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Increasing hatchery production of juvenile red king crabs (*Paralithodes camtschaticus*) through size grading

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ABSTRACT

Cannibalism is problematic in hatchery production of many crustaceans and can be exacerbated by differential growth, size variability, and asynchronous molting. We conducted two hatchery experiments in Seward, Alaska, USA to investigate effects of size grading on survival and growth of juvenile red king crabs (*Paralithodes camtschaticus*). We reared larvae and subsequent juveniles until juveniles were eight weeks post-settlement. For each experiment, these eight-week old juvenile crabs (approximately 2.0 to 4.5 mm carapace width) were sorted using a 3.3 mm mesh screen into: "small," "large," and "ungraded" size classes. In the diet experiment, the three size classes were stocked at a density of 600 crabs m^{-2} and reared either on a control diet of commercial mariculture feeds or the control diet supplemented with astaxanthin and calcium for 53 days. In the density experiment, the three size classes were stocked at densities of 400, 900, and 1400 crabs m^{-2} and fed the control diet plus astaxanthin and calcium for 31 days. Survival in both experiments was strongly influenced by size grading. Generally, small crabs had higher survival than large and ungraded crabs. Diet was not a significant factor in weight or survival. Small crabs had relatively high survival at all stocking densities, but all size classes had decreased survival with increasing density, likely from cannibalism. Size graded crabs reared at elevated densities yielded improved biomass per rearing area (g m^{-2}) compared to ungraded populations, suggesting lower survival rates may achieve the goal of optimizing hatchery production. Coupled with appropriate stocking densities, size grading could be used in laboratory and hatchery rearing protocols for red king crab and other likely cannibalistic crustaceans to maximize survival, improve hatchery efficiency, and increase the financial viability of large-scale stock enhancement or aquaculture programs.

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1. Introduction

Aquaculture-based stock enhancement can be used to sustain or improve fisheries (Leber et al., 2004; Lorenzen et al., 2010); however, cannibalism is a bottleneck in the production of juvenile fish and crustaceans (Aileen et al., 2000; Alston, 1991; Hecht and Appelbaum, 1988; Hecht and Pienaar, 1993; Liao et al., 2001; Marshall et al., 2005; Sotelano et al., 2012; Zmora et al., 2005). Size variation within a cohort is ubiquitous in fish and crustacean aquaculture (see Brett, 1979; Jobling and Baardvik, 1994; Wickins and Lee, 2002 for a review) and exacerbates cannibalism in the hatchery because of strong resource competition among individuals (Karplus et al., 1986) and size hierarchy effects (see Brown, 1946; Koebele, 1985; Magnuson, 1962; Noakes, 1978 for a review). Although cannibalism likely occurs naturally in the field, cannibalism is likely exacerbated by conditions associated with hatchery culture such as artificial diets, high stocking densities, absence of natural substrates, and elevated temperatures. Improvements in culturing technology are needed to help overcome cannibalism and improve the commercial viability of stock enhancement programs.

Size grading (rearing small and large individuals separately) is commonly used in aquaculture of many species, including crabs (Marshall et al., 2005; Zmora et al., 2005), crayfish (Ahvenharju et al., 2005), freshwater prawns (Daniels and D'Abramo, 1994; Siddiqui et al., 1997; Tidwell et al., 2003), abalone (Heath and Moss, 2009; Mgaya and Mercer, 1995), eels (Karipoglou and Nathanailides, 2009), and fish (Barki et al., 2000; Carmichael, 1994; Wallat et al., 2005) to improve survival, growth, and feeding efficiency. Size grading reduces aggressive interactions by disrupting negative effects of dominant, larger competitors that suppress the growth and survival of subdominant, smaller individuals (Karplus et al., 1986; Tidwell et al., 2003). Maintaining small individuals in the population may be important to conserve natural genetic and phenotypic variation (Frost et al., 2006), because some individuals may have a genetic predisposition for small sizes or slow growth rates (Frost et al., 2006; Gu et al., 1995). The genetic contribution of

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those individuals would be reduced if hatcheries select against slow growers. Regular size grading may increase survival or growth rates of smaller individuals from compensatory effects (Jobling, 2010; Ricker, 1979).

Most crab and lobster stock enhancement programs require hundreds of thousands or even millions of individuals for release (Aiken and Waddy, 1995; Bannister and Addison, 1998; Comeau, 2006; Secor et al., 2002; Stevens, 2006a,b; Zohar et al., 2008). In Japan, annual production of juvenile swimming crabs (*Portunus trituberculatus*) is approximately 60 million with yearly releases ranging from 28 to 42 million (Secor et al., 2002). Stock enhancement has been proposed as a possible population recovery tool for depressed red king crab (*Paralithodes camtschaticus*) stocks in Alaska, USA and will likely also require annual releases of millions to support a viable fishery (Stevens, 2006b). Hatchery production of juvenile red king crabs is limited by cannibalism and slow growth, which can be mediated with artificial substrates, diet modification, and temperature (Borisov et al., 2007; Daly et al., 2009, 2012; Long et al., 2012; Stevens and Swiney, 2005; Stoner, 2009; Stoner et al., 2010a,b). Additional rearing technologies must be developed for a large-scale stock enhancement program to be economically feasible. This study aimed to determine the effects of size grading by investigating survival and growth of size-graded juvenile red king crab reared using different diets and stocking densities.

2. Materials and methods

2.1. Broodstock and larval rearing

Twenty ovigerous females were captured with baited commercial pots in Bristol Bay, Alaska during November 2008 and 2009 for experiments the following spring and summer. Crabs were transported to the Alutiiq Pride Shellfish Hatchery in Seward, Alaska, and placed in 2000 L tanks containing flow-through ambient seawater and each fed 20 g chopped herring and squid twice per week. Once hatching began (April 2009 and 2010), larvae from eight females were mixed and raised in 1200 L cylindrical tanks until the first juvenile instar (C1) stage. Zoeal larvae were daily fed San Francisco Bay strain *Artemia* nauplii, which were enriched with DC DHA Selco® (INVE Aquaculture, UT, USA) enrichment media in 100 L cylindrical tanks for 24 h.

2.2. Nursery grow-out

We collected recently-settled, first stage (C1) juvenile crabs from larval rearing tanks, mixed them randomly and mass reared them in three 2000 L cylindrical nursery tanks for eight weeks. Nursery tanks contained artificial seaweed and commercial fishing gillnet (7.6 cm mesh size) to reduce agonistic interactions among conspecifics (Daly et al., 2009). Crabs were fed the control diet approximately 2% body dry weight daily and excess feed and wastes were removed weekly. The control diet consisted of Cyclop-eeze® (Argent Chemical Laboratories, WA, USA), Otohime B1 and B2 (Reed Mariculture, CA, USA), frozen enriched *Artemia* nauplii, and Zeigler™ shrimp feed (Zeigler Bros, Inc., PA, USA). Each feed type was alternated daily. Cyclop-eeze® is a frozen copepod (~800 μm length) high in carotenoids and omega-3 highly unsaturated fatty acids (HUFAs). Otohime B is a high protein diet developed for marine fish and consists of 200–360 μm (B1) and 360–620 μm (B2) sinking pellets. Newly hatched San Francisco Bay strain *Artemia* nauplii (~450 μm length) have high levels of lipids and unsaturated fatty acids (Tizol-Correa et al., 2006). *Artemia* nauplii were enriched with DC DHA Selco® (INVE Aquaculture, UT, USA) enrichment media for 24 h to enhance their nutritional quality and then frozen. The frozen enriched *Artemia* nauplii (~750 μm length) were negatively buoyant and available for benthic crab consumption. 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 (Meade and Watts, 1995).

2.3. Size grading

After the eight-week nursery grow-out period, crabs exhibited a size range of approximately 2.0 to 4.5 mm carapace width (CW). We collected crabs from mass rearing nursery tanks and sorted by size using a 3.3 mm mesh screen. We sorted equal numbers of crabs (~100) at a time to standardize any potential physical damage. Crabs that fell through the screen are referred to as "small", while crabs retained on top of the screen are referred to as "large". The ungraded treatment represented approximately 50% small and 50% large crabs.

2.4. Limb loss

To determine physical effects of the sorting process, we collected 100 pre-sorted crabs from the nursery tanks and examined them for missing limbs. The same crabs were then sorted by size using screens (described above) and reexamined for limb loss. The numbers of crabs with at least one missing limb and the total number of missing limbs per crab were recorded. The limb loss assessment was replicated three times.

2.5. Experiment 1: diet and size grading effects

We initiated the first experiment in summer 2009 after the eight-week nursery grow-out period. Two factors (three size-grading treatments and two diet treatments) resulted in six treatments that were each replicated six times. Crabs were sorted into small, large, and ungraded size classes as described above. Crabs were stocked at 600 crabs m^{-2} in flat bottomed 58 cm tall by 58 cm diameter cylindrical containers with a 100 μm mesh screen on the bottom, a surface area of approximately 0.25 m^2, and volume of approximately 65 L, hereafter called silos. Nine silos were placed in each of four larger 3200 L rectangular tanks and treatment replicates were randomly assigned among tanks. All silos contained equal amounts (approximately 100 g (0.88 m^2)) of commercial fishing gillnet (7.6 cm mesh size). The gillnet twine consisted of nine woven nylon monofilaments for a total diameter of approximately 1.0 mm and surface area of 88 cm^2 g^{-1}. Gillnet improves survival by providing complex structure with interstitial spaces reducing crab contact with each other (Daly et al., 2009). All silos were supplied with flow-through ambient seawater entering from the top with a flow rate of approximately 1.5 L min^{-1}. Incoming seawater was sourced from a deep-water (~75 m) intake at ambient temperature and was filtered to 5 μm, UV sterilized, and carbon filtered. Temperatures ranged from approximately 8 °C to 12 °C.

Crabs were fed the control diet (described above) and the control diet supplemented with astaxanthin and calcium. Astaxanthin improves survival and growth of hatchery-cultured juvenile red king crabs (Daly et al., 2012), and additional calcium may enhance growth or survival as observed in other species (Hossain and Furuichi, 2000a, b). Supplements consisted of two ground cuttlebones (~12 g each) and 2 g dry powdered NatuRose™ (1.5% pure astaxanthin) mixed with 25 g Zeigler™ shrimp feed and 25 g Otohime B1 and bound with 2 egg whites (~35 g each). Once bound with egg whites, supplements were ground producing particles approximately 400–1000 μm. Supplements were administered in lieu of the control diet twice weekly. Crabs were fed approximately 2% body dry weight daily and excess feed and waste were removed weekly.

The duration of the experiment was 53 days to allow crabs to molt at least once (Stoner et al., 2010a). Survival was assessed by counting all crabs within each replicate at the start and end of the experiment. Growth was assessed by weighing to the nearest 0.01 g (blotted wet weight) ten randomly selected crabs from each replicate at the end of the experiment. Exuvia were examined to determine when molting to the next instar stage occurred.