# Techniques for Culture of King Crab at the Alutiiq Pride Shellfish Hatchery, 2009

Draft Study Plan

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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 stock enhancement of Alaska king crab species for the purpose of population rehabilitation. Large-scale larval culture that was conducted at the Alutiiq Pride Shellfish Hatchery in 2007 and 2008 using red king crab (*Paralithodes camtschaticus)* and blue king crab (*Paralithodes platypus*) had varied success. In 2007, experiments yielded overall survival to the first juvenile stage of less than 1% for both species. In 2008, production experiments yielded and average of 31% survival to the glaucothoe stage and 15% to the first juvenile stage. Improvements in culture technology were made in 2008, and fine tuning larval and juvenile husbandry techniques is an ongoing process. Research in 2008 elucidated possible causes of mortality in the previous year, allowing for adjustments in larval rearing techniques that improved larval survival. Two production scale tanks exceeded 50% survival to the glaucothoe stage at a stocking density of 50 larvae $L^{-1}$. With further refinement of rearing techniques, 50% survival to the first juvenile stage is achievable on a large scale. Experiments in 2009 will continue to fine tune variables that yielded highest larval survival in 2008. Specifically, variations in diets and densities will be tested. This research will give further insight into the feasibility of mass larval culture of king crab in terms of methodology, crab survival, and costs incurred which are critical to assess the feasibility of stock enhancement.

## INTRODUCTION

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 U.S. and worldwide (Secor 2002, Stevens 2006c). However, before implementation, research is needed to assess the feasibility, effectiveness, and possible consequences of a stock enhancement program (Leber 1999, 2002). The Alaska King Crab Research, 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 stock enhancement for king crabs in Alaska. Commercial harvest of Alaska 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 APSH in Seward, Alaska.

The life histories of red king crab (*Paralithodes camtschaticus)* and blue king crab (*Paralithodes platypus*) are similar (Stevens et al. 2008), with differences that may be the result of the different habitats of the two species. Female red king crab reproduce annually and hold their fertilized eggs for 11 months, releasing them as zoeae over a period of 2-3 weeks between January and March, depending on environmental and physiological factors (Stevens and Swiney 2007). In contrast, large-sized female blue king crab reproduce biannually and hold their fertilized eggs for approximately 14 months (Somerton and Macintosh 1985, Jensen and Armstrong 1989), releasing larvae over a period of 3-7 weeks between January and May (Stevens 2006a, 2006b). Both species release planktotrophic larvae that molt through their four zoeal stages (ZI-ZIV) lasting approximately 40 days, followed by a non-feeding glaucothoe (G) stage that lasts 30-40 days (Sato 1958, Hoffman 1968). Survival of red king crab larvae from ZI to glaucothoe in the wild was estimated from plankton samples and ranged from 0.7% to 3% over four years (Shirley and Shirley 1989a). The glaucothoe stage seeks structurally complex habitat and metamorphoses into a first-instar juvenile crab (C1) (Stevens and Kittaka 1998, Stevens 2003). Both species have a relatively long juvenile period and mature at approximately 5-7 years of age and recruit to the fishery at 7-9 years of age (Jensen and Armstrong 1989, Stevens 1990, Zhou et al. 1998, Loher et al. 2001, Stevens et al. 2008).

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 1989b, Persselin 2006a, Stevens et al. 2008). 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 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; 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 stock enhancement may be possible for this species (Davis et al. 2005b).

Studies on hatchery-scale larval culture of red and blue king crabs were conducted in 2007 and 2008 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. Survival of red king crab larvae cultured at densities of 25, 40, 42, 67, 77, 96, 100, and 194 larvae $L^{-1}$ appeared to be negatively influenced by initial stocking density. Larval survival decreased dramatically throughout the zoeal and glaucothoe stages to less than 1% by the first juvenile instar (C1) in all treatments. Blue king crab larvae were cultured in 2007 at densities of 10, 30, and 100 larvae $L^{-1}$ and had survival to glaucothoe of 10.5%, 12.2%, and 9.6%, respectively, although survival decreased throughout the glaucothoe stage to less than 1% by the first juvenile instar (C1) in all treatments. Due to the low survival in hatchery-scale culture of both red and blue king crab, 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 (Fig. 1). Highest mortality occurred during the Z4 stage (Table 1). Diets consisted of enriched San Francisco Bay (SFB) artemia. Some tanks were also fed *Chaetocerus* 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 *Chaetocerus* sp. algae at 50,000 cells $ml^{-1}$, and treated with EDTA once daily. Because the tank was not replicated, definitive conclusions about specific variables are difficult to make. Production tanks yielded over 120,000 healthy glaucothoe. From these, approximately 35,000 first stage juveniles were produced, which are currently stocked in juvenile rearing experiments.

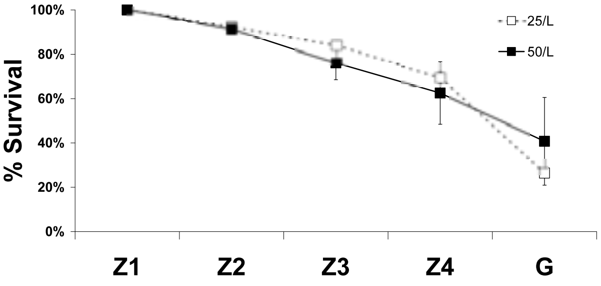


**Fig. 1.** Percent survival of red king crab reared at two stocking densities in production scale tanks.

**Table 1:** Survival from one larval stage to the next.

| | Z1 to Z2 | Z2 to Z3 | Z3 to Z4 | Z4 to G |
|---|---|---|---|---|
| 25/L | 93% | 91% | 83% | 38% |
| 50/L | 91% | 83% | 80% | 55% |

Experiments in sixteen 190 L tanks investigated effects of stocking densities. All tanks were fed unenriched SFB artemia in addition to *Isochrysis* sp. algae and yielded overall survival of approximately 15% to glaucothoe (Fig. 2). Highest mortality occurred during the Z4 stage (Table 2). When compared to production scale tanks, lower glaucothoe survival in experimental tanks may be due to a suboptimal diet. Enriched SFB artemia appears to be superior to unenriched. Overall lipid size decreases from Z2 to Z4 stages when fed unenriched SFB artemia (Fig. 3). Lipid size and number of lipids may be a health indicator in king crab larvae and was measured via microscopy under 100 x magnification.

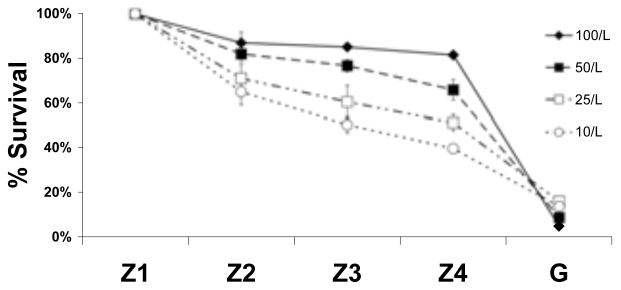


**Fig 2.** Percent survival of red king crab reared at four stocking densities.

**Table 2:** Survival from one larval stage to the next.

| | Z1 to Z2 | Z2 to Z3 | Z3 to Z4 | Z4 to G |
|---|---|---|---|---|
| 10/L | 65% | 80% | 78% | 34% |
| 25/L | 71% | 85% | 87% | 32% |
| 50/L | 82% | 94% | 85% | 13% |
| 100/L | 87% | 99% | 94% | 6% |

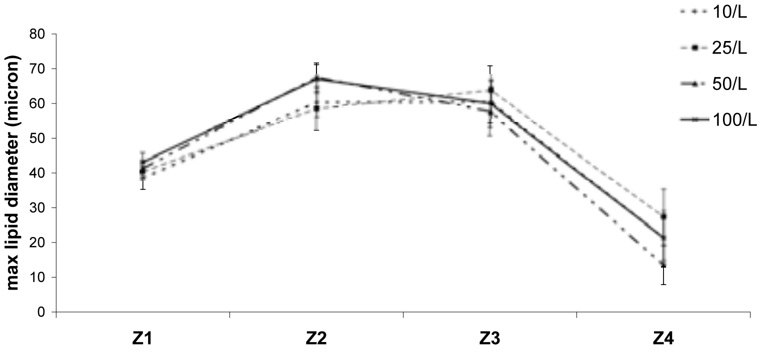


**Fig 3.** Maximum diameter of lipids seen in larval red king crab reared at four stocking densities.

Higher stocking densities appear to yield high survival to Z4 (Fig. 2). However, filamentous bacteria become problematic in mid Z3 stage in densities as high as 100 larvae $L^{-1}$ (Fig. 4) and may contribute to mass mortality events. In lower densities such as 25 and 50 larvae $L^{-1}$, bacteria appear to be more manageable, however may still contribute to larval mortality. Observations in 2008 strongly suggest that 50 larvae $L^{-1}$ is an ideal stocking density, although 75 larvae $L^{-1}$ is thought to be reasonable (A. Epelbaum, pers comm.).

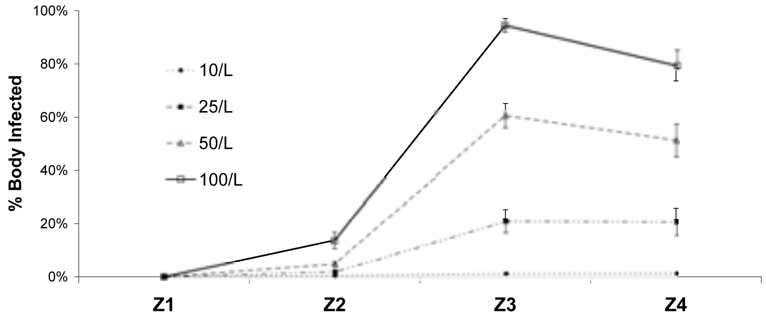


**Fig. 4.** Bacteria load seen in larval red king crab reared at four stocking densities.

Blue king crab hatchery-scale culture in 2008 also proved successful; however, three broodstock released ~50,000 larvae each limiting the numbers of treatments. Experiments in twelve 190 L tanks investigated effects of water temperature on survival and intermolt duration. All tanks were fed enriched SFB artemia. Half of the tanks had ambient seawater (~6.5°C) and half had warmed seawater (~10°C). Survival was similar between water temperature treatments and had an overall survival of 47% to the glaucothoe stage (Fig. 5), however intermolt duration was shorter in tanks with warmer water (Fig. 6). Larvae reared in ambient water took 35 days to reach glaucothoe, while larvae reared in warmer water took 25.5 days. Approximately 7,000 first stage juveniles were produced with this experiment.

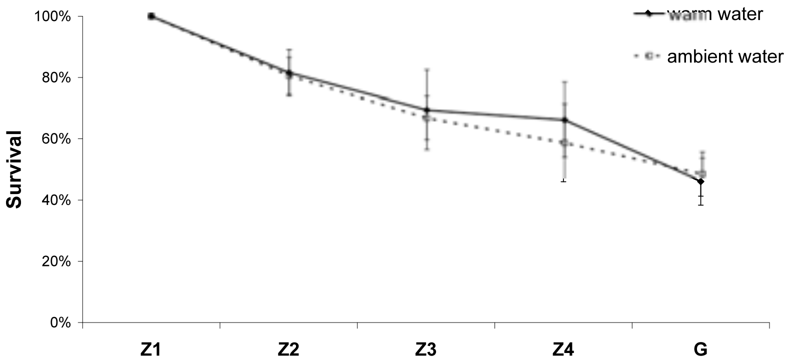


**Fig. 5.** Percent survival of blue king crab when reared at two temperatures.

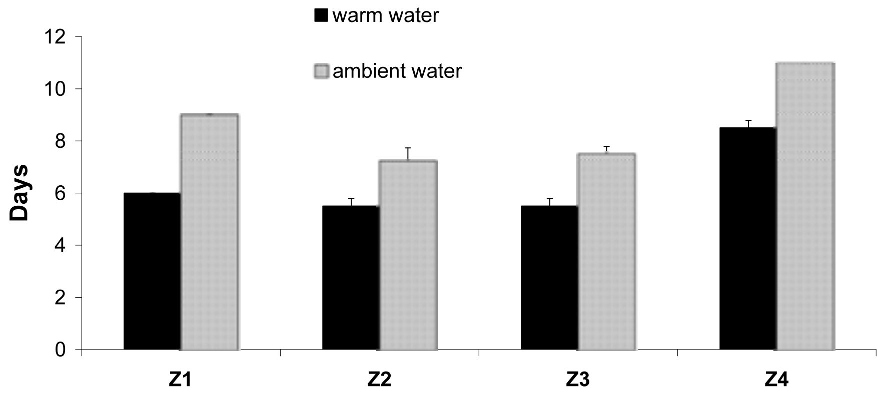


**Fig. 6.** Intermolt duration of blue king crab reared at two temperatures.

A combination of factors including improved diets, water quality, rearing temperatures, hatchery infrastructure improvements, and the knowledge gained by biologists contributed to the increased survival from 2007 to 2008. These improvements in hatchery rearing technology have led to superior husbandry techniques for king crab rearing.

## OBJECTIVES AND METHODS

Experiments in 2009 will further explore effects of diet, stocking density, and water temperature on survival, bacteria load in rearing tanks, and intermolt period. We plan on fine tuning king crab diet by investigating alternative strains of artemia. The Great Salt Lake (GSL) strain is roughly 1/3 of the cost of SFB artemia and may be a cost effective alternative when rearing king crab on a large scale. SFB strain is thought to have a better nutritional profile than GSL, however enriching GSL may compensate any lack of nutrition it may have. The benefits of using SFB versus GSL artemia have not been demonstrated conclusively. We also plan to address effects of increased water temperature on intermolt duration and how that directly relates to bacterial load. By increasing water temperature, larvae are able to molt more quickly and thus shed filamentous bacteria before it develops to a degree that may result in mortality. With colder temperatures and longer intermolt periods, bacteria are able to become more established, eventually interfering with the larvae’s ability to swim, feed, and respire. We propose to rear crab larvae in 8-10°C seawater in an attempt to decrease intermolt period and filamentous bacteria load.

The objectives of the hatchery-scale culture experiments in 2009 are the following:

- Determine optimal stocking density to rear larval king crab.
- Determine effects of two strains of artemia as a diet for king crab larvae.
- Determine effects of microalgae as a dietary supplement for larval king crab.
- Decrease larval intermolt duration and bacterial load of king crab by increasing water temperature.
- Determine effects of EDTA on survival of king crab larvae.
- Determine optimal substrate for glaucothoe settlement.
- Determine optimal diet for juvenile red king crab.
- Determine optimal stocking density for juvenile red king crab.

## *BROODSTOCK ACQUISITION AND HATCHING*

Currently 20 ovigerous red king crab, collected from Little Diomede under CF-08-025, are being held at the Seward Marine Center. Because these crabs are relatively small, they have low fecundity and will likely yield less than 50,000 larvae crab$^{-1}$, which does not provide adequate numbers for hatchery scale experiments. As many as 90,000 Z1s are planned to be stocked in a single production tank. In addition, we anticipate possible mortality from holding these females in the lab for a year. Therefore, we propose to obtain 20 ovigerous females from St. Matthew Island to allow opportunities for experimentation, including investigations that compare larval hatch timing and survival from different source populations. It is likely that the larvae from the two source populations will hatch at different times, and therefore, we plan to run multiple red king crab larval rearing trials in 2009. These additional runs are needed because the larval rearing trials in 2008 were limited by the small number of larvae that hatched that year.

## ***EXPERIMENT 1****: LARVAL REARING AT DIFFERENT DENSITIES AND DIETS*

Hatchery-scale larval culture could be more space efficient and therefore more cost effective if larvae can be cultured at high densities. Also, GSL artemia may be an inexpensive alternative to SFB artemia and may be more economic when rearing king crab larvae at the hatchery scale. The following experiment will be conducted using *P. platypus* at the mass culture scale (190 L).

**HI$_0$:** There will be no difference in survival of king crab larvae when reared at a density 50 or 75 larvae L$^{-1}$.

**HI$_a$:** Survival differs among tanks at different densities.

**HII$_0$:** There will be no difference in survival of king crab larvae when fed SFB enriched artemia or GSL enriched artemia.

**HII$_a$:** Survival differs among tanks with different diets

Larvae will be collected from at least three females and mixed randomly. Survival will be monitored at each larval stage for two densities of 50 and 75 larvae L$^{-1}$ and two diets or SFB and GSL enriched artemia with four replicates each. Larvae will be placed in circular, conical bottomed, polyethylene 190 L tanks at the Alutiiq Pride Shellfish Hatchery's Mariculture Technical Center. Incoming water filtration will include particle filtration to 5 µm, 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 µm screens to retain larvae and food in the tanks. Each morning, the 105 µm screens will be replaced with 500 µm screens to allow artemia from the previous day to flush out of the tanks. When the tanks are flushed of artemia, the 105 um screens will be replaced and all larvae will be fed enriched SFB and GSL artemia nauplii grown for 24 hours and enriched with DC DHA Selco for an additional 24 hrs. Red king crab zoeae require more food as they develop through their larval stages (Epelbaum and Kovatcheva 2005). Feeding density of artemia will be adjusted according to larval stage such that Z1 are fed at a density of 1.5 ml$^{-1}$, Z2 are fed at a density of 2.5 ml$^{-1}$, Z3 are fed at a density of 3.5 ml$^{-1}$, and Z4 are fed at a density of 4.5 ml$^{-1}$. These densities fall into suggested feeding ranges based on daily feeding rates of *P. camtschaticus* in laboratory conditions (Epelbaum and Kovatcheva 2005). We propose to rear crab larvae slightly warmer than ambient (~8-10°C) in an attempt to shorten intermolt duration and filamentous bacteria load. Feeding will be terminated when all zoeae in the tank molt to the glaucothoe stage.

### ***EXPERIMENT 2:*** *PRODUCTION SCALE MASS LARVAL REARING*

Production scale experiments are highly efficient at producing large numbers of juvenile king crabs. The following experiment will be conducted using *P. platypus* at the mass culture scale (1,200 L).

**$HIII_0$:** There will be no difference in survival of king crab larvae when *Chaetocerus* sp. algae and/or EDTA is added.
**$HIII_a$:** Survival differs among tanks with different treatments.

Larvae will be collected as described above and stocked at a density of 50 larvae $L^{-1}$ in nine 1,200 L tanks. Three tanks will only be fed enriched artemia, three tanks will be fed enriched artemia with a dose of EDTA, and three tanks will be fed enriched artemia and *Chaetocerous* sp. algae at a density of 50,000 cells $ml^{-1}$ with a dose of EDTA. EDTA is 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. We propose to rear crab larvae slightly warmer than ambient (~8-10°C) in an attempt to decrease intermolt period and filamentous bacteria load. Feeding will be terminated when all zoeae in the tank molt to the glaucothoe stage.

### *LARVAL HEALTH ASSESSMENTS*

Larval health will be monitored during each larval stage in both of the previously described experiments. Lipid content, gut content, and bacteria load will be examined via microscopy 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 percent body coverage on the larvae. Sampling will occur during the late intermolt as bacterial infection is most severe shortly prior to molting.

### ***EXPERIMENT 3:*** *POST-LARVAL AND JUVENILE REARING*

Newly settled king crabs are too small for any known method of physical tagging used on other species of juvenile crabs (e.g. coded wire tags, elastomer tags, Davis et al. 2004); therefore, further experimentation requires grow-out to a larger size. Minimizing intermolt duration is beneficial when rearing king crab at a production scale as it minimizes cost and labor and may increase survival. This study will test post-larval duration in the presence of different artificial substrates and the suitability of various diets and stocking densities using indoor rearing tanks as nurseries for mass culturing red king crab juveniles. Optimal temperatures (10-12°C) for juvenile king crab will be maintained to attempt to decrease intermolt duration.

**$HIV_0$:** There will be no difference in post-larval duration and survival in the presence of different artificial substrates.
**$HIV_a$:** Post-larval duration differs among tanks with different artificial substrate.

**$HV_0$:** There will be no difference in survival of juvenile king crab when reared at a density of 1000, 2000, 4000 crabs $m^{-2}$.
**$HV_a$:** Survival differs among tanks at different densities.

**$HVI_0$:** There will be no difference in survival of juvenile king crab when fed mixed artificial diets, calcium enriched mixed artificial diets, natural diets, and calcium enriched natural diets.
**$HVI_a$:** Survival differs among tanks using different diets.

Post-larval duration and survival will be determined using glaucothoe from the larval density/diet experiment. Glaucothoe will be held in the same respective larval rearing tanks. Substrates will consist of artificial seaweed and gill net. Seaweed will be placed vertically and horizontally in the tanks, while gillnet will be placed at the bottom of the tanks. Survival to C1 and post-larval duration will be determined.

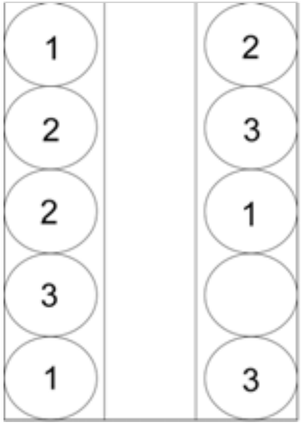
To determine the optimal conditions for raising juvenile crabs in a mass culture system, recently settled (C1) crabs from the larval density, diet and production experiments will be collected, mixed randomly and placed in containers (silos) at three different densities. Artificial diets will consist of a mix of Otohime B1, frozen enriched Artemia, commercial shrimp nursery feed (recommended by O. Zmora). Otohime B1 is 200-320 µm in size, consists of ground krill, fish and squid and is commonly used in marine mariculture. Otohime B2 is 360-620 µm in size and may be supplemented with B1 as the crabs grow. Calcium enrichment will consist of krill powder, ground cuttlebone, and egg white. Natural diets will consist of chopped herring, squid, sea star, and king crab.

The duration of the experiment will be 6 weeks (or determined otherwise) to determine overall survival of early juveniles for practical enhancement purposes. Survival and growth of juveniles will be assessed by counting, weighing (wet vs. dry: to be determined) and measuring (CW, CL) the crabs at each intermolt. Difference in survival of juveniles between density treatments will be determined by ANOVA after 6 weeks (or to be determined).

Each silo is a cylindrical container with a mesh screen bottom with surface area of approximately 0.25 $m^2$. When immersed in the SS tanks, each silo can be operated at a depth of up to about 20 cm, yielding a seawater volume of approximately 50 L $silo^{-1}$.

Each silo will have a small size mesh (100 µm) on bottom to allow water exchange but retain food. Optimal substrate determined from current red king crab juvenile experiments will be standardized (by weight or surface area) and placed in each silo.
Total C1s needed: 21,000.

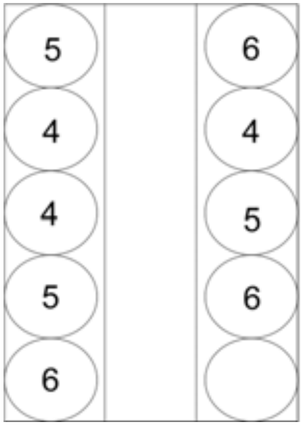
**Unit 1**



Diet: Artificial

Density: 1,000, 2,000, 4,000 m$^{-2}$

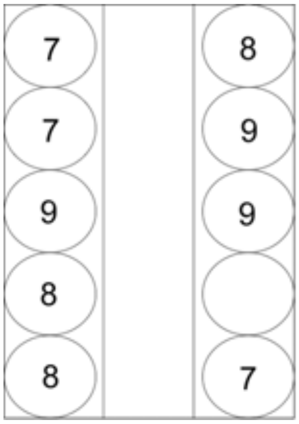
**Unit 2**



Diet: Artificial w/ calcium

Density: 1,000, 2,000, 4,000 m$^{-2}$

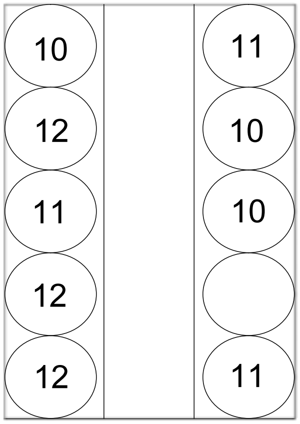
**Unit 3**



Diet: Natural

Density: 1,000, 2,000, 4,000 m$^{-2}$

**Unit 4**



Diet: Natural w/ calcium

Density: 1,000, 2,000, 4,000 m$^{-2}$

### *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 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.

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