (DWT) measurements on individual crabs will be made to the nearest 1.0 µg using a microbalance (Sartorius R16OP). Crabs will first be rinsed in 3% ammonium formate solution to remove excess salt before being transferred to 5.0 cm² pre-weighed aluminum foils and an oven set at 70°C for 48 h. Foils will be removed from the oven and then stored in a desiccator and reweighed within 1 hr. Ash weights will be measured similarly after drying in a muffle furnace for 12 h at 450°C. DWTs will be calculated by subtracting the weight of the pre-weighed foils, while organic weights will be calculated by subtracting the ash weight from the previously calculated DWTs.

**Statistical analyses**

Dry weight, organic weight, total lipids, lipid classes and fatty acids will be compared among diet treatments using analysis of variance (ANOVA). A nested ANOVA will be used to examine the results of the stress and visual tests. All percentage data will be arcsine square root transformed in order to meet the assumptions of the model. Lipid and weight variables will be compared with growth, survival, and hatchery health indices in a multivariate principal component analysis (PCA) in order to simplify the data set without losing any of the original variation (Meglen, 1992; Copeman et al., 2008, Copeman and Parrish 2004). These univariate and multivariate approaches will indicate which of the >60 fatty acid and lipid class parameters are most important in determining juvenile red king crab health.
### Project Timeline

<table>
<thead>
<tr>
<th>Milestones</th>
<th>First year</th>
<th>Second year</th>
<th>Third year</th>
<th>Fourth year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collect and freeze hatchery-reared red and blue king crab glaucothoe and juveniles</td>
<td>X</td>
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<tr>
<td>Collect wild larvae &amp; conduct lipid analyses</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>Collect &amp; maintain broodstock</td>
<td>X</td>
<td></td>
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<tr>
<td>Conduct larval nutrition experiment, including hatchery health indices, hatchery stress test, and lipid analyses</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>Data analysis &amp; manuscript preparation</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td>Monthly outreach “newsflash”</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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</table>

Figure 1. Total red king crab first stage juveniles (C1s) produced by AKCRRAB in its

Figure 2. Juvenile red king crab cultured at the Alutiiq Pride Shellfish Hatchery
III. OUTREACH COMPONENT

We have conducted extensive outreach for the Alaska King Crab Research, Rehabilitation and Biology (AKCRRAB) program since the program’s inception in 2006. Outreach activities have included press releases, a blog, a Newsflash with updates on the project that is e-mailed monthly to a wide variety of audiences, information sheets, and a website with detailed information on the project http://seagrant.uaf.edu/research/projects/initiatives/king_crab/general. We will continue with these activities, working closely with Alaska Sea Grant.

Objectives 1 & 2 & 3:
1. Understand bioenergetics of hatchery and wild crabs,
2. Perform dietary experiments & compare lipid profile to hatchery health indices.

Target Audience – Aquaculture industry, ecologists, academics involved with the development of alternative aquaculture species, fishery managers, other interested stakeholders

Intended Learning Outcomes – An understanding of improved hatchery technology for rearing king crab and other crab species, development of nutritionally based practical hatchery condition index, information of the dietary lipid class and fatty acid requirements of larval, glaucothoe, and juveniles of red king crab.

Procedures to Achieve Intended Outcomes –
- Manuscripts will be submitted to academic journals where they will be publicly accessible.
- Project personnel will conduct an “Open House” which will be advertised in the community for commercial fishing groups, students, and other interested members of the community. This open house will be conducted when larval rearing is in full swing so that members of the public may see king crab aquaculture “in action”. In the past, these open house activities have been very well attended, with representation from the fishing industry, the Governor’s office, state legislators and the press.
- Scientific conferences will be attended and lectures will be given at local community colleges.
- Research will be highlighted through a monthly newsflash sent to a list composed of various academics, lobbyists, and commercial fisherman interested in king crab enhancement in Alaska.
- Updates on research progress will be made available on the Alaska Sea Grant website.

Objective 4:
Inform interested ecologists, industry, fishery managers, and other stakeholders of advancements in king crab aquaculture.

Target Audience – Aquaculture industry, researchers, fishery managers, ecologists, other interested stakeholders

Intended Learning Outcomes – An understanding of king crab enhancement in Alaska as a potential tool to rebuild depressed stocks

Intended Management and/or Behavioral Outcomes – Fisheries managers will have a better understanding of the feasibility of a large-scale enhancement effort.

Procedures to Achieve Intended Outcomes –
- Same as above for Objectives 1&2
- One Alaska Sea Grant publication will be produced, titled, “Manual for large scale hatchery culture of king crabs”, which will serve as a useful guidebook for culturing cool-water crustacean species. Specifically, this outreach publication will provide...
detailed instruction on best practices for hatchery culturing of crabs, using Alaskan king crab as the model. The publication will specifically provide results from this project as it will provide instruction for evaluating relative health of hatchery-cultured larvae and crabs, a lipid index for larval quality, and will make recommendations for dietary enrichments to increase production of healthy larvae. Jim Swingle will lead the authorship and publication of this volume.

- The AKCRRAB group will continue to meet with lobbyists and fishery managers to discuss the possibility of using stock enhancement as a rehabilitation tool.

### Outreach timeline

<table>
<thead>
<tr>
<th>Activity</th>
<th>Year 1</th>
<th>Year 2</th>
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<td>Feb- Apr</td>
<td>May- Jul</td>
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<tr>
<td>Open house</td>
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<td>Website updates</td>
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<tr>
<td>Publication submission</td>
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<tr>
<td>Newsflash</td>
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<td>X</td>
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</table>

### IV. COORDINATION WITH PROJECT PARTNERS

Alutiiq Pride Shellfish Hatchery, Seward, Alaska: Contact – Jeff Hetrick, Hatchery Manager, jjh@seward.net

The Chugach Regional Resources Commission, through the Alutiiq Pride Shellfish Hatchery, has provided hatchery facilities for the AKCRRAB program since 2006. The hatchery is a major collaborator in this effort and will continue to provide research space and operational costs for king crab rearing.

Alaska Department of Fish and Game, Region 1, Commercial Fisheries Division, Juneau, Alaska: Contact – Gretchen Bishop, gretchen.bishop@alaska.gov

Alaska Department of Fish and Game collected (for a companion National Sea Grant Aquaculture grant) ovigerous female red king crabs during their Fall 2010 crab surveys and is available to do so again for this project in 2012. Permits for collection of adult and juvenile crabs and for maintenance of these crabs were obtained from the Alaska Department of Fish and Game in 2010 and will be requested for this project for 2012-2014.

NOAA National Marine Fisheries Service, Behavioral Ecology Laboratory, Hatfield Marine Science Center, Newport, Oregon: Contact – Dr. Cliff Ryer, Cliff.Ryer@noaa.gov

We will contract with Louise Copeman, who is a well-published authority on the use of lipid and fatty acid analyses in aquaculture research and trophic ecology. She has
conducted research at the NOAA Hatfield Marine Science Center for several years and will have full access to the chemical laboratory at the Hatfield Marine Science Center as required for the proposed project. NOAA will provide space and equipment for lipid extractions, dry weights, and any other procedures for which they possess the appropriate facilities.

Alaska King Crab Research, Rehabilitation, and Biology (AKCRRAB) program, Alaska Sea Grant: Contact – Dr. David Christie, David.Christie@alaska.edu

AKCRRAB is a partnership with Alaska Sea Grant, regional fishermen's groups, coastal communities, NOAA Fisheries, the Alutiiq Pride Shellfish Hatchery and Chugach Regional Resources Commission, and the University of Alaska Fairbanks, School of Fisheries and Ocean Sciences. The program is supported by the City of Kodiak, Kodiak Island Borough, Alaska Crab Coalition, United Fishermen’s Marketing Association, Pribilof Islands Collaborative, Gulf of Alaska Coastal Communities Coalition, Central Bering Sea Fishermen’s Association, the Aleutian Pribilof Island Community Development Association, and other organizations and individuals. A steering committee guides the overall program, while a science team conducts the research program. This coalition of state, federal, and stakeholder groups views the king crab enhancement as important to the region's long-term economic development and sustainability.

V. AVAILABLE RESOURCES

Dr. Ginny Eckert (University of Alaska Fairbanks) is the Principal Investigator for this project with overall administrative and supervisory responsibilities. Dr. Eckert has broad expertise in larval and juvenile ecology of commercially important invertebrates and is the Science Director for AKCRRAB. She will supervise the graduate student and Jim Swingle.

Dr. Louise Copeman (Contractor to University of Alaska Fairbanks, based at the NOAA Alaska Fishery Science Center Newport Laboratory) is an expert in live-food enrichment, larval fish nutrition and the use of lipids in ecological food web studies. She will lead lipid sampling and analyses as well as preparation of manuscripts for peer-reviewed journals.

Graduate Student to be determined (University of Alaska Fairbanks) will conduct the hatchery-based research and outreach activities. AKCRRAB has a long history of successfully training graduate students, including Ben Daly, Celeste Leroux, Jodi Pirtle, Miranda Westphal, and Scott Vulstek.

James Swingle (University of Alaska Fairbanks) is the Alaska Sea Grant Research Biologist who will lead the larval rearing. He has worked for the AKCRRAB project since 2006 to develop large scale hatchery and nursery protocols for rearing red and blue king crab. He is an expert in shellfish larviculture. Prior to joining the AKCRRAB project, he worked for sixteen years in research and production hatcheries culturing commercially valuable shellfish species including oysters, clams, mussels, and shrimp.
VI. OUTCOMES/EXPECTED RESULTS
The outcome of this project will be the ability to develop an aquaculture live-food enrichment that is specifically formulated for Alaskan king crab. The result of this project will be included in a master’s thesis and be prepared as peer reviewed manuscripts. We anticipate producing two manuscripts, one on the lipid comparison to hatchery health indices titled, “The effect of live-food with variable ratios of essential fatty acids on the hatchery health index, stress resistance, growth, survival and lipid composition of larval red king crab,” and one on the hatchery-wild comparison titled, “A comparison of the lipid classes and fatty acid composition of hatchery reared and wild juvenile red king crab: toward developing appropriate hatchery live-food enrichments.” Potential target journals for these publications include Marine Ecology Progress Series, Aquaculture, Fisheries Bulletin, Aquaculture Research, Journal of Experimental Marine Biology and Ecology, or Canadian Journal of Fisheries and Aquatic Sciences.
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Persselin, S. 2006. Enriched Artemia nauplii as diet for red (Paralithodes camtschaticus) and blue (P. platypus) king crab larvae in the laboratory. J. Shell. Res. 25, 762.


