Aquaculture 424–425 (2014) 86–94

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Storage and utilization of lipid classes and fatty acids during the early ontogeny of blue king crab, *Paralithodes platypus*

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ARTICLE INFO

Article history:
Received 9 October 2013
Received in revised form 9 December 2013
Accepted 11 December 2013
Available online 22 December 2013

Keywords:
Aquaculture
Larvae
Crustacean
Fatty acids
Lipids
Nutrition

ABSTRACT

Stock enhancement, through the release of cultured juveniles, has been suggested as a possible recovery tool for depleted red (*Paralithodes camtschaticus*) and blue (*Paralithodes platypus*) king crab populations in Alaska, USA. Considerable progress has been made in the past decade in red king crab culture technology, but similar technologies are less developed for blue king crabs. We examined changes in organic content, lipid classes, and fatty acids (FAs) during the larval (zoeae), post-larval (glaucothoe), and first juvenile stages to understand energetics, nutritional requirements, and potential dietary deficiencies of this new aquaculture species. Total lipids increased throughout the larval stages and then showed a decrease in both total lipids and neutral storage lipids (triacylglycerols) during the non-feeding glaucothoe phase. The proportions of C_{18} polyunsaturated FAs (PUFAs) dramatically increased from the first zoeal (Z1) to the second zoeal stage (Z2), reflecting the uptake of enriched *Artemia* FAs. Both the selective retention of $C_{20\ \&\ 22}$ PUFAs relative to their diet and the proportional increase in these PUFAs during the non-feeding glaucothoe stage suggest that they are essential for early growth and survival. Analytical measures of total lipids were highly correlated with a hatchery-based visual assessment of maximum lipid droplet size, an index of relative nutritional health. Formulated hatchery larval diets containing high levels of $C_{20\ \&\ 22}$ PUFAs may optimize nutrition in early ontogeny for blue king crabs, but future research should refine specific ratios and absolute amounts.

1. Introduction

King crabs are iconic in Alaska for their importance to commercial, sport, and subsistence harvests. Commercial harvest of blue king crabs (*Paralithodes platypus*) peaked during the early 1980's but population declines caused fishery closures in the late 1990's. The Saint Matthew stock was declared rebuilt in 2009 and briefly reopened to commercial fishing until low abundance estimates caused a fishery closure in 2013 (NPFMC, 2013). The Pribilof Islands stock remains closed to commercial fishing today because of low estimates of population abundance (NPFMC, 2013).

Stock enhancement through the release of cultured juveniles has been suggested as a potential rehabilitation tool for many species worldwide (see Bell et al., 2005; Blankenship and Leber, 1995; Leber et al., 2004; Kitada et al., 2009 for a review), including lobsters (Bannister and Addison, 1998; van der Meeren, 2005) and crabs (Secor et al., 2002; Stevens, 2006). King crabs are suitable candidates for stock enhancement, because they are some of the most commercially valuable crustaceans in the world, and recruitment limitation has been proposed to explain their lack of recovery in the absence of fishing (Blau, 1986). King crab husbandry research has been underway for the last 25 years in Russia, Japan and the United States (Kovatcheva et al., 2006; Persselin, 2006; Shirley and Shirley, 1989; Stevens, 2006; Stevens et al., 2008b) and has built extensively on successful rearing techniques used with other crab species i.e. Chinese mitten crab (*Eriocheir sinensis*, Li et al., 2001) and blue crab (*Callinectes sapidus*, Secor et al., 2002; Zmora et al., 2005). Most recently, the Alutiiq Pride Shellfish Hatchery in Seward, Alaska, has been performing large-scale larval rearing experiments which have successfully investigated rearing needs of red king crab (Daly et al., 2009; Swingle et al., 2013) and blue king crab larvae and juveniles (Daly and Swingle, 2013; Stoner et al., 2013). Established techniques for larval rearing from hatching to the glaucothoe stage have been refined and hatchery infrastructure improved, resulting in improved red king crab survival (Swingle et al., 2013; Swingle et al., unpublished data). Yet similar technologies for blue king crab are less developed (but see Stevens et al., 2008a, 2008b), mainly because of difficulty obtaining brookstock and larval mortality, attributed to sensitivity to hatchery conditions.

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http://dx.doi.org/10.1016/j.aquaculture.2013.12.025

Red and blue king crabs have similar life histories including four planktotrophic larval (zoeal) stages, a semi-benthic post-larval (glaucothoe) stage, and benthic juvenile and adult stages (Marukawa, 1933). Larvae spend several months in the water column (McMurray et al., 1984), which implies that larvae may be transported significant distances from the release location before reaching the settling stage. As such, recruitment of juveniles to a given area may depend on larvae hatched elsewhere, and could explain why some stocks have not responded to traditional management strategies such as area closures. Glaucothoe are non-feeding and exhibit a condition termed "secondary lecithotrophy" in which they utilize internal energy reserves that have been derived from planktonic organic matter ingested and accumulated in earlier larval stages (Anger and Hayd, 2009). However, despite recent production success, glaucothoe rearing is still a hatchery production bottleneck for both species. Glaucothoe rely on nutritional reserves acquired during zoeal stages (Z1 to Z4) to successfully molt to the first juvenile instar stage (C1), which is likely physiologically stressful as individuals undergo significant morphological changes. The bioenergetics and nutritional requirements to successfully complete this transition in body form have not been documented. High levels of mortality during the glaucothoe stage may be due to inadequate nutritional reserves obtained during the larval stages.

Lipids are vital to marine organisms as an energy source and as important structural components of cell membranes (Sargent et al., 1989; Arts et al., 2001), where they maintain membrane fluidity in environments with cold or variable temperatures (Cossins et al., 1997). Lipids are a major source of energy in juvenile and larval crustaceans (Coutteau et al., 1996) with triacylglycerols (TAGs), sterols (STs), and phospholipids (PLs) representing over 80% of the lipid pool (Ouellet and Taggart, 1992; Coutteau et al., 1996). Elevated levels of neutral storage TAGs are crucial to growth and molting success (Wen et al., 2006). These TAGs are often seen as lipid droplets that visibly accumulate in specific tissues, such as the hepatopancreas (Yepiz-Plascencia et al., 2002). The ratio of storage to membrane lipids (TAG/ST) has previously been found to be a good indicator of nutritional condition in a number of larval finfish and crustaceans (Copeman et al., 2002, 2008; Fraser, 1989; Harding and Fraser, 1999).

Certain lipids (i.e. sterols and PUFAs) are essential to larval crustaceans, as they are required preformed in the diet and are vital to normal growth, development and survival (Irvin et al., 2010; Sargent et al., 1999). Fatty acids (FAs) are the major constituent of both TAGs and PLs and the importance of polyunsaturated FAs (PUFAs) has been extensively investigated in marine larval nutrition (Sargent et al., 1999; Watanabe and Kiron, 1994). Specifically, docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (AA, 20:4n-6) are essential FAs (EFAs) for many crustaceans (Holme et al., 2007; Merican and Shim, 1996), however live-foods commonly used for culturing larvae, including *Artemia*, are naturally low in these EFAs (Navarro et al., 1999). As such, enrichment of live foods with EFAs prior to feeding is often necessary (Sorgeloos et al., 2001).

Levels of EFAs in crustaceans can be a good indicator of overall health, as seen in Pacific white shrimp (*Litopenaeus vannamei*) which showed increased tolerance to handling stress when fed a diet enriched with EFAs (Mercier et al., 2009). The importance of dietary PUFAs to the culture of juvenile crustaceans has been investigated for several crab (Suprayudi et al., 2004; Zmora et al., 2005), lobster (Limbourn and Nichols, 2009), and shrimp (Lavens and Sorgeloos, 2000) species. Hatchery biologists have developed a method for semi-subjectively assessing red and blue king crab larval and glaucothoe condition by visually examining the size and number of lipid droplets (Swingle et al., 2013); however, there is currently little understanding of what drives variation in king crab condition. The aim of our study is to provide blue king crab nutritional information at early developmental stages that can be used to gauge physiological condition and identify possible dietary deficiencies. Further, we aimed to validate visual lipid condition indices used in the hatchery.

2. Materials and methods

2.1. Broodstock acquisition

Ovigerous female blue king crabs were collected during the commercial fishery near St. Matthew Island in November–December 2011 by the F/V SeaBrooke. The crabs arrived in Dutch Harbor on December 5, 2011 and were shipped to the University of Alaska's Seward Marine Center, in Seward Alaska on the same day. Crabs were held in a 2000 L round bottomed fiberglass tank for a period of about three months before hatching began in late March 2012. Continuous seawater at a rate of 15 L min^{-1}, average temperature of 5 °C and salinity of 31.5‰ was supplied at a constant flow to broodstock tanks. Crabs were fed ad libitum a mixture of chopped squid and herring twice weekly as in Swingle et al. (2013).

2.2. Hatching and larval rearing

When hatching was first observed, crabs were transferred to the Alutiiq Pride Shellfish Hatchery. Methods for hatching and tank management are described in detail in Swingle et al. (2013). Briefly, larvae were collected from five females, mixed randomly, and stocked at a density of 25 larvae L^{-1} in a single 1200 L tank on April 6, 2012. *Artemia* (hatched for 24 h, then enriched for an additional 24 h with DC DHA Selco) were fed at a density of 2 ml^{-1} once daily (approximately 5 PM) which falls into suggested feeding ranges based on daily feeding rates of *Paralithodes camtschaticus* in laboratory conditions (Epelbaum and Kovatcheva, 2005). Food was retained and flushed on a daily cycle using a semi-static flow-through technique and 500 μm screens (flow-through 7 AM to 5 PM, static 5 PM to 7 AM). Feeding was terminated when all zoeae in the tank molted to the glaucothoe stage.

Glaucothoe rearing was conducted 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. Silos were placed in a larger 3200 L rectangular tank. 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}. Silos were supplied with flow-through 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 and was filtered to 5 μm, UV sterilized, and carbon filtered. Temperatures were maintained at 8–10 °C. Glaucothoe reached the C1 stage on May 22, 2012.

2.3. Health assessments

Larval health assessments were made on newly hatched Z1, at each consecutive larval stage, and at the early glaucothoe stage. During larval health assessments, ten randomly sampled larvae from each tank were examined under a compound microscope at 40× and 100× magnifications. Counts were made on lipid droplets ≥25 μm in diameter that were visible in the anterior region of the larval gut under 40× total magnifications 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× total magnification.

2.4. Weight and lipid analyses

Blue king crabs were sampled for weight, total lipids, lipid classes and FAs during the mid-molt period of each of four zoea stages (Z1–Z4), twice during the glaucothoe stage (early and late) and once during the first juvenile stage (C1). Ten dry weight (DWT) measurements were made on samples of each developmental stage. Crabs were pooled for weight analyses with higher numbers of individuals per sample at earlier developmental stages: 10 individuals at Z1to 2 individuals at