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Temperature effects on larval survival, larval period, and health of hatchery-reared red king crab, *Paralithodes camtschaticus*

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ABSTRACT

We tested the effects of two rearing temperatures (8 and 11 °C) on survival, larval period, and health of red king crab (*Paralithodes camtschaticus*) larvae in eight hatchery-scale 1200 L tanks as part of a stock enhancement feasibility study. Larvae were stocked at a density of 50 larvae L^{-1}. Survival did not vary from the newly hatched first stage zoea to the glaucothoe and first juvenile crab stage (C1) between the two temperatures. Survival averaged 50% from stocking to the glaucothoe stage and 20% from stocking to C1. Overall, 480,000 Z1s were stocked and 96,200 C1s were produced. Larval periods were significantly shorter at the higher temperature, averaging 21 days from stocking to glaucothoe and 35 days from stocking to C1 at 11 °C compared to 30 days to glaucothoe and 50 days to C1 at 8 °C. Specific attributes of larval health measured in this study included the size and relative number of lipid droplets in the anterior region of the larval gut as a measure of stored energy reserves and percentage of the surface of the larval exoskeleton fouled with filamentous bacteria. Quantitative assessment of accumulated lipids as measured by maximum lipid droplet diameters and relative numbers of lipid droplets in the anterior region of the gut was not significantly different between larvae reared at the two temperatures, suggesting that larvae were able to accumulate similar quantities of lipid energy reserves at both temperatures. Filamentous bacterial fouling was greater during the late Z4 stage in the larvae reared at 8 °C compared to those reared at 11 °C, which was likely due to the longer intermolt duration at the lower temperature allowing more time for bacterial accumulation on the exoskeleton. We found that red king crab larvae can be cultured in a substantially shorter time period without compromising survival or health by increasing rearing temperature from 8 to 11 °C.

1. Introduction

Historically, king crab has been one of Alaska's most valuable fisheries; however, many once lucrative king crab fisheries are now closed because of low stock abundance. The red king crab (*Paralithodes camtschaticus*) fishery around Kodiak Island which peaked with a commercial harvest of 94 million pounds in 1965, has been closed for almost 30 years because of low stock abundance (Bechtol and Kruse, 2009). Further, red king crab fisheries in Cook Inlet, Prince William Sound, the Aleutians, and the Alaska Peninsula have remained closed to commercial fishing for extended periods and remain closed today. Despite prolonged fishery closures, red king crab stocks in many regions have failed to recover, causing economic hardship for fishermen, processors, and coastal communities. In response, the Alaska King Crab Research, Rehabilitation, and Biology (AKCRRAB) program was initiated in 2006 to develop hatchery techniques for producing king crab juveniles and to explore the feasibility of using stock enhancement to replenish depressed king crab stocks.

In nature, red king crabs metamorphose through four planktonic larval (zoeal) stages (Marukawa, 1933) over a period of 60 to 80 days depending on environmental conditions (Kovatcheva et al., 2006). The zoeal stages consume both phytoplankton and zooplankton (Paul and Paul, 1980) before molting into the post-larval, nonfeeding glaucothoe stage (Abrunhosa and Kittaka, 1997a, 1997b; Epelbaum, 2002). Glaucothoe are thigmotactic and settle to the bottom seeking structurally complex habitat such as bryozoans and hydroids (Stevens, 2003) to which they cling prior to molting into the benthic first juvenile crab stage (C1) measuring approximately 1.7 mm carapace width and weighing approximately 4.5 mg (Daly et al., 2009). In nature, the duration of the glaucothoe stage is believed to be 20 to 30 days (Kovatcheva et al., 2006). Planktonic larvae are subject to many mortality factors including starvation (Paul et al., 1979), predation (Haflinger and McRoy, 1983), and unfavorable currents (Jackson and Strathmann, 1981) which can result in dispersal to unsuitable habitat, resulting in low survival to C1. Estimates of survival from hatch to the glaucothoe stage in nature range from 0.7 to 3% (Shirley and Shirley, 1989a) with

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survival from hatch to the first juvenile stage estimated to be less than 0.1% (Kovatcheva et al., 2006).

Hatchery rearing of king crab through this critical phase of the life cycle followed by out-planting into suitable habitat has the potential to replenish depleted stocks and is currently in progress for shrimp, crab, and lobster species worldwide (Hamasaki and Kitada, 2008; Secor et al., 2002; Stevens, 2006). Techniques for hatchery scale larval rearing were developed 30 years ago for swimming crab (*Portunus trituberculatus*) in Japan, more recently for Chinese mitten crab (*Eriocheir sinensis*) in China (Li et al., 2001; Zhang et al., 1998), and blue crab (*Callinectes sapidus*) in Chesapeake Bay, USA (Secor et al., 2002; Zmora et al., 2005). Larval rearing research on king crab has been conducted in Japan (Nakanishi, 1987; Stevens, 2006), Russia (Kovatcheva et al., 2006), and on a small scale in Alaska (Persselin, 2006; Shirley and Shirley, 1989b; Stevens et al., 2008). As research continues, it is critical to develop cost effective larval rearing protocols that maximize production of healthy juveniles in a hatchery setting for potential out-planting.

Rearing temperature has profound effects on larval survival. The range of optimal water temperatures for red king crab larvae estimated from vitality levels encompassed the broad interval between 3 and 18 °C with survival rates highest at temperatures of 8 to 13 °C (Nakanishi, 1987). Kurata (1960) found the temperature tolerance of red king crab larvae to be from 0.5 to 15 °C, and Shirley and Shirley (1989b) found 96 hour Z1 survival to be uniformly high for larvae reared below 15 °C. Kovatcheva et al. (2006) recommended rearing temperatures of 7–8 °C for culture of red king crab larvae and 10–11 °C for glaucothoe. We compared larval rearing temperatures of 8 and 11 °C and measured the effects of these temperatures on survival, larval period, and health of red king crab reared in hatchery scale tanks.

2. Materials and methods

2.1. Broodstock acquisition and husbandry

Eighteen ovigerous females (broodstock) were captured in late November 2009 in Bristol Bay by the commercial fishing vessel F/V Stormbird using baited pots. Broodstock were transported to the University of Alaska's Seward Marine Center, in Seward, Alaska on December 1, 2009, where they were held in 2000 L round flat bottomed fiberglass tanks at a density of nine crab $tank^{-1}$ for a period of about three months before hatching began. Continuous seawater flow through at a constant flow rate of approximately 15 L min^{-1} was provided to each holding tank. Average (±SE) holding temperature was 5.87 ± 0.04 °C and salinity was stable at 31.5‰. Crabs were fed *ad libitum* a mixture of chopped squid and herring twice weekly at a ration of approximately 20 g feed $crab^{-1}$ $feeding^{-1}$. All broodstock survived until hatching began on February 26, 2010.

2.2. Hatching tank management

Once hatching began, broodstock were transferred to Alaska's only commercial shellfish hatchery, the Alutiiq Pride Shellfish Hatchery, adjacent to the Seward Marine Center, where the larval rearing experiments were conducted. Individual females were placed into separate cylindrical hatching bins (0.58 m diameter, 0.58 m deep) with 100 μm mesh bottoms. Ten hatching bins were contained in a 3000 L rectangular hatching tank which was supplied with continuous ambient seawater at 15 L min^{-1}. Seawater entered each bin from above and downwelled through the bin and out the 100 μm mesh screen at the base of the bin. The depth of seawater in each bin was 0.25 m and the volume of seawater in each bin was approximately 66 L. Mean hatching tank temperature averaged 6.0 °C and salinity was steady at 32‰.

2.3. Larval rearing

Larval rearing experiments were conducted in eight 1200 L fiberglass molded tanks (Reiff Manufacturing) with gently sloping conical bottoms. Four tanks were maintained at 8 °C (8.23 ± 0.01 °C, min: 8.00 °C, max: 8.52 °C) and four were maintained at 11 °C (10.75 ± 0.04 °C, min: 9.85 °C, max: 11.98 °C) from stocking to the first juvenile stage. All tanks were stocked with newly hatched stage 1 zoeae (Z1) at a density of 50 larvae L^{-1} (60,000 larvae $tank^{-1}$) from a minimum of six broodstock to minimize potential maternal effects on larval quality. At stocking, larvae were acclimated in the larval rearing tanks from 6 °C (hatching tank and initial larval rearing tank temperature) to 8 and 11 °C (experimental larval rearing temperatures) at a rate of 2 °C h^{-1} This was accomplished by gradually adding 8 or 11 °C seawater to the gently aerated flow through tanks. During larval rearing, each tank received continuous flow through of seawater at a mean flow rate of 6.5 L min^{-1}. Salinity averaged ~32‰ and fluctuated less than 1‰ over the course of the experiment. All tanks were fed *ad libitum* a diet of live San Francisco Bay strain *Artemia* which had been enriched with DC DHA Selco® (Inve Aquaculture) for 24 h prior to feeding. Crab larvae were fed twice daily at 8 AM and 8 PM at a ration of approximately two enriched *Artemia* mL^{-1} of larval rearing tank volume at each feeding. At this feeding rate, density of uneaten enriched *Artemia* in the larval rearing tanks always remained above 0.5 mL^{-1} 12 h after the morning feeding (immediately prior to the 8 PM feeding) indicating that the larvae were being fed to satiation. In addition, these feed rates were consistent with *Artemia* rations fed red king crab larvae in previous studies (Kovatcheva et al., 2006; Nakanishi, 1987). From 8 AM until 8 PM, a 100 μm mesh seawater exchange sieve allowed for seawater flow through while retaining enriched *Artemia* and larvae in the tank. Then at 8 PM, the 100 μm mesh sieve was replaced with a 500 μm mesh sieve which allowed waste and uneaten *Artemia* to be gradually flushed from the tank overnight. Aeration was continuously supplied using an air stone positioned in the bottom center of each tank and kept the crab larvae and enriched *Artemia* evenly suspended in the water column. Each tank received a once daily addition of 12 g disodium EDTA (ethylenediaminetetraacetic acid) yielding an initial concentration of 10 ppm. EDTA is a synthetic chelator that increases survival of hatchery-cultured crustaceans such as marine shrimp (Wyban and Sweeney, 1991). EDTA is believed to protect crustacean larvae from toxic effects of heavy metals and may have inhibitory effects on some types of bacteria (Brock and Main, 1994; Castille and Lawrence, 1981). Because the cost of EDTA is relatively low and its use is widespread in crustacean hatcheries, we added it as a potential safeguard.

Percent survival from stocking of Z1s to each subsequent larval stage was estimated using volumetric sampling. Intermolt duration (days) of each larval stage was determined by visually inspecting tanks for freshly shed larval exoskeletons which were clearly visible in the tanks at molting. Larval health assessments were made on newly hatched Z1s at stocking, during mid and late intermolt of each larval stage, and at the early glaucothoe stage. During larval health assessments, a random sample of ten larvae from each tank was examined under a compound microscope at 40× and 100× magnification. For each larva examined, the relative number of lipid droplets was scored and the diameter of the maximum lipid droplet present was measured. Scores of 0, 1, 2, and 3 were given for 0, one to five, six to 10, and greater than 10 lipid droplets ≥ 25 μm in diameter, respectively. Counts were made on lipid droplets ≥ 25 μm in diameter visible in the anterior region of the larval gut under 40× magnification in a single focal plane. The diameter of the largest lipid droplet was measured to the nearest 5 μm using an ocular micrometer in the eyepiece of the microscope at 100× magnification. Also while examining larvae during health assessments under 100× magnification, the exoskeleton of each larva was examined and the percentage of the larval surface fouled with filamentous bacteria was estimated and recorded.