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Molting, growth, and energetics of newly-settled blue king crab: Effects of temperature and comparisons with red king crab

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ABSTRACT

Populations of blue king crab (BKC) (*Paralithodes platypus* Brandt, 1850) have declined in Alaskan waters over recent decades, and substantial effort is being made to rehabilitate the once important fishery with releases of hatchery-reared juveniles. However, little is known about the species' first year of post-settlement life. This study was conducted to evaluate how temperature mediates growth and energy allocation beginning with the first benthic instar (stage C1). Juvenile BKC were reared in four temperatures (1.5 to 12 °C) for a period of 60 days in low-density populations (150 crabs m^{-2}) and 120 days in individual cultures. Growth rate increased rapidly up to 8 °C, and then leveled off. At 60 days, most of the crabs in 1.5 °C remained at stage C1, most in 4.5 °C were C2, and most in 8 °C were C3, while those in 12 °C were highly variable and ranged from C2 to C5. Growth records for individuals revealed an inverse exponential relationship between water temperature and intermolt period (up to 8 °C). A small decrease in molt increment at 12 °C resulted in crabs 6% smaller than those at 8 °C. Total lipid content increased with temperature in C2 BKC, but the response was variable and not significant in later stages. The proportion of storage class lipids (triacylglycerols) increased with an increase in temperature and polar lipids decreased. Concentrations of essential fatty acids were relatively constant over all temperature treatments, indicating that temperature and growth rate did not affect the biochemical condition of juvenile BKC. Survival rates of BKC (>95%) were similar across temperatures and were much higher than rates observed for red king crab (RKC) (*Paralithodes camchaticus* Tilesius 1815) (65–72%) in identical experiments. Growth rates of the two species were nearly identical up to 8 °C, but RKC grew faster than BKC at temperatures greater than 8 °C, with more molts resulting in larger individuals. Fatty acid (FA) signatures supported the lipid class data and showed that BKC had higher proportions of FA associated with energy storage while RKC had higher proportions of polyunsaturated FAs associated with membranes. These results indicate that BKC are the hardier species, and it shows little sign of cannibalism in culture (unlike RKC), but RKC grow faster at high temperature and are less vulnerable to warming climate. These data help to model temperature-dependent recruitment processes in the field and assist in the design of diets and hatchery conditions for production of seed stocks intended for field release.

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1. Introduction

Multi-decadal shifts in oceanographic conditions are well studied in the Gulf of Alaska and the Bering Sea (Hollowed et al., 2001; Hunt et al., 2002; Peterson and Schwing, 2003), and these shifts can cause important changes in the distribution and abundance of both marine fishes and invertebrates (Anderson and Piatt, 1999; Hollowed et al., 2012; Perry et al., 2005). Longer-term trends in sea surface warming and loss of sea ice have also been documented in Alaska (Grebmeier et al., 2006; Sigler et al., 2011; Stabeno et al., 2007), and the potential impacts on economically important species are large (Logerwell et al., 2011; Orensanz et al., 2004). Both positive and negative impacts on fisheries are possible under the scenario of changing climate because of the complexity of changes in temperature, water chemistry, primary productivity, and shifts in species ranges and food web structure (Ainsworth et al., 2011). Temperature has a fundamental and dominant role in the behavior, physiology, growth, and survival of ectothermic animals living in high latitudes and these direct effects are the focus of this study.

King crabs represent some of the most valuable fishery resources harvested from the cold waters of Alaska (Orensanz et al., 1998; Otto, 1989), and two species comprise most of the catch, red king crab (RKC) (*Paralithodes camtschaticus* Tilesius, 1815) and blue king crab

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(BKC) (*Paralithodes platypus* Brandt, 1850). BKC has been secondary to RKC in value; the largest populations of BKC are more northerly and may be more sensitive to warming conditions in the Bering Sea than RKC. Details on the distribution and fishing histories for BKC are provided by others (Herter et al., 2011; Stevens et al., 2008a). Briefly, BKC live in isolated populations in the Bering Sea, Gulf of Alaska, southeast Alaska, and in the western Pacific Ocean near Japan and Russia. In the United States, the vast majority of BKC have been landed from fishing grounds near St. Matthew Island and the Pribilof Islands with a peak catch (value = $12 million) occurring in 1997. However, catch rates in these areas showed signs of decline in the 1980s, and the populations decreased precipitously in the 1990s (Stevens, 2006). The collapse of king crab fisheries in Alaska has been attributed to both over-harvest and unfavorable environmental conditions for recruitment (Dew, 2010; Dew and McConnaughey, 2005; Orensanz et al., 1998). After long closures of the BKC fishery, some stocks are rebuilding, and short-term openings and annual catch limits (400–800 MT y^{-1}) have been re-established at St. Matthew Island since 2009. The fishery for BKC remains closed in all other areas of Alaska.

We have relatively good understanding of fecundity, embryonic development, and hatching in BKC (Herter et al., 2011; Stevens et al., 2008a,b), and the larvae have been cultured in Alaskan laboratories since 2004 (Persselin, 2006). Despite the fact that BKC are being cultured with the primary intent of restoring wild populations through releases of hatchery-reared juveniles, relatively little is known about their first few years of post-settlement life (see Armstrong et al., 1985; Tapella et al., 2009), particularly with respect to how temperature affects survival, growth, and energy allocation. Consequently, this study was designed in the context of two broad themes — understanding how growth and survival of early juvenile BKC may be affected by warming trends in Alaska, and determining how temperature might be used in developing the best possible methods for culturing crabs in a hatchery setting.

Temperature is generally believed to be the most important extrinsic factor affecting growth in crustaceans (Hartnoll, 1982, 2001). The largest impact of temperature is on intermolt period, (i.e., the duration between two successive molts), and there are many examples of decreasing intermolt period with increasing temperature. Temperature can also influence the molt increment (i.e., the change in size that occurs between one instar and the next). Effects of temperature on molt increment are variable, with many crustaceans showing no variation over a wide range of temperature (reviewed by Hartnoll, 1982), although RKC demonstrated a small increase in molt increment with increasing temperature (Stoner et al., 2010a).

Lipids and fatty acids (FAs) are vital to developing marine organisms as they provide a dense source of energy ($kcal \cdot g^{-1}$) and are important structural components of membranes (Copeman and Parrish, 2003; Sargent, 1989). Prior work with RKC juveniles has shown that triacylglycerols (TAGs) are the major lipid storage class (Stoner et al., 2010a), in agreement with other studies on crustacean larvae and juveniles (Nates and McKenney, 2000). Further, Copeman et al. (2012) found that TAG levels in RKC cycled within an inter-molt period, with rapid accumulation shown seven days following a molt. Other major lipid classes in crabs include sterols (STs) and phospholipids (PLs), which form important building blocks of cellular membranes. Relative improvements in both larval and juvenile condition in marine crustaceans, such as American lobster (*Homarus americanum* H. Milne Edwards, 1837) and RKC (Copeman et al., 2012) have been attributed to elevated total lipid, TAG per dry weight, and TAG/ST ratios (Fraser, 1989).

The importance of dietary polyunsaturated fatty acids (PUFAs) to the culture of juvenile crustaceans has been investigated for crabs (Zmora et al., 2005), lobster (Limbourn and Nichols, 2009) and shrimp (Lavens and Sorgeloos, 2000). Crustaceans generally require PUFAs such as docosahexaenoic acid (DHA, 22:6n−3), eicosapentaenoic acid (EPA, 20:5n−3), and arachidonic acid (AA, 20:4n−6) at minimum dietary levels because they cannot be formed de novo from shorter chain dietary precursors (Merican and Shim, 1996). Cultured RKC fed on enriched *Artemia* have lower proportions of essential PUFAs (DHA, EPA, AA) than those collected from the wild (Copeman et al., 2012) and it is thought that nutritional deficiencies play a role in elevated mortality during the early life history stages of crabs reared in hatcheries (Daly et al., 2009). The effects of temperature and dietary FAs on juvenile crab vitality are likely interactive. Previous studies show that high levels of essential PUFAs are retained in marine organisms at low temperatures (Dunstan et al., 1999; Hall et al., 2000, 2002) with elevated PUFAs in cellular membranes being important for thermal adaptation.

This study was designed to evaluate the role of water temperature on survival, molting frequency, growth, and lipid storage in the earliest benthic stages of BKC. Four temperatures were tested (1.5° to 12 °C), spanning the normal range of distribution encountered by newly-settled BKC in the field. Lipid and FA analyses were made at each crab molt stage to explore the effects of temperature and ontogeny on crab condition and energy allocation. The results of these experiments are relevant to both aquaculture and changing climate conditions in Alaska, and are compared with somewhat different growth dynamics observed in RKC.

2. Methods

2.1. Experimental animals

Blue king crabs for this study were supplied by the Alutiiq Pride Shellfish Hatchery in Seward, Alaska. Nineteen ovigerous female BKC were collected with baited pots set near St. Matthew Island, Alaska, in November and December 2011, air transported to Seward, and maintained at the hatchery on chopped herring and squid until their larvae were released in March 2012. Larvae released from at least three females were mixed randomly and reared in three 190 l tanks until the glaucothoe stage was achieved, fed daily with *Artemia* nauplii enriched with DC DHA Selco enrichment media.[1] Glaucothoe were reared in 65 l cylindrical tanks until they molted to the first benthic crab stage (C1).

The juvenile crabs (4 ± 1 days post-settlement) were shipped in insulated containers of seawater (4–5 °C) to the Hatfield Marine Science Center (Newport, Oregon) on 22 May 2012. Upon arrival the crabs were sorted for uniform size representing the C1 stage (~2 mm carapace width), with high mobility and in premium condition. Individuals meeting these criteria were dispersed immediately to the experimental tank apparatus (see below), all set at 6 °C. An additional subset of 25 crabs meeting the same criteria were set aside for measurement.

Beginning the next day temperatures were adjusted by 2 °C per day to the target temperatures of 1.5, 4.5, 8 and 12 °C and design temperatures were achieved within 5 days. For the duration of the acclimation and experimental periods all of the crabs were fed daily on a diet of Cyclop-Eeze and Otohime pelletized food (size B), insuring that fresh food was always present. Uneaten food was removed and replenished weekly. Earlier studies (Daly et al., 2009; Stoner et al., 2010a) have shown that this diet provides for high growth rates in juvenile RKC.

2.2. Experimental apparatus and culture procedures

Methodology described by Stoner et al. (2010a), was followed for the BKC temperature experiments. The crabs were cultured in two different systems. In the first, populations of 40 C1 stage crabs were held in rectangular polyethylene tanks (42 cm wide, 63 cm long, 30 cm

[1] Reference to a trade name does not imply endorsement by the National Marine Fisheries Service, NOAA.

deep) for a density of 150 crabs m^{-2} of tank bottom area. Each tank was supplied with a continuous flow (35 ml s^{-1}) of sand-filtered seawater. Two loose bundles of BioFil filter material (~1 l each) and two loose bundles of black polypropylene gill netting material (1.5 l each) in each tank provided refuge for molting crabs. Four replicate tanks were set up for each of the three coldest temperatures (1.5, 4.5, and 8 °C), and three replicates for 12 °C. In the second system, crabs were held individually in each of the experimental temperatures so that molting schedules could be observed directly and to remove any effects of cannibalism on growth and survival. Individual cells were mesh-sided columns (3.0 mm mesh, 10 cm diam., 17.5 cm high) with a bottom of black plastic glued into place. The size of our holding compartments is well above size ranges known to inhibit growth and survival for recently settled RKC (Swiney et al., in press). A separate liner with 0.5 mm nylon mesh was used to retain the small crabs. Sets of mesh cells were set into polyethylene tanks identical to those used for the population treatments. The tanks were equipped with a floor of plastic latticework beneath which a manifold of PVC plumbing provided a continuous upwelling flow of temperature-conditioned seawater to the cells which stood upright on the false floor with 10 cm of the cells below the water line. Temperature and oxygen levels inside the cells were always equivalent to the levels in the surrounding water bath. Two tanks of individual cells (14 per tank) were designated for each temperature treatment. The cells were systematically moved within each tank on a weekly basis to minimize any location effects.

The population tanks were surveyed for survivors 30 days and 60 days after temperatures reached target levels. On each of these dates the crabs were counted and measured for carapace width (CW). The individual cultures were examined daily for the occurrence of molting, and each crab was measured 4 to 7 days following a molt. While the population-based trials were terminated at 60 days, the growth experiment with individuals was continued an additional 60 days to achieve molts to stage C2 in the coldest treatment.

Digital photographs of crabs were taken to obtain accurate measures of carapace width. Photographs of live crabs were captured with a dissecting microscope equipped with a calibrated digital camera and Image Pro software. The primary measurement taken was carapace width without lateral spines (CW) as defined by Epelbaum et al. (2006) and following the earlier study with RKC (Stoner et al., 2010a). Live crabs were held in a small Petri dish surrounded by seawater ice to maintain the experimental temperature during the measurement. Mean CW at the beginning of the experiment was 1.96 mm (SD = 0.08 mm).

2.3. Crab weights and chemical analyses

BKC cultured individually were sampled for weight, total lipids, lipid classes and FAs at the mid-molt period of C2 (all temperatures), C3 (4.5, 8 and 12 °C) and C4 (8 and 12 °C). Timing for sampling each individual was determined on the basis of the preceding intermolt period. This provided standardization for differences in lipid composition that occur over the molt cycle (Copeman et al., 2012). Two individuals were pooled for the C2 stage in order to obtain sufficient material for lipid class and FA analyses (>200 μg), while individual crabs were analyzed for later molt stages. Four lipid samples were taken from each temperature treatment at each of the above listed sampling periods.

Lipids were extracted in chloroform/methanol according to Parrish (1987) using a modified Folch procedure (Folch et al., 1957). Lipid classes were determined using thin layer chromatography with flame ionization detection (TLC/FID) with a MARK V Iatroscan (Iatron Laboratories, Inc., Tokyo, Japan) as described by MacFarlane and Norton (1999). Three μl of crab extracts were spotted on duplicate silica gel coated Chromarods. Lipids were then focused in acetone and separated into steryl or wax esters, triacyclglycerols (TAGs), free fatty acids, sterols (STs), and polar lipids (PLs) in a one-stage 45-min development bath of 246:54:0.020 hexane:diethyl ether:formic acid. Prior to the selection of this method, five crab samples were developed using a three-phase development system (Copeman et al., 2012; Parrish, 1987). The one-stage development system was chosen because 95% of the lipids were found to be TAGs, STs, or PLs. Data peaks were integrated using Peak Simple software (ver. 3.67, SRI Inc.) and the signal detected in millivolts was quantified using external lipid standards curves for each lipid class (Sigma, St. Louis, MO, USA). Lipid classes were expressed both in relative (mg g^{-1} dry weight) and absolute amounts (μg $animal^{-1}$).

Total lipid was analyzed for fatty acid (FA) composition using the same samples. Fatty acid methyl esters (FAME) were prepared by transesterification with 14% BF_3 in methanol at 85 °C for 90 min (Morrison and Smith, 1964). FAMEs were analyzed on an HP 7890 GC FID equipped with an autosampler and a DB wax+ GC column (Agilent Technologies, Inc., U.S.A.). The column was 30 m in length, with an internal diameter of 0.25 μm. The column temperature began at 65 °C and held this temperature for 0.5 min. Temperature was increased to 195 °C (40 °C min^{-1}), held for 15 min then increased again (2 °C min^{-1}) to a final temperature of 220 °C. Final temperature was held for 1 min. The carrier gas was hydrogen, flowing at a rate of 2 $ml \cdot min^{-1}$. Injector temperature was set at 250 °C and the detector temperature was constant at 250 °C. Peaks were identified using retention times based upon standards purchased from Supelco (37 component FAME, BAME, PUFA 1, PUFA 3). Nu-Check Prep GLC 487 quantitative FA mixed standard was used to develop correction factors for individual FAs. Chromatograms were integrated using Chem Station (version A.01.02, Agilent).

Dry weight (DWT) measurements on individual crabs were made to the nearest 1.0 μg using a microbalance (Sartorius R16OP). Crabs were first rinsed in 3% ammonium formate solution to remove excess salt before being transferred to 5.0-cm^2 pre-weighed aluminum foils and an oven set at 70 °C for 48 h. Foils were removed from the oven, stored in a desiccator, and reweighed within 1 h. Ash weights were measured similarly after drying in a muffle furnace for 12 h at 450 °C.

2.4. Analysis

Survival, growth, and other basic metrics observed in this study were analyzed with standard methods of analysis of variance followed by Fisher's LSD test for multiple comparisons. Appropriate transformations of the data were made as necessary following Levene's test for homogeneity of variance. Linear and non-linear relationships between temperature and growth were analyzed using Systat 13 and curves were plotted with SigmaPlot 12.

Principal component analysis (PCA) was used to simplify multivariate FA data by transforming correlated variables into a set of uncorrelated principal components (Minitab, version 15; Meglen, 1992). This technique was employed using seven highly discriminatory FA variables from juvenile BKC and RKC (Stoner et al., 2010a). The first two principal components (PC1, PC2) accounted for 83% of the variance among samples, which allowed a display of the major trends within the data set without significant loss of the total original variation. PCA lipid loading coefficients are defined as the correlation coefficients between the original lipid variables and the PCA axis. PCA scores are defined as the position of the original variables along the new PCA axis (Meglen, 1992). Lipid variables were chosen based on biological significance and the degree of variance explained by a given lipid class or FA.

3. Results

3.1. Overall crab survival and growth

Blue king crab survivals were very high, over 90%, in all of the treatments over the 60-day monitoring period. The effect of temperature on

survival in population cultures was relatively small (Table 1), and differences were not significant at either 30 (ANOVA, $F_{3,8}=1.600$, $p=0.264$) or 60 days ($F_{3,8}=2.730$, $p=0.114$). Overall survival at the end of the experiment was 3.0% higher in individually cultured crabs than in populations, but there were no consistent differences in survival among the temperature treatments (Table 1).

Average growth rate of BKC in individual cells over the 60-day experiment increased in a linear pattern with increasing temperature up to 8 °C then leveled off (Fig. 1). ANOVA with $\log_{10}$-transformed growth data showed that the effect of temperature was significant ($F_{3,75}=143.97$, $p<0.0001$), and all of the treatments were significantly different from one another (Fisher's LSD test, $p<0.0001$), except 8° and 12 °C treatments where the differences were not significant ($p=0.222$). The growth rates in populations were identical to those observed in cells (Fig. 1).

The mean size of crabs after 60 days increased with temperature up to 8 °C (Fig. 2) in concordance with the observed differences in growth rates. Mean size varied significantly among temperature treatments in both population ($F_{3,8}=1081.04$, $p<0.0001$) and individual cultures ($F_{3,75}=145.94$, $p<0.0001$). The sizes of crabs were significantly different for all combinations of temperatures (Fisher's LSD test, $p<0.001$), except 8° and 12 °C treatments where the differences were not significant for either populations ($p=0.120$) or cells ($p=0.219$). There was no difference in size between population and cell cultures at any temperature (Fig. 2).

3.2. Growth in population cultures

Size-frequency distribution for BKC at the end of the 60-day growth period (Fig. 3) provided insight into the structure of the populations with regard to molt stages. Carapace width of crabs known to be in the first benthic instar (C1) averaged 1.96 mm, with no individuals >2.1 mm. A strong cohort of individuals between 2.1 and 2.7 mm was considered to be stage C2, and break-points at 2.7, 3.4, and 4.1 mm appeared to represent the lower boundaries for stages C3, C4, and C5, respectively. The robustness of these cohort definitions were confirmed by growth patterns observed in crabs cultured individually (see below). Based upon these boundaries, the populations from each experimental temperature could be partitioned into juvenile stages (Fig. 4). At 60 days, just 6.1% of the juveniles in the 1.5 °C treatment had molted to stage C2, and the rest remained at stage C1. All of the crabs in 4.5 °C except for one were at stage C2, most in 8 °C were C3, and crabs in 12 °C were distributed over stages C2 to C4, plus one individual estimated to be at stage C5.

3.3. Growth of individuals

Growth records for individual crabs provided definitive data for intermolt period and molt increment. When crab size is shown as a function of time (Fig. 5), it is clear that the primary effect of temperature on growth was a decrease in intermolt period with increasing temperature. The effect was significant for the molt from C1 to C2 (ANOVA, $F_{3,104}=930.42$, $p<0.0001$) (Table 2). The differences among the treatments were all significant (Fisher's LSD test, $p<0.001$) and obvious in the plots. An inverse exponential relationship occurred between temperature and intermolt period (Fig. 6) for the molt from C1 to C2. Molt increments for stages C1 to C2 ranged from 19.3% to 22.0% (Table 2), with no significant treatment effect ($F_{3,104}=1.129$, $p<0.344$).

Molts from C2 to C3 occurred at the three highest temperatures, and as observed in the earlier molt transition, the intermolt period decreased as temperatures increased from 4.5 to 12 °C (Table 2) ($F_{2,47}=1464.4$, $p<0.0001$), and all three treatments were significantly different (Fisher's LSD test, $p<0.001$). In this transition, molt increments decreased slightly with increasing temperature but the differences were not significant ($F_{2,47}=1.213$, $p=0.307$). Temperature-related differences in intermolt period continued into stage C4 (observed at 12 and 8 °C) ($F_{1,17}=109.74$, $p<0.0001$), while the molt increment did not vary significantly ($F_{1,17}=0.022$, $p=0.884$) (Table 2). Small differences in molt increment between the temperature treatments, while not significant, resulted in stage C4 crabs in 12 °C being 6.0% smaller

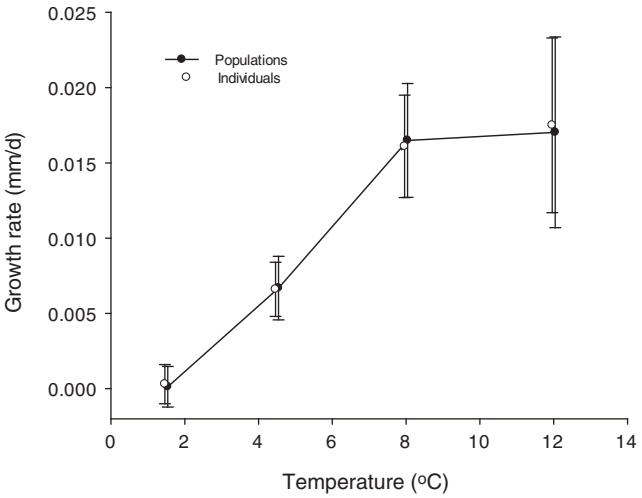

Fig. 1. Growth rates in the carapace width of blue king crabs cultured in populations and individually for 60 days at four experimental temperatures. Values are mean ± SD for all of the surviving crabs.

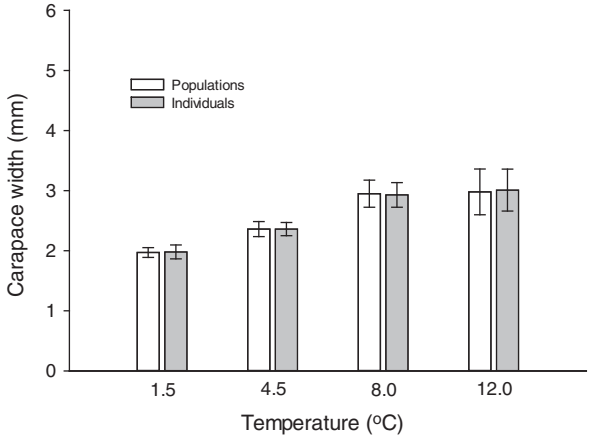

Fig. 2. Carapace width of age-0 blue king crabs cultured in four temperatures, in populations and individually and at the end of the 60-day growth period. Values are mean ± SD for all of the surviving crabs in individual cultures, while the values for population cultures represent the mean size for replicate populations (mean of means ± SD).

Table 1
Percentage survival of blue king crabs when held in populations and individually in four different experimental temperatures. Survival was recorded 30 and 60 days after design temperatures were achieved. Forty C1 stage crabs were started in each replicate population. Values for population results are mean ± standard deviation ($n=3$). Twenty-eight crabs were held in separate culture containers for each temperature treatment for individual cultures.

Temperature (°C)	Populations (30 days)	Populations (60 days)	Individuals (30 days)	Individuals (60 days)
1.5	99.2 ± 1.4	97.5 ± 2.5	100	96.4
4.5	99.2 ± 1.4	91.7 ± 1.4	100	100
8.0	95.8 ± 3.8	95.0 ± 5.0	96.4	96.4
12.0	99.2 ± 1.4	97.5 ± 0.0	100	100
Overall	98.3 ± 2.5	95.2 ± 3.8	99.1 ± 1.8	98.2 ± 2.1

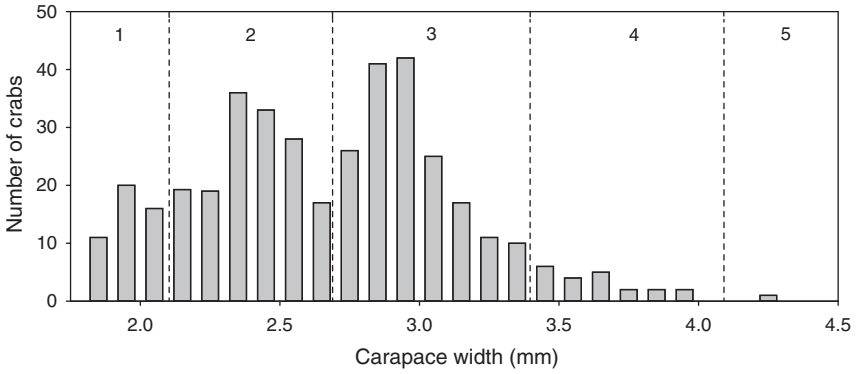

Fig. 3. Size-frequency distribution of blue king crabs in population cultures at the end of the 60-day growth period. Numbers at the top of the figure represent the juvenile molt stages from C1 to C6, confirmed by observations on individuals held in individual cultures.

than C4 crabs in 8 °C after 110 days of culture. This reflects an apparent additive effect of repeated low molt increment for crabs in 12 °C over three molting events evident in Fig. 5. Differences among individuals were also apparent. For example, among the crabs in the 8 °C treatment, size at C4 was inversely correlated with total time to stage C4 ($R = -0.871$, $p < 0.002$). That is, crabs molting on a more frequent schedule had the largest molt increments and grew to the largest size-at-stage.

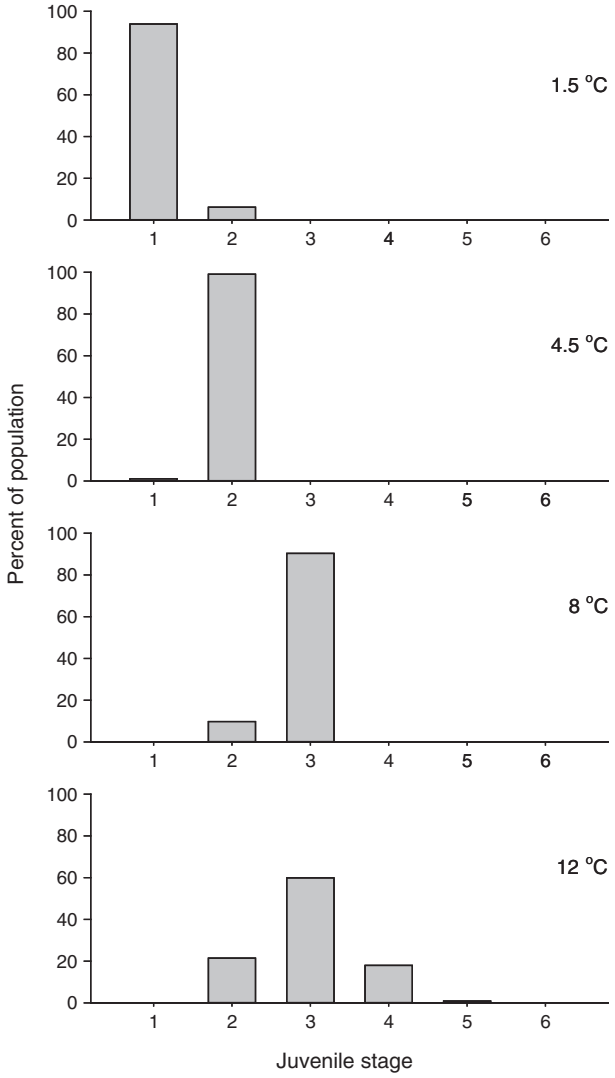

Fig. 4. Distribution of juvenile blue king crabs in population cultures at the end of the 60-day growth period in four experimental temperatures, by molt stage. Histograms represent the total counts for all of the replicate tanks.

3.4. Weights, lipid classes and fatty acids of individuals

Culture temperature had no significant effect on wet weight (ANOVA, $F_{3,43} = 0.20$, $p = 0.894$), dry weight ($F_{3,11} = 0.30$, $p = 0.827$) or ash weight ($F_{3,11} = 0.30$, $p = 0.827$) of stage C2 BKC. However, the percentage of DWT present as organic material (% organic) was significantly lower in C2 crabs reared at 1.5 °C than those reared at 12 °C (% organic, ANOVA, $F_{3,11} = 7.16$, $p = 0.012$) (Table 3). At the end of the experiment BKC ranged from just 4.3 mg DWT in C2 crabs reared at 1.5 °C to 13.8 mg in C4 crabs reared at 8 °C. After the C2 stage there were no significant differences in crab weights among temperature treatments within a given molt stage. Stage C2 crabs reared at 8 and 12 °C had significantly more lipid per unit dry weight (35.8 μg mg^{-1}) than those in 1.5 °C (19.7 μg mg^{-1}) while those in 4 °C had an intermediate level (Table 3, Fig. 7A). Total lipid per body weight did not increase significantly after the C2 stage, averaging ~30 μg mg^{-1} through C3 and C4 stages. Individual variability within temperature treatments was large in the C3 and C4 stages compared with the C2 stage (Fig. 7B).

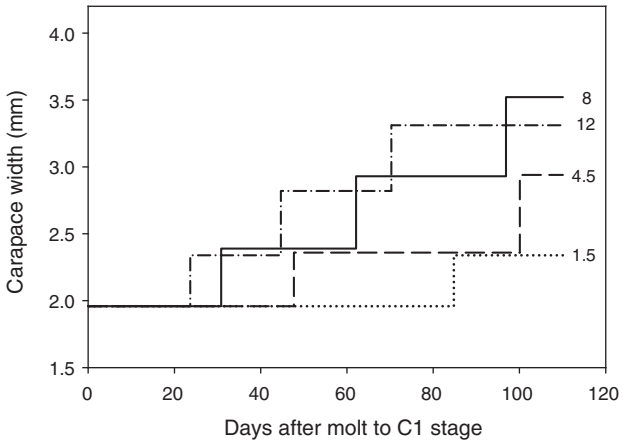

Fig. 5. Growth plots for juvenile blue king crabs cultured individually at four different experimental temperatures. The plots show the average time to molt and size at each successive molt stage. All of the crabs at the beginning of the observation period (time 0) were in stage C1 and had carapace widths averaging about 1.96 mm. All of the crabs in 12° and 8 °C were cultured to stage C4, in 4.5 °C to stage C3, and in 1.5 °C to stage C2. Horizontal differences in the steps reveal treatment differences in intermolt period, and vertical offsets show differences in growth increment (see Table 2).

Table 2
Intermolt periods and growth intervals for blue king crabs held individually at four different experimental temperatures. Values are mean ± standard deviation, shown for molts from stages C1 to C2, C2 to C3, and C3 to C4.

Molt stages and temperature (°C)	*n*	Intermolt period (days)	Molt increment (% CW)	Size at new stage (mm)
Molt from C1 to C2				
1.5	25	85 ± 9	19.4 ± 7.3	2.34 ± 0.14
4.5	28	48 ± 2	20.3 ± 5.6	2.36 ± 0.11
8.0	27	31 ± 1	22.0 ± 6.5	2.39 ± 0.13
12.0	28	24 ± 1	19.3 ± 4.6	2.34 ± 0.09
Molt from C2 to C3				
4.5	17	53 ± 2	24.4 ± 5.3	2.94 ± 0.15
8.0	16	32 ± 1	22.6 ± 3.3	2.93 ± 0.20
12.0	17	21 ± 1	22.3 ± 4.0	2.82 ± 0.12
Molt from C3 to C4				
8.0	9	35 ± 2	18.2 ± 7.7	3.52 ± 0.42
12.0	10	26 ± 2	17.8 ± 2.8	3.31 ± 0.16

Total lipid content per crab ranged from 101 μg crab^{-1} at 1.5 °C to 170 μg crab^{-1} at 12 °C during the middle of the C2 stage. The difference between C2 crabs reared at 12 °C and the two coldest temperatures was significant, while crabs at 8 °C showed an intermediate level of lipid content ($F_{3,12} = 6.21$, $p = 0.009$, Fig. 7C). However, within-treatment variability was high in stage C3 and stage C4 crabs and the differences among temperature treatments were not significant at those later stages (Fig. 7D).

Treatment-related differences in proportions of storage lipids (triacylglycerols, TAGs) and polar lipids (PLs) (Fig. 8) were significantly different in stage C2 crabs. The proportion of TAGs was significantly higher in crabs reared at 12 °C (49.8%) and declined to just 20.9% in crabs at the coldest temperature ($F_{3,12} = 25.02$, $p < 0.001$) (Table 3, Fig. 8A). The proportions of TAGs and PLs remained relatively constant throughout the C3 and C4 molt stages, and no temperature effects were significant after the C2 stage (Fig. 8B, C).

Fatty acid (FA) composition was relatively uniform across temperature treatments and molt stages (Table 4). Average ΣSFA was ~22.5%, and the most abundant SFA was 16:0 which accounted for ~13% of the total FAs. Average ΣMUFA was ~33%, and the most important MUFA was 18:1 n−9 which represented ~14% of the total. Crabs had ~42% ΣPUFAs with the essential FAs EPA (20:5n−3, ~12%), DHA (22:6n−3, ~13%), and AA (20:4n−6, ~1.5%) all present at expected levels. Proportions of DHA were higher in C4 crabs while levels of AA were lower than seen in C2 and C3 stages (Table 4).

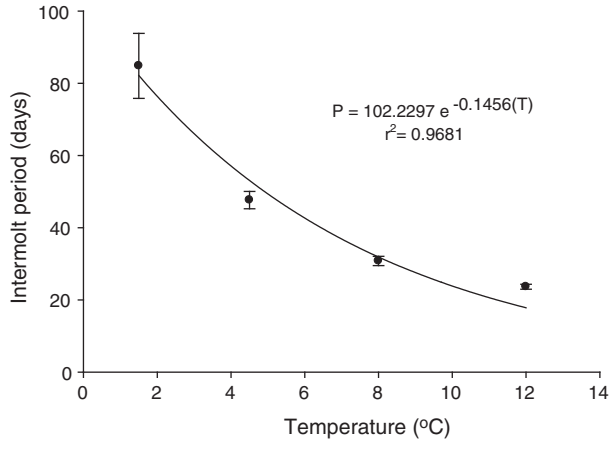

Fig. 6. Intermolt period shown for blue king crab molting from juvenile stages C1 to C2 at four different temperatures. Values are mean for individually cultured crab ± SD.

PCA for seven FAs simplified the patterns in the FA composition of BKC and allowed for a comparison with RKC juveniles (from Stoner et al., 2010a) (Fig. 9). The first two principal components accounted for 83% of the variability in FA composition of the crabs. PC1 explained most of the variation (54%) (Fig. 9A) and showed the essential PUFAs (20:4n−6, 20:5n−3, and 22:6n−3) clustered on the positive side of the axis while 16:1n−7 and 18:2n−6 were located on the negative. RKC had higher proportions of PUFAs, on the positive side of PC 1 (Fig. 9B), while BKC had higher proportions of MUFAs and C18 PUFAs. Neither molt stage nor temperature (not shown) had a large effect on the clustering of samples, with the exception of C4 BKC. PC2 separated large BKC (both 8 and 12 °C treatments) from other molt stages because they had higher proportions of 22:6n−3 and 18:2n−6.

4. Discussion

4.1. Effects of temperature on survival and growth

The upper and lower temperature tolerance limits have been studied for a variety of crab species and for different stages of development, particularly in light of warming environment (Kelley et al. 2011; Storch et al., 2011; Walther et al., 2009; Weiss et al., 2009). However, we observed no significant variation in the survival of BKC juveniles over the range of temperatures tested (1.5 to 12 °C). In fact, despite some evidence for stress at the high temperature treatment (see below), newly settled BKC juveniles appear to have a broad range of temperature tolerance. Recent experiments conducted at NOAA's Kodiak Fisheries Research Center indicate that the lethal temperature for BKC (24 h LT50 value) is approximately 21.3 °C, well above the range tested for growth, and only slightly lower than the value for RKC juveniles (24.3 °C) (C. Long, Alaska Fisheries Science Center, Kodiak, pers. comm.). The laboratory observations on acute effects indicate that mortality did not rise substantially until temperatures >20 °C were reached, and it is unlikely that acute effects of temperature would be a primary source of mortality in Alaska even at high temperatures observed occasionally during summer months in shallow nearshore waters (12 to 14 °C) (pers. observ.). Laboratory studies with other cold-water lithodid crabs provide for some comparisons with BKC. For example, Anger et al. (2004) observed that 18 °C was lethal to the zoea of *Lithodes santolla* Jaquinot, 1844, but like BKC, there were no consistent temperature-related differences in survival among six other temperature treatments ranging from 1 to 15 °C. A later study of temperature-related growth and survival in *L. santolla* and *Paralomis granulosa* Molina, 1782 (Calcagno et al., 2005) showed that juvenile survival decreased with increasing temperature. Given the wide range of temperature tolerance in BKC we believe that future research should focus on chronic rather than acute effects.

Survival of BKC in our cultures was substantially higher than that observed in comparable experiments with RKC (Stoner et al., 2010a). While 95% and 98% of BKC survived the 60-day growth period in population and individual cultures, respectively, just 66% and 71% of RKC survived under identical laboratory conditions. Some of the species difference may be attributed to the regular occurrence of cannibalism in RKC (Daly et al., 2009; Long et al., 2012; Stoner et al., 2010b) and an apparent low incidence of cannibalism in BKC (this study), but cannibalism cannot explain the species difference observed in crabs cultured individually. Rather, BKC appear to be hardier than RKC under similar laboratory conditions. Stevens et al. (2008a) noted the same for larval stages, and survival of BKC juveniles is routinely higher than RKC juveniles at the Alutiiq Pride Shellfish Hatchery where both species are reared in mass cultures (B. Daly, Alaska Fisheries Science Center, Kodiak, pers. comm.).

In contrast with survival rates, growth in age-0 BKC was strongly affected by water temperature as would be predicted for most

Table 3
Total lipids and lipid classes of blue king crab juveniles reared in individual cultures at four different temperatures from C2 to C4 molt stages. Values are mean ± standard error.

	C2 1.5 °C	C2 4 °C	C2 8 °C	C2 12 °C	C3 4 °C	C3 8 °C	C3 12 °C	C4 8 °C	C4 12 °C
	$n=11$	$n=11$	$n=11$	$n=11$	$n=7$	$n=7$	$n=7$	$n=7$	$n=7$
CW (mm)	2.4	2.3	2.4	2.4	2.9	2.9	2.8	3.5	3.3
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.1)	(0.0)	(0.1)	(0.1)
Wet Weight (mg)	13.3	13.1	13.5	13.5	23.5	24.5	22.0	41.3	34.5
	(0.5)	(0.5)	(0.4)	(0.5)	(0.6)	(1.2)	(0.6)	(3.5)	(1.4)
	$n=3$	$n=3$	$n=3$	$n=3$	$n=3$	$n=3$	$n=3$	$n=3$	$n=3$
Dry weight (mg)	4.3	4.7	4.8	4.6	8.9	8.8	7.6	13.8	11.8
	(0.1)	(0.4)	(0.6)	(0.2)	(0.7)	(0.3)	(0.2)	(1.9)	(0.6)
% Moisture[a]	62.2	63.6	64.7	65.3	62.4	64.7	66.0	63.3	62.6
	(0.9)	(0.2)	(0.5)	(0.8)	(0.2)	(0.6)	(1.6)	(2.7)	(0.9)
% Organic[b]	44.6	48.9	48.6	51.1	45.4	47.6	50.1	49.9	46.8
	(0.5)	(0.4)	(0.6)	(1.8)	(0.9)	(1.6)	(2.0)	(3.7)	(1.2)
	$n=4$	$n=4$	$n=4$	$n=4$	$n=4$	$n=4$	$n=4$	$n=4$	$n=4$
Total lipid ($\mu g \cdot ind^{-1}$)	101.2	106.6	131.8	170.4	257.9	294.1	249.9	498.8	355.4
	(9.6)	(13.4)	(8.8)	(17.2)	(21.3)	(71.7)	(54.7)	(122.2)	(86.2)
Total lipid ($\mu g \cdot mg^{-1}$ DWT)	19.7	22.8	27.9	35.8	29.2	33.0	33.3	28.0	20.6
	(1.7)	(2.6)	(2.5)	(1.9)	(2.6)	(4.9)	(5.2)	(3.6)	(5.1)
Triacylglycerols (TAGs)	20.9	26.0	38.9	49.8	43.3	51.4	38.8	49.8	45.9
	(0.7)	(1.9)	(3.9)	(2.8)	(1.1)	(4.7)	(3.6)	(3.7)	(11.3)
Sterols	4.3	2.5	1.8	3.2	2.0	2.0	3.7	3.1	3.6
(STs)	(0.8)	(1.12)	(0.2)	(0.3)	(0.7)	(0.5)	(0.2)	(0.6)	(0.6)
Polar lipids	74.8	71.5	59.3	47.0	54.7	46.6	57.5	47.1	50.5
(PLs)	(1.4)	(1.8)	(3.9)	(2.9)	(1.0)	(4.3)	(3.4)	(3.1)	(10.7)

[a] Percent moisture = (wet wt − dry wt/wet wt) ∗ 100.
[b] Percent organic = (dry wt − ash wt/dry wt) ∗ 100.

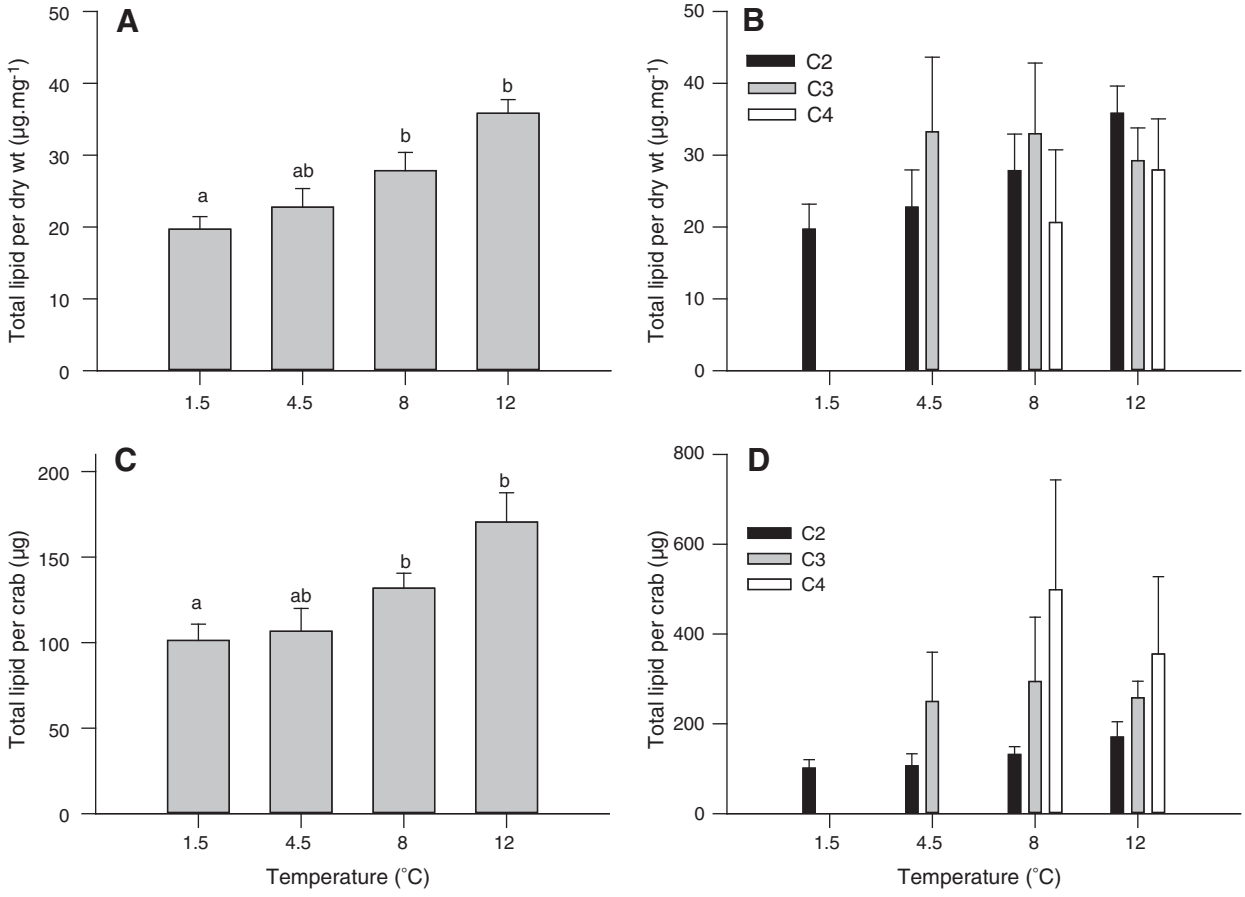

Fig. 7. Comparison of total lipids per DWT in (A) C2 crabs (B) C2, C3 and C4 crabs as well as the total lipid per crab in (C) C2 crabs and (D) C2, C3 and C4 crabs reared individually at four different temperatures. $n=4 \pm$ SE.

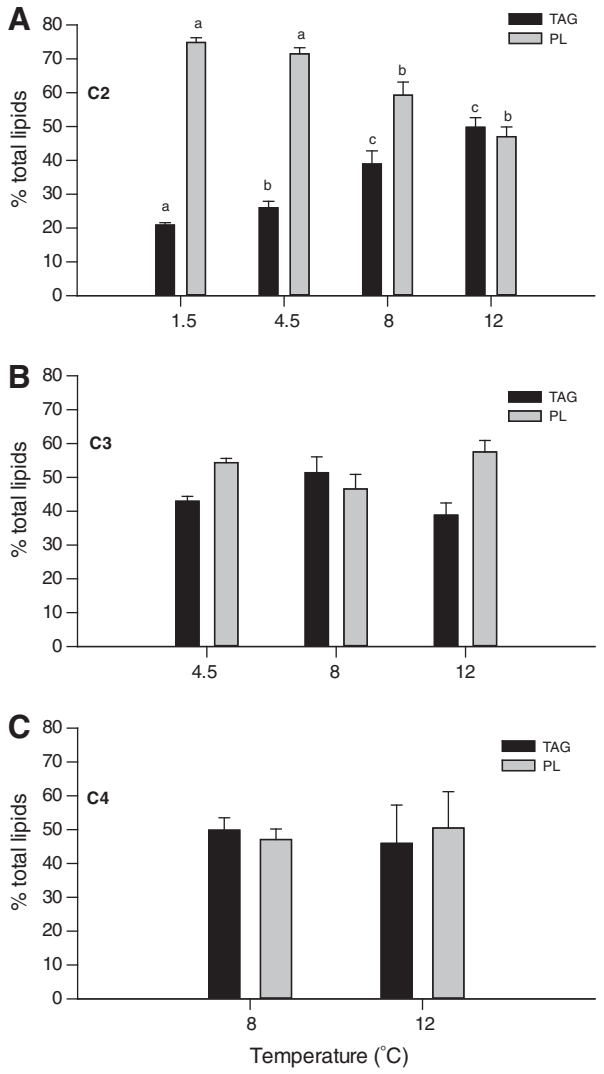

Fig. 8. Comparison of the triacylglycerols (TAG) and polar lipids (PL) in (A) C2 crabs, (B) C3 crabs and (C) C4 crabs reared individually at four different temperatures. $n = 4 \pm$ SE.

juvenile crustaceans (Hartnoll, 1982). A similar rise in growth rate with increasing temperature has been reported for other lithodid crabs (Anger et al., 2004; Calcagno et al., 2005), and it is known that feeding rates in RKC increase with temperature (Stevens, 2012, Stoner et al., 2010b) contributing to enhanced growth. Overall, BKC demonstrated an exponential increase in size across several molts, and the production of substantially larger crabs after two months in warm temperatures compared with almost no observed growth at 1.5 °C. After three months in culture, BKC cultured in 8.0 °C had molted three times and were 50% larger than BKC in 1.5 °C which molted just once. The primary mechanism for higher growth was decreasing intermolt period with increasing temperature up to 8 °C. However, 12 °C may be near the upper threshold for BKC growth and evidence for at least mild stress is provided by the decreasing molt increment associated with molts to C3 and C4 in 12 °C, and stage C4 crabs reared at that temperature were 6% smaller and more variable in size than C4 crabs reared in 8 °C. This divergence would likely increase over time with subsequent molts.

In his extensive review of growth processes in crustaceans, Hartnoll (1982) listed many species that demonstrate a decrease in molt increment with temperature, but only two showed molt increments that increased with increasing temperature. In fact, effects of temperature on molt increment are relatively uncommon. Juvenile RKC appear to be among the exceptional cases showing a near linear relationship between molt increment and temperature over the range from 1.5 to 12 °C (Stoner et al., 2010a), and may be among the few species where both intermolt period and molt increment are affected by temperature. BKC appear to be more typical with relatively constant molt increments, at least up to the point of stress observed at 12 °C. On the other hand, molt increments probably decline with BKC size. While we observed growth between 19.3 and 24.4% in molts to C2 and C3 stages, Hawkes et al. (1987) reported molt increments of just 7.2 to 13.5% for larger BKC (96 to 125 mm carapace length).

4.2. Effects of temperature on condition and lipid storage

BKC cultured to stage C2 in high temperatures exhibited significantly higher condition than those in low temperature based upon measures of organic weight, total lipid content, and percentage of TAG storage lipids. However, after the C2 stage no significant temperature-related differences in crab condition were observed. The reason for this lack of difference in later molt stages is likely two-fold: individual variation increased with increasing molt stage, and the range of temperature treatments necessarily decreased with each increasing molt stage. Further studies with larger samples sizes for biochemical indices may be useful to assess potential differences in condition due to temperature at older stages. However, the slow growth of crabs at very low temperatures makes this type of experiment difficult. For example, it would take more than a year to culture BKC from stage C1 to stage C4 at 1.5 °C.

Total lipids per dry weight in BKC (19.7 to 35.8 $\mu g \cdot mg^{-1}$) were similar to those reported for RKC reared in identical experiments with identical diets (Stoner et al., 2010a). Further, these total lipid levels were on the low end of the range compared to other crustaceans such as the mud crab *Rhithropanopeus harrisii* Gould 1841 (50 $\mu g\ mg^{-1}$) (Nates and McKenney, 2000) and rock lobster *Panulirus cygnus* George 1962 (70 $\mu g\ mg^{-1}$) (Liddy et al., 2005). However, the proportion of TAGs in BKC (20.9% to 51.4%) was substantially higher than the values reported for similar sized RKC, both cultured (3% to 17%) (Stoner et al., 2010a) and wild (~25%) (Copeman et al., 2012). Neutral lipid levels, especially TAGs, are useful indicators of physiological condition in larval and juvenile crustaceans (Nates and McKenney, 2000; Ouellet and Taggart, 1992). Higher proportions of TAGs along with higher survival indicates that BKC were in better condition than RKC reared in identical experiments (Stoner et al., 2010a).

Increasing proportions of TAGs in BKC (reared to stage C2) associated with higher temperature follow a similar increase in the proportion of TAG observed in RKC cultured to stage C4 (Stoner et al., 2010a). Comparable amounts of total lipid on a weight basis, as well as higher proportions of storage lipids, indicate that fast growth at warm temperatures may have a positive effect on storage lipid through unknown mechanisms, and did not have a negative impact on the condition of BKC, at least in the earliest juvenile stages. However, this conclusion contrasts with the slight reduction in growth increment associated with 12 °C.

The fatty acids DHA, EPA, and AA play important roles in the early survival and growth of marine larvae (Copeman et al., 2002), and dietary deficiencies are known to reduce survival, increase intermolt periods, and produce smaller size in cultured crabs (Suprayudi et al., 2004). Here we report the first data on the EFA composition of cultured BKC juveniles (DHA = 13.8%, EPA = 11.3%, AA = 1.4%). These levels correspond with those found in juvenile Arctic lyre crab (*Hyas coarctatus* Linnaeus 1758) collected in nearshore waters off Labrador, Canada (DHA = 16.4%, EPA = 22.4%, AA = 2.4%) (Copeman and Parrish, 2003). Further, Copeman et al. (2012) found that cultured RKC have lower levels of EFA than those in wild juveniles (DHA = 14%, EPA = 24%, AA = 3.5%). Specifically, lower levels of EPA and AA may be a source of dietary deficiency in cultured king crabs reared on live enriched

Table 4
Percentage of total fatty acids (%) in BKC juveniles reared in individual cultures at four different temperatures during C2 to C4 molt stages. Values are mean ± standard error in brackets. $n = 4$.

	C2 1.5 °C	C2 4 °C	C2 8 °C	C2 12 °C	C3 4 °C	C3 8 °C	C3 12 °C	C4 8 °C	C4 12 °C
14:0	2.5 (0.1)	2.8 (0.1)	2.9 (0.1)	3.0 (0.0)	3.1 (0.1)	3.4 (0.2)	3.2 (0.1)	3.3 (0.1)	3.5 (0.3)
16:0	14.3 (0.7)	14.1 (0.7)	12.9 (0.4)	12.7 (0.2)	12.8 (0.1)	12.7 (0.4)	13.7 (0.5)	14.5 (0.2)	14.6 (0.8)
18:0	3.7 (0.2)	3.7 (0.2)	3.4 (0.1)	3.2 (0.2)	2.9 (0.1)	2.9 (0.1)	3.1 (0.2)	3.1 (0.1)	3.2 (0.3)
ΣSFA[a]	**23.7** (**0.9**)	**24.5** (**1.0**)	**23.1** (**0.5**)	**22.7** (**0.4**)	**22.4** (**0.3**)	**22.6** (**0.6**)	**23.0** (**0.6**)	**23.8** (**0.5**)	**23.3** (**0.5**)
16:1 n−7	5.0 (0.3)	5.7 (0.1)	5.5 (0.1)	5.0 (0.0)	5.8 (0.1)	5.4 (0.2)	4.6 (0.1)	5.3 (0.2)	5.2 (0.2)
18:1 n−9	14.2 (0.2)	14.4 (0.4)	14.4 (0.2)	14.4 (0.1)	13.7 (0.2)	14.4 (0.4)	14.7 (0.3)	15.7 (0.6)	15.6 (0.5)
18:1 n−7	8.4 (0.4)	7.9 (0.1)	7.1 (0.1)	6.3 (0.1)	7.2 (0.1)	6.7 (0.3)	5.9 (0.1)	7.2 (0.1)	6.4 (0.4)
20:1 n−11	1.8 (0.1)	1.9 (0.1)	1.9 (0.0)	1.8 (0.1)	2.1 (0.0)	2.3 (0.2)	2.3 (0.0)	1.6 (0.6)	1.9 (0.7)
20:1 n−9	1.7 (0.1)	1.8 (0.1)	1.9 (0.0)	1.8 (0.0)	1.9 (0.0)	2.0 (0.1)	2.0 (0.0)	1.6 (0.5)	1.7 (0.6)
22:1 n−11(13)	1.5 (0.5)	2.0 (0.1)	2.3 (0.2)	2.4 (0.2)	2.4 (0.2)	2.7 (0.3)	3.0 (0.2)	0.0 (0.0)	0.0 (0.0)
ΣMUFA[b]	**34.1** (**0.7**)	**35.7** (**0.4**)	**35.6** (**0.6**)	**34.4** (**0.4**)	**35.3** (**0.5**)	**35.9** (**1.5**)	**34.7** (**0.4**)	**32.1** (**1.4**)	**31.3** (**1.5**)
16:2 n−4	1.4 (0.2)	1.6 (0.1)	2.0 (0.1)	2.0 (0.1)	2.0 (0.1)	1.9 (0.0)	1.8 (0.1)	2.0 (0.1)	2.0 (0.1)
18:2 n−6	5.0 (0.1)	5.0 (0.1)	5.6 (0.1)	6.4 (0.2)	5.8 (0.2)	5.9 (0.4)	6.3 (0.2)	7.4 (0.2)	7.4 (0.3)
18:3 n−3	2.2 (0.1)	2.0 (0.1)	2.7 (0.1)	3.1 (0.1)	2.4 (0.2)	2.5 (0.3)	2.5 (0.1)	3.2 (0.2)	3.1 (0.4)
18:4 n−3	1.0 (0.1)	1.1 (0.1)	1.1 (0.1)	1.3 (0.1)	1.5 (0.1)	1.7 (0.1)	1.5 (0.1)	1.6 (0.1)	1.5 (0.5)
20:4 n−6	2.0 (0.1)	1.9 (0.0)	1.6 (0.1)	1.5 (0.0)	1.7 (0.0)	1.5 (0.1)	1.4 (0.0)	0.8 (0.5)	0.5 (0.5)
20:5 n−3	13.6 (0.4)	12.0 (0.3)	10.6 (0.3)	9.7 (0.2)	11.6 (0.2)	10.3 (0.3)	10.2 (0.3)	11.6 (0.8)	12.4 (1.1)
22:6 n−3	13.0 (0.1)	12.3 (0.5)	12.4 (0.3)	13.1 (0.2)	13.2 (0.3)	12.8 (0.5)	13.7 (0.2)	16.3 (0.9)	17.8 (1.0)
ΣPUFA[c]	**42.2** (**0.5**)	**39.8** (**1.3**)	**41.4** (**0.7**)	**43.2** (**0.6**)	**42.4** (**0.4**)	**41.6** (**2.0**)	**42.4** (**0.4**)	**44.1** (**1.3**)	**45.4** (**1.6**)

Bold font shows the total proportions of Saturated Fatty Acids (SFA), Mono-Unsaturated Fatty Acids (MUFA), and Poly-Unsaturated Fatty Acids (PUFA), including those listed in the footnotes.

[a] Also contains <2% of i-15:0, *ai*-15:0, 15:0, i16:0, ai16:0, i17:0, ai17:0, 17:0, 20:0, 22:0, 23:0, 24:0.

[b] Also contains <2% of 14:1, 15:1, 16:1n−11, 16:1n−9, 16:1n−5, 17:1, 18:1n−11, 18:1n−9, 18:1n16, 18:1n−5, 20:1n−9, 20:1n−11, 20:1n−7, 22:1n−11(13), 22:1n−9, 22:1n−7, 24:1.

[c] Also contains <2% 16:3n−4, 16:4n−3, 16:4n−1, 18:2n−4, 18:3n−6, 18:3n−4, 18:3n−3, 18:4n−3, 18:4n−1, 18:5n−3, 20:2a, 20:2b, 20:2n−6, 20:3n−6, 20:3n−3, 20:4n−3, 22:4n−6, 22:4n−3.

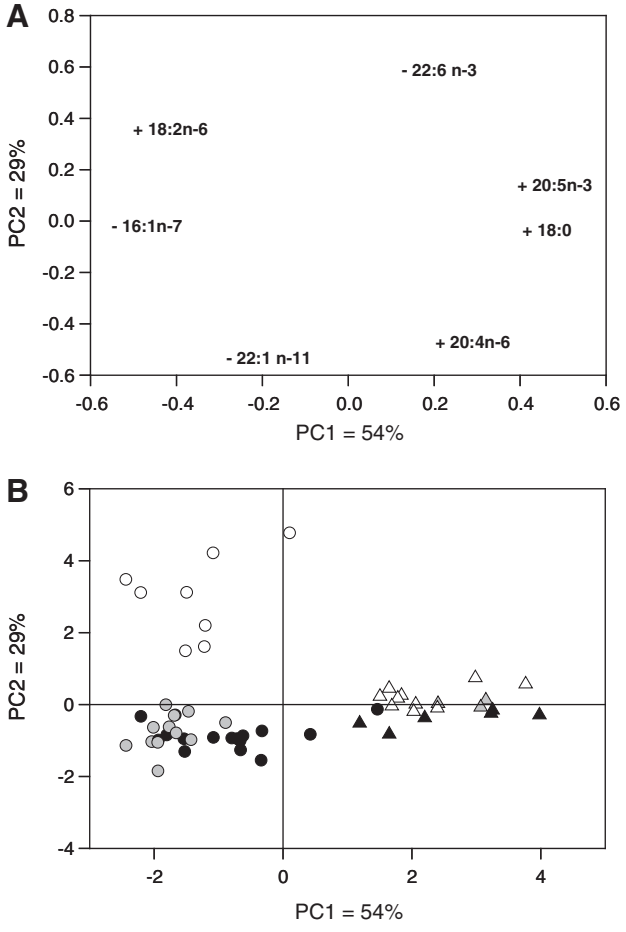

Fig 9. Analysis of the first two principal components of FA data from RKC and BKC juveniles reared individually at four different temperatures over 3 molt stages. The FAs used were: 20:4n−6, 22:6n−3, 20:5n−3, 18:2n−6, 18:0, 16:1n−7, 22:1n−11. Lipid parameter coefficients (+ or −) represent orientation along the third principal component axis while RKC and BKC scores are shown for the first two principal components.

Artemia. However, high survival and growth in our study indicate that our mixed diet of Cyclop-Eeze and Otohime pellets adequately met the EFA requirements for king crab juveniles.

Principal component analyses easily distinguished BKC from RKC based upon seven FAs. Generally, BKC have a higher proportion of MUFAs and short chain C_{18} PUFAs that are associated with increased storage lipids (TAG). Conversely, RKC had higher proportions of EFA that are classically associated with polar lipids. The physiological role of polar phospholipids in marine organisms requires a high specificity for EFA within this lipid class (Bell and Tocher, 1989). For example, Bell and Dick (1991) found that PLs in cod had the common molecular species 16:0/22:6 and 16:0/20:5. Conversely, higher levels of TAG found in BKC show little specificity for FAs explaining elevated levels of MUFAs and short chain PUFAs in this species. Elevated proportions of DHA in C4 BKC distinguished them from earlier molt stages. This change may reflect a shift in preference to a larger food item, from Otohime pellets to Cyclop-Eeze, but further analyses will be required to determine the mechanism for shifting FA composition.

4.3. Implications for BKC culture

BKC juveniles were extraordinarily hardy in laboratory culture, with survival rates far exceeding those for RKC (Stoner et al., 2010a). This is a positive attribute of BKC with respect to hatchery culture and the ultimate goal of rebuilding king crab stocks in Alaska through releases of hatchery-reared juveniles. Also, while cannibalism tends to increase with water temperature in other species such as *Callinectes sapidus* Rathbun 1896 (Hines and Ruiz, 1995) and RKC (Borisov et al., 2007; Stoner et al., 2010b), there was no such indication with BKC, and cannibalism among early stage juveniles appears to be a relatively minor problem. We expect that this trait will carry over to the field upon release.

A positive relationship between water temperature and growth rate is a useful attribute for culture of juvenile crabs. Exponential growth observed between 1.5 and 8.0 °C means that BKC can be reared to release stage in a shorter time at warm temperatures, reducing cost for facilities and labor. However, it is also apparent that

8 °C may be near the optimal temperature for culture, and growth cannot be pushed faster as is the case for RKC (Stoner et al., 2010a). In fact, the growth curves for BKC and RKC were nearly identical up to 8 °C, but growth performance in the two species diverges quickly above that temperature (Fig. 10). RKC are now routinely reared at the Alutiiq Pride Shellfish Hatchery at 12 °C (B. Daly, pers. comm.), but because BKC cannot be pushed beyond 8 °C it takes much longer to produce the larger juvenile stages desired for field release. A full cost–benefit comparison for seed stock production of the two species will need to include information on both growth and survival.

In implementing a program of hatchery supplementation to promote population recovery, it will be important to be aware of potential energetic, morphological, or behavioral deficits that might be imposed by unnaturally high temperatures. Our results on total lipid and TAG content indicate that the physiological condition of BKC reared at warm temperature was high. In fact, lipid levels in BKC were higher than those in RKC, thus BKC may have an energetic survival advantage. However, there are prominent examples of lower field survival in hatchery-reared animals than wild stock, caused by behavioral and morphological deficits (Olla et al., 1998; Stoner and Davis, 1994). In cultured blue crab (*C. sapidus*), deficiencies in spine patterns, color, and predator-avoidance behavior have all been identified as important obstacles to post-release survival (Davis et al., 2004; Young et al., 2008). Fortunately, hatchery rearing techniques and conditioning of juveniles intended for release (Young et al., 2008), along with improvements in release strategies can increase survival rates for hatchery-reared crabs (Hines et al., 2008; Johnson et al., 2008).

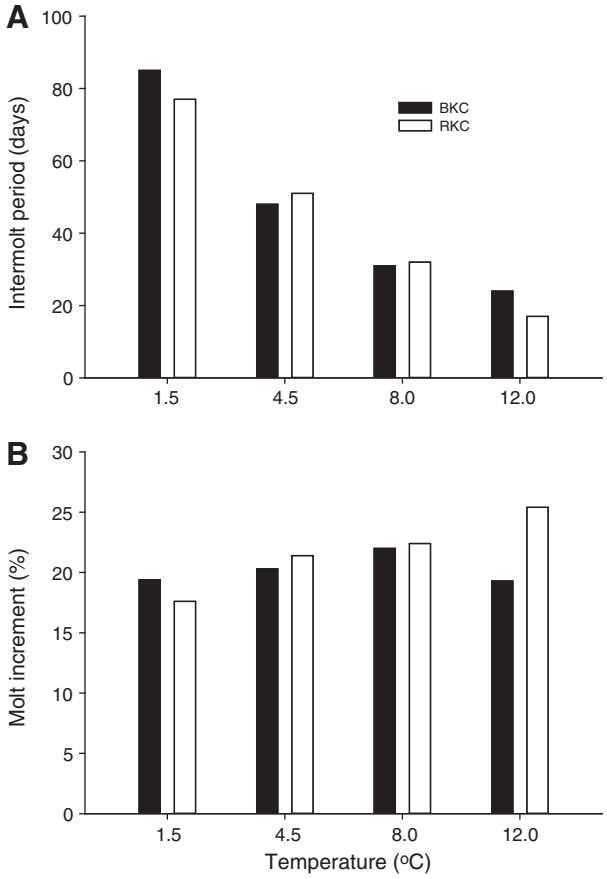

Fig. 10. Comparison of intermolt periods (A) and molt increments (B) for blue king crab (BKC) and red king crab (RKC) cultured individually at four different temperatures. Mean values are shown for the transition from stages C1 to C2 in experiments reported in the present study for BKC and in Stoner et al. (2010a) for RKC.

In sum, BKC have some advantage over RKC in terms of survival, lipid storage, and low incidence of cannibalism. However, BKC have a lower temperature tolerance, and owing to slower growth at high temperature, they would need to be held in the hatchery for a longer time than RKC to achieve similar size. To prevent accumulation of hatchery-induced deficits in behavior and morphology the best strategy for stock enhancement may be to release large numbers of BKC immediately after metamorphosis to the first juvenile stage. New experiments will be required to assess the success of BKC juveniles in the field.

4.4. King crabs in a changing environment

Increases in water temperature and loss of sea ice associated with climate change are now well documented in Alaska (Grebmeier et al., 2006; Royer and Grosch, 2006; Sigler et al., 2011; Stabeno et al., 2007), and it is clear that these changes will have direct and indirect effects on a wide range of marine taxa. While recruitment and productivity of commercially significant crabs near the boundaries of their thermal tolerance can be limited by environmental variation (Johnston et al., 2011), recent experiments on acute thermal stress discussed earlier indicate that effects of warming conditions are likely to be more subtle for age-0 stages of both BKC and RKC. There were no apparent adverse chronic effects of temperatures as high as 12 °C for RKC (Stoner et al., 2010a), and accelerated growth might have a positive, indirect effect whereby crabs can more quickly escape predation. BKC could benefit from the same acceleration of growth, but only up to 8 °C. Chronic effects of elevated temperature are more likely in BKC than RKC despite the fact that BKC appear to be more hardy. It should be noted, however, that new experiments with European green crab (*Carcinus maenas* Linnaeus, 1758) show that crabs can acclimate to different temperature regimes and develop different temperature tolerances over multiple generations (Kelley et al., 2011). The same may be true for king crabs.

How altered growth rates and lipid storage patterns in young BKC might translate to subsequent recruitment to the fishery remains poorly understood. First, large juveniles and adults tend to live in deeper water than age-0 crab and are probably less susceptible to warming of near-surface water. Second, predator–prey relationships are mediated by temperature in complex direct and indirect ways (Freitas et al., 2007; Orensanz et al., 2004; Sigler et al., 2011) because the behaviors of both predator and prey shift with metabolic demands. Temperature has a profound influence on the metabolism, growth, and trophic interactions of fish and invertebrate members of the shelf community in Alaska. This study contributes to an understanding of the factors that will determine the cumulative impacts on the community in a warming environment.

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