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Effects of diet, stocking density, and substrate on survival and growth of hatchery-cultured red king crab (*Paralithodes camtschaticus*) juveniles in Alaska, USA

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ABSTRACT

Juvenile red king crab (*Paralithodes camtschaticus*) mass rearing was conducted in Seward, Alaska, USA in a king crab stock enhancement feasibility study. Hatchery-raised juveniles were cultured from larvae of 12 ovigerous females collected from Bristol Bay, Alaska, USA. Juvenile instars were cultured in nursery grow-out containers in two phases: (1) C1–C3 juveniles and (2) C3–C6 juveniles. Experiments lasted for 42 and 44 days, respectively, and tested the suitability of various diets, stocking densities and substrates in terms of survival rate and growth. The first experiment (C1–C3) compared fully-factorial treatments of three diets (Cyclop-eeze®, enriched *Artemia* nauplii, or Zeigler™ shrimp feed), three stocking densities (500 m^{-2}, 1000 m^{-2}, or 2000 m^{-2}), and two substrates (none or a combination of artificial seaweed, gillnet, and mechanical biofilter medium). The second experiment (C3–C6) used a mixed diet and compared fully-factorial treatments of two stocking densities of 800 m^{-2} and 1600 m^{-2} and two substrates (as above). No one food produced the highest survival and growth to C3. Cyclop-eeze® yielded highest survival (62.7%) with low growth (wet weight 8.63 mg and CW 2.04 mm). Crabs on shrimp nursery feed had the highest wet weight (10.0 mg) and CW (2.14 mm) but with low survival (44.5%). The highest stocking density resulted in a decrease in survival in both experiments. Lower stocking densities of 500 m^{-2} and 800 m^{-2} had relatively high survival of 58.7% and 48.7%, respectively, while the 1600 m^{-2} and 2000 m^{-2} densities had survival of 30.5% and 44.7%, respectively. Growth appeared to be inhibited at the highest density (2000 m^{-2}) in the C1 to C3 stages, as crabs at this density were smaller and weighed less than at the 500 m^{-2} or 1000 m^{-2} density. Complex artificial substrate increased survival and reduced growth in both experiments, likely due to reduced cannibalism and increased time spent foraging. Among substrate treatments, highest abundances of crabs were observed on artificial seaweed, which may more closely resemble preferred natural substrate. These results suggest that culturing red king crab juveniles at low to intermediate stocking densities with a mixed diet in the presence of artificial substrate provides good survival. Intermediate densities may provide for greater production without great loss in survival.

1. Introduction

King crabs are some of the most commercially valuable crustaceans in the world. Historically, red king crab (*Paralithodes camtschaticus*) was one of the most important fisheries in Alaska USA. Alaskan red king crab populations have fluctuated over the past 30 years (Stevens et al., 2001). Despite fishery closures in the 1980s prompted by population crashes, stocks have not recovered. The cause of this population fluctuation and diminished stock size is unclear; however, recruitment limitation (Blau, 1986), egg predation (Kuris et al., 1991), disease, overfishing (Orensanz et al., 1998), and climate change (Zheng and Kruze, 2000; Stevens, 2006a) have been proposed to explain the lack of recovery. Stock enhancement has the potential to be an effective tool for rehabilitation of depleted stocks and for fishery management and is currently in progress for crab and lobster species worldwide (Secor et al., 2002; Davis et al., 2005; Stevens, 2006b). Progress has been made in king crab rearing technology (Nakanishi and Naryu, 1981; Nakanishi, 1987, 1988; Epelbaum et al., 2006; Kovatcheva et al., 2006; Stevens, 2006a,b), which makes it possible to explore the feasibility for stock enhancement.

Many studies have investigated red (*P. camtschaticus*) and blue king crab (*Paralithodes platypus*) culture on a small scale (Paul and Paul, 1980; Nakanishi and Naryu, 1981; Nakanishi, 1987; Shirley and Shirley, 1989; Epelbaum et al., 2006; Kovatcheva et al., 2006; Persselin, 2006a,b; Stevens et al., 2008); however, many principles that apply to small scale culture do not carry over when dealing with larger systems (Hamasaki et al., 2007). Small scale rearing is often conducted in a controlled environment where physical and biological variables such as temperature, water quality and food availability can be easy to manipulate. In

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large rearing tanks that contain more biomass, these variables are more difficult to control. Although technologies for mass production were developed for other crustaceans (Bannister and Addison, 1998; Nicosia and Lavalli, 1999; Tlusty et al., 2005; Zmora et al., 2005), only few cases of successful hatchery scale production for red king crab exist (Nakanishi, 1987; Epelbaum et al., 2006; Kovatcheva et al., 2006), mainly due to issues associated with the complex early life history of this cold water species, such as a long larval period, slow growth, and cannibalism.

Juvenile red king crab grow-out nursery technology is necessary for large-scale culture at high densities. Grow-out technology has been developed for several lobster species (Sastry, 1976; Yoshimura, 2000; Beal et al., 2002; Tlusty, 2004; Van Wyk and Davis, 2006); however, it is relatively new for red king crab. Many of the logistical challenges of rearing juvenile lobsters such as cannibalism and understanding optimal diets and stocking densities are analogous to juvenile red king crab rearing. Improving hatchery technology is essential to provide cultured animals to researchers studying early life history of red king crabs. Grow-out technology is also necessary for the development of an effective stock enhancement effort and release program. Studies investigating competency issues such as behavioral or morphological modifications induced by artificial feeding regimes and sensory deprivation during rearing are also essential for such a program (Mills et al., 2008). Release programs may require hatchery-raised crabs to reach a minimum size to be physically tagged to distinguish them from wild animals or to increase survival of deployed animals which may be due to ontogenetic shifts in anti-predator mechanisms.

Red king crabs go through four zoeal stages (Marukawa, 1933) before molting to the non-feeding, semi-benthic glaucothoe stage. Glaucothoe then molt into the first juvenile instar (C1) where they take an adult-like form. Juvenile red king crabs are thigmotactic and are typically found in habitats with complex biogenic structure such as hydroids, polychaete tube reefs, and bryozoans (Sundberg and Clausen, 1977; Dew et al., 1991; Loher and Armstrong, 2000; Stevens, 2003; Stevens and Swiney, 2005). Preference for structurally complex habitats may be a behavioral mechanism to avoid predation. In order to grow, juveniles must pass through ecdysis leaving the newly molted crab soft and vulnerable to predators for 2 to 4 days until the new exoskeleton hardens (Kovatcheva et al., 2006).

Historically, complex larval rearing protocols and cannibalism at the juvenile stages have prevented the development of large-scale hatchery technology for rearing juvenile red king crab. The objective of this study was to test the feasibility of mass culturing juvenile red king crab in nursery grow-out conditions through the use of various diets, stocking densities, and substrates using indoor rearing tanks.

2. Materials and methods

2.1. Broodstock and larval rearing

Twelve ovigerous females were captured with pots in Bristol Bay, Alaska USA during November 2007. Crabs were brought to the Alutiiq Pride Shellfish Hatchery in Seward, Alaska, USA and placed in 2000 L tanks containing flow-through ambient seawater ranging from 3.4 to 8 °C with a mean ± SE of 5.15 ± 0.1 °C and fed 20 g chopped herring and squid per crab twice per week. Once hatching began, females were placed in separate bins to isolate larvae. Larvae from each female were mixed and raised in 1200 L cylindrical tanks until the first juvenile instar (C1) stage. Larvae were fed enriched San Francisco Bay strain *Artemia* nauplii daily. *Artemia* nauplii were enriched with DC DHA Selco® (INVE Aquaculture, UT, USA) enrichment media in 100 L cylindrical tanks for 24 h.

2.2. Experiment 1: C1–C3

A total of 15,750 juvenile (C1) red king crabs were used in this first experiment over a 42 day period starting on May 28, 2008. Water temperature ranged from 6.7 °C to 9.9 °C (mean ± SE 8.15 ± 0.1 °C). Salinity was stable at 31–32‰. Recently-settled juvenile (C1) crabs were collected from larval rearing tanks, mixed randomly and placed in containers, hereafter called silos. Each silo is a flat bottomed 58 cm tall by 58 cm diameter cylindrical container with a 100 µm mesh screen on the bottom with a surface area of approximately 0.25 m^2 and a volume of approximately 65 L. Ten silos were placed in each of six larger 3200 L rectangular tanks. Three factors (diet, density and substrate) were varied resulting in 18 treatments that were each replicated three times. Diets included Cyclop-eeze® (Argent Chemical Laboratories, WA, USA), frozen enriched *Artemia* nauplii (hereafter *Artemia*), and Zeigler™ (Zeigler Bros, Inc., PA, USA) shrimp nursery feed. Stocking densities tested were 500 m^{-2}, 1000 m^{-2}, and 2000 m^{-2} with substrate either present or absent. Since the mesh-bottomed silos allowed water exchange within the larger tanks, each silo within the same large tank received the same diet treatment. Density and substrate treatments were randomly assigned within the larger rectangular tanks. All silos were flow-through with water entering from the top with a flow rate of approximately 1.5 L min^{-1}. Incoming seawater was sourced from a deep-water intake at ambient temperature and was UV sterilized and filtered to 5 µm. Survival was assessed by counting all crabs at the start and end of each experiment. Growth was assessed by weighing (wet weight) and measuring (carapace width) six haphazardly selected crabs from each treatment at the start and end of the experiment. Wet weight was determined by blotting crabs to remove excess water and then individually weighing each crab. Carapace width was determined with an ocular micrometer under 40× magnification. Carapace width was measured because the orientation of the small, motile crabs allowed more consistent measurements than carapace length. Exuvia were examined to determine when molting to the next instar stage occurred.

We used commercially-available diets that are commonly used in aquaculture. Cyclop-eeze® is a frozen whole adult copepod approximately 800 µm in length reared in arctic lakes. This feed is high in carotenoids and other essential components of a juvenile king crab diet such as omega-3 Highly Unsaturated Fatty Acids (HUFAs) and is used in many aquaculture applications including crustacean larval rearing (Lieberman, 2001). We prepared enriched *Artemia* by hatching San Francisco Bay strain *Artemia*, enriching them with DC DHA Selco® enrichment media for 24 h and freezing. Newly hatched instar 1 *Artemia* nauplii are crustaceans approximately 400 µm in length that have high levels of lipids and unsaturated fatty acids. The San Francisco Bay (SFB) strain has particularly high levels of HUFAs (Tizol-Correa et al., 2006). Enriching the *Artemia* nauplii enhances their nutritional quality and yields high survival during the larval stages of red king crab development at Alutiiq Pride Shellfish Hatchery (unpublished data) and juvenile American lobsters (*Homarus americanus*) (Tlusty et al., 2005). Freezing the enriched *Artemia* causes them to sink and evenly disperse allowing them to be readily available to the juvenile crabs. Zeigler™ PL Redi-Reserve commercial shrimp nursery feed consists of 400–600 µm particles and is commonly used in crustacean aquaculture due to its high levels of HUFAs (Meade and Watts, 1995). For the Cyclop-eeze® and frozen enriched *Artemia* treatments, 0.5 g wet weight was added to each silo daily. Shrimp nursery feed is a dry feed and was added at 0.1 g dry weight $silo^{-1}$ daily. Crabs were fed to satiation. Excess feed and waste were removed weekly from silos lacking substrate, whereas silos containing substrate were unable to be cleaned without disturbing the entire system and were left alone to avoid disrupting the attachment of juveniles on the various substrates.

Half of the silos contained no substrate and half contained equal volumes of three substrates: Fukui artificial seaweed (Walter and Liebezeit, 2003), EHEIM mechanical biofilter medium (Kovatcheva et al., 2006), and commercial gillnet (Menteforte and Bervera, 1994). Crabs could move freely among different substrates within a silo. Artificial seaweed consists of 12-cm polypropylene fronds attached to