

TOTAL LIPIDS, LIPID CLASSES, AND FATTY ACIDS OF NEWLY SETTLED RED KING CRAB (*PARALITHODES CAMTSCHATICUS*): COMPARISON OF HATCHERY-CULTURED AND WILD CRABS

LOUISE A. COPEMAN,^{1*} ALLAN W. STONER,² MICHELE L. OTTMAR,² BENJAMIN DALY,³ CHRISTOPHER C. PARRISH,⁴ AND GINNY L. ECKERT⁵

¹Cooperative Institute for Marine Resources Studies, Oregon State University, Hatfield Marine Science Center, 2030 S.E. Marine Science Drive, Newport OR, USA 97365; ²Fisheries Behavioral Ecology Program, Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2030 S.E. Marine Science Drive, Newport OR, USA 97365; ³School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 201 Railway Avenue, Seward AK, USA 99664; ⁴Ocean Sciences Centre, Memorial University of Newfoundland, Logy Bay NL, Canada A1C 5S7; ⁵Juneau Center, School for Fisheries and Ocean Sciences, University of Alaska Fairbanks, 17101 Point Lena Loop Road, 11305 Glacier Hwy, Juneau AK, USA 99801

ABSTRACT Little is known about the nutrition or lipid metabolism of cold-water crabs, particularly in the North Pacific. We undertook a 2-part study to understand more completely the energetics and nutritional requirements of juvenile red king crab (RKC; *Paralithodes camtschaticus*). First, we investigated changes in proximate composition, total lipids (TLs), lipid classes, and fatty acids (FAs) throughout a molt cycle (C4–C5). Trends in lipid parameters were described by a 3-part piecewise linear regression with 3 distinct stages: (1) a postmolt phase (~0–7 days), (2) an intramolt stage (~7–24 days), and (3) a premolt stage (~24–33 days). Significant intramolt differences in TLs indicated that caution should be taken when comparing crabs of unknown molt stage in future aquaculture and ecological experiments. However, little variability was found in the proportional FA composition of crabs, indicating that the intramolt stage has little effect on the interpretation of FA biomarkers. During a second investigation, we examined differences in lipid classes and FAs from cultured and wild RKC. We found significantly higher proportions of the essential fatty acids (EFAs) 20:5n-3 (EPA) and 20:4n-6 (AA) in wild crabs compared with cultured animals at the same stage. Furthermore, higher proportions of bacterial markers and lower proportions of zooplankton FA markers were found in wild than in hatchery-reared crabs. Here, we provide the first baseline data for future dietary studies on juvenile cold-water crabs. We suggest that an initial EFA ratio for DHA:EPA:AA of 5:8:1 could be used as a starting point for controlled dietary studies on the effect of EFAs on juvenile growth, molt success, and survival.

KEY WORDS: lipids, fatty acids, nutrition, molt, red king crab, *Paralithodes camtschaticus*

INTRODUCTION

Lipids are important biochemical components of marine food webs because they are carbon rich and provide a concentrated source of energy (Parrish 1988). Marine lipids are now examined routinely as biomarkers in ecological studies, as essential nutrients in aquaculture growth trials, and as tools to understand large-scale oceanographic processes (Budge et al. 2006, Litzow et al. 2006). Marine lipids are also vital nutrients for human health, and declines in seafood stocks (FAO 2010) are currently threatening food security for human populations on a global scale (Parrish et al. 2008).

Lipids and fatty acids are important to the survival and condition of numerous cultured cold-water marine fish and invertebrates (Couteau et al. 1996, Sargent et al. 1999). Specifically, lipids are a source of energy in juvenile and larval crustaceans (Kattner et al. 2003), and are crucial to elevated growth and molting success (Wen et al. 2006). The major lipid classes that affect condition in crustaceans are triacylglycerols (TAGs), sterols (STs), and phospholipids (Ouellet & Taggart 1992). Specifically, the ratios of different lipid classes within larvae (TAG/ST) have been used previously to indicate

condition in a number of finfish and crustaceans (Fraser 1989, Harding & Fraser 1999, Copeman et al. 2002, Copeman et al. 2008).

Certain dietary lipids are essential to larval crustaceans for growth, development, and survival. Dietary essential lipids cannot be synthesized in adequate amounts *de novo* within an animal from dietary precursors and are rarely found in adequate levels in commercially available feeds (Figueiredo et al. 2009). In particular, polyunsaturated fatty acids (PUFAs) have been investigated extensively in marine larval nutrition (Sargent et al. 1999), and highly unsaturated long-chain PUFAs such as docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3), and arachidonic acid (AA; 20:4n-6) are essential for many crustaceans (Merican & Shim 1996, Suprayudi et al. 2004, Zmora et al. 2005, Limbourn & Nichols 2009, Mercier et al. 2009).

In Alaska, red king crab (RKC; *Paralithodes camtschaticus*) stock collapses during the 1980s resulted in the closure of most RKC fisheries (Zheng & Kruse 2000), which remain closed despite decades of fishing moratoriums (Alaska Department of Fish and Game 2010). Stock enhancement has the potential to be an effective population recovery tool for depleted RKC stocks, and is currently underway for other crab and lobster species in the United States and worldwide (Stevens 2006, Hamasaki & Kitada 2008). Members of the Alaska King Crab

*Corresponding author. E-mail: copemanl@onid.orst.edu
DOI: 10.2983/035.031.0119

Research and Rehabilitation and Biology (or AKCRRAB) Program have been conducting large-scale king crab aquaculture for the past 5 y to evaluate the potential to enhance wild stocks by releasing juveniles into the wild (Daly 2010). The development of large-scale hatchery production technology has allowed for mass production of juvenile RKC (Daly et al. 2009, Swingle et al. unpublished), which consists of 3 steps: (1) larval rearing (zoeae 1–4), (2) postlarval rearing (glaucothoe), and (3) juvenile rearing. However, despite recent production success, the transition from nonfeeding glaucothoe to the first-feeding C1 juvenile stage is still a major source of mortality in hatchery production (Daly et al. 2009).

After molting (postmolt stage), crabs are pale and soft bodied, but within a few hours their cuticle hardens and darkens. During molt (ecdysis), crabs experience growth by sequential steps, because growth is otherwise constrained by their rigid exoskeleton (Sanchez-Paz et al. 2006). The process of molting from larvae to glaucothoe to sequential juvenile stages is likely a stressful period, as crabs undergo significant morphological and physiological changes (O'Halloran & O'Dor 1988). The nutritional requirements to complete a molt cycle successfully are significant (Lautier & Lagarrigue 1987), yet they have not been documented for juvenile RKC. Inadequate endogenous energy reserves in nonfeeding molting crabs are hypothesized to be a major source of "molt death" in small juvenile crabs (Holme et al. 2007). However, very little is currently known about how lipid storage fluctuates within a molt cycle for RKC juveniles. As with other shrimp and crab species, we predict that juvenile RKC will show 3 phases of lipid accumulation during each molt cycle. First, we predict that RKC will have no increase in lipids during the nonfeeding period just subsequent to molt (postmolt phase); second, RKC will accumulate lipids rapidly during the major feeding period in the center of their growth cycle (intramolt phase); and last, RKC will show a decrease in lipids during a nonfeeding period just preceding molt (premolting phase) (Ouellet & Taggart 1992, Zhou et al. 1998).

Inadequate dietary essential fatty acids (EFAs) may explain high levels of molt mortality observed in the hatchery at the larval and juvenile stages (Leroux per. comm.). "Nature Knows Best" has often been used as a starting point in the development of hatchery nutritional protocols for new culture species (Sargent 1995, Sargent et al. 1999); however, no explicit comparison has been conducted to investigate the differences in lipids between hatchery-cultured and wild-source RKC. Hatchery-reared crabs likely have different proportions of EFAs than wild juveniles. Understanding these insufficiencies/differences could help crab culturists improve enrichment protocols for live-food organisms currently used to culture RKC (Calcagno et al. 2005, Epelbaum & Kovatcheva 2005, Stevens 2006).

The purpose of this study is to improve our understanding of nutritional requirements of juvenile RKC. Here we investigate (1) variation in weight, lipid classes, and fatty acids in hatchery-reared crabs throughout an entire molt cycle (C4–C5); and (2) differences in lipid classes and fatty acids between hatchery-reared and wild-caught juvenile crabs for 5 different molt sizes. These data are pertinent to both fisheries ecology and aquaculture, because they will support the interpretation of wild-collected crab condition indices as well as the development of diets for hatchery-reared juvenile crabs.

METHODS

Experiment 1: Intramolt Lipid Cycle of RKC Juveniles (C4–C5)

Source of RKC and Crab Culture

RKC were supplied by the Alutiiq Pride Shellfish Hatchery in Seward, AK, as described previously in Daly et al. (2009). Briefly, female broodstock were collected in Bristol Bay, AK, during fall 2008, and were maintained at the hatchery on chopped herring and squid until their larvae were released in May 2009. Larvae from 12 females were mixed and reared in 1,200-L cylindrical tanks until the first juvenile instar (C1) was achieved (as in Daly et al. (2009)). Newly settled crabs were fed daily with *Artemia* nauplii enriched with DC (disinfecting continuously) DHA Selco enrichment media.

Stage C1 crabs were shipped to the Hatfield Marine Science Center (Newport, OR) on May 27, 2009. On arrival, crabs were sorted by size to achieve a C1 size class that had bright-red color and high activity levels, indicative of good health (Eckert, pers. comm.). Individuals meeting these criteria were placed in a batch culture tank set at $\sim 4.5^{\circ}\text{C}$. A total of 40 C1 crabs was held in a rectangular polyethylene tank (42 cm wide, 63 cm long, 30 cm deep) supplied with a continuous flow (35 mL/sec) of sand-filtered (50- μm particle size) seawater. The tank contained 5 L structural habitat, including loose bundles of BioFill filter material (PVC ribbon; Aquatic Eco-Systems, Orlando, FL) and black polypropylene gill netting. The structure provided refuge for molting crabs, which are vulnerable to cannibalism (Stoner et al. 2010a). Crabs were monitored for growth and survival during their first 2 molts as part of a companion experiment examining the effects of temperature on molting, growth, and lipid composition in juvenile RKC (Stoner et al. 2010b).

After juvenile RKC had molted to the C3 size class, 36 crabs were transferred to a second type of culture system that consisted of individual culture cells made from stiff mesh tubing cut to 17.5 cm high (for further details, see Stoner et al. (2010b)). Initially, individual RKC were placed in cells held at 4.5°C , and the temperature was gradually increased to 8.0°C during a 48-h period. The use of individual cells allowed us to monitor the molting schedule of individual crabs. We first monitored the day at which crabs molted from C3 to C4. Then, crabs were sampled at predetermined days throughout the entire C4 stage so that representative samples were collected throughout the entire 33 days between C4 and C5. Three crabs died during the experiment, leaving 16 crabs for lipid analysis and 17 crabs for dry weight and ash weight analysis.

Morphometrics

All 33 live crabs were measured for carapace width (CW; in millimeters) and wet weight (WWT; in milligrams). CW was measured from digital photographs using a dissecting microscope equipped with a calibrated digital camera and Image Pro software. We measured CW, as defined by Epelbaum et al. (2006), without lateral spines. WWT measurements on all individual crabs were made to the nearest 1.0 μg , whereas dry weight (DWT) measurements on 17 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 a 5.0-cm² preweighed aluminum foils and placed in an oven set at 70°C for 48 h. Foils were removed from the oven and then 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. DWTs were calculated by subtracting the weight of the preweighed foils, whereas organic weights were calculated by subtracting the ash weight from the previously calculated DWTs.

Lipid Classes

Sixteen crabs ranging from C4 (day 0 postmolt) to C5 (day 0 postmolt) and from ~40–60 mg WWT each were sampled for lipid class analyses. 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 with a MARK VI Iatroscan (Iatron Laboratories, Tokyo, Japan) as described by Parrish (1987). Extracts were spotted on silica gel-coated Chromarods and a 3-stage development system was used to separate lipid classes. The first separations consisted of 25-min and 20-min developments in 98.95:1:0.05 hexane:diethyl ether:formic acid. The second separation consisted of a 40-min development in 79:20:1 hexane:diethyl ether:formic acid. The last separation consisted of 15-min developments in 100% acetone followed by 10-min developments in 5:4:1 chloroform:methanol:water. Data peaks were integrated using Peak Simple software (version 3.67; SRI Inc.), and the signal (detected in millivolts) was quantified using lipid standards (Sigma, St. Louis, MO). Lipid classes were expressed both in relative (milligrams per gram WWT) and absolute (micrograms per animal) amounts.

Fatty Acids

Total lipids extracts were then analyzed for fatty acid composition. Fatty acid methyl esters (FAMES) were prepared by transesterification with 14% boron trifluoride (BF₃) in methanol at 85°C for 90 min (Budge 1999, Morrison & Smith 1964). The average Iatroscan-determined derivatization efficiency for marine samples is ~85%. FAMES were analyzed on an HP 6890 gas chromatograph with flame ionization detection equipped with a 7683 autosampler and a ZB wax + gas chromatography column (Phenomenex). The column was 30 m in length, with an internal diameter of 0.32 mm and a 0.25- μ m film. The oven temperature began at 65°C for 0.5 min and then the temperature was increased to 195°C (40°C/min), held for 15 min more, then increased again (2°C/min) to a final temperature of 220°C. Final temperature was held for 3.25 min. The carrier gas was hydrogen, flowing at a rate of 2 mL/min. Injector temperature started at 150°C and increased (200°C/min) to a final temperature of 250°C. The detector temperature was constant at 260°C. Peaks were identified using retention times based on standards purchased from Supelco (37-component FAME, BAME, PUFA 1, PUFA 3). Chromatograms were integrated using Galaxie Chromatography Data System (version 1.9.3.2; Varian).

Statistics

We used SigmaStat 10 to fit nonlinear piecewise regression functions to describe the relationship between RKC molt stage (36 days, independent variable) and dependent weight and lipid measures. This regression allows multiline fit equations to be defined over different independent variable (x) intervals. We expected to see 3 intervals: (1) a nonfeeding postmolt period, (2)

a feeding intramolt period, and (3) a nonfeeding premolt as described previously in RKC (Zhou et al. 1998) and shrimp (Ouellet & Taggart 1992). The equations used in the piecewise, 3-segment regressions are

Interval 1 (lipid or weight parameter, y)

$$= \{y_1(T_1 - t) + y_2(t - t_1)\} / (T_1 - t_1), t_1 < t < T_1$$

Interval 2 (lipid or weight parameter, y)

$$= \{y_2(T_2 - t) + y_3(t - T_1)\} / (T_2 - T_1), T_1 < t < T_2$$

Interval 3 (lipid or weight parameter, y)

$$= \{y_3(t_3 - t) + y_4(t - T_2)\} / (t_3 - T_2), T_2 < t < t_3$$

where t_1 is day 0, t is days, t_3 is day at the end of the experiment (33), T_1 is the calculated time at the end of the first segment (nonfeeding postmolt period), T_2 is the calculated time at the end of the second segment (feeding intramolt period), y_1 is lipid or weight parameter at $t_{1,(\text{time } 0)}$, y_2 is lipid or weight parameter at $T_{1,(\text{calculated})}$, y_3 is lipid or weight parameter at $T_{2,(\text{calculated})}$, and y_4 is lipid or weight parameter at $t_{3,(\text{end of the experiment day } 33)}$.

We report the r^2 values (proportion of variability in a data set that is accounted for by the statistical model) as well as the significant break points in the relationship (T_1 , T_2 , t values, $P < 0.05$).

Experiment 2: Comparison of Lipid Classes and Fatty Acids of Hatchery and Wild Juvenile RKCs

Hatchery-Cultured Crabs

Twenty ovigerous females were captured with baited pots in Bristol Bay, AK, during November 2009 and brought to the Alutiq Pride Shellfish Hatchery in Seward, AK (see experiment 1 for husbandry of broodstock and larvae).

Recently settled juvenile (C1) crabs were collected from larval rearing tanks, mixed randomly, and mass reared in two 2,000-L cylindrical nursery tanks for 67 days. The tanks were flow-through at approximately 10 L/min. Average culture temperature was 9°C and ranged from 8–12°C. Artificial seaweed was added to the nursery tanks to reduce agonistic interactions among conspecifics. A rich food variety was used in an attempt to provide crabs with all possible essential nutrients. Crabs were fed commercially available feeds including Cyclop-eeze (Argent Chemical Laboratories, WA), Otohime B1 and B2 fish feed (Reed Mericulture, CA), frozen and enriched *Artemia* nauplii, and Zeigler (Zeigler Bros., Inc., PA) shrimp feed, which have been used to culture juvenile RKCs successfully (Daly et al. 2009). Cyclop-eeze is a frozen whole-adult copepod (~800 μ m in length) that is high in carotenoids and omega-3 highly unsaturated fatty acids. Otohime B is a high-protein shrimp diet consisting of 200–360- μ m micropellets (B1) and 360–620- μ m micropellets (B2). Newly hatched San Francisco Bay strain *Artemia* nauplii (~400 μ m in length) have high levels of lipids and C₁₈ unsaturated fatty acids (Tizol-Correa et al. 2006). The San Francisco Bay strain *Artemia* nauplii were enriched with DC DHA Selco enrichment media for 24 h to enhance their nutritional quality. The enriched *Artemia* nauplii (~750 μ m) were frozen, which caused them to sink to be available for crab consumption. Zeigler PL Redi-Reserve commercial shrimp feed

consists of 400–600- μm particles and is commonly used in crustacean aquaculture because of its high levels of highly unsaturated fatty acids (Meade & Watts 1995). One feed type was administered daily. Crabs were fed approximately 2% body weight (DWT) daily.

Specimens for lipid analyses were collected arbitrarily from culture tanks and were held in clear water for 24 h to deplete. They were size sorted according to molt stage into C1, C2, C3, and age-1 juvenile stages. The number of animals per sample was greater for smaller molt stages than larger molt stages—C1 (5 animals), C2 (4 animals), C3 (3 animals), and age 1 (1 animal)—so that each lipid sample had a mass greater than 5 mg WWT. This ensured that adequate material was available for both lipid class and fatty acid analyses. Crabs were frozen immediately at -20°C , and were shipped on dry ice to Newport, OR, to be extracted within 3 mo of the original sample date.

Wild Crab Collections

Recently settled RKC were captured from Auke Bay ($58^{\circ}22' \text{N}$, $134^{\circ}40' \text{W}$), a small embayment in southeast Alaska located approximately 20 km north of Juneau, during summer 2010 using larval settlement collectors. The artificial collectors have an outer skin of tubular plastic netting stuffed with conditioned gill net and have been used successfully to collect passively young-of-year RKC in Alaska (Blau & Byersdorfer 1994). The collectors were deployed in May 2010 by divers along a 6-m depth contour. Collectors were attached to a 30-m ground line anchored at both ends. Along the ground line, the collectors were spaced 2 m apart. The collectors were retrieved in July 2010, and crabs were held in the laboratory for 24 h to deplete. They were then size sorted according to molt stage, C2–C4 as well as age-1 crabs based on known growth curves

(Stoner et al. 2010b). No wild C1 crabs were recovered from the artificial collectors. Crabs were then frozen at -20°C and later shipped to Newport, OR, on dry ice. Measurements of size, weight, lipid classes, and fatty acids are identical to those described earlier for experiment 1.

Statistical Comparison of Wild and Cultured Crabs

Cultured and wild crabs from molt stages 2, 3, and 10 were compared using 2-way ANOVA to examine the effect of sources (cultured or wild) and molt stage on RKC lipid content (SYSTAT 12 for Windows). However, there was, in general, a significant interaction between molt stage and culture type ($F_{2,24} = 12.9$, $P < 0.001$). Therefore, we used standard 2-sample t -tests to compare select lipid components between wild and cultured animals within a given molt stage. To avoid Type I error, our P values were Bonferroni corrected based on the number of lipid comparisons (n) made within each molt stage. Significance was set at $\alpha = 0.05/n$, which resulted in $\alpha = 0.005$ for lipid class analyses and $\alpha = 0.006$ for fatty acid analyses.

Principal component analysis (PCA) was used to simplify multivariate fatty acid and lipid class data by transforming correlated variables into a set of uncorrelated principal components (Minitab, version 15 (Meglen 1992)). This technique was used using 9 highly discriminatory fatty acid variables from wild and cultured juvenile RKC, at 5 developmental stages. The first 2 principal components (PC1 and PC2) accounted for 87% 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 fatty acid loading coefficients are defined as the correlation coefficients between the original fatty acid variable and the PCA axis. PCA scores are defined as the position of the sample along the new PCA axis (Meglen

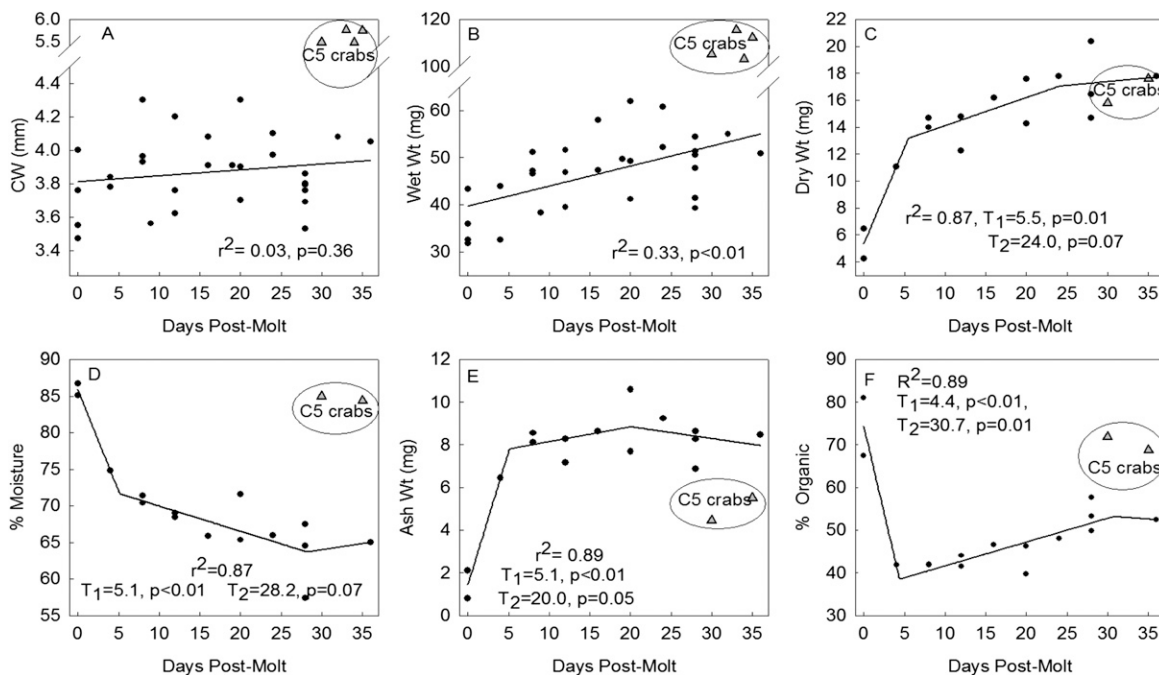


Figure 1. (A–F) Relationship between carapace width (A), wet weight (B), dry weight (C), percent moisture (D), ash weight (E), and percent organic weight (F) with days past molt for C4 juvenile red king crabs. Data from newly molted C5 crabs (triangles inside ellipses) are also shown. Relationships are described using linear regression (A, B; $n = 33$) and piecewise nonlinear regression (C–F; $n = 15$). CW, carapace width; Wt, weight.

1992). Fatty acids were chosen based on biological significance and the degree of variance explained by a given fatty acid.

RESULTS

Experiment 1: Intramolt Lipid Cycle of RKC Juveniles (C4–C5)

CW and WWT did not vary significantly ($r^2 = 0.03$ and $r^2 = 0.33$, respectively) with days past C4 molt (Fig. 1A, B). There was a dramatic increase in both CW and WWT from the C4–C5 molt stage. Average CW was 3.9 ± 0.2 mm in C4 crabs and 5.6 ± 0.2 mm in C5 crabs, whereas the average WWT was 46.6 ± 8.1 mg in C4s and 108.9 ± 5.8 mg in C5s. Average DWT was described by a 3-part piecewise regression ($r^2 = 0.87$) with significant breaks in the relationship at $T_1 = 5.5$ day and $T_2 = 24$ days. DWT increased rapidly during the first 5 days, from a low of ~ 5 mg at day 0 to ~ 13 mg at day 5. During the middle of the molt cycle, DWT continued to increase, but at a slower rate, increasing from ~ 13 mg at day 5 to ~ 17 mg at day 24. During premolt, days 24–32, there was little change in the DWT of the RKC (Fig. 1C). The percentage moisture in RKC showed an opposite trend to DWT; however, it was also well described by a 3-part piecewise regression ($r^2 = 0.87$) with significant breaks in the relationship at $T_1 = 5.1$ days and $T_2 = 28.2$ days. RKC juveniles showed a high level of water content just after molting, with $\sim 85\%$ moisture that decreased rapidly until day 5, when RKC had $\sim 72\%$ water (Fig. 1D). From day 5–day 28, the levels of moisture continued to decrease, but more gradually, to a low of $\sim 64\%$ at day 28. Ash weight mimicked the trends for DWT ($r^2 = 0.89$, $T_1 = 5.1$ days, $T_2 = 20$ days), whereas percent organic matter showed the opposite trend. There was a significant decrease from 75% of the dry mass as organic material at day 0 to $\sim 37\%$ at day 4 ($r^2 = 0.89$, $T_1 = 4.4$ days, $T_2 = 30.7$ days). The values for 2 newly molted C5s are shown to agree well with values for newly molted C4 RKC for both percentage moisture and organic weight (Fig. 1D–F).

Crabs contained, on average, 720 μg total lipid per crab and ~ 13 $\mu\text{g}/\text{mg}$ WWT (Table 1), with the 2 major lipid classes being TAG (20.3%) and PL (64.7%). Crabs contained, on average, 538 μg of total fatty acids per animal. The sum of the saturated fatty acids (ΣSFAs) made up 16.7% of the total fatty acids with 16:0 as the major SFA accounting for 15.3%. The sum of the monounsaturated fatty acids (ΣMUFAs) was, on average, 28.1% with 18:1n-9 and 18:1n-7, comprising 11.8% and 8.1%, respectively. ΣPUFAs were 49.1% of the total, and RKC contained high levels of both DHA (22:6n-3, 17.5%) and EPA (20:5n-3, 18.4%). The other essential fatty acid, AA (20:4n-6), was 2% of the total. Bacterial fatty acids made up of odd and branched chains were, on average, 2.9%, whereas shorter chain C_{18} PUFAs made up 6.3%. The ratio of DHA:EPA, an important nutritional indicator, was 1:1 (Table 1).

The relationship between both total lipids per animal (measured in micrograms) as well as total lipids per WWT (measured in micrograms per milligram) with days past molt were described by a 3-part piecewise regression (Fig. 2A, B). Total lipids per crab ($r^2 = 0.84$) showed significant changes in the relationship with days past molt at $T_1 = 7.1$ days and $T_2 = 22.7$ days. Initially, lipids decreased from ~ 390 μg per animal to ~ 200 μg at day 7. Then crabs showed a rapid accumulation of lipids from day 7 until day 22, with a high of ~ 950 μg . After day 22, no further increase was seen in the average level of total

TABLE 1.

Major lipid classes and fatty acids in all RKC examined throughout their intramolt cycle from C4 to C5.

Total lipids per crab ($\mu\text{g}/\text{animal}$)	720.3 \pm 359.2
Lipid classes expressed as % of total lipids	
Hydrocarbons	1.2 \pm 1.8
Ethyl ketones	0.7 \pm 2.4
Triacylglycerols	20.3 \pm 10.2
Free fatty acids	6.0 \pm 2.1
Sterols	4.6 \pm 1.5
Acetone mobile polar lipids	2.3 \pm 1.7
Phospholipids	64.7 \pm 9.2
TAG:ST	4.6 \pm 2.7
Total fatty acids per crab ($\mu\text{g}/\text{animal}$)	538.0 \pm 281.3
Percentage of total FAs	
14:0	1.3 \pm 0.2
16:0	15.3 \pm 0.6
18:0	3.7 \pm 0.3
ΣSFA^*	21.7 \pm 0.8
16:1n-7	2.4 \pm 0.3
18:1n-9	11.8 \pm 0.8
18:1n-7	8.1 \pm 0.7
20:1n-9	1.8 \pm 0.3
$\Sigma\text{MUFA}^\dagger$	28.1 \pm 1.4
18:2n-6	3.9 \pm 0.2
18:3n-3	2.4 \pm 0.7
20:4n-6	2.4 \pm 0.2
20:5n-3	18.4 \pm 1.1
22:6n-3	17.5 \pm 0.7
$\Sigma\text{PUFA}^\ddagger$	49.1 \pm 1.6
Σ Bacterial	2.9 \pm 0.5
Σ 18:2n-6 + 18:3n-3	6.3 \pm 0.9
22:6n-3/20:5n-3	1.0 \pm 0.2

* Also contains 15:0, 16:0, 17:0, 18:0, 19:0, 20:0, 21:0, 22:0, 23:0, and 24:0.

† Also contains 14:1, 15:1, 16:1n-11, 16:1n-9, 16:1n-5, 17:1, 18:1n-11, 18:1n-6, 18:1n-5, 20:1n-11, 20:1n-7, 22:1n-11(13), 22:1n-9, 22:1n-7, and 24:1.

‡ Also contains 16:2n-4, 16:3n-4, 16:4n-3, 16:4n-1, 18:2n-4, 18:3n-6, 18:3n-4, 18:4n-3, 18:4n-1, 18:5n-3, 20:2n-6, 20:3n-6, 20:3n-3, 20:4n-3, 22:4n-6, and 22:4n-3.

Bacterial FAs: Σ 15:0, 16:0, 17:0, 18:0, 19:0, 20:0, 21:0, and 22:0. Lipid classes $> 0.5\%$ and FAs $> 1.0\%$ are shown, $n = 15$, mean \pm SD.

lipids per RKC. A similar relationship was observed for total lipids per WWT ($r^2 = 0.92$) with $T_1 = 14.5$ days and $T_2 = 20.0$ days (Fig. 2B). The neutral lipid storage class (TAG) showed the same trends as total lipids ($r^2 = 0.86$) with $T_1 = 6.8$ days and $T_2 = 24.8$ days (Fig. 2C). However, the polar lipid class (PL) showed the opposite trend ($r^2 = 0.86$), with proportions decreasing rapidly from $\sim 79\%$ at day 4 to a low of $\sim 54\%$ at day 25 (Fig. 2D).

Total fatty acids showed the same pattern as total lipids, with a 3-part piecewise regression describing the relationship between total fatty acids and days post-C4 molt ($r^2 = 0.86$). A significant change in the relationship was observed at $T_1 = 10.1$ days and $T_2 = 20.0$ days (Fig. 3A). Total fatty acids increased from a low of ~ 175 μg at day 10 to a high of ~ 600 μg at day 20, and then gradually increased until day 33 to ~ 800 μg . The proportion of SFAs, MUFAs, and PUFAs remained stable

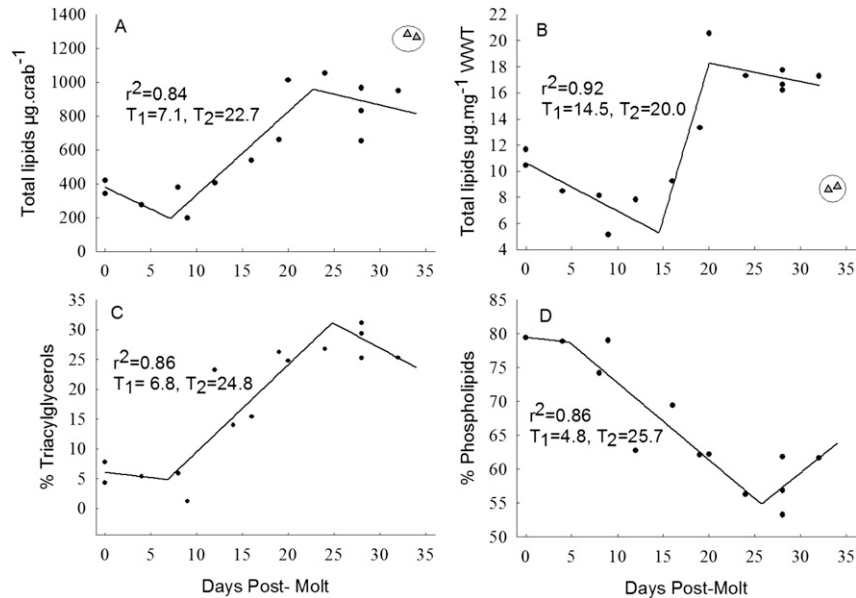


Figure 2. (A–D) Relationship between total lipids per crab (A), total lipids per weight wet (B), percent triacylglycerols (C), and percent phospholipids (D) with days past molt in C4 red king crab juveniles. The relationship between lipid parameters and days past molt were described using piecewise nonlinear regression ($n = 16$). T_1 is the calculated time at the end of first segment (nonfeeding post molt period) and T_2 is the calculated time at the end of the second segment (feeding intramolt period). Data from newly molted C5 crabs (triangles inside ellipses) are also shown. WWT, wet weight.

throughout the entire molt cycle and did not show a biologically meaningful relationship with days post-C4 molt, with coefficients of variation of 0.16, 0.12, and 0.007, respectively (Fig. 3B).

Experiment 2: Comparison of the Lipid Classes and Fatty Acids of Hatchery and Wild Juvenile RKC

Total lipids per crab varied considerably with molt stage from a low of 108 μg in C1 cultured crabs to a high of 12,035 μg in age-1 wild crabs. However, total lipids per WWT did not increase with molt stage, with C1 crabs having, on average, 16.8 $\mu\text{g}/\text{mg}$ WWT and age-1 crabs showing ~ 7.5 $\mu\text{g}/\text{mg}$ WWT (Table 2). TAG was the major neutral lipid, with no significant difference seen between wild and cultured animals at the same molt stage. Values for TAG ranged from a low of 21% in wild C3 crabs to a high of 39% in cultured age-1 animals. Significantly higher levels of FFAs were found in C2 and C3 cultured crabs compared with wild crabs at the same molt stage (Table 2). However, this significant difference in FFAs was not seen between age-1 wild and cultured animals. Overall, crabs contained 21.4% of their fatty acids as SFAs, and there were no differences in the Σ FFAs between wild and cultured crabs within the same molt stage (Fig. 4A). Both C2 and C3 cultured RKC showed higher proportions of MUFAs than that found in wild crabs of the same age (Fig. 4B), whereas wild C2 crabs had significantly higher proportions of PUFAs than those seen in cultured crabs (Fig. 4C). Wild crabs at the C2, C3, and age-1 stages had higher proportions of 20:4n-6 (AA) and 20:5n-3 (EPA) than cultured crabs (Fig. 4D, E), but there was no difference in the proportion of 22:6n-3 (DHA) between similarly aged wild and cultured crabs (Fig. 4F). The proportion of bacterial markers in wild C2 and C3 crabs was significantly higher than in cultured crabs of the same molt stage, whereas the DHA:EPA ratio was higher in cultured than wild crabs (Fig. 4G, H). Lastly, the proportion of short-chain C_{18} PUFAs

(18:3n-3 + 18:2n-6) was higher in cultured crabs for all molt comparisons than seen in wild crabs (Fig. 4I, Table 3).

PCA allowed the description of 87% of the variance in the data using only the first 2 principal components (Fig. 5). Nine lipid factors were chosen to describe the data based on the degree of variability explained and their biological importance. The first principal component (PC1) explained 72% of the variability in the data set and represented an axis that separated cultured from wild RKC juveniles. Toward the “wild” RKC negative side of the axis, two C_{20} PUFAs (20:5n-3 and 20:4n-6) were represented along with increased bacterial markers. On the positive “cultured” side of the axis, there were higher proportions of the MUFAs 20:1n-9 and 18:1n-9, as well as 2 short-chain C_{18} PUFAs (18:2n-6 and 18:3n-3). PC2 explained 15% of the variability in the data, and separated samples based on molt stage. There was more variability in wild crab samples than in cultured crabs along PC2, with negative values indicating increased lipid per WWT, and positive values indicating increases in the long-chain PUFA 22:6n-3. The trend in cultured crabs showed C1s located further negatively with increased lipids per WWT. Cultured crabs showed a general decrease in lipids per weight with age, as demonstrated by the trended from negatively located C1s and C2s toward positively located age-1 crabs. However, wild crabs showed an opposite trend, with age-1 crabs located toward the negative side of PC2, indicating increased lipids per WWT, with both C3 and C4 crabs located positively, indicating higher proportions of 22:6n-3 and lower total lipids.

DISCUSSION

Experiment 1: Intramolt Lipid Cycle of RKC Juveniles

Biochemical changes that occur during molting cycles are an overriding physiological factor determining condition, especially in larval and juvenile crustaceans that have low energy

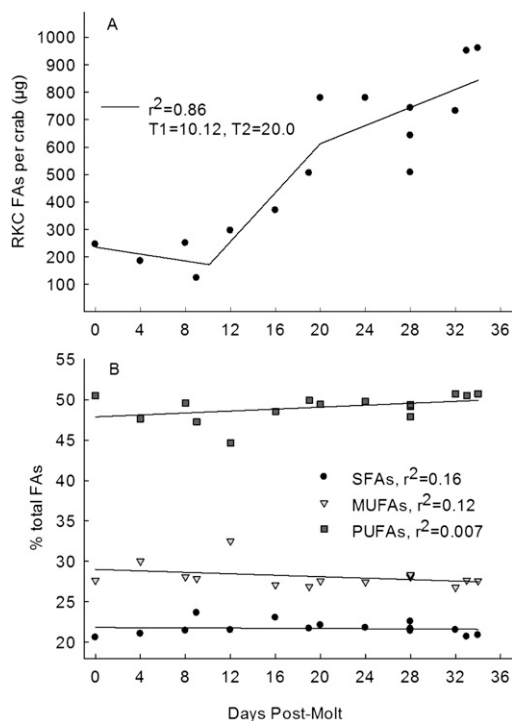


Figure 3. (A, B) Relationship between total FAs (A) and percent PUFAs, percent MUFAs, and percent SFAs (B) with days past molt in C4 red king crab (RKC) juveniles. The relationship between total FAs (A) and days past molt was described using piecewise nonlinear regression, whereas the relationships between FA proportions (B) and days past molt were described using linear regression ($n = 14$). FA, fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

reserves (Sasaki et al. 1986, Ouellet & Taggart 1992). Our study indicates that RKC proximate composition, total lipids, and total fatty acids undergo changes in association with ecdysis. We observed 3 biochemical phases during the molt cycle that were typified by a short postmolt period (~5–10 days), an

intramolt period (~15–20 days), and a premolt period (~5–10 days). Our results agree with the general pattern of energy accumulation and use throughout crustacean molt cycles, as we observed no increase in lipids during the postmolt period, a rapid accumulation of lipid during the feeding intramolt stage, and lastly, a decrease in lipids during a nonfeeding premolt stage (Ouellet & Taggart 1992, Zhou et al. 1998, Sanchez-Paz et al. 2006). Therefore, the interpretation of bioenergetic data for both ecological and aquaculture applications on RKC must consider the cyclical molt-directed nature of energy accumulation and utilization.

CW and WWT remained relatively stable throughout the molt cycle; however, dramatic changes in moisture content, DWT, and ash weight occurred during the postmolt period (Fig. 1). RKC total lipids decrease during postmolt, whereas both TAG and PL proportions remained relatively stable (Fig. 2). Adult RKC have been shown to reduce feeding significantly, for up to 8 days directly after ecdysis, and have an even longer period of reduced feeding during their premolt stage (Zhou et al. 1998). Our study also showed decreasing lipid accumulation beginning at ~11 days premolt, likely indicating reduced feeding prior to molting.

TAG is the most common lipid storage class for larval fish, bivalves, and crustaceans, and has been found to accumulate in larvae and juveniles when their exogenous energy supplies exceed their immediate metabolic demands (Gallager et al. 1986, Sasaki et al. 1986, Harding & Fraser 1999). TAG was the major neutral lipid storage class found in RKC juveniles and it showed a dramatic increase during the intramolt feeding period (5–30%), explaining the majority of the increase in total lipids observed during intramolt (Fig. 2). Absolute TAG content alone is not an appropriate condition index for marine organisms because it varies significantly with larval size (Fraser 1989). A more appropriate measure of condition is the amount of TAG per DWT in an animal; however, it is often impractical to determine DWT in individual larvae/juveniles that will be processed for lipid analyses. This is because of their small size and the risk of hydrolysis and oxidation during the freeze-drying

TABLE 2.

Total lipids and major lipid classes in 5 molt stages of both wild-caught and hatchery-cultured RKC juveniles.

	C1		C2		C3		C4		Age 1	
	Cultured	Cultured	Wild	Cultured	Wild	Wild	Cultured	Wild		
Total lipids (µg/animal)	108.8 ± 26.5	131.1 ± 13.4	118.6 ± 38.1	151.8 ± 51.9	112.4 ± 28.0	207.1 ± 167.4	8.1 × 10 ³ ± 1.6 × 10 ³	12.0 × 10 ³ ± 9.6 × 10 ³		
Total lipids (µg/mg WWT)	16.7 ± 3.4	17.1 ± 3.6	12.0 ± 4.2	10.4 ± 2.6	5.3 ± 1.7	5.4 ± 4.4	5.9 ± 2.4	8.9 ± 4.4		
% Total lipids										
Hydrocarbons	0.3 ± 0.4	0.1 ± 0.2	0.2 ± 0.3	0.2 ± 0.4	—	—	1.4 ± 0.8	1.6 ± 1.3		
Sterol/wax esters	0.4 ± 0.6	0.2 ± 0.4	—	—	2.8 ± 6.3	1.4 ± 2.2	—	—		
TAG	24.3 ± 11.5	24.5 ± 6.7	29.4 ± 7.3	24.6 ± 4.8	21.3 ± 14.1	27.7 ± 20.2	39.2 ± 17.6	23.4 ± 13.7		
FFA	30.6 ± 12.2	33.5 ± 7.8	2.9 ± 0.8*	21.4 ± 3.1	0.8 ± 1.1*	3.4 ± 3.0	1.3 ± 1.3	2.1 ± 2.0		
Sterols	8.4 ± 1.6	8.8 ± 1.4	10.6 ± 6.3	11.1 ± 4.2	18.1 ± 3.7	20.5 ± 22.6	13.9 ± 5.3	11.9 ± 4.6		
AMPL	5.9 ± 1.6	4.4 ± 2.0	6.2 ± 6.0	8.7 ± 4.5	8.4 ± 7.8	10.9 ± 8.9	9.1 ± 4.9	12.4 ± 5.2		
PL	29.3 ± 7.6	25.3 ± 3.2	50.8 ± 19.1	34.0 ± 12.2	48.1 ± 12.5	32.3 ± 21.2	35.1 ± 21.2	47.6 ± 17.5		
TAG/ST	2.9 ± 1.3	2.8 ± 0.9	3.1 ± 0.9	2.4 ± 0.9	1.3 ± 1.0	2.4 ± 2.5	3.3 ± 2.3	2.4 ± 1.8		

* Significant difference in the lipid composition of wild and cultured crabs within the same molt class, Bonferroni corrected P value < 0.005. Data are the mean of 5 RKC ± SD.

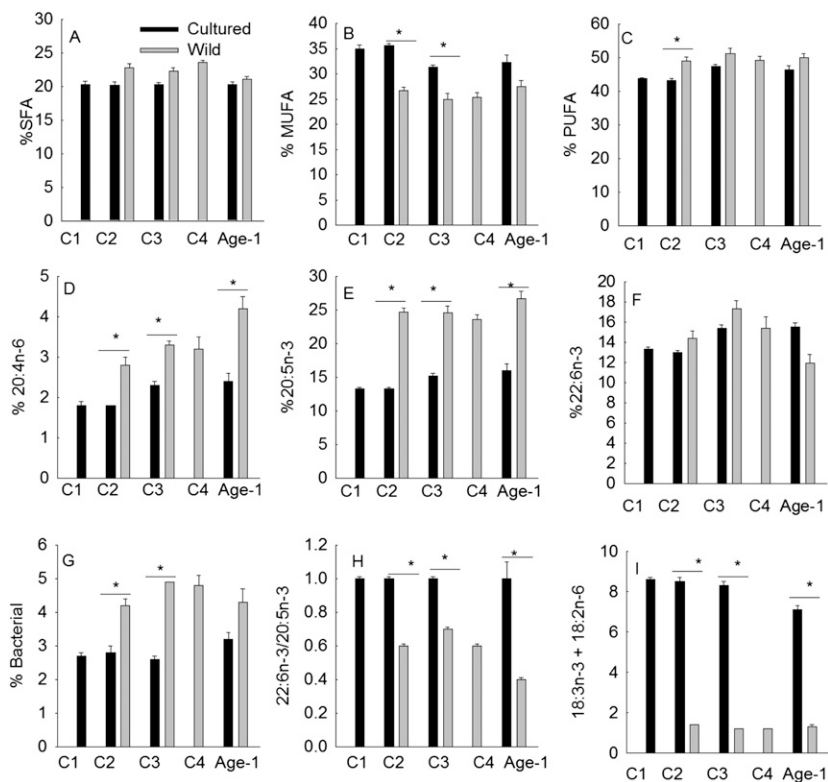


Figure 4. (A–I) Proportion of saturated fatty acid (A), monounsaturated fatty acid (B), polyunsaturated fatty acid (C), 20:4n-6 (D), 20:5n-3 (E), 22:6n-3 (F), bacterial (G), 22:6n-3/20:5n-3 (H), and $\Sigma 18:3n-3 + 18:2n-6$ (I) in wild and cultured C1 to age-1 red king crab juveniles. *Significant difference between wild and cultured crabs of the same molt stage, family error rate of $P < 0.05$. Error bars are SEs, $n = 5$. MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

and weighing procedures. Instead, the use of a TAG-to-ST ratio has been proposed because STs are directly proportional to body DWT and are not significantly catabolized during periods of starvation (Sasaki et al. 1986, Fraser 1989). Furthermore, STs are easily measured on the Iatroskan using the same solvent development system as for TAG (Parrish 1987).

The TAG:ST condition factor in all C4 RKC was variable, but on average was $4.4:1 \pm 1.8$ (Table 1). This ratio mimicked the increase in TAG seen throughout the molt cycle, with a postmolt ratio of $\sim 2.5:1$ and a premolt ratio of $\sim 7:1$. Larval American lobsters (*Homarus americanus*) from Georges Banks and the Gulf of Maine also have significant variability in this condition index (Harding & Fraser 1999). The TAG:ST condition index for American lobster increased from 0.4:1 during molt stage 1, to 8:1 during molt stage 5. High levels of variability in the TAG:ST index within a given molt (i.e., RKC C4) indicates that caution should be taken when applying this index to ecological questions using wild samples. Comparisons of condition in crabs from different field locations without knowing the phase of their molt cycle at the time of collection is problematic. Furthermore, the increase in TAG-to-ST ratios with each successive molt indicates that animals of different molt stages (e.g., C3 and C5) should not be compared using this index.

PLs play an important role in the nutrition of marine crustaceans (Sanchez-Paz et al. 2006, Wu et al. 2007). PLs along with protein are major components of cell membranes, and they mediate cell transmembrane signaling. Furthermore, PLs are an emulsifier and is important for digestion during early

stages of crustacean development (Couteau et al. 1996). There was a proportional decrease in the relative amount of PL in juvenile RKC from $\sim 80\%$ at postmolt to 55% at the end of the intramolt period, reflecting the relative increase in TAG. However, this was not an absolute decrease because the amount of PL on a per-WWT basis increased from 4 $\mu\text{g}/\text{mg}$ at the end of the postmolt period to 11 $\mu\text{g}/\text{mg}$ WWT at the end of the intramolt period (data not shown).

Total fatty acids (measured in micrograms per animal) showed the same pattern as total lipids per animal, with a large increase during the intramolt period; however, there was little change in the relative proportions (measured as a percentage) of ΣSFA , ΣMUFA , and ΣPUFA (Fig. 3). This result is surprising given that the lipid class composition of RKC changed from 80% PL during the postmolt period to 55% PL during the premolt period. In general, PLs contain higher proportions of ΣPUFA and lower levels of ΣMUFA , in marine organisms (Sargent et al. 1999). This may indicate a reduced level of membrane specificity in RKC compared with other marine larvae (Copeman et al. 2002), or it could indicate that their diet had an abundance of EFAs so that both neutral and polar lipids contained high levels of ΣPUFA . In general, under reduced feed conditions, poor nutrition, or during starvation, ΣPUFA are conserved in the PL of both marine fish and crustaceans (Sargent et al. 1999, Copeman & Parrish 2002, Sheen & Wu 2002).

The lack of variability in the relative composition of RKC fatty acids (measured as a percentage; Fig. 3) during the intramolt period indicates that the use of fatty acid trophic

TABLE 3.
Major fatty acids (>1%) in 5 molt stages of wild-caught and hatchery-cultured RKC juveniles.

	C1		C2		C3		C4		Age 1	
	Cultured	Cultured	Wild	Cultured	Wild	Wild	Cultured	Wild		
Total fatty acids per wet weight ($\mu\text{g}/\text{mg}$)	13.0 \pm 1.5	14.4 \pm 1.3	8.6 \pm 1.5	7.7 \pm 1.0	3.3 \pm 0.6	3.6 \pm 1.6	4.0 \pm 0.7	5.8 \pm 1.6		
14:0	1.6 \pm 0.3	1.7 \pm 0.1	1.9 \pm 0.6	1.2 \pm 0.2	1.2 \pm 0.6	2.2 \pm 0.9	1.6 \pm 0.3	2.4 \pm 0.8		
16:0	13.5 \pm 0.8	13.4 \pm 0.7	13.6 \pm 1.2	13.9 \pm 0.5	13.3 \pm 0.2	13.4 \pm 0.2	13.1 \pm 0.9	12.4 \pm 0.8		
18:0	3.0 \pm 0.3	3.1 \pm 0.3	4.9 \pm 0.6	3.5 \pm 0.3	5.2 \pm 0.5	4.9 \pm 0.3	3.8 \pm 0.3	4.4 \pm 0.4		
Σ SFA*	20.3 \pm 1.1	20.2 \pm 1.2	22.8 \pm 1.3	20.3 \pm 0.6	22.3 \pm 1.0	23.6 \pm 0.6	20.3 \pm 0.9	21.1 \pm 0.9		
16:1n-7	4.9 \pm 0.4	5.1 \pm 0.3	6.8 \pm 1.5	3.4 \pm 0.5	4.7 \pm 1.7	6.9 \pm 2.4	3.9 \pm 0.9	6.6 \pm 1.5		
17:1	0.8 \pm 0.2	0.9 \pm 0.2	1.6 \pm 0.5	0.9 \pm 0.3	2.1 \pm 0.2	1.4 \pm 0.7	1.3 \pm 0.4	1.6 \pm 0.8		
18:1n-9	13.3 \pm 0.4	13.5 \pm 0.6	7.1 \pm 2.2	13.0 \pm 0.5	6.3 \pm 0.5	5.5 \pm 0.5	15.4 \pm 1.3	6.8 \pm 0.8		
18:1n-7	7.0 \pm 0.1	6.9 \pm 0.2	8.7 \pm 0.5	7.2 \pm 0.3	8.6 \pm 0.3	7.9 \pm 0.8	6.8 \pm 0.3	7.5 \pm 1.0		
20:1n-11	1.2 \pm 0.2	1.3 \pm 0.2	0.3 \pm 0.1	0.9 \pm 0.1	0.4 \pm 0.1	0.4 \pm 0.3	0.7 \pm 0.3	1.3 \pm 1.0		
20:1n-9	2.7 \pm 0.4	2.8 \pm 0.2	0.5 \pm 0.1	2.3 \pm 0.4	0.6 \pm 0.2	0.4 \pm 0.2	1.3 \pm 0.2	0.8 \pm 0.2		
22:1n-11	3.2 \pm 0.3	3.3 \pm 0.5	—	2.2 \pm 0.4	—	—	0.9 \pm 0.4	—		
Σ MUFA†	35.0 \pm 1.3	35.6 \pm 0.8	26.7 \pm 1.4	31.3 \pm 1.2	25.0 \pm 2.4	25.3 \pm 2.1	32.3 \pm 3.1	27.5 \pm 2.8		
18:2n-6	6.7 \pm 0.2	6.7 \pm 0.3	1.1 \pm 0.1	6.5 \pm 0.4	0.9 \pm 0.0	0.9 \pm 0.1	5.3 \pm 0.4	1.0 \pm 0.1		
18:3n-3	1.9 \pm 0.1	1.8 \pm 0.2	0.3 \pm 0.1	1.8 \pm 0.1	0.3 \pm 0.1	0.3 \pm 0.0	1.8 \pm 0.2	0.3 \pm 0.1		
18:4n-3	0.9 \pm 0.1	0.9 \pm 0.1	1.0 \pm 0.3	0.6 \pm 0.1	0.7 \pm 0.3	0.9 \pm 0.7	0.7 \pm 0.2	0.8 \pm 0.3		
20:4n-6	1.8 \pm 0.1	1.8 \pm 0.1	2.8 \pm 0.3	2.3 \pm 0.2	3.3 \pm 0.3	3.2 \pm 0.7	2.4 \pm 0.4	4.2 \pm 0.7		
20:5n-3	13.3 \pm 0.4	13.3 \pm 0.5	24.7 \pm 1.3	15.4 \pm 1.0	24.6 \pm 2.1	23.6 \pm 1.6	16.0 \pm 2.4	26.7 \pm 2.4		
22:6n-3	13.3 \pm 0.4	13.0 \pm 0.4	14.4 \pm 1.4	15.4 \pm 0.7	17.3 \pm 1.8	15.4 \pm 2.4	15.5 \pm 0.9	11.9 \pm 2.1		
Σ PUFA‡	43.7 \pm 0.7	43.1 \pm 1.4	49.0 \pm 2.5	47.4 \pm 1.0	51.2 \pm 3.3	49.2 \pm 2.7	46.4 \pm 2.6	49.9 \pm 2.6		
Bacterial	2.7 \pm 0.2	2.8 \pm 0.4	4.2 \pm 0.6	2.6 \pm 0.3	4.9 \pm 0.1	4.8 \pm 0.7	3.2 \pm 0.4	4.3 \pm 0.9		
DHA:EPA	1.0 \pm 0.0	1.0 \pm 0.0	0.6 \pm 0.1	1.0 \pm 0.0	0.7 \pm 0.0	0.6 \pm 0.1	1.0 \pm 0.1	0.4 \pm 0.1		
Zooplankton	7.9 \pm 0.8	8.3 \pm 0.8	1.8 \pm 0.4	5.9 \pm 1.0	2.7 \pm 0.9	2.7 \pm 1.0	3.9 \pm 1.1	4.1 \pm 2.0		
Σ 18:2n-6 + 18:3n-3	8.6 \pm 0.3	8.5 \pm 0.4	1.4 \pm 0.1	8.3 \pm 0.5	1.2 \pm 0.1	1.2 \pm 0.0	7.1 \pm 0.4	1.3 \pm 0.1		

* Also contains *i15:0*, *ai15:0*, 15:0, *i16:0*, *ai16:0*, *i17:0*, *ai17:0*, 17:0, 20:0, 22:0, 23:0, and 24:0.

† Also contains 14:1, 15:1, 16:1n-11, 16:1n-9, 16:1n-5, 17:1, 18:1n-11, 18:1n-6, 18:1n-5, 20:1n-11, 20:1n-7, 22:1n-11(13), 22:1n-9, 22:1n-7, and 24:1.

‡ Also contains 16:2n-4, 16:3n-4, 16:4n-3, 16:4n-1, 18:2n-4, 18:3n-6, 18:3n-4, 18:4n-3, 18:4n-1, 18:5n-3, 20:2n-6, 20:3n-6, 20:3n-3, 20:4n-3, 22:4n-6, and 22:4n-3.

Bacterial fatty acids are the Σ 15:0, *ai15:0*, *i15:0*, *i16:0*, *ai16:0*, 15:1, 17:0, and 17:1.

Zooplankton is Σ 20:1 + 22:1.

Data are the mean of 5 RKC \pm SD.

markers in ecological studies will not be significantly affected by the intramolt stage. In general, our reported levels of Σ PUFA, Σ MUFA, and Σ SFA in RKC larvae are in agreement with those from previous studies (Stoner et al. 2010b).

Experiment 2: A Comparison of Lipids in Hatchery and Wild Juvenile RKC

Hatchery-cultured and wild RKC of the same molt stage differed in lipid composition likely as a result of both dietary and husbandry practices. The amount of lipid per WWT reported here (\sim 5–17 $\mu\text{g}/\text{mg}$) for hatchery and wild RKC is in agreement with previous studies. Alaskan cultured RKC and blue king crab (*Paralithodes platypus*) larvae have been previously reported to contain 9 μg lipid/mg WWT and 19 μg lipid/mg WWT, respectively (Leroux per. comm.). Cultured spider crabs contain \sim 40 $\mu\text{g}/\text{mg}$ lipid/DWT, which is comparable with 20 $\mu\text{g}/\text{mg}$ WWT, assuming 50% moisture (Andres et al. 2011). Previously, wild-caught cold-water Arctic lyre crabs and hermit crabs were shown to contain comparable amounts of 3.2 μg lipid/mg WWT and 14.3 μg lipid/mg WWT, respectively (Copeman & Parrish 2003).

The lipid class composition of wild and cultured RKC was generally similar (Table 2), which indicates that the mixture of enriched live food and gel food were meeting the basic nutritional requirements of juvenile crabs. High FFA levels (21–31%, Table 2) in cultured age-0 RKC at stages C1–C3 were unexpected. In experiment 1, we found that the average level of FFA in RKC juveniles from 2009 was only 6%, whereas Stoner et al. (2010b) reported FFA levels ranging from 1.9–5.9% in RKC reared by the same Alaskan hatchery. In addition, Copeman and Parrish (2003) measured FFA in 15 different species of cold-water benthic invertebrates and found levels that ranged from 0.6–20% of total fatty acids. It was originally thought that FFA levels less than 25% were acceptable for phytoplankton and zooplankton samples (Parrish 1988), but more recently, Berge et al. (1995) demonstrated that most of the FFA in diatom samples (*Skeletonema costatum*) was the result of lipolysis of PL during routine sampling procedures. Our RKC samples with elevated FFA also had reduced proportions of PL, indicating that some of the FFA here likely resulted from lipolysis of PL during sampling or shipping from a remote location in Alaska. Most interestingly, age-1 crabs that were handled in the same manner did not show elevated levels in

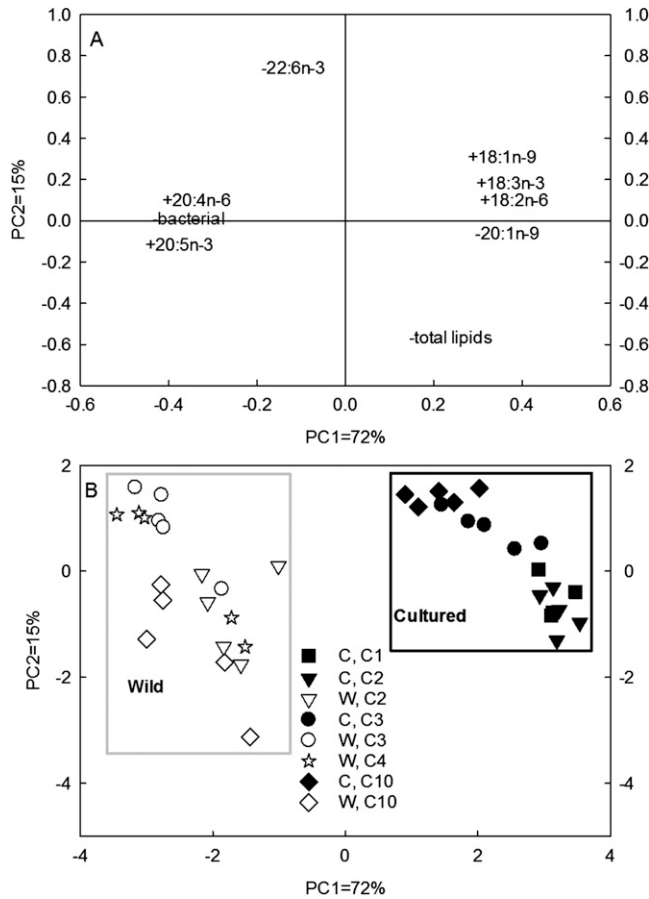


Figure 5. (A, B) Principal component analysis of lipid class and fatty acid proportions in cultured and wild red king crab (RKC) juveniles from 5 different molt stages. Lipid loading coefficients for PC1 (A) and PC2 (B) scores for cultured and wild RKC juveniles. Analysis of the first 2 principal components of lipid data used the following: 20:4n-6, 22:6n-3, 20:5n-3, total lipids per wet weight (measured in micrograms per milligram), Σ bacterial fatty acid markers, 18:3n-3, 18:2n-6, 18:1n-9, and 20:1n-9. Bacterial markers included Σ -i-15:0, ai-15:0, 15:0, 15:1, i-16:0, ai-16:0, i-17:0, ai-17:0, 17:0, 17:1, and 18:1n-6. Groups were determined by cluster analysis. Lipid parameter coefficients (+ or -) represent orientation along the third principal component axis whereas RKC scores are shown for the first 2 principal components. Cultured crabs (C) and wild crabs (W) are shown for molt stages C1–C10.

hatchery-cultured individuals. Clearly, extreme care must be taken when working with RKC to keep samples cold throughout the sampling, shipping, and extraction procedures.

A comparison of the proportions of EFAs in both wild and cultured crabs displayed differences in all molt stages (Table 3). In general, wild crabs had higher proportions of AA (20:4n-6) and EPA (20:5n-3) than those found in cultured crabs, whereas there was no significant difference in the percentage of DHA (22:6n-3) found in crabs throughout the 5 different molt stages. EPA and AA are important fatty acids both for inclusion in membranes and for the production of biologically active compounds called eicosanoids. These “localized hormones,” such as prostaglandins, thromboxanes, and leukotrienes, play a wide variety of physiological roles in marine organisms that can range from ionic regulation to stress responses (Sargent 1995, Reddy et al. 2004). Their role in cold-water crabs is yet to be investigated

in detail. Sheen and Wu (2002) found that the warm-water juvenile mud crabs (*Scylla serrata*) conserved AA, EPA, and DHA in their polar lipid and whole-body tissues during starvation. Furthermore, after 70-day feeding trials, they found that a dietary source of DHA and AA improved weight gain compared with crabs reared on a control low-PUFA diet. In addition, Nghia et al. (2007) reported that dietary algae with high levels of AA did improve the growth and molting success of larval mud crabs (*Scylla paramamosain*).

In the wild, DHA, EPA, and AA are synthesized by primary producers and are concentrated as they move through the food web to higher level consumers (Parrish 2009). In general, elevated levels of EPA in plankton have been correlated with diatom production, whereas elevated DHA is found at higher proportions in dinoflagellates (Dunstan et al. 1994, Parrish et al. 2000, Stevens et al. 2004). AA is also found at high levels in macroalgae, but in general is found in proportions of less than 4% in marine plankton (Copeman et al. 2003, Copeman et al. 2009). Little is known about the feeding habits of juvenile RKC in the wild; however, diet mediates survival and morphology of cultured RKC (Daly et al. unpub.). Recently, Pirtle and Stoner (2010) investigated habitat choice in juvenile RKC and found that juveniles associated with biogenic habitats composed of structural invertebrates and macroalgae significantly more often than with artificial structured habitats. Furthermore, foraging was especially high on bryozoans and hydroids, but additional work is required to elucidate the role that diet and nutrition play in habitat choice by age-0 RKC in the wild.

In Atlantic fish larviculture, a DHA:EPA ratio of 2:1 has been suggested as adequate for normal growth, development, and pigmentation (Sargent 1995, Sargent et al. 1999). Therefore, enrichment oils for live feeds, such as DC DHA Selco for *Artemia* sp. used in this study, have been formulated with high proportions of DHA to meet this 2:1 requirement. Nevertheless, little is known about the dietary requirements of cold-water crabs. Increased levels of DHA:EPA reduce developmental retardation and metamorphosis failure of some warm-water species (e.g., *S. paramamosain* (Nghia et al. 2007)). Chinese mitten crab (*Eriocheir sinensis*) larvae show improved stress test results and elevated survival with higher levels of dietary DHA:EPA (Sui et al. 2007). Wild RKC had lower ratios of DHA:EPA in their tissues (0.6:1), and therefore may not require such high levels of enrichment with DHA. Similarly, Pacific cod larvae grow well at a ratio of 1:1, which is lower than required for their Atlantic cogener (*Gadus morhua*, DHA:EPA > 2:1 (Copeman & Laurel 2010)). Controlled feeding studies with larval and juvenile RKC are required to determine EFA requirements for this new culture species.

PCA analyses demonstrated that the variability in lipid composition between wild and cultured crabs was higher than the variability between different molt stages (Fig. 5; C1–age 1). PC1 explained much of the variation in the data set, and showed wild crabs associated with increased EPA, AA, and bacterial markers. Bacterial fatty acids include odd and branched-chained C₁₅ and C₁₇ fatty acids. Wild RKC had about 4.5% of their total fatty acids from bacterial sources, whereas cultured crabs had lower proportions (~2%). This dietary source could be incorporated into wild RKC after feeding on biofilms that foul many of their biogenic habitats, such as macroalgae (Pirtle & Stoner 2010). Copeman et al. (2009) found bacterial levels of 8.3% in epiphytes scraped from eelgrass (*Zostera marina*)

blades, whereas Kharlamenko et al. (2001) reported ~6% in epiphytes from cold-water eelgrass habitats. Furthermore, elevated proportions of zooplankton fatty acids (~6.5%, $\Sigma 22:1 + \Sigma 20:1$) in cultured crabs compared with wild animals (~2.7) were also noted. Cultured crabs consumed both enriched *Artemia* and Cyclop-eeze, both of which have elevated levels of these long-chain MUFA zooplankton markers (Nair et al. 2007). Conversely, based on the fatty acid profiles of wild RKC juveniles, they likely consume very little zooplankton in the field after settlement onto benthic habitats.

CONCLUSIONS

This study has implications for both crab ecology and for suitable crab feed development. Juvenile RKCs showed variability throughout their intramolt cycle, which agreed with the 3-phase feeding pattern observed in adult RKCs and in other crustaceans. Ecological and aquaculture studies of RKC must consider the cyclical nature of energy accumulation and utilization associated with molting patterns. Care should be taken when comparing condition or proximate composition in crabs of unknown molt stage. Because of the high variability in crab lipids, large sample sizes are required to detect differences in condition between crabs from different locations or habitat types. No difference in the relative proportions of fatty acids in crabs throughout their molt cycle suggests that fatty acid biomarkers will not be significantly affected by the intramolt stage.

Wild and cultured crabs showed differences in their lipid composition, with wild crabs characterized by higher proportions of EPA and AA. Additional studies are required to determine the effect of these different ratios of fatty acids on

growth, molt success, and behavior of juvenile crabs. Our data provide a starting point for future nutritional work, with the essential DHA:EPA:AA ratio in wild crabs being 5:8:1. Future studies should aim at developing feeds with similar essential fatty acid ratios.

ACKNOWLEDGMENTS

Sample processing costs and salary for L. C. were provided by a joint NOAA aquaculture and a Cooperative Institute for Marine Resource Studies, Oregon State University grant (no. NA17RJ1362). We are thankful to Miranda Westphal, Jaspri Sylvan, and Melissa Rhodes-Reese at the University of Alaska for the collection and shipment of wild RKCs. We also thank Jim Swingle and Jeff Hetrick of the Alutiiq Pride Shellfish Hatchery for hatchery logistical support, and Jeff Stephan and Lu Dochterman for helping with broodstock acquisition. Our lipid data were analyzed in partnership with the Core Research Equipment and Instrument Training (CREAIT) Network of Memorial University, Newfoundland, Canada. Partnership funding for lipid analyses was provided through a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to C. P. We thank Jeanette Wells and Tara Hooper for chromatography of lipid classes and fatty acids on lipid extracts. Thanks also to Scott Haines and Paul Iseri for providing husbandry assistance in the Newport laboratory during the juvenile intramolt study. Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA. The findings and conclusions in the paper are those of the authors and do not necessarily represent the view of the National Marine Fisheries Service.

LITERATURE CITED

- Alaska Department of Fish and Game. 2010. Alaska commercial shellfish fisheries catches & exvessel values. <http://www.cf.adfg.state.ak.us/geninfo/shellfish/09value.php> </biburl>. Accessed May 23, 2010.
- Andres, M., G. Rotllant, M. Sastre & A. Estevez. 2011. Replacement of live prey by formulated diets in larval rearing of spider crab *Maja brachydactyla*. *Aquaculture* 313:50–56.
- Berge, J. P., J. P. Gouygou, J. P. Dubacq & P. Durand. 1995. Reassessment of lipid-composition of the diatom *Skeletonema costatum*. *Phytochemistry* 39:1017–1021.
- Blau, S. F. & S. C. Byersdorfer. 1994. Sausage-shaped artificial collector developed in Alaska to study young-of-year red king crabs. *Bull. Mar. Sci.* 55:878–886.
- Budge, S. M. 1999. Fatty acid biomarkers in a cold water marine environment. PhD diss. Memorial University of Newfoundland. 197 pp.
- Budge, S. M., S. J. Iverson & H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Mar. Mamm. Sci.* 22:759–801.
- Calcagno, J. A., G. A. Lovrich, S. Thatje, U. Nettelmann & K. Anger. 2005. First year growth in the lithodids *Lithodes santolla* and *Paralomis granulosa* reared at different temperatures. *J. Sea Res.* 54:221–230.
- Copeman, L. A. & B. J. Laurel. 2010. Experimental evidence of fatty acid limited growth and survival in Pacific cod (*Gadus macrocephalus*) larvae. *Mar. Ecol. Prog. Ser.* 412:259–272.
- Copeman, L. A. & C. C. Parrish. 2002. Lipid composition of malpigmented and normally pigmented newly settled yellowtail flounder, *Limanda ferruginea* (Storer). *Aquacult. Res.* 33:1209–1219.
- Copeman, L. A., C. C. Parrish, J. A. Brown & M. Harel. 2002. Effects of DHA, EPA and AA on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. *Aquaculture* 210:185–204.
- Copeman, L. A., C. C. Parrish. 2003. Marine lipids in a cold coastal ecosystem. *Marine Biology* 143(6):1213–1229.
- Copeman, L. A., C. C. Parrish, R. S. Gregory & J. Wells. 2008. Decreased lipid storage in juvenile Atlantic cod (*Gadus morhua*) during settlement in cold-water eelgrass habitat. *Mar. Biol.* 154:823–832.
- Copeman, L. A., C. C. Parrish, R. S. Gregory, R. E. Jamieson, J. Well & M. J. Whiticar. 2009. Fatty acid biomarkers in coldwater eelgrass meadows: elevated terrestrial input to the food web of age-0 Atlantic cod *Gadus morhua*. *Mar. Ecol. Prog. Ser.* 386:237–251.
- Couteau, P., M. R. Camara & P. Sorgeloos. 1996. The effect of different levels and sources of dietary phosphatidylcholine on the growth, survival, stress resistance and fatty acid composition of postlarval *Penaeus vannamei*. *Aquaculture* 147:261–273.
- Daly, B. 2010. King crab rehabilitation in Alaska. In: G. Bishop, editor. *Oncorhynchus*. Newsletter of the Alaska Chapter, American Fisheries Society, Summer 2010 (Vol. 30, No. 3) 1–4.
- Daly, B., J. S. Swingle & G. L. Eckert. 2009. Effects of diet, stocking density, and substrate on survival and growth of hatchery-cultured red king crab (*Paralithodes camtschaticus*) juveniles in Alaska, USA. *Aquaculture* 293:68–73.
- Daly, B., J. S. Swingle & G. L. Eckert. 2011. Evaluation of astaxanthin and calcium as dietary supplements for hatchery-cultured red king crab (*Paralithodes camtschaticus*) juveniles. *Aquaculture*.

- Dunstan, G. A., J. V. Volkman, S. M. Barrett, J. Leroi & S. W. Jeffrey. 1994. Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). *Phytochemistry* 35:155–161.
- Epelbaum, A. B., R. R. Borisov & N. P. Kovatcheva. 2006. Early development of the red king crab *Paralithodes camtschaticus* from the Barents Sea reared under laboratory conditions: morphology and behaviour. *J. Mar. Biol. Assoc. UK* 86:317–333.
- Epelbaum, A. B. & N. P. Kovatcheva. 2005. Daily food intakes and optimal food concentrations for red king crab (*Paralithodes camtschaticus*) larvae fed *Artemia* nauplii under laboratory conditions. *Aquacult. Nutr.* 11:455–461.
- FAO. 2010. The state of world fisheries and aquaculture 2010. Rome: Food and Agriculture Organization of the United Nations. Rome: FAO. 218 pp.
- Figueiredo, J., R. van Woessik, J. Lin & L. Narciso. 2009. *Artemia franciscana* enrichment model: how to keep them small, rich and alive? *Aquaculture* 294:212–220.
- Folch, J., M. Lees & S. G. Sloane. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 22:497–509.
- Fraser, A. J. 1989. Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. *Can. J. Fish. Aquat. Sci.* 46:1868–1873.
- Gallager, S. M., R. Mann & G. C. Sasaki. 1986. Lipid as an index of growth and viability in three species of bivalve larvae. *Aquaculture* 56:81–103.
- Hamasaki, K. & S. Kitada. 2008. Potential of stock enhancement for decapod crustaceans. *Rev. Fish. Sci.* 16:164–174.
- Harding, G. C. & A. J. Fraser. 1999. Application of the triacylglycerol/sterol condition index to the interpretation of larval lobster *Homarus americanus* distribution in close proximity to Georges Bank, Gulf of Maine. *Mar. Ecol. Prog. Ser.* 186:239–254.
- Holme, M.-H., P. C. Southgate & C. Zeng. 2007. Survival, development and growth response of mud crab, *Scylla serrata*, megalopae fed semi-purified diets containing various fish oil:corn oil ratios. *Aquaculture* 269:427–435.
- Kattner, G., M. Graeve, J. A. Calcagno, G. A. Lovrich, S. Thatje & K. Anger. 2003. Lipid, fatty acid and protein utilization during lecithotrophic larval development of *Lithodes santolla* (Molina) and *Paralomis granulose* (Jacquinot). *J. Exp. Mar. Biol. Ecol.* 292:61–74.
- Kharlamenko, V. I., S. I. Kiyashko, A. B. Imbs & D. I. Vyshkvartzev. 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. *Mar. Ecol. Prog. Ser.* 220:103–117.
- Lautier, J. & J.-G. Lagarrigue. 1987. Lipid metabolism of the female crab *Pachygrapsus marmoratus* during the molt cycle. *Biochem. Syst. Ecol.* 15:611–619.
- Limbourn, A. J. & P. D. Nichols. 2009. Lipid, fatty acid and protein content of late larval to early juvenile stages of the western rock lobster, *Panulirus cygnus*. *Comp. Biochem. Physiol. Part B* 152:292–298.
- Litzow, M. A., K. M. Bailey, F. G. Prah & R. Heintz. 2006. Climate regime shifts and reorganization of fish communities: the essential fatty acid limitation hypothesis. *Mar. Ecol. Prog. Ser.* 315:1–11.
- Meade, M. E. & S. E. Watts. 1995. Weight gain and survival of juvenile Australian crayfish *Cherax quadricarinatus* fed formulated feeds. *J. World Aquacult. Soc.* 26:469–474.
- Meglen, R. R. 1992. Examining large databases: a chemometric approach using principal component analysis. *Mar. Chem.* 39:217–237.
- Mercier, L., I. Racotta, G. Yepiz-Plascencia, A. Muhlia-Almazán, R. Civera, M. F. Quiñones-Arreola, M. Wille, P. Sorgeloos & E. Palacios. 2009. Effect of diets containing different levels of highly unsaturated fatty acids on physiological and immune responses in Pacific whiteleg shrimp *Litopenaeus vannamei* (Boone) exposed to handling stress. *Aquat. Res.* 40:1849–1863.
- Merican, Z. O. & K. F. Shim. 1996. Qualitative requirements of essential fatty acids for juvenile, *Penaeus monodon*. *Aquaculture* 147:275–291.
- Morrison, W. R. & L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride methanol. *J. Lipid Res.* 5:600–608.
- Nair, C. M., K. R. Salin & K. A. Kumar. 2007. Use of Cyclop-eeze as a substitute for *Artemia* nauplii in larval rearing of giant freshwater prawn *Macrobrachium rosenbergii* (De Man 1879). *Aquacult. Nutr.* 13:88–93.
- Nghia, T. T., M. Wille, S. Vandendriessche, Q. TheVinh & P. Sorgeloos. 2007. Influence of highly unsaturated fatty acids in live food on larviculture of mud crab *Scylla paramamosain* (Estampador 1949). *Aquacult. Res.* 38:1512–1528.
- O'Halloran, M. J. & R. K. O'Dor. 1988. Molt cycle of male snow crabs, *Chionoecetes opilio*, from observations of external features, setal changes, and feeding behavior. *J. Crustac. Biol.* 8:164–176.
- Ouellet, P. & C. T. Taggart. 1992. Lipid condition and survival in shrimp (*Pandalus borealis*) larvae. *Can. J. Fish. Aquat. Sci.* 49:368–378.
- Parrish, C. C. 1987. Separation of aquatic lipid classes by Chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. *Can. J. Fish. Aquat. Sci.* 44:722–731.
- Parrish, C. C. 1988. Dissolved and particulate marine lipid classes: a review. *Mar. Chem.* 23:17–40.
- Parrish, C. C. 2009. Essential fatty acids in aquatic food webs. In: M. T. Arts, M. T. Brett & M. J. Kainz, editors. Lipids in aquatic ecosystems. Dordrecht: Springer. pp. 309–326.
- Parrish, C. C., T. A. Abrajano, S. M. Budge, R. J. Helleur, E. D. Hudson, K. Pulchan & C. Ramos. 2000. Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In: P. J. Wangersky, editor. Marine chemistry. Heidelberg: Springer-Verlag. pp. 193–223.
- Parrish, C. C., N. J. Turner, R. E. Ommer & S. M. Solberg. 2008. Conclusions: what food security in coastal communities really means. In: C. C. Parrish, N. J. Turner & S. M. Solberg, editors. Resetting the kitchen table: food security, culture, health and resilience in coastal communities. New York: Nova Science Publishers. pp. 191–198.
- Pirtle, J. L. & A. W. Stoner. 2010. Red king crab (*Paralithodes camtschaticus*) early post-settlement habitat choice: structure, food and ontogeny. *J. Exp. Mar. Biol. Ecol.* 393:130–137.
- Reddy, P. S., P. R. Reddy & G. P. C. Nagaraju. 2004. The synthesis and effects of prostaglandins on the ovary of the crab *Oziotelphusa senex senex*. *Gen. Comp. Endo.* 135:35–41.
- Sanchez-Paz, A., F. Garcia-Carreno, A. Muhlia-Almazán, A. B. Peregrino-Uriarte, J. Hernandez-Lopez & G. Yepiz-Plascencia. 2006. Usage of energy reserves in crustaceans during starvation: status and future directions. *Insect Biochem. Mol. Biol.* 36:241–249.
- Sargent, J. R. 1995. Origins and functions of egg lipids: nutritional implications. In: N. R. Bromage & J. R. Roberts, editors. Broodstock management and egg and larval quality. Cambridge, UK: Oxford University Press. pp. 353–372.
- Sargent, J., L. McEvoy, A. Estevez, G. Bell, M. Bell, J. Henderson & D. Tocher. 1999. Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture* 179:217–229.
- Sasaki, G. C., J. M. Capuzzo & P. Biesiot. 1986. Nutritional and bioenergetic considerations in the development of the American lobster *Homarus americanus*. *Can. J. Fish. Aquat. Sci.* 43:2311–2319.
- Sheen, S. S. & S. W. Wu. 2002. Essential fatty acid requirements of juvenile mud crab, *Scylla serrata* (Forsk., 1775) (Decapoda, Scyllaridae). *Crustaceana* 75:1387–1401.
- Stevens, B. G., editor. 2006. Alaska crab stock enhancement and rehabilitation: workshop proceedings. Alaska Sea Grant College Program AK-SG-06-04. Fairbanks, AK: Alaska Sea Grant College Program, University of Fairbanks. p. 88.
- Stevens, C. J., D. Deibel & C. C. Parrish. 2004. Species-specific differences in lipid composition and omnivory in Arctic copepods collected in deep water during autumn (North Water Polynya). *Mar. Biol.* 144:905–915.
- Stoner, A. W., M. L. Ottmar & S. A. Haines. 2010a. Temperature and habitat complexity mediate cannibalism in red king crab: observations

- on activity, feeding and prey defense mechanisms. *J. Shellfish Res.* 29:1005–1012.
- Stoner, A. W., M. L. Ottmar & L. A. Copeman. 2010b. Temperature effects on the molting, growth, and lipid composition of newly-settled red king crab. *J. Exp. Mar. Biol. Ecol.* 393:138–147.
- Sui, L., M. Wille, Y. Cheng & P. Sorgeloos. 2007. The effect of dietary n-3 HUFA levels and DHA/EPA ratios on growth, survival and osmotic stress tolerance of Chinese mitten crab *Eriocheir sinensis* larvae. *Aquaculture* 272:139–150.
- Suprayudi, M. A., T. Takeuchi, & K. Hamasaki. 2004. Essential fatty acids for larval mud crab *Scylla serrata*: implications of lack of the ability to bioconvert C18 unsaturated fatty acids to highly unsaturated fatty acids. *Aquaculture* 231:403–416.
- Tizol-Correa, R., L. Carreón-Palau, B. O. Arredondo-Vega, G. Murugan, L. Torrentera, T. D. N. J. Maldonado-Montiel & A. M. Maeda-Martínez. 2006. Fatty acid composition of *Artemia* (Branchiopoda: Anostraca) cysts from tropical salterns of southern México and Cuba. *J. Crustac. Biol.* 26:503–509.
- Wen, X., L. Chen, Y. Ku & K. Zhou. 2006. Effect of feeding and lack of food on the growth, gross biochemical and fatty acid composition of juvenile crab, *Eriocheir sinensis*. *Aquaculture* 252:598–607.
- Wu, X., Y. Cheng, L. Sui, C. Zeng, P. C. Southgate & X. Yang. 2007. Effect of dietary supplementation of phospholipids and highly unsaturated fatty acids on reproductive performance and offspring quality of Chinese mitten crab, *Eriocheir sinensis* (H. Milne-Edwards), female broodstock. *Aquaculture* 272:602–613.
- Zheng, J. & G. H. Kruse. 2000. Recruitment patterns of Alaskan crabs in relation to decadal shifts in climate and physical oceanography. *ICES J. Mar. Sci.* 57:438–451.
- Zhou, S., T. C. Shirley & G. H. Kruse. 1998. Feeding and growth of the red king crab *Paralithodes camtschaticus* under laboratory conditions. *J. Crustac. Biol.* 18:337–345.
- Zmora, O., A. Findiesen, J. Stubblefield, V. Frenkel & Y. Zohar. 2005. Large-scale juvenile production of the blue crab *Callinectes sapidus*. *Aquaculture* 244:129–139.