

Red King Crab Hatchery Manual

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Alutiiq Pride Shellfish Hatchery/AKCRRAB Program
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Red King Crab Hatchery Manual

Chapter 1. Introduction

This manual describes techniques for large scale hatchery and nursery rearing of red king crab (RKC) (*Paralithodes camtschaticus*). These techniques could potentially be used to produce millions of juvenile RKC that could be released as part of a stock enhancement program to rebuild and replenish RKC stocks for the benefit of commercial, subsistence, and personal use fishermen in Alaska. The techniques described in this manual were developed and tested in hatchery scale experiments conducted at Alutiiq Pride Shellfish Hatchery (APSH) as part of the Alaska King Crab Research, Rehabilitation, and Biology (AKCRRAB) Program from 2006 through 2013. Our primary goal was to develop simple, cost effective hatchery technology for consistent mass production of healthy RKC larvae, glaucothoe, and juveniles. Though research is ongoing and rearing methods continue to be refined, this manual describes techniques which have yielded greatest success to date. The manual begins with a summary of the life history of RKC and then presents detailed methods used to mass rear the species in a hatchery. First, methods for collecting, transporting, and holding egg bearing broodstock are discussed. Techniques for assessing egg health, monitoring embryonic development, and predicting hatch timing are then described. Hatchery protocols for mass rearing larvae and glaucothoe to the first juvenile crab stage are presented and nursery culture techniques for rearing juveniles are discussed. Finally, methods for transporting juveniles are presented.

Chapter 2. Summary of the Biology and Life History of RKC

In order to successfully rear RKC in a hatchery, it is important to understand the basic biology and life history of the species. Photographs of each stage of the RKC life cycle are presented in [Figure 1](#).

Reproduction

In nature, RKC usually become sexually mature at 75 to 100 mm carapace length at approximately five years of age. The number of eggs (fecundity) per adult female range from approximately 50,000 to 450,000 with larger females typically carrying more eggs ([Table 2-1](#)). Females carry a clutch of eggs attached to spines on pleopods in a brood pouch between the ventral side of the carapace and abdominal flap. In Alaska, eggs typically hatch in the spring or early summer. Hatching of crab larvae at this time coincides with the spring and summer phytoplankton bloom when microalgae and zooplankton on which larval crab feed is most abundant ([Shirley and Shirley, 1989](#)). Once hatching begins, it usually takes several weeks for all eggs from the clutch of an individual female to hatch. Approximately one to several weeks after hatching is complete, the female molts and mates and within a day of mating extrudes a new clutch of fertilized eggs. The egg clutch remains attached to the female for about eleven months as the embryos slowly develop prior to hatching the following spring.

Table 2-1. RKC fecundities.

RKC Size (mm)	Eggs (1000's)	Location	Study
92-131 CW	15-130	Bristol Bay, AK	Wainwright et al.1991
127-169 CW	63-346	Hokkaido, Japan	Nakazawa 1912 (Cited in Fukuhara (1985))
115-168 CW	70-270	Hokkaido, Japan	Marukawa 1933
128-145 CL	148-447	Canoe Bay, AK	Wallace et al 1949
94-171 CW	55-445	Bristol Bay, AK	Rodin 1970 (Cited in Fukuhara (1985))
?-159 CW	70-280	SE Bering Sea, AK	Fukuhara 1985
98-175 CL	78-334	Cook Inlet, AK	Haynes 1968 (Calc from his regression eqn.)

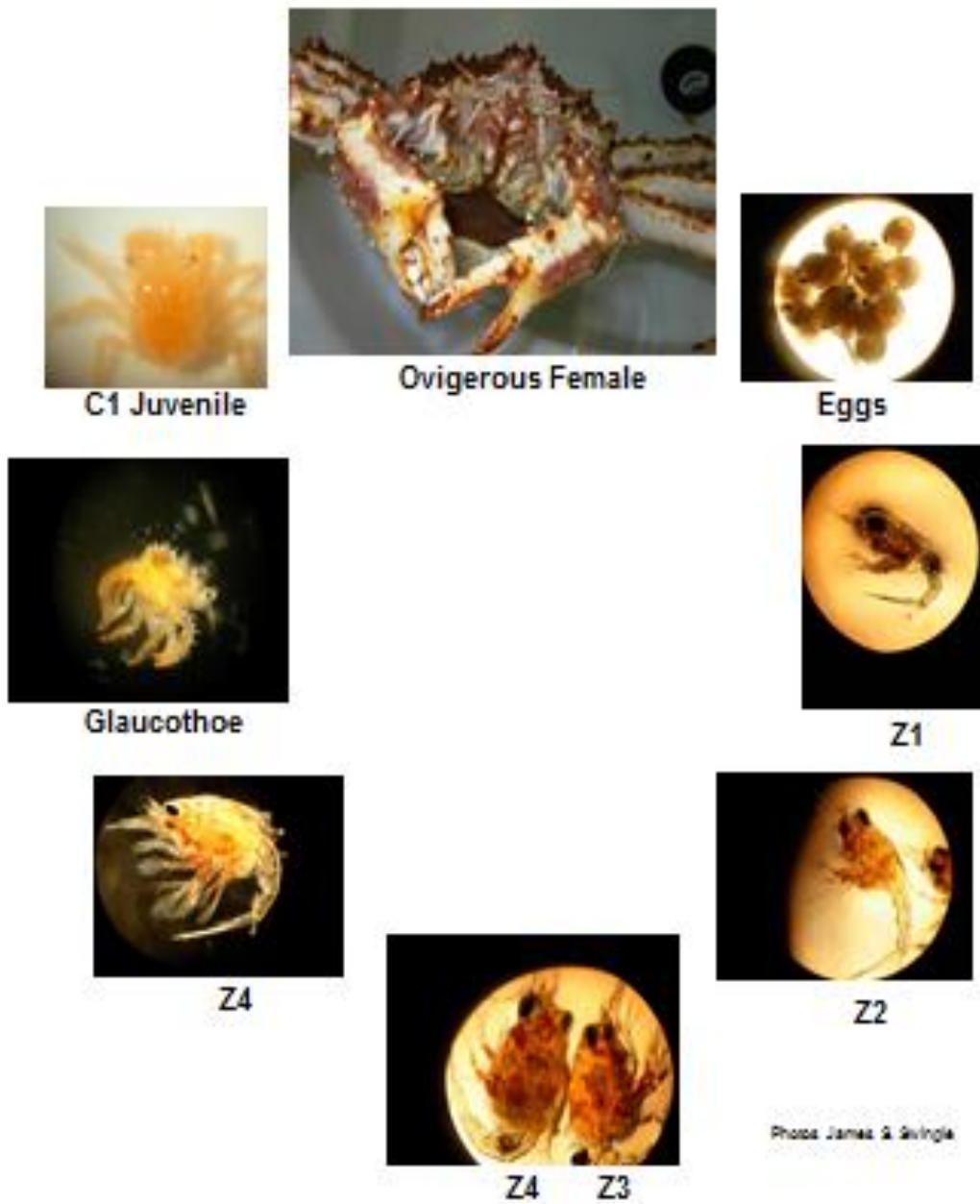
CW (Carapace Width), CL (Carapace Length)

Table modified from [Wainwright et al. 1991](#)

Larval Development

Larvae hatch as prezoaeae, a brief larval stage lasting less than an hour before molting into the first zoeal stage (Z1). There are four morphologically distinct zoeal stages (Z1-Z4) followed by a postlarval glaucothoe stage, which in turn molts into the first stage juvenile crab (C1) ([Figure 1](#)). In nature, the four zoeal stages are planktonic, vertically migrating larvae which feed on phytoplankton and zooplankton for roughly two to three months before molting into the glaucothoe stage. RKC zoeae are encounter feeders. They are not visual predators that see and

Figure 1. Red King Crab Life Cycle



then pursue their prey. Rather, as they drift and swim in the water column, zoeae create micro-currents with their swimming appendages (maxillipeds) that sweep prey items toward their mouthparts. As plankton of the appropriate size and quality is swept towards them, they seize and consume it. The glaucothoe is a non-feeding, postlarval, intermediate stage which can swim like larvae but also crawl like juveniles and adults. After a brief planktonic period, the glaucothoe settles to the bottom and seeks out suitable substrate on which it clings until molting into the first juvenile crab stage (C1). The glaucothoe stage typically lasts several weeks. After molting into the first stage juvenile, the young crab can no longer swim and finally resembles a miniature adult. At this stage, crabs resume feeding and begin the benthic stage of their life cycle. In nature, being highly vulnerable to predation, inadequate food availability, unfavorable currents, and/or other mortality factors, less than 0.1 % of RKC larvae typically survive from hatching to C1 ([Kovatcheva et al., 2006](#)). This planktonic larval period is a critical phase of the RKC life cycle having great impact on overall survival and future abundance of crab stocks.

Growth

In order to grow, RKC must molt, shedding their shell in a process termed ecdysis. During this process, the shell separates where the posterior margin of the carapace is attached to the abdominal flap and the newly molted crab backs out of its old shell. The newly molted crab absorbs seawater to stretch its soft, pliable new shell so that its internal tissues can grow into it over time. For the shell to re-harden takes about a day in small juveniles and up to two weeks in large adults. With each molt, carapace width typically increases up to 29% in early juveniles ([Kovatcheva et al., 2006](#)) and by a lesser percentage in adults ([Sato, 1958](#)). Male RKC reach commercially harvestable size (minimum 7" CW) about seven to eight years after hatching. The average weight of commercially harvested RKC in Alaska is about six to seven pounds. Historically, the largest individuals harvested in Alaska weighed approximately 25 pounds, measured six feet from leg tip to leg tip, and were estimated to be up to 25 years old ([Bliss, 1982](#)).

Chapter 3. Broodstock Acquisition

Collecting Broodstock

In this manual, the term broodstock refers to egg-bearing (ovigerous) RKC females (**Figure 2**). Broodstock can be captured in crab pots, typically at depths ranging from one hundred to several hundred feet. Since RKC females carry their eggs for approximately eleven months prior to hatching, broodstock can be captured almost year round from early summer to late spring. However, since hatching typically occurs from spring to early summer, to minimize holding time and associated costs of caring for the broodstock, the best strategy is usually to collect broodstock in late winter or early spring just prior to hatching. Capture depth varies with season as ovigerous females tend to exhibit seasonal migrations, tending to occupy shallower water during the late winter to early summer hatching-molting-mating season and deeper water the remainder of the year.

Figure 2. RKC broodstock with full clutch of eggs.



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Selecting Broodstock

If the goal is to maximize hatchery productivity, as egg bearing females are captured, they should be sorted to obtain the best quality broodstock for the hatchery. Though females as small as 75-100 mm carapace length may carry eggs, these small crabs are less desirable as broodstock for two primary reasons. First, they tend to carry fewer eggs and second, they are typically first time spawners (primiparous). In our experience, these first time spawners have sometimes molted prematurely, prior to completion of the hatching of their clutch of eggs, therefore

reducing the number of larvae available for stocking in the hatchery larval rearing tanks (LRTs). When selecting broodstock, it is generally best to target females greater than 125 mm (5”) carapace length. Females at and above this size tend to produce hatches of greater than 150,000 larvae each. Though there is natural variation, the approximate expected mean hatch of RKC broodstock of various carapace lengths is presented in [Table 3-1](#). Once broodstock have been received at the hatchery and their carapace lengths are measured, this table can be used as a guide to obtain rough estimates of the number of larvae likely to hatch and be available for stocking in the larval rearing tanks.

Table 3-1. Approximate mean number of embryos in the egg clutches of RKC broodstock of various carapace lengths.

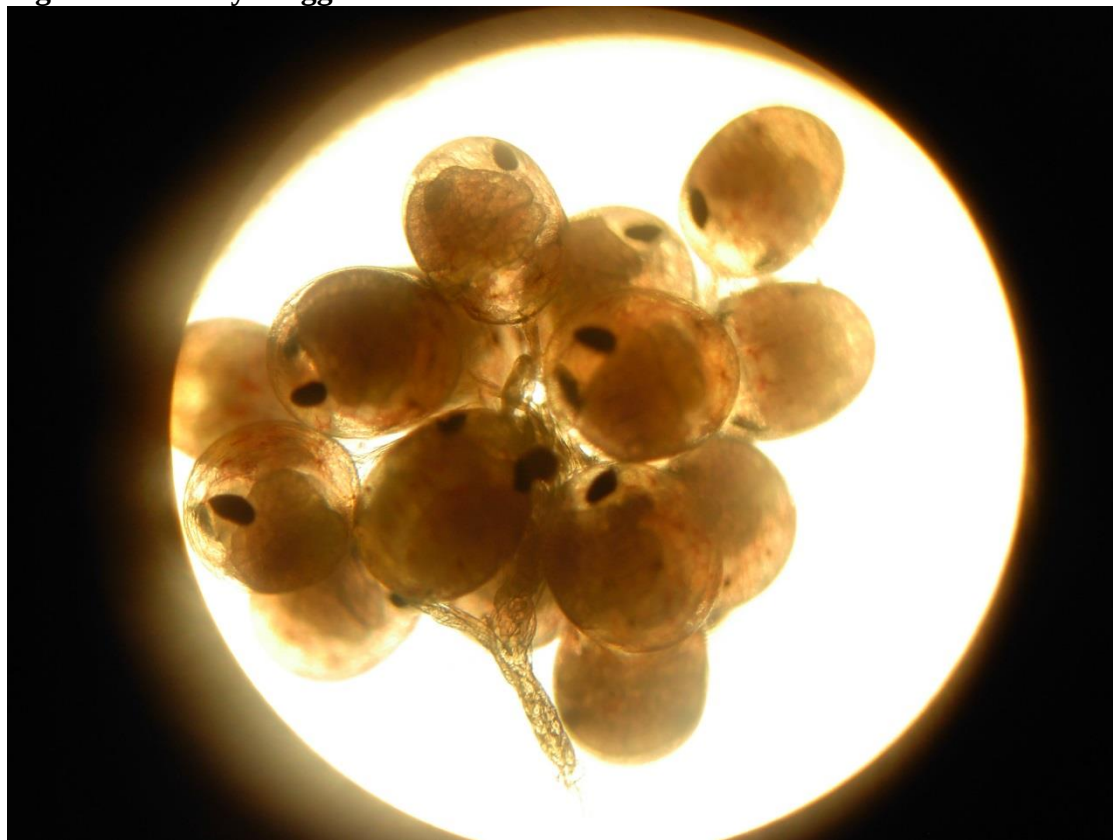
CL	~# Embryos*
(mm)	(~#)
110	100,000
120	140,000
130	180,000
140	220,000
150	260,000

*Averages interpolated and smoothed from graph in [Johnson et al. \(2002\)](#)

In addition to being a productive size, females selected as broodstock should also possess the following characteristics. Broodstock should appear healthy with undamaged shells and unbroken legs or claws. The egg clutch should be full, occupying close to 100% of the brood pouch with the egg mass thick enough to prevent full closure of the abdominal flap ([Figure 2](#)). The eggs should appear healthy, being clean, shiny, and uniform in color, typically purple to beige depending on stage of development. Females with egg clutches containing dead eggs, which appear off white or opaque, should not be collected. When broodstock are collected from late winter to spring, their eggs (approximately 1 mm in length) should be examined with a magnifying glass to determine whether the eggs are eyed. Eyed eggs possess two small black pigmented eyespots visible in the developing embryo through the semi-transparent egg membrane ([Figure 3](#)). The presence of eyed eggs indicates the eggs were fertilized at extrusion, have been carried by the female for over six months, and are likely to hatch in spring or early summer.

Alternatively, if the overriding hatchery goal is to maximize genetic diversity of the juveniles produced for out-stocking rather than hatchery productivity, then all healthy broodstock captured should be transported to the hatchery and no selection criteria should be applied.

Figure 3. RKC eyed eggs.



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Transporting Broodstock

Broodstock can be transported in seawater filled tanks (live wells) or coolers utilizing a waterless shipping technique. Live wells are typically used to keep the crab alive on board the fishing boat. The live well is a holding tank through which seawater is continuously pumped to maintain good water quality. Egg bearing females with desirable characteristics (described in the previous section) are removed from the crab pots and placed in a live well. The live well should be well oxygenated (dissolved oxygen >6 ppm), close to full strength salinity (30-35 ‰), and kept at a steady temperature of about 4 °C. Individual egg bearing females can be kept separate in the live well by placing them in a burlap bag closed with a zip tie before immersing them in the tank for transport. Once off-loaded from the capture vessel, the second method of live transport can be used to deliver broodstock to the hatchery. This method entails waterless shipping (shipping without seawater), in which broodstock are placed on their backs in a cooler between layers of damp seawater soaked burlap bags. The temperature in the cooler is maintained at about 4 °C during transport by placing 1-lb ice packs lying flat in the bottom of the cooler. The ice packs are covered with two layers of damp burlap to prevent direct contact with the shell of the crabs being shipped. One pound of ice should be used for about every 12 quarts of cooler capacity. We have repeatedly used 100 quart plastic coolers containing eight one-pound ice packs to successfully ship (100% survival) six RKC broodstock (mean weight ~1.5 kg) per cooler. Shipping time was eight hours from when the broodstock were packed until being received and placed in their

holding tanks at the hatchery. The broodstock are packed on their backs two to three layers deep with damp seawater-soaked burlap above and below each layer of crab. It may be possible to successfully use this method for longer transport times, as [Donaldson and Byersdorfer \(2005\)](#) state that crab transported in a similar manner can survive up to four days, though it is unclear whether their attached eggs could survive that long out of water.

Chapter 4. Hatchery Seawater Supply and Filtration System

Hatchery Seawater Supply

The seawater source for a RKC hatchery must deliver clean seawater of stable temperature and salinity. Seawater should be drawn from relatively deep water below the thermocline, where temperature and salinity are relatively constant and therefore suitable for broodstock holding and larval, glaucothoe, and juvenile rearing. Ideally, incoming seawater temperature should range from 4-10 °C, though at a cost seawater outside of this temperature range can be heated or chilled for use in the hatchery. Salinity should range from 30 to 35 ‰. Temperature and salinity should be stable, varying less than 1 °C and 1 ‰ daily. Clean, clear seawater is required, though the hatchery seawater filtration system can remove some turbidity. The seawater must not contain toxic compounds such as pesticides, herbicides, creosote, petrochemicals, or heavy metals. At APSH, seawater used for king crab culture was drawn from the Seward Marine Center's (SMC) intake pipe at 75 m depth in Resurrection Bay and then pumped to the hatchery. Daily seawater temperatures and salinities were stable, varying less than 1 °C and 1 ‰. Mean monthly temperatures of seawater drawn from the 75 m intake point and pumped to the hatchery are presented in Table 4-1. Salinity remained constant at 31 to 32 ‰ year round.

Table 4-1. Mean monthly temperatures (°C) of seawater pumped from 75 m deep SMC intake to APSH.

Jan	5.2
Feb	4.4
March	4.2
April	4.1
May	5.1
June	6.9
July	9.4
Aug	11.4
Sept	11.9
Oct	10.8
Nov	8.4
Dec	6.7

Source: SMC daily temperature log 2006 – 2011 (Courtesy Phyllis Shoemaker)

Hatchery Seawater Filtration System

Seawater filtration requirements typically vary depending on the water quality from site to site. At APSH, unfiltered SW proved adequate for broodstock holding, though silt in the incoming seawater tended to accumulate on the broodstock holding tank bottom over time. At most sites, seawater should be pumped through sand and bag filters to remove silt and other particulates greater than 25 µm before the seawater enters the broodstock holding tanks.

At APSH, the seawater filtration system used for the hatching tanks, and larval, glaucothoe, and juvenile rearing consisted of a parallel series of filters (Figure 4). Seawater pumped from the 75 m deep intake passed in sequence through a sand filter, 25 µm bag filter, 5 µm bag filter, UV sterilizer, and finally a carbon filter before being piped to the hatching and rearing tanks. Each component of the seawater filtration system serves a particular function. The sand filter removes particulates from seawater larger than approximately 100 µm. The 25 and 5 µm bag filters then

remove smaller particulates down to 25 and 5 μm , respectively. The UV sterilizer then acts to deactivate a portion of potential pathogens such as protozoans, fungi, bacteria, and viruses. Finally, the activated carbon filter removes by adsorption low levels of substances including organic contaminants, pesticides, polyaromatic hydrocarbons, trihalomethanes, phenols, chlorine, ozone, perchlorates, hydrogen peroxide, bromine, some heavy metals, and other chemicals which can potentially harm crab larvae.

Figure 4. RKC hatchery seawater filtration system.



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Filtration System Maintenance

The seawater filtration system must be properly maintained to ensure good water quality and stable flow rates. Over time, particulates removed by the filtration system reduce the flow rate of seawater through the system. For this reason, sand and bag filters must be periodically cleaned. Sand filters are typically cleaned daily by back-washing for several minutes. This is done by resetting the valves on the sand filter to temporarily reverse flow through the filter and thereby flush accumulated particulates such as silt out of the filter and down the drain. Likewise, the bag filters need to be periodically removed from their housings and rinsed clean with a freshwater hose. Usually, a bag filter can be cleaned and reused for about a month before needing replacement. UV bulbs in the sterilizer typically are effective for about eight months before needing replacement. Bulbs should be checked daily through the indicator windows to make sure none have prematurely burned out and extra bulbs should be available on site for immediate replacement when needed. The activated carbon in the carbon filter should be changed about every three months to ensure it is functioning properly. If the activated carbon is replaced too infrequently, it can become saturated with adsorbed contaminants and can no longer effectively

remove harmful materials from the seawater. A filtration system maintenance schedule is summarized in **Table 4-2**.

Table 4-2. Seawater filtration system maintenance schedule.

Sand Filters	Backwash Daily
Bag Filters	Rinse Daily
UV Sterilizer	Check daily. Replace bulbs every 8 months
Carbon Filter	Replace carbon every three months

Chapter 5. Broodstock Management

Stocking Broodstock Holding Tanks

Several days before broodstock are received at the hatchery, the broodstock holding tanks should be scrubbed clean with Clorox® bleach and Alconox® detergent, thoroughly rinsed, and allowed to dry for before filling with filtered seawater. Upon arrival at the hatchery site, the broodstock crabs should be placed into clean holding tanks containing filtered seawater between 3-6 °C, 30-35 ‰ salinity, and well oxygenated with a dissolved oxygen concentration greater than 6 ppm. When first placing a crab in a holding tank, the crab should be held under water facing up to allow air bubbles to escape from under her abdominal flap. This will prevent any air from being trapped around the female's egg mass. With adequate seawater flow, good water quality, and careful monitoring, up to 18 broodstock can be held long term in a six foot diameter holding tank. This is a holding density of approximately seven crabs per square meter of tank bottom area.

Broodstock Measurements and Tagging

After newly received broodstock have been in the holding tank about a week and have fully acclimated and recovered from shipping stress, broodstock are measured, weighed, and tagged. This information is recorded in the Broodstock Receiving Log ([Appendix 1](#)). The carapace length and width are measured using calipers. After excess seawater drips away, the crab is weighed to the nearest gram on an electronic balance. A numbered plastic tag is then secured to the third or fourth leg near the base of the carapace with a zip tie ([Figure 5](#)). Spines on the leg prevent the tag from slipping off and the tag will remain attached to the crab until it molts, usually several days or weeks after all eggs have hatched. By tagging each broodstock with an identifying number, records can be kept on the reproductive performance of each individual in the Hatch Log ([Appendix 4](#)), including the date hatching begins, hatch duration (days from beginning to end), approximate number of larvae hatched daily, and date of molt.

Figure 5. Tagged RKC broodstock.



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Broodstock Holding Tank Design and Operation Specifications

A six foot diameter flat-bottom cylindrical tank operated at a depth of about 30" and volume of 2000 L works well for long term holding of RKC broodstock (Figure 6). A continuous flow of seawater through each broodstock holding tank of 15 LPM maintains stable temperature (± 1 °C daily), dissolved oxygen concentration above 6 ppm, unionized ammonia below 0.05 ppm, and clear seawater. Aeration from a blower enters the tank through vinyl tubing connected to two air stone diffusers mounted about 12" below the water surface along each side of the tank. Air stones are kept off bottom to prevent the crabs from getting tangled in the attached tubing. Maintaining the volume of seawater at 2000 L provides a margin of safety for the crabs, providing a reserve of dissolved oxygen and a more stable temperature in the event of a temporary interruption in seawater flow or aeration due to mechanical failure of a pump or blower. In a single six foot diameter tank operated under these parameters, we held 12 RKC broodstock for over six months with no mortality, and believe up to 18 broodstock could be held safely in this manner, equivalent to a broodstock holding density of about seven crabs per square meter of tank bottom area. Crabs appear highly tolerant of crowding and when well fed, rarely exhibit antagonistic behavior. In fact, broodstock were occasionally observed stacked on top of one another in the holding tank, leaving most of the available tank bottom area unoccupied. We successfully held broodstock at ambient seawater temperatures, ranging from 3 to 9 °C for over six months with no mortality. Daily temperatures were stable, varying less than 1°C and salinity was stable at 31 to 32 ‰ over the entire holding period. A tank chiller is installed above the tank and should be used if needed to prevent broodstock holding temperatures from exceeding 9 °C. Tanks were kept covered with 2" thick Styrofoam lids.

Figure 6. RKC broodstock holding tank.



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Feeding Broodstock

Broodstock are fed a mixture of chopped squid and herring twice weekly to satiation. At a holding temperature of 5 °C, during the first few weeks after acclimating to the conditions in the holding tank, each broodstock (mean weight ~1.5 kg) consumes approximately 40 grams of mixed chopped herring and squid each feeding. After several weeks, individual consumption rates drop to about 20 g of feed per feeding and remain at this level until several weeks before hatching. This is roughly equivalent to a wet weight feed ration of approximately 3% crab biomass per week.

Prior to feeding, frozen squid and herring are chopped into 1" chunks using a sharp butcher knife and a cutting board. The chopped feed is weighed on a scale and allowed to thaw before feeding. Thawed feed sinks to the bottom of the tank where it can be consumed by the crabs, while frozen chunks of herring and squid tend to float and can be lost down the surface drain. When placing feed in the tank, it is evenly spread to disperse it over the tank bottom.

The amount of food consumed should be carefully monitored and recorded. The goal is to feed the crabs to satiation. Gross overfeeding degrades water quality in the holding tank as well as wastes money on uneaten feed. Underfeeding should be avoided so that broodstock obtain sufficient food to maintain good health and full stomachs and remain non-aggressive toward each other. Feeding rate should be adjusted such that when feeding twice weekly, just a little uneaten feed remains prior to the next feeding.

Broodstock Holding Tank Cleaning

While broodstock are being held in holding tanks, the tanks should be cleaned at least twice per week. Just before each feeding, any remaining uneaten feed, crab feces, and other waste should be siphoned out of the holding tanks. The siphon used to clean the tanks consists of a five foot long piece of 1.5" diameter PVC pipe attached to twelve feet of 1.5" diameter clear vinyl tubing. A 300 μ m mesh bag is tied to the outlet end of the siphon hose to collect uneaten feed and other waste. After tank cleaning is complete, waste collected in the mesh bag is discarded and the 300 μ m mesh bag is rinsed clean and put away. The concrete floor area around the tanks is rinsed clean with a freshwater hose. It typically takes about five minutes to siphon clean each broodstock holding tank.

Broodstock Tank Monitoring and Record Keeping

Broodstock in the holding tanks should be checked at least twice a day. Typical broodstock behavior in the holding tank is to crawl slowly or remain still on the tank bottom, moving only their mouthparts and antennae. Observing these movements is the simplest way to tell the crabs are alive. Each broodstock crab should be carefully observed to ensure that no mortalities have occurred. If a broodstock crab has died, it should be immediately removed from the tank to prevent its decomposition from degrading water quality and harming other broodstock in the tank. In addition, when checking on the broodstock, the holding tank temperature, salinity, oxygen concentration, and incoming flow rate should be measured and recorded routinely at 8 AM and 4 PM daily. Each parameter should be maintained near the optimal levels for broodstock holding ([Table 5-1](#)) and recorded in the Broodstock Holding Log ([Appendix 2](#)). In addition, each time a tank is fed and cleaned, these activities should be recorded in the log. Any mortality, molting, or other pertinent observations should also be recorded. Careful monitoring and daily record keeping are essential for maintaining healthy broodstock to ultimately produce healthy larvae for stocking the hatchery larval rearing tanks.

Table 5-1. Broodstock holding tank management summary.

Tank Specifications	6' diameter, 42" deep, round, flat-bottom tank with 2" center drain and 30" tall standpipe
Temp	6 °C (opt) 3-9 °C (acceptable range) Stable daily within 1 °C
Salinity	30-35 ‰ (acceptable range) Stable daily within 1 ‰
Dissolved Oxygen	> 6 ppm (>70% Saturation)
Unionized NH3	<0.05 ppm
SW Clarity	Clear. No cloudiness or turbidity.
pH	6.5 - 8.5
Seawater Volume	2000 L
Seawater Depth	30"
Continuous SW Flow Rate	15 lpm (10 times holding tank volume per day)
Aeration	Two 1.5" air stones at 5 lpm each
Tank Monitoring Frequency	8 AM and 4 PM every day.
Tank Monitoring Method	Check, measure, and maintain seawater quality at optimum. Measure temperature, salinity, dissolved oxygen concentration, and seawater flow rate twice daily Check and maintain aeration. Confirm each broodstock alive and remove any mortalities Observe feed consumption rate. Adjust if necessary. Record measurements and observations in Broodstock Holding Log.
Feed Frequency	Twice per week
Feed Type	Chopped and thawed herring and squid (~ 1" chunks)
Feed Ration	~1.5% wet wt feed to wet wt crab per feeding
Tank Cleaning Frequency	Twice per week just before each feeding
Tank Cleaning Method	Siphon waste and uneaten feed out of holding tank
Holding Density	Up to 7 Broodstock per square meter tank bottom area

Chapter 6. Monitoring Embryonic Development and Predicting Hatch Timing

Monitoring Embryonic Development

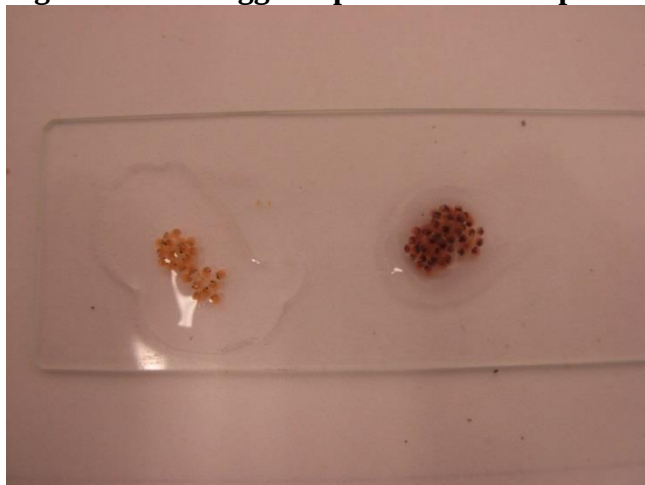
As broodstock are held prior to hatching their eggs, the health and stage of their developing embryos are routinely monitored. Once a week, a sample of approximately 50-100 eggs is removed with forceps from the forward margin of the egg clutch of each female and placed in a spot plate depression in clean seawater (Figure 7). Once in the lab, the egg samples are transferred onto a glass microscope slide in a drop of clean seawater (Figure 8). The slide is placed on a white background with overhead lighting and the color of the eggs is recorded. The eggs are then examined under a compound microscope at 40X and 100X magnifications. The presence and amount of filamentous bacteria (Figure 9) and/or stalked protozoans (Figure 10) on the surface of the eggs is recorded. All observations are recorded in the Egg Health Assessment Log (Appendix 3).

Figure 7. RKC egg samples in spot plate.



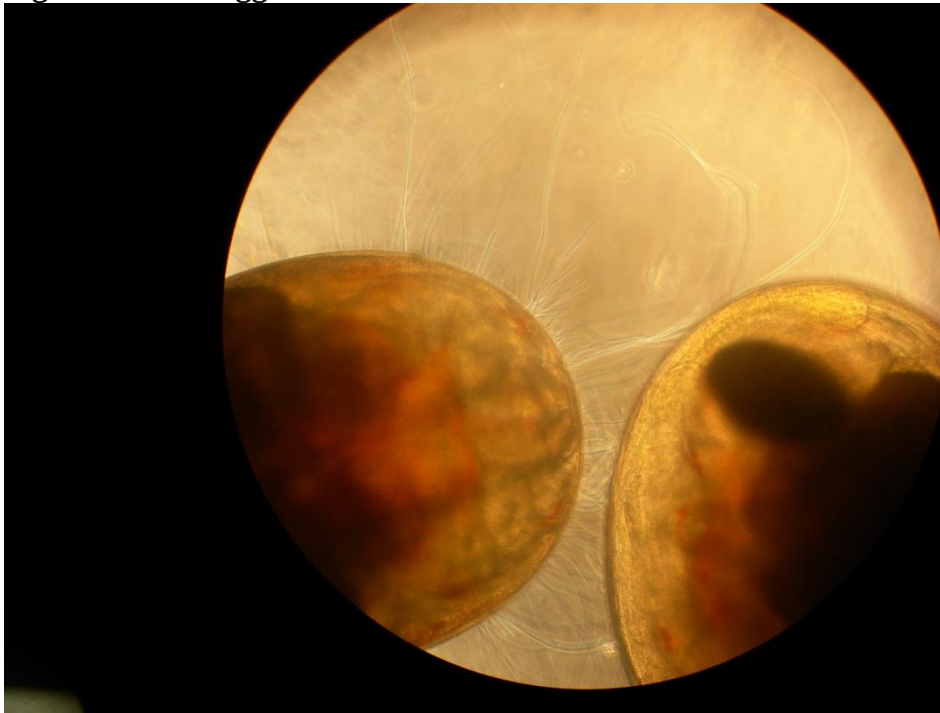
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Figure 8. RKC egg samples on microscope slide.



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Figure 9. RKC eggs with filamentous bacteria.



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Figure 10. RKC egg with stalked protozoans at base.



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The stage of embryonic development is also recorded in the Egg Health Assessment Log. Stages of RKC embryonic development from fertilization through hatch are described in detail and depicted in diagrams by Nakanishi (1987) for eggs incubated at 3 °C (Figure 11). First cellular division in the egg is observed four days after fertilization (Figure 11a) and from day 4 through day 39, the developing egg is multi-cellular in appearance as embryonic development proceeds through the morula and blastula stages (Figures 11 b and c). By day 39 post fertilization, the boundaries between individual cells within the egg become no longer distinguishable marking the beginning of the gastrula stage (Figure 11d). At day 92, the blastodisc becomes visible on the yolk and by day 109, the embryo becomes visibly distinct from the yolk, marking the beginning of the metanauplius stage (Figure 11e). On day 201, observable pigment first forms in the compound eyes of the developing embryos marking the beginning of “eyed egg” stage of development (Figure 11f). After eggs become eyed, two readily observable key features can be monitored as embryonic development continues. These are the length of yolk relative to the length of the egg and the length of the black pigmented eyespots, both of which can be used to estimate hatch timing (described in the following section). Key stages of RKC egg and embryonic development timing at 3 °C observed by Nakanishi (1987) are summarized in Table 6-1.

Table 6-1. RKC egg and embryonic development at 3 °C.

Days	
Post	Developmental
Fertilization	Stage
0	Eggs extruded and attached to pleopods
4	First cellular division observed in egg
39	Cellular boundaries no longer visible in egg – Gastrula stage begins
109	Embryo visible and clearly distinct from yolk - Metanauplius stage begins
201	Pigmented eyespots become visible – “Eyed egg” stage begins
331	Hatching begins

Predicting Hatch Timing

At APSH, we utilize a simple but effective method for predicting approximate hatch timing by microscopic measurement of the length of the black pigmented eyespot in the developing embryos. Similar methods have been shown effective for predicting hatch timing in lobsters (Perkins, 1972) and blue king crab (Stevens et al., 2008). Using a pair of forceps, a sample of approximately 50-100 eggs is removed from the forward margin of the egg clutch and placed on a glass microscope slide in a drop of seawater. The egg sample is then observed under a compound microscope. The small cluster of eggs is examined and the maximum length of embryonic black pigmented eyespots, which are clearly visible through the transparent egg membrane, is measured using the ocular micrometer in the eyepiece of the microscope (Figure 12). It is important to measure maximum eyespots which are oriented perpendicular to the field of view in sharp focus to obtain an accurate measurement. The maximum embryo eyespot length from the egg sample of each female is measured. Once the maximum eyespot length in the embryos from all the broodstock averaged 300 µm, significant hatch (a total of >1000 Z1s hatched per day) was ~50 days away (± two weeks), when broodstock holding temperature

averaged 4-5 °C in the three months prior to hatching, At hatching, the RKC embryonic eyespot is approximately 370-380 microns in length.

Figure 11. RKC embryonic developmental stages at 3 °C (Nakanishi, 1987).

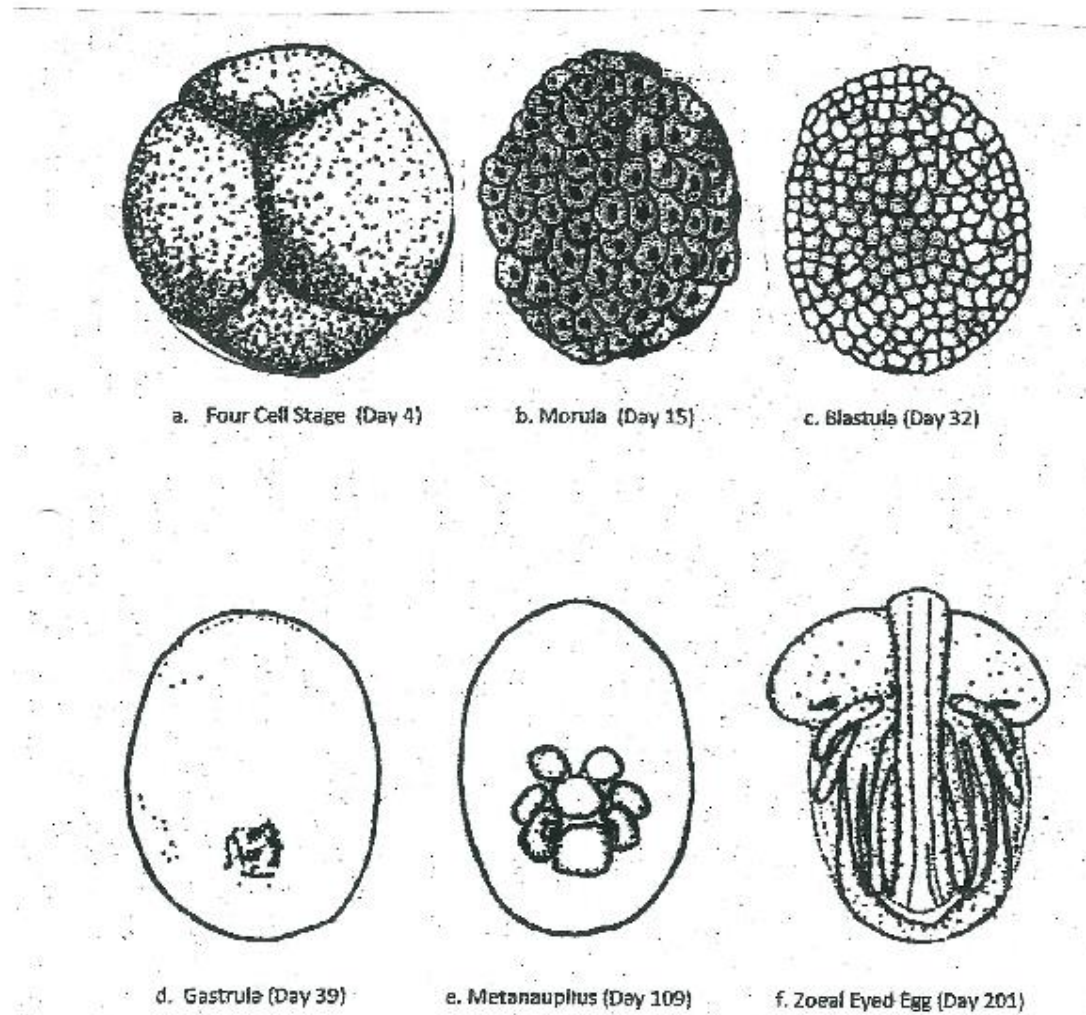
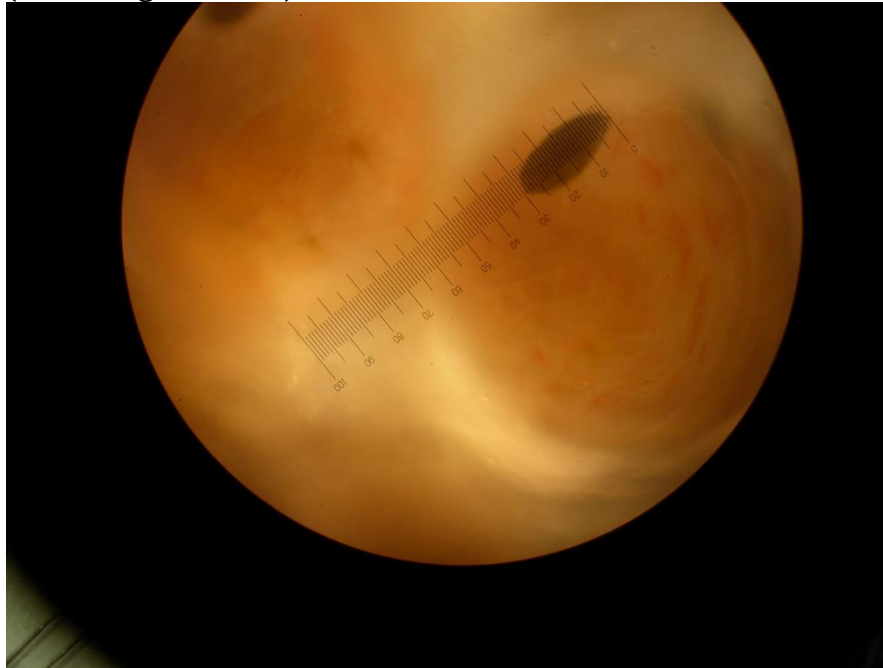


Figure 12. 300 μm long embryonic eyespot measured with ocular micrometer (100X magnification).

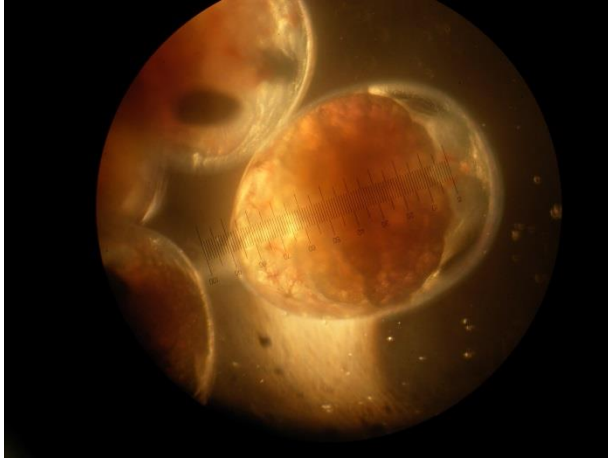


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Measuring maximum eyespot lengths to predict when hatch will begin is a simple but powerful tool for the hatchery manager, allowing orderly preparations for the beginning of the larval rearing phase of production. To date, we have been able to roughly estimate hatch timing for embryos developing at 4-5 °C over the last couple of months prior to hatching. It has been shown that embryos develop faster at elevated broodstock holding temperatures. In laboratory experiments, [Nakanishi \(1987\)](#) found that the time from egg fertilization to hatching for RKC broodstock held at 3 °C was 11 months, while [Omi and Mizushima \(1980\)](#) found that hatching occurred in 8 to 10 months from broodstock held at 9 °C. [Shirley et al. \(1990\)](#) found that hatching in RKC broodstock held at 3, 6, 9, and 12 °C occurred at 305, 240, 225, and 207 days post fertilization, respectively. Further research needs to be conducted to further refine hatch timing estimates for broodstock held at variable temperatures and determine the viability of the larvae produced.

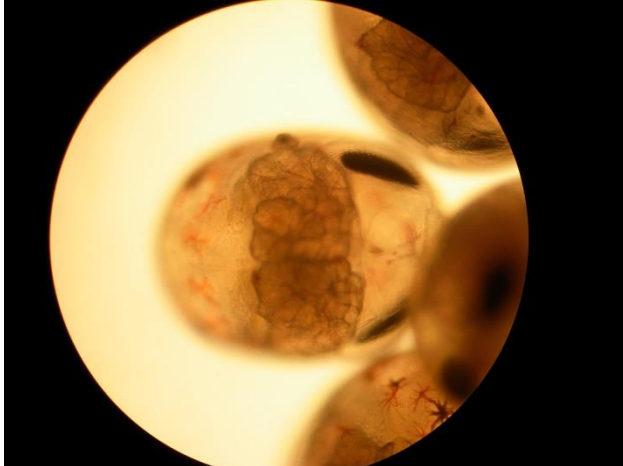
In addition to eyespot length, the length of the yolk relative to the length of the egg can also be used to estimate hatch timing. Like the eyespots, yolk is easily observable through the transparent egg membrane and can be measured using the ocular micrometer in the eyepiece of a microscope ([Figures 13 & 14](#)). As the embryo develops, the length of the yolk relative to the length of the egg decreases. At an egg incubation temperature of 3 °C, [Nakanishi \(1987\)](#) found yolk length is approximately 75% of egg length ten weeks prior to hatch, 50% of egg length six weeks before hatching, and declines to about 25% of egg length in the week just prior to hatch. Our observations at APSH on eggs developing at 4-5 °C were similar. We found that at an egg incubation temperature of 4-5 °C, the ratio of yolk length to egg length was 75%, 50%, and 25% at eight, four, and one week prior to hatch, respectively.

Figure 13. Yolk length approximately 65% of egg length.



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Figure 14. Yolk length approximately 30% of egg length.



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Changes in broodstock behavior can also indicate hatching is imminent. Typically, several weeks before hatching begins, the feed consumption rate of the broodstock declines. Further, females within several days of hatching increase the frequency of egg ventilation, lowering their abdominal flap and vigorously fanning their clutch of eggs several times per hour.

Hatch Detection

As broodstock are held, it is essential to identify when hatching begins. A simple and effective method for detecting hatch in a broodstock holding tank is to place a 300 μm mesh bag over the outflow drainpipe. Freshly hatched RKC larvae are approximately 5 mm in total length, reddish orange in color, and can be easily seen when retained in the hatch detection bag attached to the outflow drain. Every day as part of the broodstock monitoring routine, the hatch detection bag should be inspected for the presence of larvae. If no larvae are seen, the bag should be

temporarily removed from the outflow drainpipe, rinsed clean with freshwater and reattached to be checked again the following day. Daily rinsing removes decomposing feed and other wastes which can accumulate in the hatch detection bag, clogging it and making it more difficult to see freshly hatched larvae. When hatching is detected, the broodstock should be transferred from the broodstock holding tanks to the hatching tanks.

It is interesting to note that over the first seven years of the AKCRRAB project, RKC broodstock collected from Kodiak, Bristol Bay, and Juneau always began significant hatch in their holding tanks in late February, early to mid-March, and early April, respectively ([Table 6-2](#)).

Table 6-2. RKC broodstock capture date, mean holding temperature, holding period, and date significant hatch began each AKCRRAB project year.

		Broodstock	Broodstock	Date
Capture		Mean Holding	Holding	Significant*
Date	Stock	Temp (°C)	Period (mo)	Hatch Began
July 2006	Kodiak	8	7	2-22-07
Nov 2007	Bristol Bay	6	4	3-15-08
Nov 2008	Bristol Bay	6	4	3-21-09
Nov 2009	Bristol Bay	6	4	3-5-10
Oct 2010	Juneau	6	6	4-4-11
Nov 2010	Bristol Bay	6	4	3-12-11
Oct 2012	Juneau	6	5	4-1-13
Oct 2012	Kodiak	6	4	2-20-13

*Significant hatch defined as total hatch of greater than 1000 larvae per day.

Note: All RKC broodstock were held at ambient temperature each project year.

Chapter 7. Hatching Tank Management

Broodstock Transfer to the Hatching Tank

Once larvae begin hatching, crabs should be transferred from the broodstock holding tank to individual chambers (hatching bins) in the hatching tank (Figure 15). The hatching tank contains 10 individual hatching bins. One crab is placed in each hatching bin (Figure 16). To avoid temperature shock or stress to the broodstock and their eggs, at transfer initial temperature and salinity in the hatching and holding tanks should be the same. Broodstock are held in the hatching bins for three to four weeks as their eggs hatch. Broodstock are not fed while in the hatching tank to maintain highest possible water quality. Adding food would increase the bacterial load in the hatching tank seawater and is unnecessary as we have observed that broodstock feed very little from two weeks prior to beginning of hatch until about a week after their post hatch molt.

Figure 15. 3000 L 10-bin hatching tank.



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Figure 16. Broodstock in individual hatching bin.



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Hatching Tank Specifications and Seawater Quality Parameters

The hatching tank consists of a 3000 L rectangular tank (11' long x 5' wide x 2' deep) into which 10 cylindrical hatching bins are partially immersed ([Figures 15 & 16](#)). Each 23" diameter, 23" deep hatching bin has a 100 µm Nitex® mesh screen attached to its base. The 100 µm mesh screen retains larvae as they hatch and allows good water exchange as seawater down-wells from above through the screen at the base of the bin. Each hatching bin is partially immersed to a depth of 10" in the hatching tank, providing an operating volume of approximately 70 L of seawater in each bin. Continuous flow-through of seawater is delivered to each bin through a perforated ½" diameter, 10' long PVC spray bar positioned across the top of the bins. The spray bar delivers continuous seawater flow of 1.5 lpm to each bin (15 lpm to the entire hatching tank). This flow rate provides good water quality and maintains stable temperature in the hatching tank. At a hatchery room temperature of 12 °C, this flow rate and tank volume maintained the target seawater hatching tank temperature at near the ambient incoming seawater temperature of 5-6 °C with daily fluctuations of less than 1 °C. Salinity should remain between 30-35 ‰ and vary less than 1 ‰ daily.

Hatch Pattern

Though variation exists, a typical RKC broodstock (~130 mm CW and 1.5 kg) with a full clutch of eggs will produce about 180,000 larvae over a three to four week period of hatch. The typical hatch pattern is as follows. During the first several days of hatch, only several hundred to several thousand larvae are hatched per day. Then daily hatch tends to gradually increase and by about day 7, a productive female will be hatching between 10,000 and 20,000 larvae per day. Daily hatch rate typically remains at about this level until about day 21 when the number of larvae hatched per day tends to begin declining, with several thousand to several hundred larvae per day released the last few days of hatch. Though some eggs hatch during the day, the majority of hatching tends to occur at night. The total number of larvae hatched per female is largely dependent on size, with larger females typically hatching more larvae than smaller ones ([See Tables 2-1 and 3-1 for details](#)). At the completion of hatch, a brown, spongy looking material composed of remnants of egg casings remains attached to the underside of the female in her brood pouch. This material remains attached until she molts out of her old shell, which usually occurs several days to several weeks after hatching is complete.

Collecting Larvae from the Hatching Bins

Newly hatched larvae are collected from the hatching bins every morning and their numbers estimated before stocking the larval rearing tanks. Larvae are typically collected from the hatching bins each morning in the following way. First, the broodstock crab is removed from the bin and placed temporarily in a tub containing seawater from the hatching tank, to ensure the broodstock and eggs experiences no temperature or salinity shock. Broodstock waste (feces) in the hatching bin is then siphoned out. The seawater containing the larvae within the bin is then stirred vigorously in a circular motion. After about three minutes, the swirling motion of seawater within the bin stops, and any weak or dead larvae along with any unhatched eggs that had sloughed off of the female's egg mass settle in the bottom center of the bin where it is easily siphoned out and discarded. The bin cleaning siphon is composed of an 18" long piece of ½" PVC pipe with a 10' long piece of clear vinyl tubing snugly inserted into one end. Healthy larvae will be actively swimming off the bottom and along the wall of the bin. The hatching bin, now containing only actively swimming larvae, is then gently lifted out of the hatching tank. The

healthy larvae are then gently rinsed from the hatching bin into a 100 L aerated counting container that contains a 6" cushion of seawater the same temperature and salinity as the hatching tank. We utilize a 33 gallon heavy duty gray plastic food grade Rubbermaid trash bin as the larval counting container. Each time larvae or broodstock are transferred from one tank to another, care is taken to avoid temperature or salinity shocks, which can potentially stress or kill the crab. The counting container is then topped off to a volume of 100 L. Larvae can be held temporarily at high density in a well aerated counting container while volumetric sample counts are made to estimate the total number of larvae. As a rule of thumb, the concentration of larvae in the counting container should not exceed 1000 per liter and larvae should remain in the counting container at high density no longer than 30 minutes.

Volumetric Larval Counts and Population Estimates

Larval counts and population estimates are made using a volumetric method. First the counting container with 100 L of seawater and the larvae to be counted is vigorously aerated to evenly mix and ensure a homogenous suspension of the larvae. Five 100 ml samples of seawater containing suspended larvae are then scooped from the vigorously aerated container. The number of larvae in each 100 ml sample is then counted by pouring the sample through a sample sieve which is partially immersed in a Petri dish. The sample sieve is a 2" diameter, 1" deep section of PVC pipe with a 500 μ m mesh screen attached to its base ([Figure 17](#)).

Figure 17. Sample sieve in petri dish for collecting and counting RKC larvae.



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During the counting process, the sample sieve is partially immersed in a seawater filled Petri dish to provide a cushion of seawater for the counted larvae. By providing a cushion of seawater in the Petri dish, the larvae are shielded from direct or overly forceful contact with the sample sieve screen and are not harmed during the counting process. The sample sieve has a grid drawn on its bottom screen dividing it into four equal parts to facilitate counting. The sample is poured about

20 larvae at a time into the sample sieve until all the larvae in the 100 ml sample are counted. The sample sieve containing larvae to be counted can be lifted from the Petri dish and held inverted toward an overhead light source. The temporarily immobilized larvae adhere to the damp screen of the sample sieve and can be easily counted in this way. After each count is made, the sample sieve containing the counted larvae is immersed back into the counting container to return the unharmed larvae. Once larvae in all five 100 ml samples have been counted, the five sample counts are averaged and then divided by 100 to estimate the number of larvae per ml in the counting container. The total number of larvae in the counting container is then estimated by multiplying by 100,000 (the total volume in ml of the 100 L counting container). To obtain a reasonably accurate population estimate, it is important that each sample count contains at least 30 larvae. The goal is to count enough larvae to obtain a statistically valid estimate while not counting so many larvae that the counts become overly burdensome. To achieve this goal, the sample volume can be adjusted so that the number of larvae collected in each sample is in the 30-50 range. If the counting container is sufficiently aerated to keep the larvae evenly suspended during the sampling process, individual counts will generally not vary by more than 20%.

Once all sample counts are made, the following equation can be used to estimate the number of larvae in the counting container:

Estimated Total Number of Larvae in the Counting Container = (Average Number of Larvae in the Sample Counts / Average Sample Volume (ml)) x Counting Container Volume (ml)

Once counts are complete and population estimates are calculated, the larvae can be stocked into the larval rearing tank (LRT). The larvae are transferred using a 5L beaker with handle to scoop and pour the volume of seawater containing the desired number of suspended larvae from the vigorously aerated and well mixed counting container into the LRT.

Chapter 8. Larval Rearing (Z1 to Glaucothoe)

Larval Rearing Overview

During the larval rearing phase of hatchery production, the goal is to maximize cost effective production of healthy glaucothoe which will ultimately molt into healthy first stage juvenile crab (C1s). To date (July 2013) the protocols described in this manual have been successfully utilized in a 1200 L LRT to obtain a maximum survival of 67% from Z1 to glaucothoe, producing a yield of 33 glaucothoe per liter of LRT volume over a 26 day larval rearing period. Using these larval rearing protocols in 2013, we averaged 59% survival to the glaucothoe stage, a mean yield of 30 glaucothoe per liter of LRT volume. Larval rearing begins on the day of hatch with stocking of stage 1 zoeae into LRTs and ends with harvest of the glaucothoe stage. Subsequent rearing of glaucothoe to the first stage juvenile (C1) is the second phase of hatchery production (termed glaucothoe rearing) and is described in Chapter 9 of this manual. Nursery rearing (the culture of the juvenile stages from C1 onward) is described in Chapter 10.

Larval Rearing Seawater Quality Parameters

High quality filtered seawater is necessary for consistent successful operation of a RKC hatchery. Seawater hatchery supply and filtration requirements were previously discussed in [Chapter 4](#) of this manual. Optimum larval rearing seawater quality and environmental parameters are summarized in [Table 8-1](#). Larval rearing temperature should be maintained between 8-11 °C and vary less than 1 °C daily. Salinity should be maintained between 30-35 ‰ and vary less than 1 ‰ daily. Though these are the optimum conditions, larvae can tolerate daily variations of 2 °C with no apparent harmful effects if the changes are gradual. For instance, if the temperature in the LRT gradually rises from 8 to 10 °C from morning to afternoon and then gradually falls back to 8 °C the following morning, the larvae are not harmed. However, abrupt changes in temperature and salinity, referred to as temperature or salinity shock, should be avoided as these can stress or kill larvae. For this reason, whenever larvae are moved from one container to another, such as into a counting container or into a new tank, care must be taken to ensure temperature and salinity in the source and receiving tanks are the same. Dissolved oxygen concentration in the LRT should remain above 6 ppm and is ensured by providing continuous aeration and periodic seawater flow-through to the LRT (described in greater detail in following sections). Oxygen concentration can be measured with a YSI® dissolved oxygen probe and meter. Nitrogenous wastes in the form of unionized ammonia should not exceed 0.05 ppm. Unionized ammonia is kept below this level in the LRT by periodic flow-through of seawater (described in a following section). Levels of unionized ammonia can be monitored with a LaMotte® ammonia test kit. The acceptable range of seawater pH in the LRT is 7.5 to 8.5, which can be measured with a pH meter. Moderate lighting above the LRTs is acceptable. Intense lighting (greater than 1000 lux) at the water surface can stress the larvae and should be avoided. A light meter can be used to measure light intensity just above the surface of the LRT. Daily larval rearing records are kept in the larval rearing log ([Appendix 5](#)).

Table 8-1. RKC larval rearing seawater quality parameters.

Temp	8-11 °C Stable daily within 1 °C
Salinity	30-35 ‰ (acceptable range) Stable daily within 1 ‰
Dissolved Oxygen	> 6 ppm
Unionized NH₃	<0.05 ppm
SW Clarity	Clear. No Cloudiness or turbidity
SW Smell	No Odor
Tank Lighting	<1000 lux at surface
pH	7.5 - 8.5

Larval Rearing Tank Specifications

A 1200 L, 52” high, 48” diameter molded fiberglass tank with a smooth interior and a gently sloped conical bottom works well for larval rearing of RKC. Nine production scale LRTs are depicted in [Figure 18](#).

Figure 18. Larval rearing tanks (LRTs).



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Larval Rearing Tank Preparation

Several days prior to stocking an LRT, it should be cleaned, disinfected, and allowed to dry. To clean the tank, a mop, brush, or sponge is used to thoroughly scrub the tank with freshwater and a mix of Clorox bleach and Alconox® detergent. After a thorough rinse, the tank should then be disinfected overnight by filling it with seawater and adding 50 ppm hypochlorite (1 L Clorox® per 1000 L seawater). While the chlorinated tank is being disinfected, air-stones, air tubing, flow-through seawater exchange sieves and other items can be immersed in the tank overnight to also be disinfected. The following morning the chlorinated tank is drained, rinsed, and left to dry. Likewise, all disinfected items are thoroughly rinsed with freshwater and stored on a clean shelf prior to use. Chlorination and drying are both effective means of killing potential pathogens.

Characteristics of Healthy Z1s

Prior to stocking the LRTs, Z1s to be stocked should be carefully observed and their health assessed. Healthy RKC Z1s are typically red to orange in color and actively swim in the water column. Z1s typically orient themselves in the water column with their heads down and tails up, and usually swim backwards, though they are also capable of forward motion. Maximum swimming speed is ~1" per second. Healthy zoeae are positively phototactic and swim toward light.

When observed under the microscope, several other characteristics of healthy Z1s can be seen. Using an ocular micrometer in the eyepiece of the microscope, measurements of the larvae can be made. A Z1 on the day of hatch typically measures 5.2 mm in total length (from the forward tip of the rostrum to the posterior tip of the tail spines). When placed on a microscope slide in a drop of seawater, most zoeae lie on their sides in a partially flexed fetal position, making it relatively simple to measure the carapace height of the larvae (Figure 19). Healthy Z1s generally measure approximately 0.9 to 1 mm in carapace height on the day of hatch. Their shells are clean and transparent. Through their transparent shell, spherical lipid droplets can be seen in the forward region of the gut. On the day of hatch prior to first feeding, a healthy RKC Z1 typically has multiple lipid droplets with maximum diameters ranging from 30 to 60 μm . These lipid droplets contain stored energy which can be utilized by the newly hatched larvae prior to first feeding. If unfed, larvae can live approximately one week before starvation occurs. However, in the hatchery, larvae are stocked and fed on the day of hatch and begin feeding within several hours. Though it is best to begin feeding on the day of hatch, hatchery reared larvae generally do well if feeding is begun no later than two days after hatching.

Figure 19. Carapace height of RKC Z1.



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Stocking of Z1s

Prior to stocking, the cleaned and disinfected LRT is again thoroughly rinsed and then filled with filtered seawater ([Chapter 4](#)) of the same temperature and salinity as that of the tank containing the Z1s to be stocked. Maintaining the same temperature and salinity in the source and receiving tanks prevents the larvae from experiencing a temperature or salinity shock at transfer, which could potentially cause stress or mortality. The primary air stone is placed in the bottom center of the seawater filled LRT and the air valve adjusted to provide aeration at ~7 lpm. A clean 8" diameter, 500 μ m mesh seawater exchange sieve is then installed in the LRT ([Figure 20](#)).

Healthy larvae to be stocked are then scooped with a 5 L plastic beaker from the vigorously aerated, evenly mixed 100 L counting container (described in [Chapter 7](#)) until the volume of seawater containing 60,000 Z1s is transferred from the counting container to the LRT. This yields an initial stocking density of 50 Z1s per liter in the 1200 L LRT. It is best to stock all tanks with Z1s from a single day's hatch. This keeps the molting of the larvae as synchronous as possible and also achieves greatest size uniformity, both of which reduce the potential for cannibalism which has been a cause of larval mortality identified during previous attempts to rear the larvae of RKC ([Kovatcheva et al., 2006](#)). Immediately following molting from one zoeal stage to the next, the larvae are temporarily soft and until their exoskeletons reharden, they are potentially more susceptible to cannibalism. Synchronous molting of the group of larvae therefore lowers the potential for cannibalism. Stocking the LRT with a single day's hatch of Z1s also helps at the conclusion of the larval rearing run by keeping the molt timing to glaucothoe as synchronous as possible, which aids in efficient harvest of glaucothoe and stocking of the glaucothoe rearing tank

Figure 20. Seawater exchange sieve in LRT.



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Remember that the goal of larval rearing is consistent, cost effective production of maximum number of healthy glaucothoe per liter of LRT volume. By stocking 50 Z1s per liter and following the protocols described in this chapter, we achieved our greatest mean yield of glaucothoe (30 per liter) and 59% survival from stocking of Z1s to harvest of glaucothoe.

Larval Feeding Protocol

RKC larvae are first fed within an hour of being stocked in the LRT. RKC larvae are fed a diet of *Chaetoceros muelleri* (*C.m.*) and *Thalassiosira weissflogii* (*T.w.*) microalgae and DC DHA Selco® (also known as A1 DHA Selco) enriched *Artemia* (brine shrimp). The *C.m.* and *T.w.* are added to the LRT at concentrations of 15,000 and 5,000 cells per ml, respectively, once daily each morning. Both *C.m.* and *T.w.* are directly consumed by the larvae. We confirmed this in beaker experiments. Unfed, freshly hatched Z1s were placed in beakers containing only seawater and separate beakers containing either *C.m.* or *T.w.* After 48 hours, the Z1s in the beakers containing microalgae exhibited definite yellow color in the anterior portion of their gut, while the guts of the Z1s in the unfed beaker remained empty and colorless. Both *C.m.* and *T.w.* have good nutritional profiles and grow well and consistently in standard algae production rooms of shellfish hatcheries (Figure 21). *C.m.* is the most commonly utilized microalgae in commercial shrimp hatcheries around the world. In addition to providing direct nutritional benefits, microalgae provide additional benefits such as improving water quality by taking up nitrogenous wastes. Microalgae may also have a beneficial probiotic effect by excluding or out-competing potentially harmful bacteria. Algal exudates may help control microbes in the larval rearing tank water and in the larval gut. It is also believed consumption of algae may stimulate the nonspecific immune system of the larvae (Lavens and Sorgeloos, 1996).

Figure 21. Microalgae production room.



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Though microalgae provides nutrition, the primary food source for the crab larvae is enriched *Artemia*, which is fed twice daily at 8 AM and 4 PM. After hatching, *Artemia* is enriched for 24 hours with DC DHA Selco® before being fed to the crab larvae (Figure 22). Detailed methods for hatching and enriching *Artemia* are presented in Appendix 7 of this manual. DC DHA Selco® is produced by Inve Aquaculture Company and is an emulsified off-the-shelf product containing high levels of essential fatty acids EPA and DHA and vitamins. DHA and EPA are highly unsaturated fatty acids (HUFA's) which are important components of the diet of RKC larvae and must be consumed directly since crab larvae are incapable of synthesizing them from other fatty acids. DC DHA Selco® is widely used to enrich *Artemia* fed to marine shrimp larvae in commercial hatcheries worldwide.

Figure 22. Hatching and enrichment tanks in the *Artemia* production room.



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Carefully managing the feeding protocol is critically important. Concentrations of enriched *Artemia* fed must be carefully monitored and adjusted at each larval stage. The goal is to feed the crab larvae to satiation, ensuring *Artemia* are continuously available to the crab larvae in an amount adequate to minimize capture time. It is also important to avoid over feeding the larvae which wastes money and degrades water quality by increasing bacterial load and nitrogenous wastes in the LRT. Table 8-2 serves as a guide indicating the approximate concentration of enriched *Artemia* to feed at each larval stage.

Table 8-2. Larval feeding guide.

Concentrations of enriched *Artemia* to be fed daily at each larval stage in an LRT stocked with 50 RKC larvae per liter.

Larval Stage	8:00 AM (# Art/ml)	4:00 PM (# Art/ml)	Daily Total Art/ml
Z1	1	1	2
Z2	1	2	3
Z3	1.5	2	3.5
Z4	2	2	4

A good rule of thumb is to manage feed in the larval rearing tank such that concentration of enriched *Artemia* in the LRT never drops below 0.5 per ml prior to the next feeding. Following this strategy ensures larvae can feed continuously to satiation and also keeps time larvae spend capturing *Artemia* to a minimum. RKC larvae are encounter feeders, not visual predators that see and then pursue their prey. Rather, as they drift and swim in the water column, RKC zoeae create micro-currents with their swimming appendages (maxillipeds) that sweep prey items toward their mouthparts. As a prey item of the appropriate size and quality such as a 24 hour-enriched *Artemia* is swept towards them, they seize and consume it.

Over the first seven years of AKCRRAB hatchery scale larval rearing research, highest mean survivals from stocking to glaucothoe and C1 were achieved using the diet of microalgae and enriched *Artemia* described above. Using this technique during 2013 in six 1200 L tanks stocked at 50 Z1s per liter, survival from Z1 to glaucothoe averaged 59% and survival from Z1 to C1 averaged 33%. However, it should be noted that we have also achieved reasonable survivals in previous years utilizing a diet of enriched *Artemia* alone (no microalgae). In 2009 and 2010, using a diet of enriched *Artemia* alone in a total of thirteen 1200 L tanks stocked at 50 Z1s per liter, survival from Z1 to glaucothoe averaged 50% and survival from Z1 to C1 averaged 20%.

Monitoring and Adjusting Feed Amounts (Residual Feed Counts)

Though [Table 8-2](#) serves as a guide for the concentrations of enriched *Artemia* to be fed during each larval stage of the hatchery production run, feed consumption rates should none the less be monitored daily and adjusted as necessary to avoid underfeeding or overfeeding of the larvae. This is best achieved by making residual feed counts of the concentration of enriched *Artemia* remaining in the LRT prior to each feeding.

The procedure for making residual feed counts is as follows. A 20 ml sample of seawater is scooped from the aerated, well mixed LRT and poured through a 100 µm sample sieve. The number of enriched *Artemia* retained on the 100 µm mesh screen is then counted and divided by 20 to determine the residual concentration (number of *Artemia* per ml) remaining in the LRT. Three sample counts should be made and the average calculated.

Ideally, just prior to each afternoon feeding, the residual feed count average should be between 0.5 and 1 *Artemia* per ml. If the afternoon residual *Artemia* count is above 1 per ml, this suggests the crab larvae are being overfed and future feed concentrations should be adjusted downward.

Making routine residual feed counts allows for fine tuning the feeding rate and is an important aspect of successful hatchery management.

LRT Water Exchange

Good seawater quality is required for successful larval rearing of RKC. To achieve this, we incorporated a protocol and larval rearing tank design which employs a semi-static flow-through system of seawater exchange. Between 8 AM and 4 PM, the tank is operated as a static system, with no seawater flow-through. Since microalgae is added to the LRTs at 8 AM, this allows the RKC larvae an eight hour period to consume it without any being flushed from the tank during that period. Then from 4 PM until 8 AM the following morning, we provide continuous seawater flow-through at a rate of 6 lpm. Seawater enters the tank at the surface and passes out of the tank through an 8" diameter, 500 µm mesh seawater exchange sieve (Figure 20). The 500 µm exchange sieve retains the larvae in the tank while allowing uneaten microalgae, *Artemia*, and waste to be gradually flushed from the tank overnight. A primary reason for flow-through seawater exchange is to keep the amount of nitrogenous waste (measured as unionized ammonia) below levels at which it would become harmful to the larvae. Though specific RKC larval tolerance to unionized ammonia (NH₃) has not been determined, concentrations below 0.05 ppm have been found safe for marine shrimp larvae. Using this semi-static seawater exchange method, testing of seawater in the LRTs with a LaMotte® ammonia test kit confirmed that unionized ammonia concentrations remained below 0.05 ppm throughout the RKC larval rearing period.

A risk involved in all flow-through hatchery systems is the potential for the seawater exchange sieve to become clogged, causing the LRT to overflow resulting in the loss of larvae down the drain. The smaller the mesh size, the more likely the screen is to clog. To reduce the likelihood of the 500 µm mesh exchange sieve from clogging, it is mounted on an extended 1" diameter PVC pipe to position it over the bubbles rising up from the center air stone in the bottom of the tank. In addition, a second air stone is hung over the exchange sieve and positioned about two feet directly below the sieve. Aeration rate from this second air stone is set at about 5 lpm. As bubbles rise from the air stones to the surface, these brush against the vertical face of the exchange sieve, sweeping away debris such as crab excrement, partially consumed *Artemia*, and molted shells, reducing the potential for screen clogging and tank overflow. As a final safeguard, a 300 µm mesh bag can be attached to the outlet drain of each LRT. The 300 µm mesh bag remains immersed in a seawater filled dishpan at the base of the outflow drain and will catch the larvae in the event of a clogged or torn seawater exchange sieve.

LRT Aeration

Aeration to each LRT is provided through two 1.5" square silica air stone diffusers, one positioned on the bottom of the tank about 6" from the center and the other about two feet below the exchange sieve as described in the paragraph above. Aeration rate through the primary air stone is maintained continuously at a ~7 lpm and the secondary air stone at ~5 lpm throughout

the larval rearing period. This level of aeration creates moderate turbulence and provides a number of critical benefits. First, it provides adequate dissolved oxygen levels (greater than 6 ppm) throughout the larval rearing run. Second, it keeps the live crab larvae, microalgae, and *Artemia* well suspended and evenly mixed. As described earlier, RKC larvae are encounter feeders, seizing prey when they come into contact with them, rather than visually seeking them out. Therefore, keeping both the crab larvae and their food evenly suspended the LRT water column with moderate aeration minimizes prey search time and maximizes feeding efficiency. Further, moderate aeration prevents waste from accumulating on the tank bottom by keeping uneaten feed and waste suspended so that these materials are more effectively flushed from the LRT through the flow-through 500 µm seawater exchange sieve. Moderate aeration keeps live, healthy crab larvae evenly suspended and dispersed in the water column, reducing the potential for cannibalism. Finally, by positioning the air stones in the described manner and delivering this level of aeration, weak, unhealthy, dead, and dying larvae will settle in a distinct pile in or near the bottom center of the tank so that they can be siphoned out. Each tank is carefully inspected for piles of dead or dying larvae on the tank bottom each morning, noon, and evening and any observed are promptly siphoned out of the tank and discarded. This is a critically important part of larval rearing tank management.

Daily LRT Cleaning Protocol

The LRTs are routinely cleaned on a daily basis to help maintain good water quality and tank hygiene, essential for the successful hatchery rearing of healthy larvae. Each morning, any uneaten *Artemia* or waste that has settled in the bottom of the 500 µm seawater exchange sieve is siphoned out and discarded. This is accomplished by inserting the PVC end of the exchange sieve siphon into the exchange sieve and siphoning out the waste while the sieve is still installed in the LRT. The exchange sieve siphon consists of an 18" long piece of ½" PVC pipe with a ten foot long piece of clear vinyl tubing inserted snugly into one end. In addition to routinely cleaning the exchange sieves, any waste or dead larvae observed accumulating on the LRT bottom should be siphoned out of the tank several times a day as needed. A 15' long piece of 1/2" clear vinyl tubing attached with three plastic zip ties to a 6' long piece of 1" diameter PVC pipe which serves as a handle works well as a tank cleaning siphon (Figure 23). It is especially important to remove dead larvae from the LRT as these can become a substrate for bacterial growth and proliferation. If dead larvae are left in the LRT, they eventually decompose and fragment into smaller pieces. These bacteria filled fragments can then be consumed by other larvae in the tank causing bacterial pathogens such as *Vibrio* to quickly spread and result in high mortality in the LRT.

Figure 23. Tank cleaning siphon.



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Periodic Complete LRT Cleaning

In addition to the daily tank cleaning previously described, during the approximately one month long larval rearing run, it may be necessary to periodically completely clean the larval rearing tanks. The LRTs are cleaned as necessary to maintain healthy conditions but not too often as this would subject the larvae to unnecessary handling stress. Complete cleaning of the LRTs as the larvae are about half way through the Z3 stage may be necessary if the tank bottom or walls become excessively fouled. Tanks are cleaned at mid-intermolt to avoid soft shelled, newly molted larvae, as these would be more susceptible to handling stress injury.

Before a complete cleaning of an LRT, the larvae within the tank must first be removed by drain harvesting. The first step in this process is to lower the volume of the LRT from 1200 L to about 300 L by siphoning seawater out of the LRT through a 12" diameter, 500 μ m mesh siphon sieve attached to a 15' long piece of 1" diameter clear vinyl tubing (Figure 24).

Figure 24. LRT siphon sieve.



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The sieve spreads the force of the out-flowing seawater over a large surface area. This prevents the larvae from being forcefully sucked against or damaged by contact with the siphon sieve

screen as the water level in the tank is being lowered. Larvae which come into contact with the sieve screen can drift away rather than being held against the screen by excessively forceful current. Once the water level has been lowered to approximately 300 L, the siphon sieve is removed from the LRT and the remaining 300 L of seawater containing the larvae is then drain harvested through a 7" deep, 8" diameter, 500 μ m harvest sieve partially immersed in a 4" deep seawater filled harvest tub (Figure 25). The seawater filled harvest tub provides a cushion of water protecting the larvae from direct or excessively forceful impact on the screen of the harvest sieve.

Figure 25. LRT harvest sieve.



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Once all larvae have been collected in the harvest sieve, it is gently lifted from the seawater-filled harvest tub and the larvae are gently rinsed into an aerated, partially filled 100 L temporary holding container (a 33 gallon Rubbermaid food grade trash can). This container was partially filled to a depth of at least 6" prior to transfer to provide a cushion of water for the larvae, again protecting them from physical injury at transfer. Once all larvae are rinsed into the holding container, it is filled to 100 L and moderately aerated, while the larval rearing tank from which they came is being cleaned. The temperature and salinity of the seawater in the holding container is the same as the LRT to prevent temperature or salinity shock or stress to the larvae.

Transferred larvae can be safely held in the temporary holding tank at densities of up to 1000 per liter for 30 minutes while the larval rearing tank from which they were removed is brushed clean with a mixture of Alconox® detergent and Clorox bleach, thoroughly rinsed with freshwater, and then refilled, again with seawater of the same temperature and salinity. The larvae can then be poured one 5 L beaker at a time back into their clean LRT.

It should be noted that from time to time, our 1200 L LRTs have run clean enough that survivals of 50% or above to glaucothoe have been achieved without requiring a complete tank cleaning between stocking of Z1s and the glaucothoe stage. This is the best case scenario in that less labor is required and the larvae are not be subjected to the handling stress that accompanies a complete tank cleaning.

Larval Growth and Development

During the larval rearing period, the larvae pass through four morphologically distinct zoeal stages, termed Z1 through Z4, before finally molting into the glaucothoe stage. Photos of each larval stage are presented in [Figure 1](#). The key features for distinguishing between the larval stages are illustrated in [Figure 26](#) and summarized in [Table 8-3](#).

Figure 26. Red king crab larval, glaucothoe, and first juvenile crab stage (Sato, 1958).

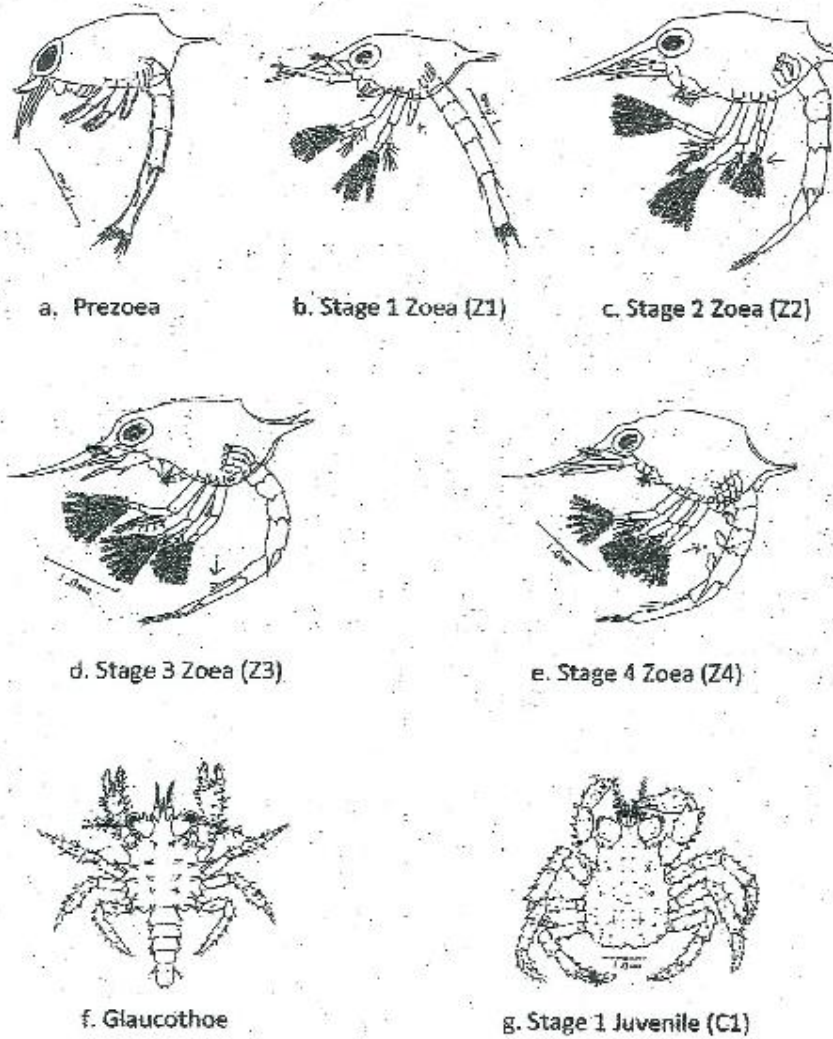


Table 8-3. Distinguishing characteristics of RKC larval stages.

	Spines on		
Larval	3rd Pair		
Stage	Maxillapeds	Uropods	Pleopods
Z1	Absent	Absent	Absent
Z2	Present	Absent	Absent
Z3	Present	Present	Absent
Z4	Present	Present	Present

At stocking the total length of Z1s from the forward tip of the rostrum to the posterior tip of the tail spines is ~5.2 mm. Carapace height of first day Z1s is typically 0.9 to 1.0 mm. Near the end of the hatchery run, healthy late stage Z4s have increased to about 7.5 mm in total length and exhibit carapace heights of approximately 1.6 mm. These measurements can be made using an ocular micrometer in the eyepiece of a compound microscope. Carapace height is easily measured as larvae placed in a drop of seawater on a microscope slide almost always orient themselves on their sides partially flexed in a semi-fetal position providing a good lateral view of the larvae.

Larval Rearing Period and Intermolt Durations

Larval rearing period (the time from stocking of Z1s to the beginning of the postlarval, glaucothoe stage) is strongly influenced by temperature (Table 8-4). In our first year of production trials, we utilized ambient, unheated seawater which averaged 5.9 °C. The time from stocking to the glaucothoe stage averaged 47 days. In subsequent years, we tested higher larval rearing temperatures. Our best survivals, averaging approximately 50% from stocking to glaucothoe occurred over the range of 8 to 11 °C. At 8 °C, mean larval rearing period from stocking to glaucothoe averaged 30 days while at 11 °C, total larval rearing period to glaucothoe was reduced to only 21 days. In production trials carried out in 2009 and 2010, we averaged approximately 50% survival (yield of 25 glaucothoe per liter) at both 8 and 11 °C. Likewise, in 2013, at a mean rearing temperature of approximately 9.5 °C, in six 1200 L LRTs we averaged 59% survival from stocking of Z1s to the glaucothoe stage with a larval period from stocking of Z1s to first glaucothoe averaging 26 days. Over the 8 to 11 °C temperature range for a cohort of RKC larvae of the same age all stocked on a single day, it typically takes three days from the appearance of first glaucothoe until essentially all (>90%) of the larvae have molted into the glaucothoe stage.

Table 8-4. Intermolt period of each larval stage at 8 and 11 °C.

	Intermolt	Intermolt
	Period	Period
Larval	at 8 °C	at 11 °C
Stage	(Days)	(Days)
Z1	6	4
Z2	7	5
Z3	7	5
Z4	10	7
Z1 to G	30	21

Larval Population Estimates

During the larval rearing run, population estimates are made at stocking of newly hatched Z1s and during each subsequent larval stage, typically mid Z2, mid Z3, mid Z4, and at the beginning of the glaucothoe stage (once >90% have molted into the glaucothoe stage). These estimates can be made directly from the LRT using the volumetric method. First, the incoming flow-through seawater is turned off and the exchange sieve is removed and replaced with a plug (consisting of a 1" long piece of 1" diameter PVC pipe with a cap). Then a weighted air stone with its air tubing attached to a portable compressor is lowered to the bottom center of the 1200 L LRT to provide vigorous aeration (~20 lpm) to evenly suspend all the larvae in the tank. Then, five 1 L samples are scooped from the evenly mixed tank. The larvae from each 1 L sample are then poured through a 500 µm sample sieve partially immersed in a petri dish to keep the larvae in a cushion of seawater. The larvae from each 1 L volumetric sample are then counted in the sample sieves and the average of the five counts yields the average number of larvae per liter in the tank. This value is then multiplied by the total volume of the 1200 L tank to yield the population estimate of total number of larvae in the tank. In a successful larval rearing run initially stocked at 50 larvae per liter, average survivals we typically observe are approximately 90%, 80%, 60%, and 50% at mid Z2, mid Z3, mid Z4, and early glaucothoe, respectively. When stocking the LRTs at 50 larvae per liter, consistent survival from Z1 to glaucothoe at or above 50% is our production goal. This is equivalent to a target production yield of at least 25 healthy glaucothoe per liter of LRT volume.

Larval Health Monitoring

In commercial and stock enhancement hatcheries, daily assessment of larval health is a routine and important component of hatchery management. Daily observation of the larvae is essential for monitoring the progress and general health of the developing larvae. Observing the larvae under a compound microscope can help identify problems which might occur, allowing hatchery personnel to take actions to limit potential mortality or at least better understand the causes of mortality. For example, observing development of filamentous bacteria on the larvae would likely prompt the hatchery manager to adjust the larval rearing protocol to respond by increasing water exchange rate, adjusting feed levels, reducing larval density, ensuring optimum temperature, or some practical combination of these and/or other factors to attempt to improve the health of the larvae.

Larval Health Assessment Protocol

Health assessments are made using the following protocol. A beaker is used to scoop a random sample of at least 10 larvae from the well aerated, evenly mixed LRT. The larvae are then poured through a 500 µm sample sieve on a cushion of seawater by keeping the sieve partially immersed in a Petri dish. A random sample of ten larvae is then pipetted out of the sample sieve and transferred in drops of seawater to a microscope slide. Each crab larva is then observed under 40X and 100X magnification and evaluated for gut fullness, gut lipid droplet number and maximum diameter, deformities, attached debris, and filamentous bacteria. Observations are recorded in the Larval Health Assessment Log ([Appendix 6](#)). A brief discussion of each larval health assessment category follows.

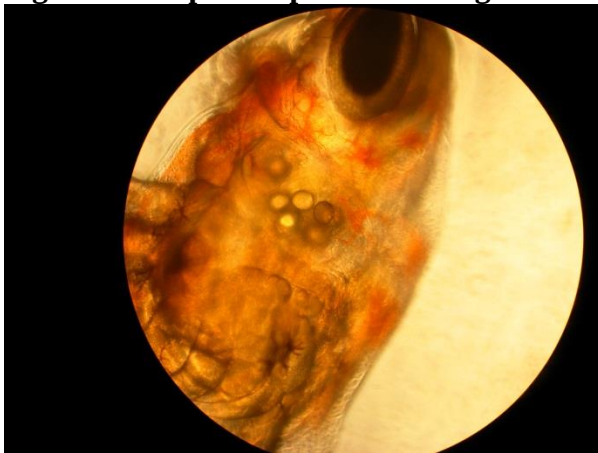
Gut Fullness

Microscopic examination of the larval gut is useful to determine whether the larvae are consuming food. Unfed larvae exhibit clear, transparent, empty guts. Well-fed larvae exhibit tinted material similar in color to the food they have ingested in the forward portion of the gut. Even continuously well fed larvae often exhibit an apparently empty posterior region of the intestine, suggesting relatively rapid clearance of this portion of the digestive tract. One can attempt to quantify relative gut fullness as follows: empty, almost empty, moderately full, or full scored as 0, 1, 2, or 3 respectively. However, since observations on gut fullness are somewhat subjective, it is probably better expressed simply as gut empty or partially full.

Lipid Droplet Number and Maximum Diameter

Microscopic observation of larvae is also useful for assessing larval energy reserves and feeding success by counting the number and measuring the maximum diameter of lipid droplets present in the forward region of the larval gut ([Figure 27](#)). The maximum number of total lipid droplets greater than 25 μm in diameter in a single focal plane at 40X magnification should be counted as an indicator of the relative energy reserves possessed by the larvae.

Figure 27. Lipid droplets in the region of the RKC foregut.



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In addition, the ocular micrometer is used to measure the diameter of the largest lipid droplet observed in the larval gut. As discussed earlier, even freshly hatched, unfed larvae exhibit lipid droplets which were derived from yolk in the eggs. [Table 8-5](#) indicates the mean number and maximum diameters of lipid droplets typically observed in unfed freshly hatched Z1s and well fed larvae at stages Z1 through early glaucothoe. Note that the maximum lipid diameters increase with increasing larval stage. Lipids are a source of stored energy for RKC larvae. The presence of large and numerous lipid droplets tends to indicate larvae are obtaining not only enough energy from their diet for growth and metabolism but are also able to store excess energy in the form of lipids for later use. Therefore, number and maximum diameter of lipids serve as a simple and useful indicator of relative zoeal health and feeding success. Maximum lipid droplet diameters corresponding to those specified in [Table 8-5](#) indicate the larvae are feeding well, while smaller and fewer lipids suggest larvae are underfed or not consuming the feed.

Table 8-5. Typical microscopic lipid droplet profiles observed in well fed, healthy RKC larvae.

	Mean #	Mean of Max. Lipid Droplet Diameter	Max Diameter of Lipid Droplets Observed
Larval Stage	>25 µm*	(µm)	(µm)
Unfed Z1 at Hatching	6	45	80
Late Z1	8	60	85
Late Z2	9	90	125
Late Z3	10	120	155
Late Z4	10	150	200
Day 1 Glaucothoe	12	150	200

* Number of lipids observed in single focal plane under 40X magnification. This is an index of lipid droplet abundance, not a complete count of all lipid droplets present.

To test whether maximum lipid diameter and feeding success are correlated, a simple observational experiment was performed. Ten randomly collected Z2s were transferred from a well fed 1200 L LRT into an aerated 1 L beaker of 12 °C seawater without food and daily measurements of maximum lipid diameters were made. At transfer to the unfed beaker, mean maximum lipid size of the Z2s was 70 µm (range 60-90 µm). After 48 hours without food, mean maximum lipid diameter had declined to 47 µm (range 35-60 µm) and after four days without food no visible lipid droplets remained in any of the Z2s in the unfed beaker. After four days without food in the 12 °C beaker, all larvae were still alive but pale and swimming weakly. Meanwhile in the 1200 L LRT from which the sample Z2s came which continued to be fed to satiation, mean maximum lipid diameter had increased to 74 µm (range 65-90 µm) after two days and 76 µm (range 30-100 µm) after 4 days, further suggesting that lipid droplet size monitoring is a useful measure of feeding success and stored energy reserves.

Deformities

Microscopic observation of the larvae is also used to look for deformities such as bent, twisted, or malformed tail spines. Bent or twisted spines may indicate molting difficulty in the larvae. Damaged or broken delicate structures such as the rostrum and carapace spines should also be looked for and if present, may indicate mechanical injury from handling stress or possible difficulty during the molting process.

Attached Debris

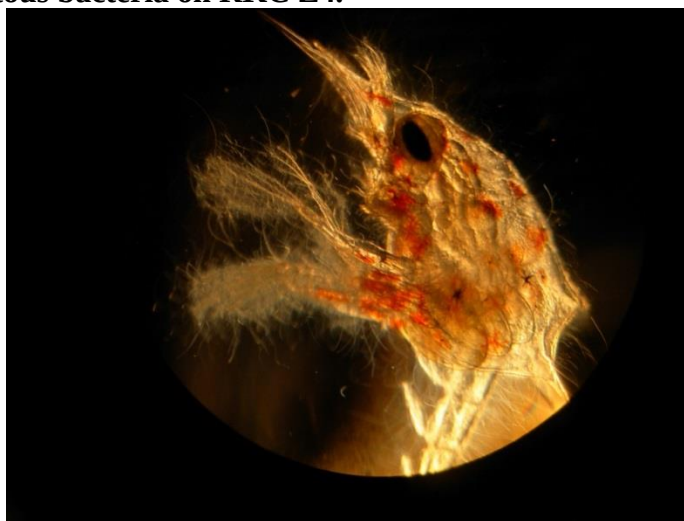
Microscopic examination can also reveal the presence of attached debris on the exoskeletons of the larvae. Material adhering to the larval shell or appendages can potentially interfere with the larva's ability to swim and feed and therefore reduce its chances for survival. If attached debris is observed on the surface of the larvae, the type of material should be identified and the percent of the larval surface fouled should be estimated, with findings recorded in the Larval Health Assessment Log ([Appendix 6](#)). The most commonly observed type of attached debris is usually clumped or unhealthy algae cells which tend to adhere to the shell and spines of larvae. If this is observed, additional effort should be made to ensure all microalgae being added to the LRT is healthy and of high quality. Attached debris on the larvae might also be related to substandard

water quality and should prompt the hatchery manager to increase seawater exchange rate and carefully inspect the seawater filtration system, making adjustments if necessary.

Filamentous Bacteria

When microscopically assessing larval health, a key feature to look for is the presence of filamentous bacteria. The filamentous bacteria we have encountered on RKC larvae appear to be a *Leucothrix*-like bacterium, possibly *Leucothrix mucor*. It appears as fine, hair-like strands that grow attached to the exoskeleton, appendages, and gills of the larvae (Figure 28). It is often first observed as tiny (<50 µm long) strands at the tip of the rostrum and on the fine spines of the larval swimming appendages (setae of the maxillipeds). Each day after first appearing on the outer surface of the larval shell, the bacterial strands tend to lengthen and spread over progressively greater areas of the larval exoskeleton. As the presence and severity of filamentous bacteria increases, it can eventually interfere with the larva's ability to swim, feed, and respire, and if the larva is unable to molt out of it, can eventually result in larval mortality.

Figure 28. Filamentous bacteria on RKC Z4.



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The general pattern of filamentous bacteria (FB) development we have observed on the RKC larval stages is as follows. Typically no FB is seen on Z1s. Stage Z2 and Z3 larvae generally develop minor FB by about 6 days post molt (dpm) but molt out of it before it becomes problematic. In stage Z4, minor FB is typically first observed five dpm and becomes progressively severe with increasing length of bacterial strands and percent of larval exoskeleton covered with each passing day. In a heavily infected RKC larva, it is not uncommon for bacterial strands to reach greater than 500 µm in length and cover virtually 100% of the larval shell and appendages. Since the Z4 stage tends to have the longest intermolt period (see Table 8-4), FB is most problematic in this stage, resulting in some mortality of late stage Z4s. Further, FB likely reduces the health of Z4s which do manage to survive and molt into the glaucothoe stage. Due to the reduced feeding ability of late Z4s fouled with FB, the glaucothoe into which they molt probably have reduced energy reserves on which to survive during the approximate 20 day non-feeding glaucothoe stage, subsequently resulting in lower survival to the first juvenile stage (C1).

When filamentous bacteria is observed on larvae during microscopic health assessments, the percent of the larval surface covered and the maximum length of bacterial strands on the infected larvae is recorded in the Larval Health Assessment Log ([Appendix 6](#)).

Our most successful approach to date for reducing filamentous bacterial fouling on the RKC larvae has been to raise the rearing temperature. By raising the larval rearing temperature, we were able to reduce intermolt duration during the most problematic Z4 stage from a mean of ten days at 8 °C to a mean of seven days at 11 °C ([Table 8-4](#)). At both temperatures, fouling with strands of FB during Z4 did not begin to occur until about five days post Z3 to Z4 molt. Since the Z4 larvae reared at 11 °C molted more quickly into the glaucothoe stage, they experienced lower levels of accumulated fouling and exposure time to FB than larvae reared at lower temperatures.

Other General Indicators of Larval Health

In addition to the above criteria, larval growth, duration of intermolt periods, and behavior are also useful indicators of larval health and should be monitored and recorded during larval rearing production runs. When larvae are cultured at 8-11 °C using the protocol described in this manual, if intermolt durations are significantly longer than those specified in [Table 8-4](#), it may suggest a feeding problem or nutritional deficiency. Further, if larvae are observed to be pale, lethargic, or swimming weakly, these are also indicators of suboptimal larval health.

Harvesting Glaucothoe

At the conclusion of the larval rearing run, the number of glaucothoe produced is estimated using the volumetric technique described in the “Larval Population Estimates” section of this chapter. Glaucothoe are then drain harvested (as described in the “Periodic Complete LRT Cleaning” section of this chapter). Harvested glaucothoe can then be transferred into the glaucothoe rearing tanks where they are raised to the first juvenile crab stage (C1) using the techniques described in the “Glaucothoe Rearing” chapter of this manual.

Chapter 9. Glaucothoe Rearing (G to C1)

Glaucothoe Rearing Overview

Glaucothoe rearing is the second phase of RKC hatchery production and follows larval rearing of the zoeal stages. It comprises that portion of the rearing period from first appearance of the glaucothoe stage until molting and metamorphosis into the first juvenile crab stage (C1). RKC glaucothoe are roughly 7 mm in length and weigh roughly 4 mg.

Glaucothoe Characteristics

Red king crab glaucothoe differ from zoeae in several key ways. First, they are morphologically distinct, dramatically different in appearance from the zoeae (**Figures 1 and 26**). Second, their orientation in the water column is upright and they swim in a forward direction as opposed to the backward swimming, head down orientation exhibited by zoeae. Third, unlike the zoeal stages, glaucothoe are thigmotactic and seek out substrate on which to cling. In addition, the RKC glaucothoe stage does not feed, but rather subsists on energy reserves accumulated and stored as lipids during the zoeal stages of development. As such, the rearing protocols for glaucothoe are distinctly different from those of the preceding zoeal larval rearing period.

Glaucothoe Rearing Protocols

It should be noted that our development of large scale glaucothoe rearing protocols is continuing and has not to date yielded survival rates we believe possible. To date, we have developed two primary methods for the glaucothoe rearing stage of production: the “tank” method and “bin” method. The tank method yielded roughly 40% survival from the beginning of the glaucothoe stage to the beginning of the C1 stage in 2009 and 2010. In 2013, we again utilized the tank method but also utilized the bin method for glaucothoe rearing and averaged 56 and 66% survival from G to C1 for each method, respectively. The protocols for both glaucothoe rearing methods are described below, along with some advantages and disadvantages of each.

Tank Glaucothoe Rearing Method

Our primary method for large scale glaucothoe rearing to date has been the tank method. This entails using the same 1200 L tanks in which the larvae were reared. Glaucothoe harvested from the LRTs are stocked into clean 1200 L tanks at a density of 25 per liter (30,000 glaucothoe per 1200 L tank). We then add Fukui artificial seaweed at a density of one 1-meter strand for every 2000 glaucothoe in the tank (15 1-m strands per 1200 L tank). The artificial seaweed is added to each glaucothoe rearing tank as a substrate for attachment of the thigmotactic post-larvae (**Figure 29**).

Figure 29. Glaucothoe attached to artificial seaweed.



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The artificial seaweed consists of green or black 12-cm polypropylene fronds attached to a one meter long center cord. When viewed under a dissecting scope, each frond resembles microscopic netting enabling easy attachment for glaucothoe. The seaweed is vertically oriented within the tanks with one end anchored at the base of the tank and the rest of the buoyant strand floating vertically toward the surface (Figure 30). To anchor one end of the artificial seaweed, we use a plastic zip tie to attach a clean 200 g stone wrapped in plastic mesh netting. Since glaucothoe do not feed, no food is added during the two to three week glaucothoe rearing period. Each 1200 L glaucothoe rearing tank is managed as a flow-through system. Seawater continuously enters the surface of the tank at 6 LPM and flows out through a 500 μ m exchange sieve (Figure 30) which retains the glaucothoe. Each tank also has two 1.5" square silica air stones, each positioned on the tank bottom about one foot away from the center of the tank. Each air stone provides gentle aeration ($\sim$ 3 lpm). Temperature is maintained at 8 to 11 °C and should be held steady varying less than 1 °C daily and salinity should remain between 30 to 35 ‰ and vary less than 1 ‰ daily. The bottom of each tank should be inspected several times daily and if dead glaucothoe are observed, these must be siphoned out of the tank to maintain good seawater quality and protect the surviving glaucothoe.

Figure 30. Glaucothoe rearing tank containing artificial seaweed.



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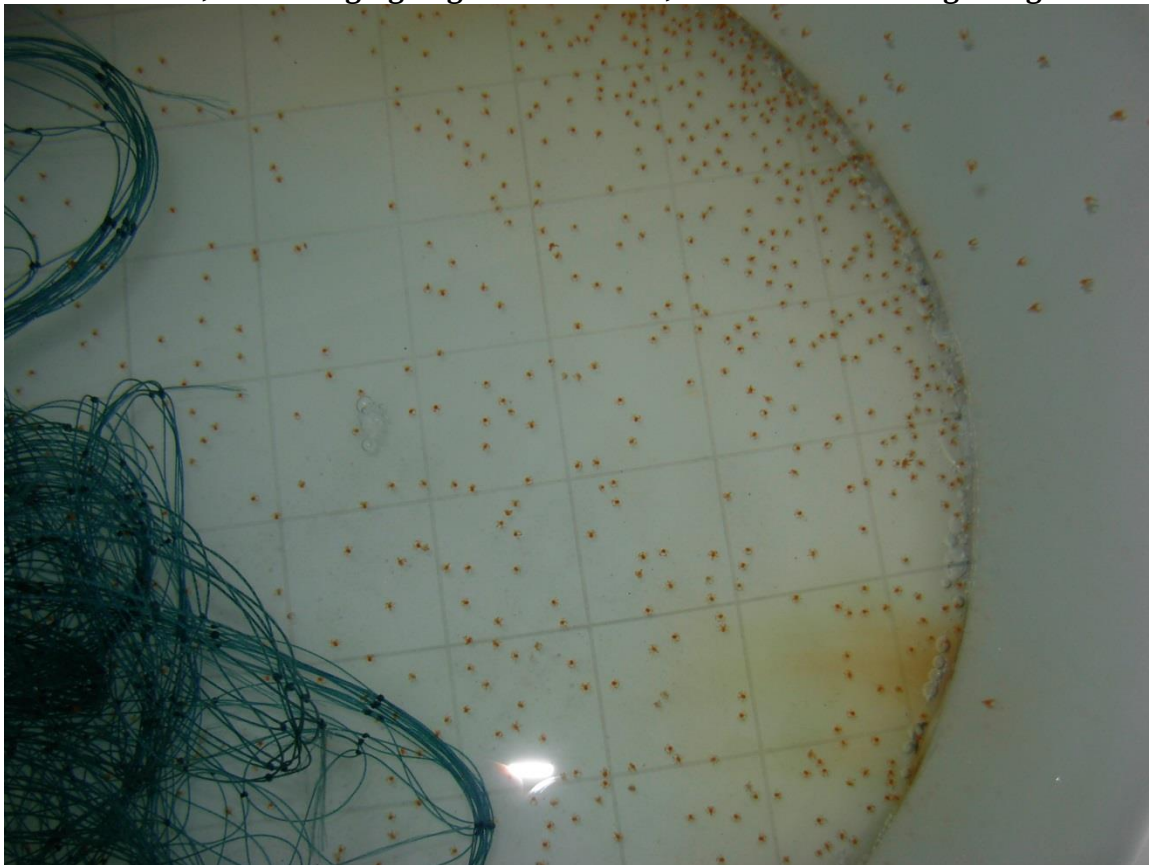
The primary advantage of the tank rearing method is economic, in that the same tanks used for larval rearing can also be used for glaucothoe rearing. The primary disadvantage is that the gently sloped conical bottom of the tank, while ideal for larval rearing is suboptimal for glaucothoe rearing. This is because the glaucothoe which settle on the bottom of the tank rather than on the artificial seaweed tend to slip down into the bottom center of the gently sloped conical tank where they form piles of glaucothoe clinging to each other, which is not conducive to high rates of survival. A slight but largely superior variation on this theme is to stock the glaucothoe into flat-bottomed round fiberglass tanks containing artificial seaweed or gillnet as substrate onto which glaucothoe can cling. We tested this protocol in 2013, stocking 21,000 glaucothoe in a flat bottomed, five foot diameter fiberglass tank containing gillnet as substrate. Survival from early G to C1 was ~50% and the undesirable clumps of glaucothoe seen in the conical bottom tanks were not observed in the flat bottom tank.

Bin Glaucothoe Rearing Method

The bin rearing method utilizes the same tank and bins described in the hatching tank chapter of this manual. Ten bins are held in a single rectangular, 3000 L tank. Each bin is 23" in diameter,

23" tall, and has a bottom composed of 100 μ m mesh screen and a surface area of ~ 0.25 m². The depth of seawater in each bin is maintained at ~ 15 " yielding a volume of ~ 100 L per bin. Seawater enters the top of each bin from a spray bar positioned above such that each bin receives a continuous flow rate of ~ 1.5 lpm. Seawater downwells out of the 100 μ m mesh screen at the bottom of each bin. Each individual bin is stocked with 2000 glaucothoe. Into each bin we add 100 g of clean gillnet as additional substrate onto which the glaucothoe can cling. Within the bins, glaucothoe are observed clinging to both the gillnet and the 100 μ m mesh screen at the base of the bin (Figure 31). Over the course of the glaucothoe rearing period, each bin is carefully inspected each day for any mortalities. When dead glaucothoe are observed, they are immediately siphoned out of the bin. To achieve this, first the gillnet upon which healthy glaucothoe are clinging is removed from the bin and placed in a 20 L bucket containing seawater of the same temperature and salinity. After the gillnet is removed, the seawater in the bin is stirred in a circular motion causing dead glaucothoe to drift into and settle in the center of the bin. Live glaucothoe remaining in the bin will be tightly clinging to the 100 μ m mesh bottom of the bin or swimming up in the water column, most frequently along the walls of the bin. Thus the dead glaucothoe can be easily siphoned out of the bin. Following siphoning, the gillnet with healthy clinging glaucothoe is put back into the bin.

Figure 31. Healthy glaucothoe in a rearing bin (some clinging to 100 μ m mesh screen at the base of the bin, some clinging to gillnet substrate, and some swimming along bin wall).



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The primary advantage of the bin rearing method is that it yields higher survival during glaucothoe rearing than the tank method. Using this method, it is also easier to see and remove

mortalities, allowing for better water quality and protection of healthy glaucothoe. The primary disadvantage is that this method requires more floor space per 1000 C1s produced than the tank rearing method.

Glaucothoe Rearing Period

Rearing period from appearance of first glaucothoe to first C1s is strongly influenced by temperature, averaging approximately 35 days at 5 °C, 20 days at 8 °C and 14 days at 11 °C. Generally for a cohort of glaucothoe of the same age, the time between the appearance of first C1s until essentially the whole group has molted from Gs to C1s is typically about five days at 8 °C.

Harvesting C1s

When harvesting C1s from the 1200 L glaucothoe rearing tank, it is typical for the majority of the early C1s to be clinging to the artificial seaweed or gillnet in the tank. These substrates with the attached C1s are removed one strand or clump at a time and the clinging C1s are shaken off the substrate into a clean flat-bottom rectangular harvest tank containing seawater. Once all the C1s on the substrates are shaken into the harvest tank, the remaining C1s in the glaucothoe rearing tank are drain harvested using the same technique described in the “Periodic Complete LRT Cleaning” section of this manual and then also transferred into the harvest tank.

Harvesting C1s from the bins is accomplished by first shaking the clinging C1s that are on gillnet off and into a flat bottom harvest tank. Many of the C1s remaining in the bin will be clinging so tightly to the 100 µm mesh screen at the base of the bin that it is difficult to use rinsing alone to dislodge them. To remove the C1s, lift the bin out of the tank and tilt it at a 45 degree angle. Then vigorously tap the screen by flicking the fingernail of the index finger against it and the C1s will release from the screen and slip down into the tilted base of the bin. They can then be easily rinsed into the harvest tank.

Once all the C1s are in the harvest tank, a rectangular-framed, 1 mm mesh aquarium net is used to scoop a small (~1 g) sample from the harvest tank. This sample will be used to determine the average live weight of the C1s harvested, which can later be used to estimate the total number of C1s harvested. To collect the sample, the front flat edge of the aquarium net is skimmed along the smooth flat bottom of the harvest tank sweeping up and collecting C1s. The excess seawater is then allowed to drain off the sample of C1s until no excess seawater is dripping from the sample or the net. Then remaining excess moisture is drained away from the crabs by touching the portion of the net in contact with the crabs to paper towels until no excess moisture can be drawn away. The “damp blotted” sample of crabs is then weighed on an electronic balance to obtain the damp blotted sample weight to the nearest 0.1 g. The number of C1s in the sample is then counted and this number divided into blotted sample weight to determine the average weight of an individual C1. Typically, early stage C1 RKC weigh ~5 mg each. Once the average C1 weight is determined, the entire group of crabs harvested is weighed in the same manner, usually roughly 10 g at a time. The total damp weight is then divided by the average individual damp weight to provide an estimate of the total number of C1s harvested. Once the total number of C1s harvested is estimated, the crabs can be packed and transported to out-planting sites or stocked into nursery tanks for production of larger, later staged juveniles.

Chapter 10. Nursery Rearing (C1 to later stage juveniles)

Nursery Rearing Overview

Nursery rearing (also known as juvenile rearing) begins when the crabs reach the first juvenile crab stage (C1) and encompasses the culture methods used for producing later stage juveniles. Juveniles appear like miniature adults and are benthic, crawling on the bottom or clinging to substrates. RKC juveniles grow by molting. The rate of molting is strongly influenced by temperature, with higher temperatures resulting in shorter intermolt periods. At about 8 °C, the early juvenile stages molt roughly once per month. After about 30 days, the first stage juvenile (C1) molts into the second stage juvenile (C2) and so on. At a nursery rearing temperature of 12 °C, the inter-molt period is much reduced. [Stoner et al., 2010](#), found that at 12 °C mean intermolt duration from C1 to C2 was 17 days, C2 to C3 was 21 days, and C3 to C4 was 22 days. The approximate carapace widths (excluding spines) and mean weights of the early stage juveniles are presented in [Table 10-1](#).

Table 10-1. Approximate carapace width (excluding spines) and live weight of RKC juvenile stages C1 – C6.

Stage	CW (mm)	Wt (mg)
C1	1.8	5
C2	2.3	10
C3	3	20
C4	4	50
C5	5	100
C6	6.5	200

Whether nursery rearing beyond C1 is a necessary phase of a stock enhancement program for RKC replenishment is as yet an unresolved question. It seems unlikely, for there are a number of advantages to releasing the C1 stage. The overriding problem during the nursery phase of culture of RKC is cannibalism which results in a high level of mortality among the juvenile crab. This problem is compounded by the economic necessity of rearing the juveniles at high density to cost effectively produce large numbers of crab (millions) required to rebuild depleted populations.

Up to the C1 stage, crab survival in the hatchery is much higher than in nature. In the hatchery, using the techniques described in the preceding chapters, survival from hatch to the beginning of the C1 stage is about 30%. In nature, due to predation, starvation, unfavorable currents which can carry larvae into unsuitable habitat, and other mortality factors, survival from hatch to the first juvenile stage is believed to be less than one in a thousand (<0.1%) ([Kovatcheva et al., 2006](#)). Therefore, from hatch to C1, survival in the hatchery is likely 300 times higher than it is in nature. During larval and glaucothoe rearing, cannibalism **IS NOT** a problem, even at high

density in the hatchery tanks. However, this situation changes once the crabs reach the first juvenile stage. From C1 onward, cannibalism **IS** a major problem within the confines of the nursery rearing tanks. At the required high stocking densities, even when the crabs are fed to satiation and provided with substrate for additional cover, cannibalism is extreme. Indeed due to cannibalism in our mass rearing nursery systems, at best we only achieve roughly 67% survival from C1 to C2, 67% survival from C2 to C3, and 50% survival per molt thereafter. In nature, where the crabs can disperse into suitable habitat and aren't held under artificially crowded conditions, survival per molt is estimated to be roughly 85% (Stevens, 2006). **Table 10-2** compares expected survivals of hatchery produced C1s released as C1s into nature versus juveniles held longer term in nursery tanks, based on the above assumptions.

Table 10-2. Expected RKC survival in nature vs nursery tanks (beginning with 100,000 hatchery produced C1s).

Stage	Nature	Nursery
C1	100,000	100,000
C2	85,000	67,000
C3	72,250	44,890
C4	61,413	22,445
C5	52,201	11,223
C6	44,371	5,611

In summary, from hatch to early C1, survival of the crabs is roughly 300 times higher in the hatchery than in nature, while from C1 onward, survival of the crabs is significantly higher in nature than in the nursery. It therefore seems obvious that the most effective stock enhancement strategy would be to release the hatchery produced crabs into carefully selected habitat at the C1 stage.

There are, however, circumstances where extended nursery culture may be appropriate. Examples might include the production of large numbers of later stage juveniles for research or commercial purposes.

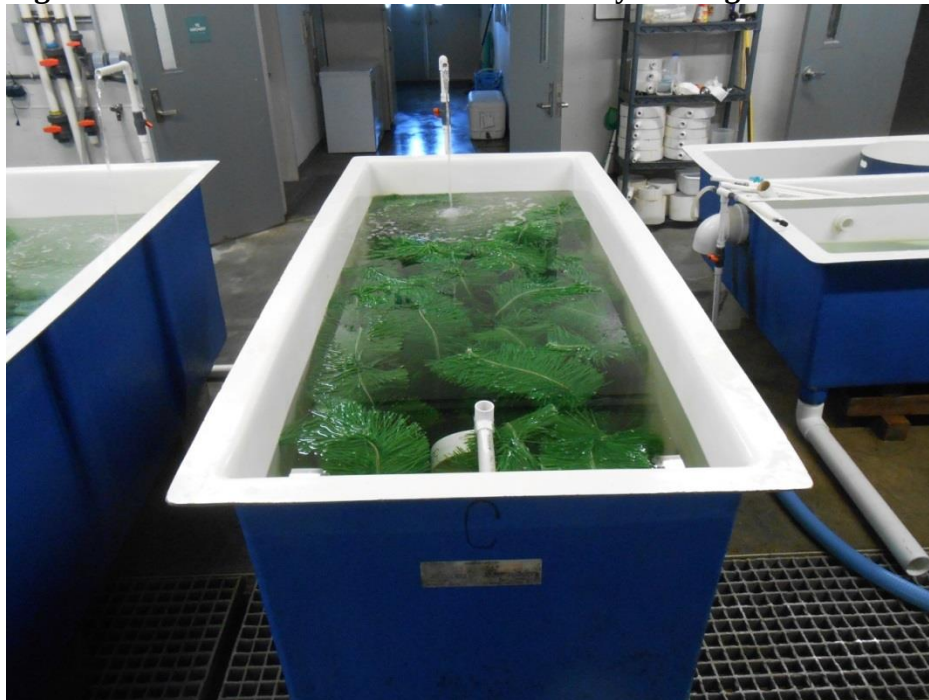
The most efficient and effective method we have yet developed for the mass rearing of juvenile RKC is in flat bottomed rectangular tanks containing artificial seaweed and/or gillnet as substrate. Specific methods for high density nursery rearing are detailed in the following sections of this chapter.

Nursery Rearing Tank Specifications

The nursery rearing tank is a flat bottomed, 84" long, 34" wide, and 33" deep rectangular fiberglass molded tank with a smooth white interior. Surface area of the bottom of the tank is

1.84 m². It has a 2" diameter drain in the bottom center of one end into which a 2" PVC standpipe is inserted. The standpipe determines the depth of seawater in the tank as seawater flows out of the tank through the standpipe. The standpipe is cut such that seawater depth in the tank is 28", resulting in an operating volume of 1300 L. The tank is mounted on a level platform such as four by four lumber or concrete blocks so that drain pipes can be installed under the tank. A flat bottom tank on a level surface is best because any slope tends to cause the juveniles to aggregate and compounds the problem of cannibalism. Artificial seaweed or gillnet is added to the tank as substrate onto which the juveniles can climb and cling (Figure 32). This provides additional three dimensional structure within the tank onto which the juveniles can spread out, allowing for a higher stocking density. The artificial seaweed is cut into individual 1 meter long strands with one end attached to a clean 200 g rock wrapped in plastic netting. The netting surrounding the rock is attached to one end of the artificial seaweed with a plastic cable tie. The rock serves as an anchor keeping one end of the artificial seaweed on the bottom of the tank while the remainder of the buoyant strand rises vertically through the water column to the surface. We add 18 strands of artificial seaweed to the 1.8 m² rectangular nursery tank. This is equivalent to one strand for every 0.1 m² bottom area of the tank. When adding gillnet as substrate, no anchor is necessary as the gillnet sinks such that some of it is in contact with the tank bottom and the rest is loosely suspended in the water column. We add about 100 g dry gillnet per 100 L of tank volume. A 100 g piece of gillnet when spread out into a single flat panel is roughly 7' long by 7' wide (49 ft²).

Figure 32. 1300 L flat bottomed RKC nursery rearing tank with artificial seaweed.



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Stocking C1s in the Nursery Rearing Tank

We stock the 1300 L rectangular nursery rearing tank with 25,000 C1s. This is high density nursery rearing, equivalent to a stocking density of ~14,000 C1s per square meter of tank bottom

area. Sample weights are used to estimate the number of C1s stocked. For example, if the mean weight of the C1s is found to be 5 mg, then we stock a total weight of 125 g into the nursery rearing tank. As we stock the tank, we spread the 125 g of C1s evenly across the tank bottom. Initially, one will observe clumps of C1s clinging to each other on the tank bottom. Within 15 minutes, healthy C1s will begin to disperse evenly across the tank bottom as the clumps break up and disappear. After the C1s have evenly dispersed, we add the 18 one meter strands of artificial seaweed or roughly 1300 g of loosely spread gillnet to the tank. Within several hours, many C1s will typically be observed climbing on and clinging to the substrate. Within several days, typically one half of the C1s will be on the substrate and the other half evenly dispersed over the tank bottom.

Nursery Seawater Quality and Flow Rate

We rear the juveniles at temperatures ranging from 5 to 12 °C and at a salinity of 30–32 ‰. [Kovatcheva et al. \(2006\)](#) recommends a rearing temperature of 10–12 °C and salinity of 32–35 ‰ for nursery rearing of juvenile RKC. Temperature and salinity should be stable, varying less than 2 °C and 1 ‰ daily. We have found a continuous seawater flow rate of 8 lpm to be adequate for maintaining good water quality in the 1300 L nursery rearing tanks. This is a flow rate equivalent to roughly nine times tank volume per day.

Nursery Feeding

We feed the RKC juveniles a mixed diet. One of four types of commercially available feeds is fed each day on an alternating basis. The four feed types we use are Cyclop-eeze® (Argent Chemical Laboratories, WA, USA), frozen enriched *Artemia*, Zeigler™ (Zeigler Bros, Inc., PA, USA) post-larval shrimp feed, and Otohime B2 (Reed Mariculture, CA, USA). All four of these feeds when stirred into seawater just prior to feeding will sink to the tank bottom where they are readily available for crab consumption. Also by adding the required daily portion of feed to ~2 L of seawater in a beaker, the feed particles can be evenly mixed so that when poured throughout the surface of the tank, the feed will spread evenly across the tank bottom.

The Cyclop-eeze® and enriched *Artemia* are stored frozen and the appropriate ration must be thawed just prior to feeding. The Zeigler™ and Otohime are stored in sealed containers in the refrigerator. Cyclop-eeze® is a frozen copepod (~800 µm length) high in carotenoids and omega-3 highly unsaturated fatty acids (HUFAs). Otohime B2 is a high protein diet developed for marine fish and consists of 360-620 µm sinking particles. Newly hatched San Francisco Bay strain *Artemia* nauplii (~450 µm length) have high levels of lipids and unsaturated fatty acids. *Artemia* nauplii are enriched ([see Appendix 7 in this manual for enrichment method details](#)) with A1 DHA Selco® (INVE Aquaculture, UT, USA) enrichment media for 24 h to enhance their nutritional quality and then frozen. The frozen enriched *Artemia* are ~750 µm length. Zeigler™ PL Redi-Reserve commercial shrimp feed (400-600 µm particles) is commonly used in crustacean aquaculture due to its high levels of Highly Unsaturated Fatty Acids.

Our goal is to feed the juvenile RKC to satiation while avoiding over feeding which can foul the tank bottom and degrade seawater quality. Our feeding rate is based on crab biomass in the tank and tank temperature. We use these parameters to establish a feeding table and then carefully

inspect the tank bottom daily to adjust the feed rates as required. For example, if the tank is observed to contain uneaten feed 24 hours after a feeding, the feed rate is adjusted slightly downward to fine tune the feeding rate. **Table 10-3** specifies the base feeding rate for juvenile RKC at three rearing temperatures. Note that Cyclop-eeze[®] and frozen enriched *Artemia* are fed at a five times higher ration than the Zeigler and Otohime. This is due to the fact that live (or once live) feeds (Cyclop-eeze and frozen enriched *Artemia*) contain a high moisture content not present in the dry feed particles (Zeigler and Otohime).

Table 10-3. Nursery feed rate guide (% biomass to be fed daily) for RKC juveniles at three temperatures. This table serves as a guide but is adjusted based on observed consumption rates.

	Cyclop-eeze or Artemia	Zeigler or Otohime
5 °C	7.5%	1.5%
8 °C	10%	2%
11 °C	15%	3%

For example, if the nursery rearing tank temperature is 8 °C and the tank contains 25,000 C1s averaging 5 mg (0.005 g) in weight, and the day's feed type is Otohime, the feed guide allows us to calculate the amount of Otohime to feed that day. First, we calculate the biomass of crab in the tank = 25,000 x 0.005 g = 125 g. Then we use the food guide to determine the amount of Otohime to feed the tank. The table shows that at 8 °C, we feed an amount of Otohime equal to 2% of the crab biomass in the tank. Therefore, we feed 125 g x 0.02 = 2.5 g of Otohime.

Nursery Tank Cleaning

The nursery tanks are cleaned periodically on an as needed basis. If feed rates are carefully managed, tank cleaning will only be required roughly once per week. However, if mortalities or an accumulation of uneaten feed or waste is observed on the tank bottom, it should be siphoned from the tank. A 15' long piece of 1/2" diameter clear vinyl tubing attached with three plastic zip ties to a 6' long, 1" diameter PVC pipe handle works well as a tank cleaning siphon (**Figure 23**). The waste siphoned from the tank is passed through a 1 mm mesh screen. The waste passes through the screen down the drain and any juveniles accidentally siphoned out will be retained on the screen and can be returned to the nursery tank.

Chapter 11. Transporting Juveniles

During the first seven years of AKCRRAB research, we have transported nearly 100,000 hatchery produced juveniles to other researchers. Our hatchery produced juvenile RKC have enabled and supported research on juvenile growth, molting, hormones, predation, behavior, out-planting, ocean acidification, and other topics. The vast majority of juveniles shipped have been C1s, though we have shipped lesser numbers of later stage juveniles, some as late as stage C6.

The technique we use for shipping juveniles has proven adequate for our needs to date. However, we need to conduct more research in order to determine the most efficient, cost effective methods for safely shipping larger numbers of juveniles (likely the C1 stage) in order to support future out-planting for stock enhancement

The transport method we use is outlined below. All materials used in shipping need to be cleaned, disinfected in 200 ppm hypochlorite, and thoroughly rinsed prior to and after each use. It is especially important that the gillnet used is thoroughly rinsed and has no residual chlorine on it before use. On the day before packing, juveniles to be shipped are not fed to allow them to clear their digestive tracts, ensuring better water quality in the shipping containers. On the morning before packing, the juveniles to be shipped are transferred into a smooth, flat bottomed holding tank without substrate so they can be easily captured using an aquarium net, weighed, and then placed into the shipping containers.

Method for packing juvenile RKC for transport

1. Place 50 g clean gillnet into a clean 1-gallon jug.
2. Add 2 L seawater to the jug. We typically use seawater in the 5-8 °C temperature range for transporting juveniles. Seawater in the transport jugs should be no more than 2 °C colder than the tank from which the juveniles are being collected.
3. Capture juveniles from holding tank with aquarium net
4. Allow excess seawater to drain off the net and juveniles and then blot off excess moisture from the bottom of the net with paper towels.
5. Weigh out 9 g juveniles into plastic 1 L beaker on electronic balance. This would be equivalent to 1800 C1s (average weight 5 mg).
6. Add seawater to beaker containing 9 g C1s and pour into the jug containing seawater and gillnet
7. Reach into the jug to grasp the top edge of the gill net and gently spread it so that it loosely fills the jug from top to bottom and side to side. While being shipped, most of the juveniles in the shipping jug will slowly disperse on and cling to the gillnet
8. Top off the jug with seawater.
9. Screw on jug cap tightly and then tape down pour spout with electrical tape and wrap electrical tape around the seam between the cap and jug.
10. Place each sealed jug into a plastic garbage bag and seal top of bag shut with a cable tie. This is a safeguard to capture any seawater in case there is any leakage from the jug during transport.

11. Place bagged jugs in cooler and add 5 1.5 lb frozen ice packs to the cooler. We use a 100 Quart cooler which will hold 6 1-gallon jugs. The cooler packed in this manner weighs approximately 90 pounds (Figure 33).
12. Seal the seam of the cooler shut with a couple of wraps of duct tape. Then further seal the lid of the cooler shut by adding a couple of wraps of duct tape around the cooler near each end.
13. Tape on the address label and shipping permit (if required) and ship to destination by express air cargo service.

Figure 33. 100-quart cooler with six 1-gallon jugs (containing juvenile crabs and gillnet) and ice packs (for keeping cooler contents at ~4 °C) during transport.



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Using this packing technique, we have consistently achieved greater than 95% transport survival when total time from packing to unpacking is 24 hours or less (Figure 34).

Figure 34. One-gallon transport jug containing juvenile RKC and gillnet upon opening following a 24 hour shipment.



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Chapter 12. Conclusion

It is hoped that the protocols described in this manual can be further tested and refined and ultimately implemented to operate a large scale stock enhancement hatchery capable of producing millions of red king crab first stage juveniles (C1s) to replenish RKC stocks for the benefit of Alaska's commercial, subsistence, and personal use fishermen. Research is ongoing at APSH to further refine and develop these techniques for large scale hatchery production of red king crab.

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Appendix 1. Broodstock Receiving Log

Species:

Capture location:

Stock:

Capture date:

Date to hatchery:

Date measured and tagged:

[illegible]

Appendix 2. Broodstock Holding Log.

Holding Tank #:

Species:

Stock:

Contains Broodstock #'s:

[illegible]

Appendix 3. Egg Health Assessment Log.

Date:

Species:

Stock:

[illegible]

Appendix 4. Hatch Tank Log

Hatching Tank #:

Species:

Stock:

[illegible]

Appendix 5. Larval Rearing Log.

LRT #:

Number Stocked:

Species:

Stocking Density:

Date Stocked:

Z1s from Females Tag #'s:

[illegible]

Appendix 6. Larval Health Assessment Log

Tank #:

Date:

Species:

Day:

	1	2	3	4	5	6	7	8	9	10
Larval Stage										
Lipid #										
Max Lipid Diam										
% Fil Bac										

Notes:

Tank #:

Date:

Species:

Day:

	1	2	3	4	5	6	7	8	9	10
Larval Stage										
Lipid #										
Max Lipid Diam										
% Fil Bac										

Notes:

Tank #:

Date:

Species:

Day:

	1	2	3	4	5	6	7	8	9	10
Larval Stage										
Lipid #										
Max Lipid Diam										
% Fil Bac										

Notes:

Tank #:

Date:

Species:

Day:

	1	2	3	4	5	6	7	8	9	10
Larval Stage										
Lipid #										
Max Lipid Diam										
% Fil Bac										

Notes:

Appendix 7. *Artemia* Hatching, Enrichment, and Counting Procedures

The methods used by the AKCRRAB Program at the APSH for producing enriched *Artemia* as a live food source for king crab larvae are summarized below.

***Artemia* Cyst Prehydration/Disinfection**

The required amount of *Artemia* cysts are weighed out (assume ~150,000 *Artemia* will be produced per gram of cysts). The cysts are then transferred to a 100 µm mesh bag and rinsed for several minutes with freshwater (FW). The rinsed cysts are then placed in a well illuminated bucket containing 10 L FW, 33 ml Clorox® bleach (6% hypochlorite), and vigorously aerated for 30 minutes. This exposes the cysts to a 200 ppm hypochlorite solution for 30 minutes and kills bacteria and fungi that may be present on the cysts. Up to a maximum of 50 g of cysts per liter can be disinfected in this manner. After 30 minutes, the disinfected cysts are poured back through the 100 µm mesh bag to collect them and then rinsed again with FW for several minutes to remove residual chlorine.

Cyst Incubation/Hatching

The rinsed, disinfected cysts are then transferred to a clean, well illuminated, cylindroconical hatching tank containing 100 L seawater (SW) which is vigorously aerated to keep all cysts suspended in the water column. Up to a maximum of 2 g of cysts per liter of hatching tank volume can be used. SW temperature should be maintained at 27-28 °C and salinity should be between 25 and 35 ‰. After 24 hours in the hatching tank, the *Artemia* are harvested. This is accomplished by first covering the tank and turning off the aeration for about 10 minutes. During this time, the *Artemia* will settle to the bottom of the hatching tank while most the cyst casings will float to the surface. After allowing 10 minutes for the *Artemia* to accumulate near the bottom drain of the hatching tank, the bottom drain valve is opened and about 10 L of SW containing most the hatched *Artemia* is collected in a bucket. The bottom drain is fitted with clear vinyl tubing allowing for visual inspection of the *Artemia* harvested so that if the first small portion of the harvest contains many un-hatched, brown cysts, these can be discarded. The freshly hatched *Artemia* are orange in color. After the first 10 L harvest, the hatching tank is allowed to sit un-aerated for ten more minutes after which a second ~10 L harvest of *Artemia* is collected in another bucket. The remainder of the hatching tank contents is then discarded down the drain. The two 10 L buckets containing the harvested *Artemia* are then poured through a 100 µm mesh bag to collect them and then rinsed for several minutes with FW.

***Artemia* Enrichment**

The thoroughly rinsed, newly harvested *Artemia* are then transferred to a well aerated 100 L enrichment tank containing SW at ~22°C and 25-35 ‰. The volume of SW used in the enrichment tank should be sufficient to ensure *Artemia* density in the tank is ~300/ml. DC DHA Selco® (also known as A1 DHA Selco) enrichment medium is then weighed out into a 5 L plastic beaker with a handle. The amount added to the beaker is 0.6 g Selco per liter of enrichment tank volume. Before adding the Selco to the enrichment tank, it is first emulsified. This is done by adding 3 L of seawater to the Selco in one beaker and

then pouring the mixture back and forth between two 5 L beakers for about a minute until the Selco is completely emulsified in the seawater. The emulsified Selco is then added to the enrichment tank containing the *Artemia*. After a 24 hour enrichment period, during which the *Artemia* feed on and pack their guts with the Selco, the enrichment tank is drain harvested through a 100 μm mesh bag. The enriched *Artemia* captured within the bag are then rinsed thoroughly with FW for about five minutes. The rinsed enriched *Artemia* are then transferred into a well aerated 10 L bucket containing 8 °C SW where they can be counted and temporarily stored until being fed to the crab larvae in the LRTs.

***Artemia* hatching (left) and enrichment tanks.**



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***Artemia* Counting Procedure**

Newly harvested enriched *Artemia* in the well aerated 10 L storage bucket are counted in the following manner. A 1 ml sample is pipetted from the uniformly mixed, aerated bucket and transferred to a 100 ml beaker prefilled with 99 ml SW. This dilutes the concentration of *Artemia* 100 fold from its concentration in the harvest bucket. The *Artemia* in the sample beaker are then evenly mixed and three 1 ml samples are pipetted out and then counted in a 100 μm mesh sample sieve. The average of these three sample counts is then multiplied by 1,000,000 to provide an estimate of the total number of enriched *Artemia* in the harvest bucket. Up to 50 million enriched *Artemia* can be stored for 12 hours in a well aerated 10 L bucket of SW maintained at 8 °C.

***Artemia* Tank Cleaning**

Prior to and following each use, *Artemia* hatching and enrichment tanks should be scrubbed with a mix of Clorox bleach and Alconox® detergent and then thoroughly rinsed with FW. All air stones, air tubing, and submersible heaters should also be kept clean and disinfected with an overnight soak in a bucket containing 10 L FW and 10 ml Clorox bleach.

Appendix 8. *Artemia* Production and Enrichment Log

[illegible]

