

GROWTH PHYSIOLOGY OF JUVENILE RED KING CRAB,  
*PARALITHODES CAMTSCHATICUS*, IN ALASKA

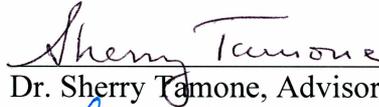
By

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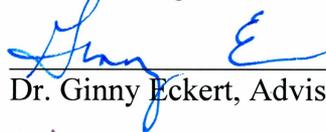
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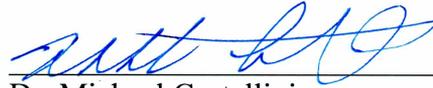


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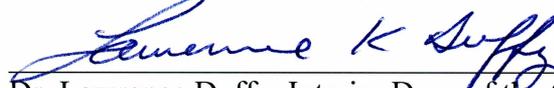


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GROWTH PHYSIOLOGY OF JUVENILE RED KING CRAB,  
*PARALITHODES CAMTSCHATICUS*, IN ALASKA

A

THESIS

Presented to the Faculty  
of the University of Alaska Fairbanks

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for the Degree of

MASTER OF SCIENCE

By

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## ABSTRACT

Lack of recovery, following collapse of the Alaskan red king crab, *Paralithodes camtschaticus*, fishery, has prompted research directed towards rehabilitating the species. To better inform rehabilitation efforts aimed at increasing survival and growth of *P. camtschaticus* in their first year of life, I compared individual growth of hatchery-raised and wild-caught juvenile crabs in the laboratory and then compared both sets of laboratory individuals with cohorts from the field. To understand molt cycles, hemolymph was collected from age-0 and age-3 crabs to quantify circulating molting hormones (ecdysteroids) and the duration of premolt. Size, growth increment, molt interval, and cumulative molt interval did not differ significantly between hatchery-raised and wild-caught crabs. No consistent differences existed in CL between hatchery, wild-laboratory and field-surveyed juveniles for most months, although spine lengths of hatchery-raised and wild-caught crabs were significantly longer than field-surveyed crabs most months. Patterns of circulating ecdysteroids resembled published profiles for other crustacean species. Peak ecdysteroid levels occurred regularly (approximately 17 d) prior to ecdysis despite varying molt intervals. Age-0 and age-3 juveniles spent approximately 39 % and 32 % of the molt cycle in premolt, respectively. Overall, hatchery-raised and wild *P. camtschaticus* were markedly similar with respect to growth.

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## DEDICATION

This thesis is dedicated to my husband, Mark, who has spent countless hours working in the laboratory and field by my side and countless more hours listening to me and supporting my work.

## INTRODUCTION

Until the collapse of the fishery, red king crab, *Paralithodes camtschaticus*, were the most valuable crustacean species in Alaska. Across Alaska, commercial *P. camtschaticus* fisheries peaked throughout the mid-1960s into the late 1970s. The largest Alaskan stock, in Bristol Bay, had a record harvest of 58,943 t during 1977 – 1980 (Zheng and Sideek, 2010). Shortly thereafter, in the early 1980s, all of the major *P. camtschaticus* stocks collapsed. Despite the absence of fishing pressure, *P. camtschaticus* stocks have failed to recover to pre-collapse abundance levels (Orensanz et al., 1998; Zheng and Kruse, 2000) and six of the eight commercial Alaskan king crab stocks are currently closed to fishing. The main commercial fishery in Bristol Bay, AK had a total allowable catch of 7,257.5 t in 2009/2010 (Zheng and Sideek, 2010).

Possible explanations for the collapse of the fishery include overfishing (Kruse et al., 1996; Orensanz et al., 1998; Dew and Austrung, 2007), handling trauma (Zhou and Shirley, 1996), bycatch in trawl fisheries (Wall et al., 1981; Armstrong et al., 1993; Dew and McConnaughey, 2005) and climate shift (Anderson and Piatt, 1999; Zheng and Kruse, 2006). In response to the lack of recovery, management focused on reducing *P. camtschaticus* in the bycatch of other fisheries. Yet, bycatch reduction measures, such as the mandatory shellfish observer program (Wall et al., 1981; Boyle and Schwenzfeier, 2002) and bycatch refuges (Somerton and June, 1984; Armstrong et al., 1993), have not led to significant increases in crab abundance. The Pacific Decadal Oscillation (PDO) was in a cold phase during the crab harvest peak and then shifted to a warm phase in 1976-77, coincident with the decline in *P. camtschaticus* abundance (Anderson and Piatt, 1999; Zheng and Kruse, 2006). The PDO influences bottom temperature (Loher and Armstrong, 2005), which results in altered timing of development, female migration, larval release, and molting (Zheng and Kruse, 2006). If larvae were released in sub-optimal locations, they may be advected away from suitable juvenile nursery areas, which may result in low recruitment into the fishery (Zheng and Kruse, 2000; Loher and Armstrong, 2005; Zheng and Kruse, 2006).

In Alaska, rehabilitation (also termed restocking, enhancement or rebuilding), is currently being explored as a potential tool for the restoration of *P. camtschaticus* populations. Rehabilitation is intended to address a bottleneck during early life stages; adult females release hundreds of thousands of larvae, but very few reach maturity and recruit into the fishery. Crab rehabilitation requires capturing ovigerous females from wild populations, allowing the females to incubate and hatch their clutches in captivity, and rearing the larval and juvenile crab in a hatchery. The hatchery can enhance larval and juvenile survival through lack of predation and adequate feeding, with the goal of maximizing survival in the wild when larvae or juveniles are released in large numbers (Davis et al., 2005; Oliver et al., 2006) into nursery habitats with the aim of replenishing the indigenous stock (Robinson and Tully, 1999; Johnson et al., 2008). Stock rehabilitation has been implemented in other commercially harvested marine invertebrate species, including giant tiger prawn, *Penaeus monodon* (Davenport et al., 1999), kuruma prawn, *Penaeus japonicus* (Hamasaki and Kitada, 2006), fleshy prawn, *Penaeus chinensis* (Wang et al., 2006), bay scallop, *Argopecten irradians irradians* (Goldberg et al., 2000), Japanese scallop, *Patinopecten yessoensis* (Uki, 2006), European lobster, *Homarus gammarus* (Beal et al., 2002), spiny lobster, *Jasus edwardsii* (Oliver et al., 2006), queen conch, *Strombus gigas* (Stoner, 1994), mud crabs, *Scylla olivacea* and *S. serrata* (Le Vay et al., 2008; Lebata et al., 2009) and Atlantic blue crabs, *Callinectes sapidus* (Davis et al., 2005). Crustacean rehabilitation efforts strive to minimize costs and maximize survival by releasing late stage larvae or young juveniles, as in the case of the American lobster, *Homarus americanus*, (Castro et al., 2001) and Atlantic blue crab, *C. sapidus* (Johnson et al., 2008). Since late stage larvae or small juveniles are the most likely to be released during a rehabilitation endeavor, the foundation of any restoration effort lies with research of early life stages.

Often, fishery management efforts are focused on the adults to be harvested, with little regard to juveniles that will eventually recruit into that fishery or that could be used to for restoration. The *P. camtschaticus* fishery is no exception to this paradigm. Recently, however, the paradigm has been changing, and efforts are focused on studying

the early life history of juvenile *P. camtschaticus* through the efforts of the Alaska King Crab Research, Rehabilitation and Biology (AKCRRAB) program. The AKCRRAB program is currently exploring rehabilitation as a prospective tool for rebuilding *P. camtschaticus* stocks. As a part of this effort, the goals of this thesis are 1) to quantify juvenile *P. camtschaticus* molt interval and molt increment, 2) to compare growth between hatchery-raised and wild-caught *P. camtschaticus* juveniles, and 3) to determine *P. camtschaticus* molting physiology through analysis of molting hormones in juveniles. In order to fully evaluate rehabilitation as a restoration tool, it is important to understand the early life history of *P. camtschaticus*, as well as any possible differences that may or may not exist in hatchery-raised crabs that may potentially be released during rehabilitation efforts.

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COMPARISON OF FIRST-YEAR GROWTH BETWEEN HATCHERY-RAISED,  
WILD-CAUGHT AND FIELD-SURVEYED JUVENILE RED KING CRAB,  
*PARALITHODES CAMTSCHATICUS*, IN ALASKA<sup>1</sup>

ABSTRACT

In an effort to better understand the early life history and to determine potential effects of hatchery larval rearing, wild-caught and hatchery-raised juvenile red king crab, *Paralithodes camtschaticus*, were reared individually in the laboratory for one year starting at the glaucothoe stage. Growth of these laboratory cohorts was compared with juvenile crabs in the field in Juneau, AK (January through August) to evaluate potential laboratory influences on growth. Size, molt interval, and cumulative molt interval did not differ significantly between hatchery-raised and wild-caught crabs, nor between male and female crabs in the laboratory. Growth increment averaged 24 % for both hatchery and wild crabs and was not significantly different between molts. There were no consistent differences in carapace length between hatchery-raised, wild-caught laboratory-reared and field-surveyed juveniles for most months. Spine lengths differed significantly between hatchery-raised, wild-caught and field-surveyed crabs for most months with laboratory crabs having longer spines than field-surveyed juveniles, although the differences were not consistent. There was no difference in mean spine length among any groups of crabs in April, May and July. Growth of juveniles was consistent among hatchery-reared and wild-caught individuals raised in the laboratory, as well as with individuals from the field, suggesting no deleterious effects of hatchery or laboratory rearing.

Keywords: molting, Lithodid, Anomuran, restoration, enhancement, comparative

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## INTRODUCTION

Sustainable fisheries require successful recruitment of juveniles to replenish stocks and maintain harvest levels. As a result, growth parameters such as growth increment and molt interval are an important parameter for consideration by fishery managers. Until the collapse of major commercial crab fisheries in the early 1980s, *Paralithodes camtschaticus* were the most valuable crustacean species in Alaska. Currently six of the eight *P. camtschaticus* stocks in Alaska are closed to fishing. The goal of this study is to inform restoration and management with regards to the early life history of juvenile *P. camtschaticus* through a comparison of growth between hatchery-raised, wild-caught laboratory-reared and wild field-surveyed juveniles.

*Paralithodes camtschaticus* have an extended juvenile phase, reaching maturity between five and seven years of age, at approximately 60 – 69 mm carapace length (CL) (Paul, 1992). *Paralithodes camtschaticus* have a meroplanktonic early life history with four planktonic zoeal stages lasting a total of about 40 d and a highly thigmotactic glaucothoal stage lasting about 30 d (Sato, 1958). Glaucothoae seek suitable habitat in which to settle and metamorphose into benthic juveniles (Sato, 1958; Loher and Armstrong, 2000; Stevens, 2003; Epelbaum et al., 2006). Newly settled *P. camtschaticus* juveniles are cryptic and prefer highly complex substrates (Stevens and Kittaka, 1998; Loher and Armstrong, 2000; Stevens, 2003; Pirtle and Stoner, 2010). At approximately one and one half to two years of age, juvenile *P. camtschaticus* aggregate in large pods that move into deep benthic habitats (Bright et al., 1960; Dew, 1990).

Juvenile crustacean growth varies as a function of rearing density, and varies between hatchery-raised and wild-caught cohorts. Individually raised juvenile American lobsters, *Homarus americanus*, grow less than those reared communally, with growth rates ranging from 11.0 – 20.9 % per molt for individually-raised (Aiken and Waddy, 1978; Gendron and Sainte-Marie, 2006) and 9.6 – 32.9 % per molt for communally-reared juvenile lobster (Hudon, 1987). Juvenile snow crab, *Chionoecetes opilio*, reared in the laboratory have significantly longer molt intervals when reared at high densities than low densities (Sainte-Marie and Lafrance, 2002). Hatchery-raised mud crab, *Scylla*

*paramamosain*, grow significantly faster than wild-caught cohorts reared in separate pond culture (Ut et al., 2007). Interestingly, hatchery-raised and wild-caught *S. paramamosain* stocked together in mixed pond culture show no significant difference in growth rate even though wild-caught crab have significantly higher wet weight to carapace width ratio than hatchery crabs at the beginning of the experiment (Ut et al., 2007).

Patterns in growth, which are consistent across juvenile decapod crustacean species, point to the dependence of growth and final attainable size on the animals' rearing conditions, origin and size. Smaller (< 5mm CL) juvenile golden king crab, *Lithodes aequispinus*, grow an average of 28.0 % per molt, whereas larger (> 10 mm) juveniles grow 33.0 % per molt (Paul and Paul, 2001). Reduced growth rate for smaller size classes is also found in juvenile southern king crab, *Paralomis granulosa*, and the false southern king crab, *Lithodes santolla* (Calcagno et al., 2005). In the laboratory, juvenile *P. camtschaticus* reared communally are > 1 mm carapace width larger after 60 d than juveniles reared individually (Stoner et al., 2010) likely because of competitive growth or competition for food resources. Similar patterns in growth are seen in juvenile *H. americanus* (Aiken and Waddy, 1978; Hudon, 1987; Gendron and Sainte-Marie, 2006).

Growth of crustaceans is inextricably linked to temperature (Hartnoll, 2001). Increased temperature results in an increased growth rate and decreased molt interval for the Chesapeake Bay blue crab, *Callinectes sapidus* (Brylawski and Miller, 2006), and the red clinging crab, *Mithraculus forceps* (Penha-Lopes et al., 2006). Early stage (C1 to C2) juvenile *P. camtschaticus* held individually grew larger with a shorter molt interval at 12 °C compared to 1.5, 4.5 or 8.0 °C over an approximately 60 – 80 d period in the laboratory (Stoner et al., 2010). Lipid analysis of small juvenile *P. camtschaticus* revealed that juveniles reared at higher temperatures (12 °C) had total lipids:dry weight ratios that were not different than cohorts reared at lower temperatures (1.5, 4.5 or 8.0 °C) showing that faster growth at higher temperatures may have a positive effect on the

storage of lipids (Stoner et al., 2010). Animals that grow larger and store lipids may have an advantage going in to winter months.

Cannibalism results in density dependence in crustacean populations and is a contributing factor in juvenile growth. Stable isotope analysis of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of sand crabs, *Portunus pelagicus*, from the same cohort revealed that larger crabs are at a higher trophic level than smaller sized crabs of the same age, suggesting that cannibalism was likely occurring; as a result larger individuals grew faster (Moller et al., 2008). In the laboratory, cannibalism accounted for approximately 80 % of mortalities in juvenile (C1 to C6) *P. camtschaticus* over the first 130 d at temperatures of 7, 10 and 13 °C (Borisov et al., 2007). For juvenile *P. camtschaticus* in captivity, cannibalism can be reduced by the addition of structure, reduced densities, varied diet, and size grading (Mortensen and Damsgard, 1996; Borisov et al., 2007; Daly et al., 2009; Daly et al., in prep). Survival for stage C1 to C3 juvenile *P. camtschaticus* in the hatchery is highest at stocking densities of 500 and 1,000 crabs/m<sup>2</sup> with added substrate compared to stocking densities of 2,000 crabs/m<sup>2</sup> and those without substrate (Daly et al., 2009). In the field, it is unlikely that early benthic juvenile *P. camtschaticus* (prior to the start of podding behaviors) would experience densities close to what is seen in a laboratory or hatchery, and presumably cannibalism is less likely to occur in the field.

Previous studies of juvenile *P. camtschaticus* growth were either conducted in the laboratory or in situ, making direct comparisons difficult. In the field, newly settled stage 1 crabs (C1) have an average CL of 2.2 mm and within a year grow to stage 9 crab (C9), measuring approximately 10.5 mm – 11.2 mm CL, for a total growth of 8.3 - 9.0 mm per year (Donaldson et al., 1992; Loher et al., 2001). C1 crabs in the laboratory grow from 1.8 mm – 1.9 mm CL to a size range of 3.7 – 4.9 mm CL (C6) in four to six and one half months (Nakanishi, 1987; Kovatcheva et al., 2006), which is less than the in situ mean size reached at four to five and one half months of 4.47 - 6.58 mm CL (Donaldson et al., 1992). The molt interval for laboratory mass-reared juvenile *P. camtschaticus* from stages C2 to C3 is estimated to be between 7 – 17 d, C3 to C4 estimated between 26 – 44 d and C4 to C5 estimated between 50 – 68 d (Kovatcheva et al., 2006). Despite previous

research efforts focused on juvenile *P. camtschaticus* growth, individual growth under ambient conditions has not yet been reported. Previous growth experiments with juvenile *P. camtschaticus* have been mainly conducted on juveniles reared en masse, at fixed temperatures in the laboratory or from an undetermined life stage. We examined growth of juvenile *P. camtschaticus*, starting with hatchery-raised and wild-caught glaucothoe by raising them individually in the lab over a period of one year at ambient southeast Alaska temperatures. Simultaneously, we surveyed and measured juvenile crabs monthly in the field to compare sizes of field-surveyed individuals with our laboratory-reared individuals to identify possible artifacts of laboratory rearing.

## METHODS

### Experimental Animals

Hatchery-reared glaucothoe were obtained from 20 ovigerous *P. camtschaticus* that were collected in Bristol Bay, AK on 15 November 2008 and shipped to the Alutiiq Pride Shellfish Hatchery in Seward, AK on 2 December 2008. The larvae hatched in March 2009 and were reared in 1,200 L continuous flow through tanks at densities of approximately 50 larvae L<sup>-1</sup> at 11 °C (Swingle et al., in prep). On 9 May 2009, *P. camtschaticus* glaucothoe were shipped to the University of Alaska Fairbanks, School of Fishery and Ocean Sciences in Juneau, AK.

Wild *P. camtschaticus* glaucothoe were collected using sausage-shaped artificial collectors (SACs) modeled after Donaldson et al. (1992). Larval collectors were placed in the coastal waters near Juneau at the Couverden Islands (N58.15163°, W135.04668°) and Indian Point (N58.37231°, W134.69411°) at depths of 6 and 9 m in late April 2009 and retrieved by SCUBA divers in mid-June through early July 2009 (Pirtle, 2010). SACs were collected in black plastic bags under water and brought to the surface where they were placed in coolers chilled with ice packs. In the lab, the SACs were deconstructed, rinsed carefully with filtered seawater, contents strained through 1 mm mesh and sorted. All hatchery-raised and wild-caught *P. camtschaticus* glaucothoe were carefully separated and sequestered in individual containers. Individuals entered into the experiment on the day that metamorphosis from glaucothoe to first stage juvenile occurred.

### Rearing Conditions

Juvenile *P. camtschaticus* were reared in individual 10.2 cm diameter by 25.4 cm tall PVC cylindrical containers with 1 mm fiberglass mesh suspended 5.1 cm above the bottom (1657.9 cm<sup>3</sup> living volume). A small amount of tangled gill net was placed inside each container to provide vertical structure. Rearing containers were set into Living Stream© tanks (LS-700; Frigid Units, Toledo, OH), each individually supplied with flow-through filtered seawater and ambient photoperiod at the University of Alaska marine labs at Auke Bay and Lena Point in Juneau. Temperature was recorded every 30

min using a HOBO water temp Pro-VR logger. Salinity measurements were provided by the National Oceanic and Atmospheric Association's (NOAA) Auke Bay Laboratory (Wing, unpublished data). Salinity and temperature were maintained at ambient levels and varied seasonally from 10.1 – 31.2 and 3 – 12 °C, respectively. Crabs received, to excess, a gelatin-bound raw seafood diet enriched with mineral calcium. A typical recipe yielded approximately 355 mL and contained the following: 3,600 mg Caltrate<sup>®</sup> calcium, 12 g Knox<sup>®</sup> gelatin, 177 mL water, 28 g Otohime<sup>™</sup>, 20 g Ocean Nutrition<sup>™</sup> brine shrimp (*Artemia spp.*), 20 g San Francisco Bay Brand<sup>™</sup> cyclops, plus approximately 250 g wet weight of available raw seafood (krill, prawn roe, salmon roe, herring roe, silversides, salmon, shrimp, squid, mussels, or sea urchin) chopped finely in a food processor. The seafood-gelatin was refrigerated and used within two weeks. Individual rearing containers were cleaned at minimum weekly or more often as needed.

#### Molt Interval & Growth Increment

Growth was monitored starting at the first juvenile molt, which occurred in May 2009 for hatchery crabs (n = 46) and July 2009 for wild crabs (n = 35). The earlier molt to first stage juvenile seen in hatchery-raised crabs was likely due to geographic differences in source populations and different larval rearing conditions in the hatchery compared to the wild. Crabs were monitored daily for molts through August 2010. Molt dates were recorded, exuvia removed, labeled and frozen in seawater until photographed. Carapaces were measured digitally using ImageJ software (version 1.42, Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>, 1997-2009; accuracy  $\pm 0.03$ ). Growth increment was determined by subtracting CL of the current molt from the preceding molt and then dividing that difference by the CL of the preceding molt  $[(CL_{i+1}-CL_i)/CL_i]$ ; where  $i$ =instar]. Molt interval was standardized to degree days, calculated by summing the average temperature from each day during individual molt intervals. Spine length (SL) was determined by subtracting carapace width at the widest point not including spines (CW) from the carapace width including the longest spines (CWS), typically the last set

of lateral spines on the carapace, and dividing by two giving single spine length  $[(CW - CWS)/2 = SL]$ .

### Field Surveys

Regular monthly field surveys were conducted, concurrent with the laboratory experiment, in order to compare growth of laboratory-raised individuals with those in the field. Field surveys were conducted during monthly low tides at two survey sites, Indian Point (between N58.37850°, W134.68900° and N58.37383°, W134.69166°) and the beach located behind the University of Alaska Southeast's marine lab, hereafter referred to as Anderson Beach (between N58.38016°, W134.64616° and N58.38250°, W134.64633°), nearshore Juneau, AK from January 2010 through December 2010. In the intertidal zone, surveys were spread vertically from approximately 0.5 m below the water line to 3 m above the water line as topography allowed (surveys never extended above the first mussel band in the intertidal zone). Surveys were typically broken up into two or three transects and were dependent on the tide level and exposed topography. Transect areas were measured using GPS coordinates. The average area surveyed at Indian Point was 703.6 m<sup>2</sup> ( $\pm$  561.3 m<sup>2</sup> SD) and at Anderson Beach the average area was 300.5 m<sup>2</sup> ( $\pm$  127.1 m<sup>2</sup> SD). Juvenile *P. camtschaticus* were counted and CL, CW and CWS (for SL measurements) were measured using either digital (accuracy  $\pm$  0.001 mm) or dial calipers (accuracy  $\pm$  0.01 mm).

### Statistics

Size, growth increment, molt interval and time (standardized to degree days) to the C9 stage were analyzed using repeated measures MANOVA with the measures of each crab at each molt stage as the repeated measure (response variables) and crab source (hatchery or wild) as the predictor variable. Either matched pairs (paired t-tests with two repeated measures) or Tukey-Kramer HSD post-hoc tests were used to discern differences. The data for the repeated measures MANOVA met the criteria that  $N - M > k + 9$  ( $N$ =total number of animals,  $M$ =number of between-subject treatments and  $k$  = number of factors in the dependent variable). The robustness of the repeated measures MANOVA depends on the assumption of sphericity, which can be unreliable (Quinn and

Keough, 2002). Therefore, we assumed that in all instances sphericity was violated and used the F-statistic from the more conservative Greenhouse-Geisser adjusted  $\epsilon$  (Quinn and Keough, 2002). To maintain robustness of the analysis, missing data accounted for less than 5 % of all data for the response variables. Individual crabs that were missing data, as well as crabs that died during the experiment, were excluded from the analysis. With the removal of crabs that did not molt to the C9 stage and crabs that had measures missing, the final sample size for hatchery crabs was  $n = 10$  and wild crabs,  $n = 23$ . Since sample sizes only permitted the MANOVA to be used up to the C9 stage, we compared the sizes of hatchery and wild crabs on 3 September 2010 at the conclusion of the experiment with ANOVA. The crabs were also sexed at this time, which allowed for a comparison of the growth of male and female juvenile crabs at each stage using repeated measures MANOVA and at the end of the experiment by ANOVA. Sex was determined by the presence or absence of a gonopore on the coxa of the third periopod.

Mean monthly CL and SL from field-surveyed crabs were compared to laboratory-reared hatchery and wild crabs using a mixed-model ANOVA for repeated measures, with crab measures each month as the repeated measure and crab source as the fixed effect. The mixed-model ANOVA was used to accommodate monthly variation in sample size for field-surveyed crabs. Differences were discerned using Tukey-Kramer HSD post-hoc tests. Since measures for field-surveyed crabs were taken on specific days each month, survey dates were used as size cutoff points for the laboratory population.

## RESULTS

### Size and Growth Increment

Crab size (mm) for C1 through C9 molts were not significantly different between hatchery and wild crabs (MANOVA, G-G  $\epsilon = 0.22$ ,  $df = 1.73$ , 44.98,  $p = 0.49$ ) (Figure 1.1). As expected, size increased over time (MANOVA, G-G  $\epsilon = 0.22$ ,  $df = 1.73$ , 44.98,  $p < 0.001$ ). The growth increment differed significantly between hatchery and wild crabs but not in a consistent manner as evidenced by a significant interaction (MANOVA, G-G  $\epsilon = 0.74$ ,  $df = 5.17$ , 134.42,  $p = 0.04$ ). Wild crabs ( $28.7\% \pm 6.0$  SD) had a larger growth increment than the hatchery crabs ( $22.3\% \pm 7.2$  SD) for the C2C3 molt (Tukey HSD,  $p = 0.02$ ), while hatchery crabs ( $28.9\% \pm 11.6$  SD) had a larger growth increment than wild crabs ( $20.7\% \pm 5.9$  SD) in the C7C8 molt (Tukey HSD,  $p = 0.02$ ). Between stages, molt increment C5C6 was significantly smaller than molt increments C2C3, C3C4, C6C7 and C8C9 (Figure 1.2a). The growth increment averaged  $24.5\% (\pm 8.5$  SD) for both hatchery and wild crabs over all molt stages (Appendix 1A).

At the conclusion of the experiment, size (mm) did not differ significantly between hatchery and wild crabs (ANOVA,  $F = 0.18$ ,  $df = 1, 31$ ,  $p = 0.67$ ). After one year, hatchery and wild crabs averaged 13.71 mm CL ( $\pm 1.45$  SD; with a minimum CL of 11.45 mm CL and a maximum of 15.59 mm CL) and 13.95 mm CL ( $\pm 1.59$  SD; with a minimum CL of 11.46 mm CL and a maximum of 18.73 mm CL), respectively. The maximum molt stages reached by hatchery crabs was C11 ( $n = 1$ ) and by wild crabs was C12 ( $n = 1$ ), with the majority of hatchery and wild crabs at the C10 stage ( $n = 6$  for hatchery and  $n = 14$  for wild) (Appendix 1A).

### Molt Interval

Molt interval (time in degree days) did not differ significantly between hatchery and wild crabs (Figure 1.2b; MANOVA, G-G  $\epsilon = 0.46$ ,  $df = 3.21$ , 99.58,  $p = 0.08$ ). Molt intervals were longer at later molt stages (MANOVA, G-G  $\epsilon = 0.46$ ,  $df = 3.21$ , 99.58,  $p < 0.0001$ ). In general, the first three molts (C1C2, C2C3 and C3C4) had shorter molt intervals than all later molts. The C4C5 molt and the C7C8 molt were of intermediate duration, while the C5C6, the C6C7 and the C8C9 molts were the longest (Figure 1.2b).

Cumulative time to molt did not differ between hatchery and wild crabs (MANOVA, G-G  $\epsilon = 0.29$ ,  $df = 2.06$ ,  $61.67$ ,  $p = 0.17$ ) (Appendix 1A).

#### Male vs. Female Crabs

There was no significant difference in size (final measurements of  $13.73 \text{ mm} \pm 1.77 \text{ SD}$  and  $14.13 \text{ mm} \pm 1.22 \text{ SD}$  for males and females respectively; MANOVA, G-G  $\epsilon = 0.22$ ,  $df = 1.79$ ,  $46.52$ ,  $p = 0.71$ ), growth increment ( $0.25 \pm 0.09 \text{ SD}$  and  $0.24 \pm 0.08 \text{ SD}$  for males and females respectively; MANOVA, G-G  $\epsilon = 0.77$ ,  $df = 5.40$ ,  $140.46$ ,  $p = 0.14$ ), molt interval (MANOVA, G-G  $\epsilon = 0.49$ ,  $df = 3.43$ ,  $102.91$ ,  $p = 0.31$ ) or cumulative time to molt (C1 to C9 molt  $2,416.0 \text{ dd} \pm 230.2 \text{ SD}$  and  $2,340.8 \text{ dd} \pm 263.8 \text{ SD}$  for males and females respectively; MANOVA, G-G  $\epsilon = 0.81$ ,  $df = 2.05$ ,  $61.58$ ,  $p = 0.45$ ) between male and female crabs (Appendix 1B).

#### Field Surveys

Juvenile *P. camtschaticus* were found at the field-survey sites from January through December, except for September when no crabs were found at either site. Crabs were found at Indian Point from January through April (with one crab found in July) and at Anderson Beach from February through December (except for September). During the months when crabs were observed, densities ranged from 0.01 to 0.43 crabs/m<sup>2</sup> (the highest density was at Anderson Beach in February and the lowest at Indian Point in March and Anderson Beach in December). Field-surveyed crabs were directly comparable to laboratory-reared hatchery and wild crabs from January through August (when the laboratory experiment ended). Overall, there were no consistent differences in CL between field-surveyed, wild-laboratory and hatchery crabs (mixed-model ANOVA,  $F = 2.45$ ,  $df = 2$ ,  $90$ ,  $p = 0.09$ ), although the similarities were not consistent, evidenced by a significant interaction between crab source and month (mixed-model ANOVA,  $F = 1.91$ ,  $df = 14$ ,  $90$ ,  $p = 0.03$ ). In January, hatchery crabs ( $6.98 \text{ mm} \pm 0.61 \text{ SD}$ ) had larger CL than both wild-caught ( $5.91 \text{ mm} \pm 0.72 \text{ SD}$ ; Tukey HSD,  $p = 0.001$ ) and field-surveyed ( $6.40 \text{ mm} \pm 1.37 \text{ SD}$ ; Tukey HSD,  $p = 0.04$ ) crabs. In March and May, both hatchery (March  $8.65 \text{ mm} \pm 1.29 \text{ SD}$ ; Tukey HSD,  $p = 0.01$  and May  $10.47 \text{ mm} \pm 1.24 \text{ SD}$ ; Tukey HSD,  $p = 0.02$ ) and field-surveyed (March  $8.18 \text{ mm} \pm 1.42 \text{ SD}$ ; Tukey HSD,

$p = 0.03$  and May  $10.83 \text{ mm} \pm 1.85 \text{ SD}$ ; Tukey HSD,  $p = 0.001$ ) crabs had larger CL than wild-caught crabs (March  $7.48 \text{ mm} \pm 1.05 \text{ SD}$  and May  $9.17 \text{ mm} \pm 1.40 \text{ SD}$ ). In August, hatchery crabs ( $13.43 \text{ mm} \pm 1.67 \text{ SD}$ ) had larger CL than field-surveyed crabs ( $12.19 \text{ mm} \pm 2.57 \text{ SD}$ ; Tukey HSD,  $p = 0.04$ ) (Figure 1.3a) (Appendix 1C).

#### Spine Lengths

Spine lengths of hatchery-raised, wild-caught and field-surveyed crabs differed overall (mixed-model ANOVA,  $F = 5.74$ ,  $df = 2$ ,  $90$ ,  $p = 0.005$ ), with hatchery (Tukey HSD,  $p = 0.03$ ) and wild-caught (Tukey HSD,  $p = 0.01$ ) crabs both significantly different from field-surveyed crabs, although the differences were not consistent which was evidenced by the significant interaction of crab source and month (mixed-model ANOVA,  $F = 2.52$ ,  $df = 14$ ,  $90$ ,  $p = 0.005$ ). In January, hatchery crabs ( $0.65 \text{ mm} \pm 0.11 \text{ SD}$ ) had longer spines than wild-caught ( $0.53 \text{ mm} \pm 0.08 \text{ SD}$ ; Tukey HSD,  $p = 0.001$ ) and field-surveyed ( $0.45 \text{ mm} \pm 0.14 \text{ SD}$ ; Tukey HSD,  $p < 0.0001$ ) crabs; additionally, wild-caught crabs had longer spines than field-surveyed crabs (Tukey HSD,  $p = 0.02$ ). Spines from both hatchery-raised crabs in February ( $0.74 \text{ mm} \pm 0.10 \text{ SD}$ ; Tukey HSD,  $p < 0.0001$ ) and March ( $0.82 \text{ mm} \pm 0.13 \text{ SD}$ ; Tukey HSD,  $p < 0.0001$ ) and wild-caught laboratory-reared crabs in February ( $0.71 \text{ mm} \pm 0.15 \text{ SD}$ ; Tukey HSD,  $p < 0.0001$ ) and March ( $0.75 \text{ mm} \pm 0.13 \text{ SD}$ ; Tukey HSD,  $p < 0.0001$ ) had longer spines than field-surveyed crabs from February ( $0.49 \text{ mm} \pm 0.19 \text{ SD}$ ) and March ( $0.55 \text{ mm} \pm 0.19 \text{ SD}$ ). Wild-caught crabs in June ( $1.21 \text{ mm} \pm 0.28 \text{ SD}$ ) and August ( $1.40 \text{ mm} \pm 0.37 \text{ SD}$ ) had longer spines than field-surveyed crabs in June ( $1.05 \text{ mm} \pm 0.26$ ; Tukey HSD,  $p = 0.04$ ) and August ( $1.14 \text{ mm} \pm 0.41$ ; Tukey HSD,  $p = 0.01$ ). There was no difference in mean SL among any groups of crabs in April, May and July (Figure 1.3b, Appendix 1C).

## DISCUSSION

Hatchery-raised and wild-caught laboratory-reared *P. camtschaticus* do not differ significantly in size from field cohorts, suggesting that *P. camtschaticus* may be reared in a hatchery or laboratory without significant influence on growth. Interestingly, juvenile *P. camtschaticus* growth appears to be similar across a diversity of studies from different parts of the world and from both field and laboratory studies. Studies from Japan (Nakanishi, 1987), Russia (Kovatcheva et al., 2006) and Alaska (Weber, 1967; Donaldson et al., 1992; Stoner et al., 2010) all observed red king crab juvenile CL in the range of 1.5 to 2 mm at the C1 stage and 3.5 to 5 mm at the C5 stage (Table 1.1). During growth studies in Unalaska and Kodiak Island, AK, the average growth reported after one year was approximately 11 mm CL, with an average of 11 molts/year in Unalaska and 9 molts/year in Kodiak at ambient temperatures (Weber, 1967; Donaldson et al., 1992). In the laboratory, our crabs molted more often but were similar in size to one-year old C9 Kodiak crabs (Donaldson et al., 1992). Our one-year old crabs reached an average size of 13.87 mm ( $\pm 1.53$  SD) CL after 10 – 11 molts/year at ambient temperatures.

In this study, juvenile crabs (both hatchery and wild; C1 to C9) had an average growth increment of 24.5 % ( $\pm 8.5$  SD), with a range of 20.3 % ( $\pm 7.9$  SD) to 26.7 % ( $\pm 10.1$  SD) at each molt, which is consistent with literature values. In Unalaska, AK juvenile crabs averaging 10 mm CL in ambient water had an overall growth increment of 23 % (Weber, 1967), while our hatchery crabs averaged 21.5 % ( $\pm 4.5$  SD) and wild crabs averaged 29.2 % ( $\pm 11.2$  SD) at comparable sizes. Juvenile crab (C1 to C9) in Kodiak at ambient temperatures in situ had growth increments ranging from 16 – 30 % with higher growth increments during the earlier stages (C1 to C4 increments ranged from 29 – 32 %) (Donaldson et al., 1992) which was a trend not seen in this study. Similarly, growth increments comparable to our data in a companion study ranging from 23.5 % ( $\pm 4.5$  SD) to 27.5 % ( $\pm 8.2$  SD) were seen in juvenile crabs (C1 to C3) reared at 12 °C (Stoner et al., 2010). Even though growth appears to be consistent across studies, temperature still has an effect on growth rates. From the C1 to C2 stage, juvenile *P. camtschaticus* growth increment increases significantly from an average 17.6 % ( $\pm 7.8$  SD) at 1.5 °C to an

average of 25.4 % ( $\pm 5.2$  SD) at 12 °C over 60 d (Stoner et al., 2010). Since juvenile *P. camtschaticus* reside in the intertidal and shallow subtidal during the early life phases (Weber, 1967), they most likely experience a wide range of temperatures. Even though it has been shown that growth rates of *P. camtschaticus* vary significantly with temperature (Stoner et al., 2010), the difference is relatively small and may not be biologically significant especially if low temperatures resulting in less growth are only experienced for short periods of time.

Growth trajectories and sizes were not different for male and female *P. camtschaticus* during the first year (Weber, 1967), which is in agreement with our results. The lack of differences between male and female crabs in the first year of growth is not surprising since the crabs are still a long way from maturity. Size differentiation between males and females in *P. camtschaticus* does not occur until crabs reached five (Weber, 1967) or six years of age (Weber, 1967; Lysenko and Gaydaev, 2005), approximately the age at which crabs reach sexual maturity (Weber, 1967; Paul, 1992).

Molt interval for individual juvenile *P. camtschaticus* is highly variable and details for molt intervals for each stage are sparse in the literature. One explanation is that there are currently no known methods for aging crabs and the only way to determine accurate age information is to rear crabs individually from a known larval or early juvenile stage. Growth studies of *P. camtschaticus* rarely rear crabs individually since it is extremely labor intensive. For the first three juvenile stages (C1 to C3), our results for both hatchery and wild crabs are comparable to previous studies (Stoner et al., 2010). For the C1 to C3 stages, hatchery crabs in this experiment averaged 248.2 dd ( $\pm 33.1$  SD) and wild crabs averaged 216.4 dd ( $\pm 8.4$  SD). A companion study of C1 to C3 crabs reared individually from the same hatchery stock averaged 240 dd ( $\pm 31$  SD) (Stoner et al., 2010).

Cumulative molt interval, or the cumulative time (in degree days) that it takes to reach each molt stage, is reported in the literature more often than molt interval at each stage, although values varied widely. Our findings do not seem to match any published results for comparable crab stages for cumulative molt intervals with variation over 300

dd in some instances. For the three molt stages that are comparable across studies, our findings for cumulative molt interval falls in between what has been reported for juvenile *P. camtschaticus*. For C2C3, the crabs in our study had accumulative molt interval of 506.4 dd ( $\pm 13.4$  SD) and 427.2 dd ( $\pm 30.6$  SD) for hatchery and wild crabs respectively compared to 900 dd (Mortensen and Damsgard, 1996) and 126 dd (Kovatcheva et al., 2006). For the C3C4 molt, we measured 744.7 dd ( $\pm 107.9$  SD) and 649.1 dd ( $\pm 46.7$  SD), for hatchery and wild crabs, respectively, compared to 1,100 dd (Mortensen and Damsgard, 1996) and 367.5 dd (Kovatcheva et al., 2006). For the final comparable molt, C4C5, we found 995.4 dd ( $\pm 131.2$  SD) and 933.6 dd ( $\pm 85.0$  SD), for hatchery and wild crabs respectively, compared to 1,400 dd (Mortensen and Damsgard, 1996) and 619.5 dd (Kovatcheva et al., 2006). Since the other reports for cumulative molt interval are given for mass-reared crabs, it is possible that the molt stages were misestimated or that it was not possible to accurately determine the molt interval for crabs reared en masse since individual molt intervals vary greatly. Another possible explanation for such wide variation in cumulative molt intervals could be the difference in crab stocks. While our crabs originate from Alaskan waters, crabs in the other published studies originate in Norway and Russia, and there could potentially be adaptive difference between these stocks.

Body spines likely improve predator defense (Young et al., 2008), and have been shown to vary in size between hatchery-reared and wild animals (Stoner, 1994; Davis et al., 2004). We did not find exception to this trend in this study. Hatchery-raised juvenile Atlantic blue crabs, *Callinectes sapidus*, grow significantly shorter spines than wild counterparts (Davis et al., 2004). Spine length, in this case, is plastic and varies as a function of exposure to their natural environment (Davis et al., 2004). Hatchery-reared queen conch, *Strombus gigas*, have lower shell weights and shell diameters including spine diameters than their wild cohorts (Stoner, 1994). Our hatchery crabs had significantly longer spines than their field counterparts for the first three months (January, February and March) that juvenile crabs were found in the intertidal. Interestingly, the wild-laboratory reared crabs had significantly longer spines than their

field cohorts in January, February, March, June and August, suggesting that spine length may be an artifact of laboratory rearing during the early phases of crab growth (Figure 4b). Additional studies are needed to determine the significance and plasticity of spine length for juvenile red king crab.

When considering stock enhancement for any species, it is important to discern any advantages or disadvantages that the hatchery animals may have. Advantages over wild counterparts may have considerable consequences for native stocks while many disadvantages make enhancement a futile endeavor. Ideally, hatchery and wild cohorts would be indistinguishable from one another in physiology as well as behavior. This study demonstrates that growth, when compared between hatchery-raised and wild-caught juveniles in the laboratory, is not significantly different from field cohorts in southeast Alaska even though the hatchery stock originated in Bristol Bay, AK. Future experiments should address growth and behavior of stock specific hatchery crabs in the wild in comparison with wild counterparts in areas where rehabilitation is proposed.

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## TABLES

Table 1.1. Comparison of published mean  $\pm$  SD carapace length (mm) for juvenile *P. camtschaticus*.

	<b>This study hatchery crabs</b>	<b>This study wild crabs</b>	<b>Donaldson et al. (1992)<sup>2</sup></b>	<b>Nakanishi (1987)<sup>3</sup></b>	<b>Kovatcheva et al. (2006)<sup>4</sup></b>
<b>C1</b>	2.09 $\pm$ 0.09	2.08 $\pm$ 0.20	2.18 $\pm$ 0.155	1.572 $\pm$ 0.042	1.81 $\pm$ 0.022
<b>C2</b>	2.61 $\pm$ 0.25	2.51 $\pm$ 0.20	2.84 $\pm$ 0.152	2.280 $\pm$ 0.322	2.01 $\pm$ 0.041
<b>C3</b>	3.18 $\pm$ 0.33	3.23 $\pm$ 0.31	3.76 $\pm$ 0.243	2.850 $\pm$ 0.080	2.43 $\pm$ 0.065
<b>C4</b>	3.94 $\pm$ 0.42	4.11 $\pm$ 0.36	4.85 $\pm$ 0.338	3.700 $\pm$ 0.140	2.85 $\pm$ 0.114
<b>C5</b>	4.91 $\pm$ 0.48	5.09 $\pm$ 0.57	5.64 $\pm$ 0.394	4.185 $\pm$ 0.207	3.41 $\pm$ 0.188
<b>C6</b>	5.93 $\pm$ 0.74	6.10 $\pm$ 0.68	6.67 $\pm$ 0.560	n/a	4.03 $\pm$ 0.179
<b>C7</b>	7.52 $\pm$ 1.12	7.67 $\pm$ 1.01	8.0 $\pm$ 0.64	n/a	n/a
<b>C8</b>	9.66 $\pm$ 1.32	9.25 $\pm$ 1.21	9.5 $\pm$ 0.77	n/a	n/a
<b>C9</b>	11.74 $\pm$ 1.72	11.89 $\pm$ 1.42	11.2 $\pm$ 0.93	n/a	n/a

<sup>2</sup>Donaldson et al. (1992) reared juvenile *P. camtschaticus* en masse in situ at ambient temperatures.

<sup>3</sup>Nakanishi (1987) reared juvenile *P. camtschaticus* en masse in the laboratory at 8°C.

<sup>4</sup>Kovatcheva et al. (2006) reared juvenile *P. camtschaticus* en masse in the laboratory at 10 - 11°C.

## FIGURES

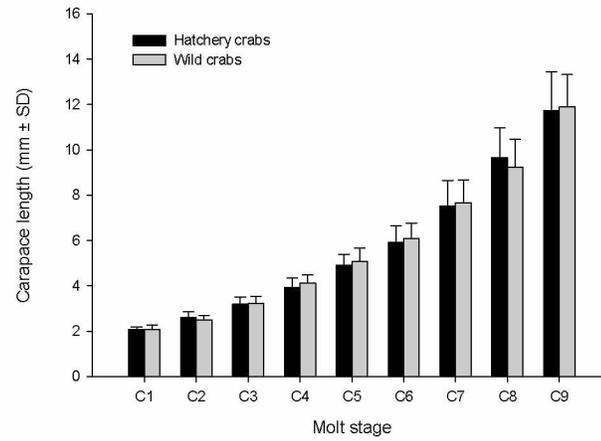


Figure 1.1. Mean  $\pm$  SD carapace length (mm) for the first nine molt stages for juvenile hatchery-raised ( $n = 10$ ) and wild-caught laboratory-reared ( $n = 23$ ) *P. camtschaticus*.

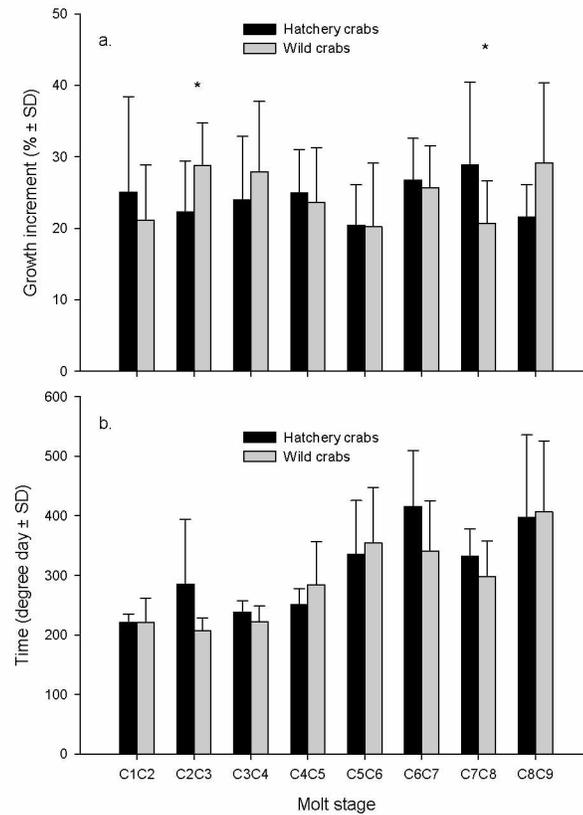


Figure 1.2. Mean  $\pm$  SD growth increment (%)  $[(CL_{i+1}(\text{mm}) - CL_i(\text{mm}))/CL_i(\text{mm})]$ ;  $i$ =instar] and molt interval (degree day) for the first nine molts of hatchery ( $n = 10$ ) and wild ( $n = 23$ ) juvenile *P. camtschaticus*. An asterisk, “\*”, denotes significant differences at  $p < 0.05$ . For the C2C3 growth increment, hatchery crabs had a significantly higher growth increment than wild crabs (1.2a). During the C7C8 molt increment, wild crabs had significantly larger growth increment than hatchery crabs (1.2a).

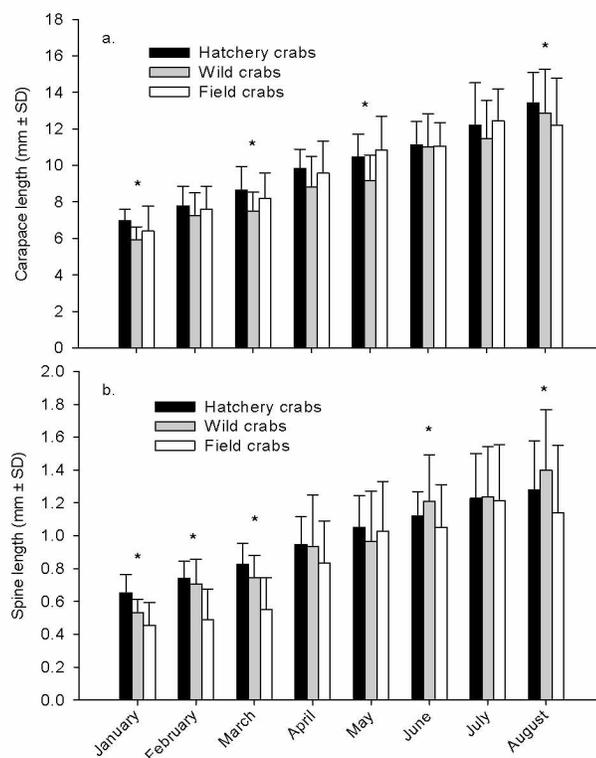


Figure 1.3. Mean  $\pm$  SD carapace length (mm) and spine length (mm) by month for hatchery ( $n = 10$ ), wild-laboratory ( $n = 23$ ) and field-surveyed (January,  $n = 12$ ; February,  $n = 41$ ; March,  $n = 50$ ; April,  $n = 57$ ; May,  $n = 19$ ; June,  $n = 23$ ; July,  $n = 24$ ; August,  $n = 9$ ) juvenile *P. camtschaticus* from January through August 2010. An asterisk, “\*”, denotes significant differences at  $p < 0.05$ . In January, hatchery crabs had significantly larger CL than wild-caught and field-surveyed crabs. In March and May, hatchery and field-surveyed crabs both had significantly larger CL wild-caught crabs. In August, hatchery crabs had significantly larger CL than field-surveyed crabs (1.3a). In January, hatchery crabs had significantly longer SL than field-surveyed and wild-caught crabs. Additionally in January, wild-caught crabs had significantly longer spines than field-surveyed crabs. In February and March, hatchery and wild-caught crabs had significantly longer spines than field-surveyed crabs. In June and August wild-caught crabs had significantly longer spines than field-surveyed crabs (1.3b).

## APPENDIX 1A

Table A1. Mean  $\pm$  SD carapace length (mm), molt interval (degree day), cumulative molt interval (degree day) and growth increment of hatchery (%) (n = 10) and wild-laboratory (n = 23) raised juvenile *P. camtschaticus* for the first nine molt stages as well as for the final measurements taken in September 2010. Growth increment was determined by  $[(CL_{i+1}(\text{mm}) - CL_i(\text{mm}))/CL_i(\text{mm})]$ ; CL = carapace length, i=instar].

		C1	C2	C3	C4	C5	C6	C7	C8	C9	Final
Hatchery crabs	n	10	10	10	10	10	10	10	10	10	10
	CL (mm $\pm$ SD)	2.09 $\pm$ 0.09	2.61 $\pm$ 0.25	3.18 $\pm$ 0.33	3.94 $\pm$ 0.42	4.91 $\pm$ 0.48	5.93 $\pm$ 0.74	7.52 $\pm$ 1.12	9.66 $\pm$ 1.32	11.74 $\pm$ 1.32	13.71 $\pm$ 1.45
	Molt Interval (dd $\pm$ SD)	-	221.2 $\pm$ 13.4	285.1 $\pm$ 108.5	238.3 $\pm$ 18.9	250.7 $\pm$ 26.5	335.8 $\pm$ 90.0	416.0 $\pm$ 93.6	332.4 $\pm$ 45.7	397.6 $\pm$ 138.1	-
	Cumulative time to molt (dd $\pm$ SD)	-	221.2 $\pm$ 13.4	506.4 $\pm$ 106.9	744.7 $\pm$ 107.9	995.4 $\pm$ 131.2	1331.2 $\pm$ 204.7	1747.2 $\pm$ 162.4	2079.7 $\pm$ 183.1	2477.3 $\pm$ 252.6	-
	Growth increment (% $\pm$ SD)	-	25.1 $\pm$ 13.3	22.3 $\pm$ 7.2	23.9 $\pm$ 8.9	25.0 $\pm$ 6.0	20.4 $\pm$ 5.7	26.8 $\pm$ 5.8	28.9 $\pm$ 11.6	21.6 $\pm$ 4.5	-
Wild crabs	n	23	23	23	23	23	23	23	23	23	23
	CL (mm $\pm$ SD)	2.08 $\pm$ 0.20	2.51 $\pm$ 0.20	3.23 $\pm$ 0.31	4.11 $\pm$ 0.36	5.09 $\pm$ 0.57	6.10 $\pm$ 0.68	7.67 $\pm$ 1.01	9.25 $\pm$ 1.21	11.89 $\pm$ 1.42	13.95 $\pm$ 1.59
	Molt Interval (dd $\pm$ SD)	-	220.5 $\pm$ 41.5	206.7 $\pm$ 22.2	222.0 $\pm$ 26.4	284.5 $\pm$ 72.5	354.2 $\pm$ 93.2	340.7 $\pm$ 84.9	297.9 $\pm$ 59.7	406.4 $\pm$ 119.5	-
	Cumulative time to molt (dd $\pm$ SD)	-	220.5 $\pm$ 41.5	427.2 $\pm$ 30.6	649.1 $\pm$ 46.7	933.6 $\pm$ 85.0	1287.7 $\pm$ 154.7	1628.4 $\pm$ 154.9	1926.2 $\pm$ 189.4	2332.7 $\pm$ 230.4	-
	Growth increment (% $\pm$ SD)	-	21.1 $\pm$ 7.8	28.7 $\pm$ 6.0	27.9 $\pm$ 9.8	23.6 $\pm$ 7.7	20.2 $\pm$ 9.0	25.7 $\pm$ 5.8	20.7 $\pm$ 5.9	29.2 $\pm$ 11.2	-

## APPENDIX 1B

Table B1. Mean  $\pm$  SD carapace length (mm), molt interval (degree day), cumulative molt interval (degreed day) and growth increment for male (%) (n = 15) and female (n = 18) juvenile *P. camtschaticus* for the first nine molt stages as well as for final measurements taken in September 2010. Growth increment was determined by  $[(CL_{i+1}(\text{mm}) - CL_i(\text{mm}))/CL_i(\text{mm})]$ ; CL = carapace length, i=instar].

		C1	C2	C3	C4	C5	C6	C7	C8	C9	Final
Male crabs	n	15	15	15	15	15	15	15	15	15	15
	CL (mm $\pm$ SD)	2.09 $\pm$ 0.19	2.53 $\pm$ 0.21	3.25 $\pm$ 0.30	4.01 $\pm$ 0.35	5.04 $\pm$ 0.59	6.08 $\pm$ 0.74	7.57 $\pm$ 1.09	9.40 $\pm$ 1.27	11.96 $\pm$ 1.41	13.73 $\pm$ 1.77
	Molt Interval (dd $\pm$ SD)	-	227.0 $\pm$ 23.6	229.0 $\pm$ 78.3	231.3 $\pm$ 15.6	261.9 $\pm$ 24.0	330.1 $\pm$ 71.2	384.2 $\pm$ 69.9	320.9 $\pm$ 46.7	432.2 $\pm$ 151.3	-
	Cumulative time to molt (dd $\pm$ SD)	-	227.0 $\pm$ 23.6	456.0 $\pm$ 76.4	687.3 $\pm$ 76.1	949.2 $\pm$ 82.6	1279.3 $\pm$ 148.9	1663.5 $\pm$ 133.4	1983.8 $\pm$ 172.4	2416.0 $\pm$ 230.2	-
	Growth increment (% $\pm$ SD)	-	0.21 $\pm$ 0.09	0.28 $\pm$ 0.06	0.24 $\pm$ 0.05	0.26 $\pm$ 0.07	0.21 $\pm$ 0.10	0.24 $\pm$ 0.06	0.25 $\pm$ 0.09	0.28 $\pm$ 0.13	-
Female crabs	n	18	18	18	18	18	18	18	18	18	18
	CL (mm $\pm$ SD)	2.06 $\pm$ 0.13	2.55 $\pm$ 0.23	3.17 $\pm$ 0.34	4.13 $\pm$ 0.42	5.02 $\pm$ 0.49	5.99 $\pm$ 0.64	7.70 $\pm$ 0.97	9.35 $\pm$ 1.24	11.68 $\pm$ 1.65	14.13 $\pm$ 1.22
	Molt Interval (dd $\pm$ SD)	-	213.7 $\pm$ 44.5	233.3 $\pm$ 66.8	223.8 $\pm$ 32.5	287.4 $\pm$ 87.9	354.6 $\pm$ 98.0	358.3 $\pm$ 94.7	297.2 $\pm$ 67.6	372.6 $\pm$ 86.8	-
	Cumulative time to molt (dd $\pm$ SD)	-	213.7 $\pm$ 44.5	447.0 $\pm$ 72.5	670.7 $\pm$ 91.8	958.1 $\pm$ 125.3	1312.7 $\pm$ 191.5	1671.0 $\pm$ 197.9	1968.2 $\pm$ 230.4	2340.8 $\pm$ 263.8	-
	Growth increment (% $\pm$ SD)	-	0.24 $\pm$ 0.11	0.24 $\pm$ 0.06	0.24 $\pm$ 0.05	0.26 $\pm$ 0.07	0.21 $\pm$ 0.10	0.24 $\pm$ 0.06	0.25 $\pm$ 0.10	0.28 $\pm$ 0.13	-

## APPENDIX 1C

Table C1. Mean  $\pm$  SD carapace length (mm) and spine length (mm) for hatchery (n = 10), wild-laboratory (n = 23) and field-surveyed juvenile *P. camtschaticus*. Neither CL nor SL was calculated for field-surveyed crabs in December due to small sample size.

		Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Hatchery crabs	n	10	10	10	10	10	10	10	10	0	0	0	0
	CL (mm $\pm$ SD)	6.98 $\pm$ 0.61	7.78 $\pm$ 1.07	8.65 $\pm$ 1.29	9.83 $\pm$ 1.06	10.47 $\pm$ 1.24	11.12 $\pm$ 1.28	12.21 $\pm$ 2.32	13.43 $\pm$ 1.67	n/a	n/a	n/a	n/a
	SL (mm $\pm$ SD)	0.65 $\pm$ 0.11	0.74 $\pm$ 0.10	0.82 $\pm$ 0.13	0.95 $\pm$ 0.17	1.05 $\pm$ 0.19	1.12 $\pm$ 0.15	1.23 $\pm$ 0.27	1.28 $\pm$ 0.30	n/a	n/a	n/a	n/a
Wild crabs	n	23	23	23	23	23	23	23	23	0	0	0	0
	CL (mm $\pm$ SD)	5.91 $\pm$ 0.72	7.27 $\pm$ 1.22	7.48 $\pm$ 1.05	8.83 $\pm$ 1.67	9.17 $\pm$ 1.40	11.01 $\pm$ 1.81	11.48 $\pm$ 2.10	12.86 $\pm$ 2.41	n/a	n/a	n/a	n/a
	SL (mm $\pm$ SD)	0.53 $\pm$ 0.08	0.71 $\pm$ 0.15	0.75 $\pm$ 0.13	0.93 $\pm$ 0.32	0.97 $\pm$ 0.30	1.21 $\pm$ 0.28	1.24 $\pm$ 0.30	1.40 $\pm$ 0.37	n/a	n/a	n/a	n/a
Field crabs	n	12	41	50	57	19	23	24	9	0	5	13	2
	CL (mm $\pm$ SD)	6.40 $\pm$ 1.37	7.59 $\pm$ 1.26	8.18 $\pm$ 1.42	9.57 $\pm$ 1.77	10.83 $\pm$ 1.85	11.06 $\pm$ 1.27	12.44 $\pm$ 1.76	12.19 $\pm$ 2.57	n/a	15.89 $\pm$ 1.03	16.78 $\pm$ 3.67	n/a
	SL (mm $\pm$ SD)	0.45 $\pm$ 0.14	0.49 $\pm$ 0.19	0.55 $\pm$ 0.19	0.83 $\pm$ 0.26	1.03 $\pm$ 0.30	1.05 $\pm$ 0.26	1.21 $\pm$ 0.34	1.14 $\pm$ 0.41	n/a	1.56 $\pm$ 0.27	1.67 $\pm$ 0.70	n/a

CIRCULATING ECDYSTEROIDS IN HEMOLYMPH OF JUVENILE RED KING  
CRAB, *PARALITHODES CAMTSCHATICUS*<sup>1</sup>

ABSTRACT

Ecdysis, or molting, is a prerequisite for growth in crustaceans and is coordinated by multiple hormones. The primary molting hormone is the steroid 20-hydroxyecdysone (20-HE) which circulates at low levels throughout most of the molt cycle, increasing significantly during premolt, during which this steroid promotes epidermal proliferation and behavioral changes associated with imminent ecdysis. Crustaceans experience reduced function and reduction in predatory avoidance behaviors during premolt and determining the amount of time spent in a state of reduced function is useful for successful aquaculture and stocking efforts. This study investigated concentrations of 20-HE throughout the molt cycle of red king crab, *Paralithodes camtschaticus*, and the duration of premolt in juveniles. Hemolymph was sampled weekly from juvenile (age-0) *P. camtschaticus* (n = 5) and ecdysteroid levels measured using an enzyme linked immunosorbant assay and ranged from approximately 1.1 to 480 ng/mL. Three of the five juveniles provided hormone data for a complete molt cycle (molt interval of 109 d ± 15 SD), with 42.6 d (± 5 SD) which represented 39.4 % (± 2.8 SE) of the entire molt cycle spent in premolt. Larger juvenile *P. camtschaticus* (age-3, n = 2) exhibited molt intervals of 209 d and 182 d with circulating ecdysteroid levels that ranged from approximately 0.9 to 612 ng/mL, with premolt durations that lasted 30.1 and 34.6 % of the entire molt cycle, respectively. Despite variable molt intervals, we found that circulating ecdysteroid peaks were fairly consistent, which occurred 17 d (± 5.7 SD) for younger crabs and 14 – 15 d for older juvenile crabs preceding ecdysis. This information on the circulating ecdysteroid profile and premolt duration for juvenile *P. camtschaticus* will better inform early life history as well as restoration efforts for this species in Alaska. Keywords: hatchery, 20-hydroxyecdysone, enhancement, premolt, growth, ecdysone

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<sup>1</sup> Westphal, M.J., Tamone, S.L., Eckert, G.L. (in prep). Circulating ecdysteroids in hemolymph of juvenile red king crab, *Paralithodes camtschaticus*. Prepared for submission in the Journal of Crustacean Biology.

## INTRODUCTION

Crustacean growth is a complex process requiring coordination by multiple hormones (Chang et al., 1993), prompting energetically demanding physical, physiological and behavioral processes (Tamm and Cobb, 1978; Chang, 1995). Crustaceans grow by synthesizing a new cuticle under their existing exoskeleton, shedding the old exoskeleton (ecdysis or molting), expanding the soft cuticle and then allowing it time to harden into a new larger exoskeleton (Skinner, 1985; Chang, 1995). The physiological process of growth in crustaceans has been reviewed and is well understood (Kincaid and Scheer, 1952; Chang, 1985; Skinner, 1985; Skinner et al., 1985; Devaraj and Natarajan, 2006). The molt cycle can be characterized by four major periods; 1) intermolt, the period during which the exoskeleton is calcified and somatic tissue growth occurs, 2) premolt, the period of active preparation for ecdysis, 3) ecdysis, or molting, and 4) post-molt, the period when soft-shelled, newly molted crabs absorb water to increase in size (Drach, 1939; Kurup, 1964).

The primary hormones that regulate crustacean molt cycles are derived primarily from the X-organ and the Y-organ endocrine glands. The X-organ-sinus gland complex is located in the eyestalk and produces many functionally diverse neuropeptides including molt inhibiting hormone (MIH) (Chang, 2001; Chung and Webster, 2003; Chung and Webster, 2005). Early studies showed that eyestalk removal accelerated ecdysis and re-implantation of sinus gland extract resolved such induction (Brown and Cunningham, 1939). Later studies identified a neuropeptide that suppresses the production of molting hormones including the steroid hormone  $\alpha$ -ecdysone from the Y-organ (Brown and Cunningham, 1939; Chang and O'Connor, 1977; Soumoff and O'Connor, 1982; Chang, 1985). The Y-organ resides in the branchial chamber (Gabe, 1956) and secretes ecdysone into the hemolymph (Chang and O'Connor, 1977) which is later hydroxylated by distal tissues into the bioactive hormone, 20-hydroxtecdysone (20-HE) (Soumoff and O'Connor, 1982). Ecdysteroids formed from dietary cholesterol are enzymatically synthesized within calcium-regulated cells within the Y-organ (Skinner, 1985; Lachaise et al., 1993; Spaziani et al., 2001). Circulating MIH negatively regulates molting

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hormones through receptor mediated events (Webster, 1993). In the absence of MIH, which can be manipulated through eyestalk ablation, circulating 20-HE increases and molting related activities and behaviors are initiated (Chang, 1985; Tamone et al., 2005).

The principal hormone that promotes ecdysis is 20-hydroxyecdysone (20-HE) and is derived from the steroid ecdysone. Levels of 20-HE change as a function of specific stages of the molt cycle (Skinner et al., 1985) and, for crustaceans, exhibit a general pattern. Patterns of circulating molting hormones for juvenile lobster, *Homarus americanus*, (Chang and Bruce, 1980), Dungeness crab, *Cancer magister*, (Thomton et al., 2006), blue crab, *Callinectes sapidus*, (Chung, 2010) and the American crayfish, *Procambarus clarkii*, (Nakatsuji et al., 2000) are similar in that 20-HE concentrations remain relatively low during intermolt, increase during premolt and decline sharply prior to ecdysis. After ecdysis, the concentrations of ecdysteroids remain relatively low until the next premolt (Chang and Bruce, 1980; Thomton et al., 2006). The increase in circulating 20-HE during premolt is responsible for molt related processes that include the proliferation of epidermal cells, synthesis of the cuticle that will later become the new exoskeleton, muscle atrophy necessary for withdrawal from the old exoskeleton (Mykles and Skinner, 1985) and limb regeneration (Skinner et al., 1985).

The amount of time a crustacean spends in premolt represents time during which the animal may be vulnerable to predation. Premolt is known to be a time during which many animals cease feeding (Lipcius and Herrnkind, 1982; Zhou et al., 1998), decrease locomotion (Lipcius and Herrnkind, 1982; Cromarty et al., 2000), decrease predator avoidance responses (Cromarty et al., 2000) and increase refuge, shelter-seeking behaviors in response to threats (Chang, 1995). Decreases in the daily activities of premolt crustaceans is thought to be caused by physical and physiological processes necessary for the impending ecdysis such as muscle atrophy (Mykles and Skinner, 1985), reduced synaptic efficacy due to markedly increased level of circulating 20-HE in the hemolymph (Cooper and Ruffner, 1998), limb regeneration, (Hopkins, 1982; Hopkins, 1983; Skinner et al., 1985), apolysis (Skinner et al., 1985), gastrolith formation (Skinner et al., 1985) and degradation of the membranous layer and endocuticle of the old

exoskeleton (Skinner et al., 1985). For adult female red king crab, *Paralithodes camtschaticus*, approximately 74 d of the approximately 365 d molt cycle (approximately 19 % of the molt cycle) is needed for the shell to harden to 90 % pre-molt hardness (Stevens, 2009). These processes associated with molting have serious biological implications for survival.

Determining the levels of circulating 20-HE in the hemolymph of juvenile *P. camtschaticus* throughout the molt cycle can be useful for determining the amount of time spent in the energetically demanding stage of premolt. Little information is available regarding the molting physiology of juvenile *P. camtschaticus*. Discerning the amount of time spent in premolt in relation to the molt interval can give valuable insight into the time during which a crab is physiologically and behaviorally influenced by increasing hormones which may affect juvenile rearing and release timing during potential restoration activities. The goals of study were to 1) describe the molt hormone profile of juvenile *P. camtschaticus* and 2) determine the percentage of the molt cycle that juvenile *P. camtschaticus* spend in premolt.

## METHODS

### Experimental Animals

Newly settled juvenile *P. camtschaticus* instars were hatched and reared at the Alutiiq Pride Shellfish Hatchery from broodstock collected in Bristol Bay, AK in November 2009. The larvae hatched in April 2010 and were reared in 1,200 L continuous flow through tanks at densities of approximately 50 larvae L<sup>-1</sup> at 11°C. On 20 August 2010, *P. camtschaticus* juveniles were shipped via Alaska Air Cargo to the University of Alaska Fairbanks, School of Fisheries and Ocean Sciences in Juneau, Alaska. Additionally, we included two larger juvenile *P. camtschaticus* (age-3) that were originally wild captured but maintained under laboratory conditions.

### Rearing Conditions

Juvenile *P. camtschaticus* (age-0) were reared individually in perforated plastic cups set into Living Stream© tanks (LS-700; Frigid Units) in running seawater with ambient temperature, salinity and photoperiod at the University of Alaska marine lab. Temperature was recorded every hour using a HOBO water temp Pro-VR logger. Crabs were fed minced raw seafood (mussels, herring or squid) ad libitum weekly. Larger juvenile *P. camtschaticus* (approximately age-3) were maintained in Living Stream tanks (Frigid Units) with flow through seawater. Crabs were fed weekly, to excess, a diet of chopped raw seafood that included fresh salmon, mussels, squid, herring, shrimp, urchins and supplemented with blocks of mineral calcium in gelatin. All rearing containers and tanks were cleaned weekly. Crabs were monitored daily for evidence of ecdysis. Carapace length (CL) was measured using dial calipers (accuracy  $\pm$  0.01 mm), prior to and at least two weeks post-molt in order to ensure that maximum size had been reached and that the exoskeleton had hardened. CL was measured from the base of the eyestalk crevice to the distal median of the carapace.

### Hemolymph Extraction

Once weekly, 2.5  $\mu$ L of hemolymph was withdrawn from the lateral carapace suture above the rear coxa of age-0 crabs (n = 5) using a 33 gauge needle attached to a Hamilton syringe (2.5  $\mu$ L volume) under a dissecting scope. Hemolymph was diluted

with 50  $\mu$ L of buffer (0.025 M Tris pH 7.5; 0.5 % BSA; AB/BSA) and frozen prior to hormone assay. Hemolymph (50  $\mu$ L) was sampled from larger juveniles ( $n = 2$ ) from the base of the rear pereopod and diluted with 150  $\mu$ L of AB/BSA. As with similar studies, samples were frozen for later hormone analysis (Tamone et al., 2005; Thomton et al., 2006).

#### Ecdysteroid Assay

Circulating levels of 20-HE were analyzed using an enzyme-linked immunosorbant assay (ELISA) developed by Kingan (1989) and modified for crustaceans by Tamone et al. (2005). Ninety-six (96) well plates were coated with goat anti-rabbit IgG (Jackson Immuno Research Laboratories) overnight and blocked for one hour at room temperature using AB/BSA. Plates were washed three times using PBS containing 0.05 % TWEEN-20. Standards of ecdysone and diluted hemolymph samples (50  $\mu$ l) were incubated overnight in the presence of primary antiserum (1:80,000 dilution; 50  $\mu$ l) and HRP conjugated ecdysone (1:4000; 50  $\mu$ l). For each age-0 crab, 2.5  $\mu$ l of hemolymph was assayed, for age-3 crabs, 0.14 to 1.71  $\mu$ l of hemolymph was assayed. The conjugated steroid and the primary antiserum were purchased from Dr. T. Kingan. After washing three times for five min with PBS containing 0.05 % TWEEN-20, the plates were developed using a TMB substrate (KPL, Inc) and color development was stopped using 1 M phosphoric acid. Absorbance was measured at 450 nm (BIORAD plate reader Model 680XR).

#### Descriptive Statistics

Premolt was determined visually by observing the ecdysteroid concentration inflection point on the molt hormone graphs. Since age-0 juveniles had premolt and intermolt levels that varied and had varying number of measures, average premolt and intermolt hormone levels were calculated using weighted mean ( $\pm$  SE). For individual age-0 and age-3 juveniles, mean ecdysteroid levels ( $\pm$  SE) were calculated.

## RESULTS

### Age-0 Juveniles

During the course of this experiment, 3 of the 5 juveniles completed two molts and therefore provided the desired hormone data over an entire molt cycle (Figure 2.1 a – c). The other two crabs contributed hormone data towards understanding premolt (Figure 2.1 d and e). Of the three crabs that provided complete molt profiles, molt intervals were 109 d ( $\pm 15$  SD). Juvenile crabs grew from 13.6 mm CL ( $\pm 1.5$  SD) to 16.0 mm ( $\pm 2.4$  SD). Crabs that molted more than once during the experiment had a growth increment of 16.3 % ( $\pm 2.4$  SD). Premolt began 46.6 d ( $\pm 5.2$  SD) prior to ecdysis and comprised 42.6 % ( $\pm 5$  SE) of the entire molt cycle. Peak concentration of 20-HE occurred 17 d ( $\pm 5.7$  SD) prior to ecdysis and averaged 410.9 ng/mL ( $\pm 22.4$  SE). Circulating concentrations of 20-HE averaged 5.98 ng/mL (weighted average  $\pm 1.44$  SE) during intermolt (Figure 2.2).

### Age-3 Juveniles

Circulating ecdysteroids were measured in the two larger juvenile *P. camtschaticus* (age-3) and are presented for comparison with younger animals. Larger juveniles showed similar patterns in their hormone concentrations over a molt cycle to the smaller juveniles. Concentrations of 20-HE increased approximately 64 d prior to ecdysis in both crabs. Both crabs showed an initial peak and decline early in premolt followed by a much larger peak in 20-HE and a decline prior to ecdysis (Figure 2.3). The timing of molting events was similar between the two crabs, with molt intervals of 209 d and 182 d. Premolt began in crab A and crab B 64 d and 63d prior to ecdysis and comprised 30.1 % and 34.6 % of the molt cycle, respectively. Peak ecdysteroid levels (378.2 ng/mL) occurred 15 d prior to ecdysis in Crab A and 14 d (612.7 ng/mL) prior to ecdysis in Crab B. Crab A demonstrated intermolt concentrations of 20-HE of 7.9 ng/mL ( $\pm 2.2$  SE), while Crab B demonstrated intermolt concentrations of 20-HE of 6.7 ng/mL ( $\pm 2.4$  SE).

## DISCUSSION

The molt interval for individual juvenile *P. camtschaticus* (age-0) can be highly variable (Chapter 1, this volume), whereas premolt timing, duration and 20-HE concentrations showed less variability. For juvenile *P. camtschaticus* (age-0), the peak concentrations of 20-HE during premolt occurred approximately 17 d ( $\pm 5.7$  SD) prior to ecdysis with similar 20-HE peak values ( $411 \text{ ng/mL} \pm 22.4 \text{ SE}$ ) (Figure 2.1 and 2.2). The pattern of molt cycle variations with predictable patterns of premolt timing and duration is similar to that seen in another anomuran, *Petrolisthes cinctipes* (Kurup, 1964). Concentrations of circulating 20-HE decreased to intermolt hormone concentrations prior to ecdysis in all small juveniles (Figure 2.1a-e). Premolt concentrations of circulating 20-HE and premolt duration of juvenile *P. camtschaticus* are similar to those published for other crustaceans (Chang and Bruce, 1980; Hopkins, 1982; Thomson et al., 2006; Chung, 2010). The peak concentration of 20-HE ranged from 378.2 to 612.7 ng/mL and was similar to premolt hormone concentrations in juvenile *H. americanus* (350 ng/mL) (Chang and Bruce, 1980), in adolescent female *H. americanus* (400 ng/mL) (Chang and Bruce, 1980), and for juvenile female *Callinectes sapidus* (290 – 330 ng/mL) (Chung, 2010).

The duration of premolt appears variable across crustaceans and the percentage of the molt cycle that a crustacean spends in premolt can range from approximately 11 % to 39.4 %. Juvenile *P. camtschaticus* in this study spent more time in premolt than either juvenile *Homarus americanus* (Chang and Bruce, 1980), *Uca pugilator* (Hopkins, 1982) or *P. cinctipes* (Kurup, 1964). We were able to get complete 20-HE molt profiles for two juvenile *P. camtschaticus* and the molt intervals appeared to be very similar. Crab A spent approximately 30.1 % (63 d) of the molt cycle in premolt and Crab B spent approximately 34.6 % (56 d) of the molt cycle in premolt. Younger juvenile *P. camtschaticus* spent 39.4 % ( $\pm 2.8 \text{ SE}$ ) in premolt, which appeared to be more time than larger *P. camtschaticus* (30.1 and 34.6 %), *P. cinctipes* (approximately 11 %) (Kurup, 1964), *H. americanus* (approximately 20 %) (Chang and Bruce, 1980), but seemed to be

about the same as intact *U. pugilator* which spend about 32 % of their molt cycle in premolt (Hopkins, 1982).

Although there are similarities in the patterns of circulating ecdysteroids of individual juvenile *P. camtschaticus* (age-0), two 20-HE peaks were observed in older (age-3) juvenile *P. camtschaticus*. For age-3 *P. camtschaticus* the first peak occurred approximately 42 d prior to ecdysis. The second peak was much larger and occurred 28 d later (approximately 14 d prior to ecdysis). This pattern was also seen in *Uca pugilator*, with a small peak occurring three weeks prior to ecdysis and a second, larger peak occurring a few days prior to ecdysis (Hopkins, 1983). The first peak may be a precursory hormonal signal to stimulate the physical process of premolt and ecdysis. The smaller juveniles in this experiment may also exhibit this dual peak but it would take more intensive sampling to be certain. In the literature, molt hormone profiles for other intact crustacean species do not illustrate this dual peak but instead the classic crustacean molt hormone profile with a single spike in circulating ecdysteroid levels that falls precipitously prior to ecdysis (Chang, 1985; Thomton et al., 2006; Chung, 2010). It may be that when looking at individual profiles, this dual peak becomes apparent but when the profiles of many animals are averaged together, the first peak becomes lost in the variation.

Studying growth hormones can elucidate distinct physiological and behavioral changes that occur as a result of circulating hormones during specific molt stages, specifically premolt and post-molt, which may help to estimate molt stage or predict ecdysis in individual crabs sampled in the field. The molting hormone, 20-HE, affects daily activities in most crustaceans (Chang and Bruce, 1980; Cooper and Ruffner, 1998). In red swamp crayfish, *Procambarus clarkii*, synaptic function in muscle fibers is reduced when exposed to the 20-HE compared to saline injections (Cooper and Ruffner, 1998). This synaptic depression causes weakened muscle contractions which may, in part, be a mechanism for reducing injury in post-molt (soft shell) crayfish by reducing the animal's ability to make large or rapid muscle movements which may damage or separate the layers of the new cuticle (Cooper and Ruffner, 1998). Physiological and physical

stressors that occur leading up to, and during, ecdysis affect behavior. Feeding rates of juvenile spiny lobster, *Panulirus argus*, decrease significantly immediately preceding and following ecdysis when compared to other stages of the molt cycle (Lipcius and Herrnkind, 1982; Briones-Fourzan et al., 2003). Similar findings are reported for adult *P. camtschaticus*, which may cease feeding twelve days prior until eight days after ecdysis (Zhou et al., 1998). High rates of submissive or avoidance behaviors, such as tail-flipping away from conspecifics or retreating when approached, in juvenile *P. argus* are exhibited preceding, during and soon after ecdysis (Lipcius and Herrnkind, 1982). Similarly, juvenile *H. americanus*, demonstrate reduced predator escape response during the post-molt phase (Cromarty et al., 2000). Juvenile *H. americanus* swim with significantly less velocity (distance/time), significantly lower swim frequency (tailflips/sec) and significantly shorter distance (distance/body weight/tailflip and distance/body length) than intermolt juvenile lobsters when threatened (Cromarty et al., 2000). Post-molt soft-shelled juvenile *H. americanus* show significantly less (nearly zero) aggression towards threatening stimulus than hard-shelled juveniles (Tamm and Cobb, 1978; Cromarty et al., 2000).

In this experiment, concentrations of circulating ecdysteroids are seemingly comparable between multiple size classes of juvenile *P. camtschaticus* although they seem to differ markedly from published concentrations measured previously in adult *P. camtschaticus* (Dvoretzky and Dvoretzky, 2010). In that study, extremely high concentrations (4-18  $\mu\text{g/ml}$ ) were measured in large (90 mm CL) and small (< 90 mm CL) adult crabs, and the authors concluded from their data that larger crabs maintained higher hormone concentrations than do smaller crabs (Dvoretzky and Dvoretzky, 2010). This is in contrast to this experiment in which levels of ecdysteroids between age classes appear to be similar. The higher levels of circulating 20-HE seen in older juvenile and adult *P. camtschaticus* compared to small juveniles in this study could be due to differences in the methodology for measuring hormones. The method (ELISA) used in this study are consistent with radioimmunoassay used in past studies (Chang and Bruce, 1980; Hopkins, 1983; Hopkins, 1986; Laufer et al., 2002; Chung, 2010) and not at all like

the analytical assay used by Dvoretzky and Dvoretzky (2010) which can detect all types of steroids in the hemolymph and not just 20-HE levels.

The hormone 20-hydroxyecdysone promotes physical, physiological and behavioral changes during premolt and many of these actions have implications in the implementation of ecological restoration of crustacean populations. Ecdysteroids in the hemolymph are responsible not only for molting and somatic growth (Chang, 1995), but also for allometric growth (Laufer et al., 2002; Tamone et al., 2007), larval metamorphosis (Buckmann and Tomaschko, 1992; Yamamoto et al., 1997), cuticular repair (Halcrow and Steel, 1992) and limb regeneration (Mykles, 2001). Levels of circulating ecdysteroids are affected by several factors such as xenobiotics (molt interfering agents) in the environment (Zou, 2005), loss of eyestalks potentially by predation, cannibalism or ablation (Tamm and Cobb, 1978; Chang and Bruce, 1980; Cromarty et al., 2000; Laufer et al., 2002; Tamone et al., 2005; Chung, 2010), disease (Laufer et al., 2005) and parasites. It is important that the physiology of molting be understood in ideal laboratory conditions in order for impacts on growth, and potentially the fishery, to be properly recognized and assessed. Understanding molting physiology is beneficial to restoration and restocking efforts where juveniles that are reared in a hatchery are reintroduced into the wild in an effort to restore broodstock populations. Having a more complete understanding of growth physiology can assist hatchery researchers in assessing the fitness of hatchery juveniles and give them a baseline by which to compare hatchery individuals to their wild cohorts. Additionally, understanding of the amount of time that juveniles spend in the energetically demanding phase of premolt, especially in young *P. camtschaticus* that can molt up to 12 times per year, could allow for a more successful restocking effort.

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## FIGURES

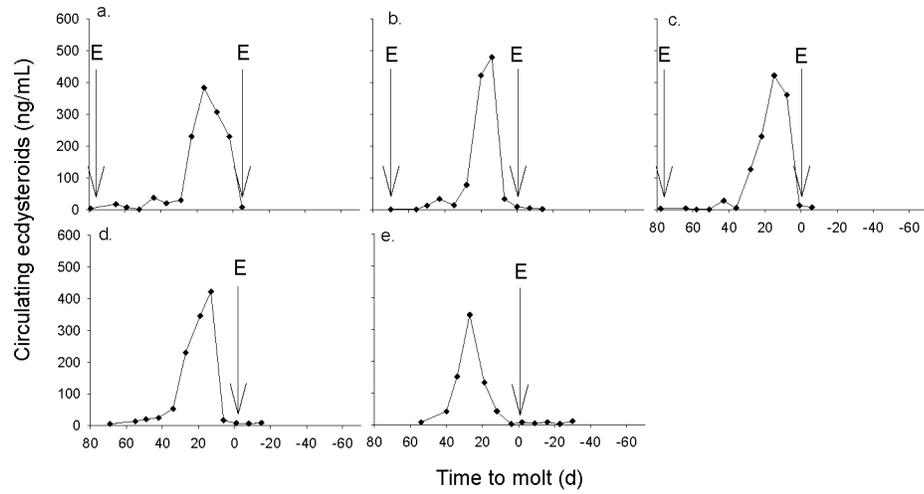


Figure 2.1. Circulating concentrations of 20-hydroxyecdysone in the hemolymph of five small juvenile *P. camtschaticus* (age-0). The first three graphs (2.1a, b and c) show crabs that completed two molts during the experiment. The final graphs (2.1d and e) show crabs that completed one molt during the experiment. “E” indicates ecdysis.

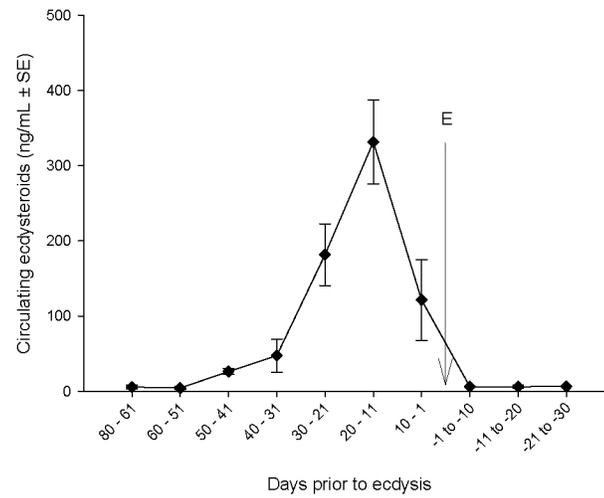


Figure 2.2. Weighted average levels ( $\pm$  SE) of circulating 20-hydroxyecdysone in the hemolymph of juvenile *P. camtschaticus* (age-0). All crabs with known molt dates ( $n = 5$ ) were used in calculations. “E” indicates ecdysis.

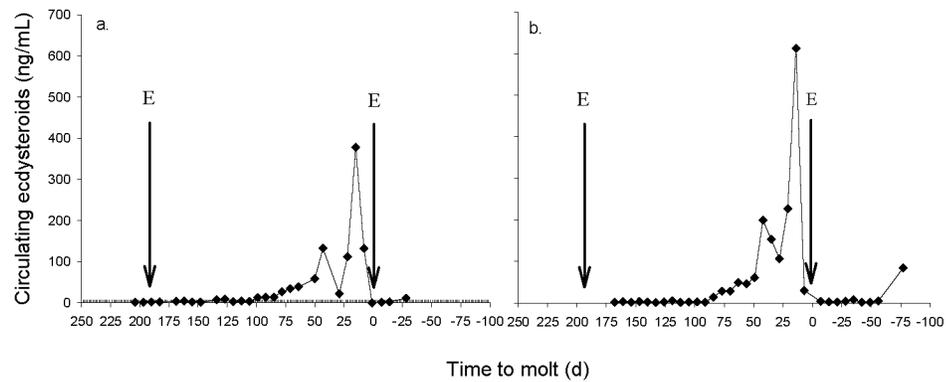


Figure 2.3. Circulating ecdysteroid levels (ng ecdysteroids per mL hemolymph) for juvenile *P. camtschaticus*, crab A (2.3a) and crab B (2.3b). Crab A (2.3a) was estimated to be approximately age-3 years with molts that occurred on 25 October 2008 and 22 May 2009. Crab B (2.3b) was estimated to be approximately age-3 years with molts that occurred on 25 November 2008 and 26 May 2009. “E” indicates ecdysis.

## CONCLUSION

The goals of this thesis were two-fold: to inform the early life history of *Paralithodes camtschaticus* as well as inform rehabilitation efforts in Alaska. In the light of exploring early life history through the lens of rehabilitation, it was important to focus mainly on the range of juvenile sizes that would most likely be released into the wild. To do this, I explored growth from an ecological as well as a physiological perspective. In many published growth experiments, the focus is mainly on growth in the laboratory with a minority of other studies focused mainly on cohort growth in the field. I had the fortune to explore both in this thesis.

Being able to explore growth in the laboratory with cohort growth in the field gave great insight and ecological perspective to juvenile red king crab growth. This was even more remarkable since the broodstock for the hatchery juveniles were collected in Bristol Bay, AK. Generally, there would be the expectation that crabs from such different origins would demonstrate differences in growth but that was not seen here. The assumption could be made therefore, that growth is more of a function of current rearing conditions rather than crab origin but other studies have shown that spatial as well as temporal differences do not seem to greatly affect growth in juvenile *P. camtschaticus*. In the literature juvenile *P. camtschaticus* growth has been reported from Japan, Russia, Norway and Alaska from 1960 through 2011 and despite such geographic and temporal variation, not to mention variable rearing conditions, there does not seem to be much difference in *P. camtschaticus* size or growth rate.

Even more remarkable in this thesis was the lack of difference in growth seen between hatchery crabs and wild-caught laboratory raised crabs and field-surveyed crabs when looked at by month. It was expected that wild-caught laboratory-reared and field-surveyed crabs would have comparable growth by month since the wild glaucothoe collected for the field was from the same site as our beach surveys. For the hatchery crabs, which metamorphosed into first-stage juveniles a full two months prior to wild-caught laboratory crabs, I was expecting that, when comparing growth by month, the hatchery crabs would be much larger and at more advanced molt stages which was not

the case. I presented data showing that there was no difference in size between hatchery-raised, wild-caught and field-surveyed crabs and when looking at molt stage by month both hatchery and wild-laboratory crabs have the same modal molt stage beginning in November (Table D1), just prior to field surveys. It is also interesting to note that juvenile *P. camtschaticus* begin to show up on the beaches in southeast Alaska in December, shortly after reaching the C6 stage. This lack of difference, along with the similarity in growth seen among published growth studies, suggests an adaptive juvenile growth strategy for this species.

The second perspective on growth for this thesis focused on the molting physiology of juvenile *P. camtschaticus*. This was important in understand the breadth of physical demands that juvenile *P. camtschaticus* undergo during a molt interval, especially during premolt. Premolt is a time during which the crab is preparing for molting (or growth) and is marked by many quiescent behaviors. Crabs during this time generally minimized movement, ceased feeding and were, in general, nearly appeared dormant, which was clearly seen during daily observations of crabs in the laboratory. It has been suggested that releasing crabs, perhaps in a rehabilitation effort, during this time may disadvantage the hatchery juveniles in the wild. Laboratory or hatchery-raised crabs placed into the wild are thought to be deficient in their behavioral ability to seek refuge from predators, since they lack experience interacting with predators in the hatchery. This may be compounded if the animal is in premolt and unable, physically, to seek refuge. Since we have shown that juvenile *P. camtschaticus* spend about 30 % of their molt cycle in premolt, it may help to determine the most appropriate time for rehabilitation release; either at very young (C1 or C2 stages) where the crabs are fairly synchronous in their molt interval or at later stages when the molt intervals are longer and the actual days spent in premolt minimized.

Even though there were not any significant differences in growth between hatchery-raised, wild-caught and field-surveyed juvenile *P. camtschaticus*, there is significant variation between individual molt intervals that was given some degree of resolution by looking at growth hormones. The first attempt to elucidate molt hormone

data was by sequential homogenization of many individuals over time instead of sequential hemolymph extraction of the same few individuals that is presented in this thesis. For the homogenization procedure, individual juvenile crabs were monitored daily for molts and then homogenized at certain intervals (five individuals homogenized at 5, 10, 15 , etc. d for a 70 d period) post-molting. What I did not realize at the time was the how the variation in individual molt interval would impact the levels of circulating ecdysteroids in the hemolymph of crabs during each of these time periods. The end result was a fairly erratic average molt hormone levels at each point in time with very large error bars for most points, which I have attributed to the individual molt interval variation (Figure D1). This variation further pointed to the need to sample hormones of the same individuals over time.

By looking at the molt hormone profiles of several individuals over time, the actual nature of molt hormones circulating in the hemolymph of juvenile *P. camtschaticus* became apparent. The molt hormones profiles of the individuals looked at in this thesis were remarkably similar between individuals so we could then come to the conclusion that the variation seen in the homogenized crab molt hormone graph was actually due to the variation in molt interval lengths. Even more remarkable was the timing of the premolt peak in ecdysteroids. For the crabs studied, no matter the length of the molt interval, the premolt peak occurred approximately 17 d prior to molting and only varied by 5.7 d. This suggests that despite the variation in molt interval, there is fairly rigid timing of the sequence of events tied to the act of molting.

This thesis is the first to compare growth of wild and hatchery juvenile *P. camtschaticus* and is therefore the first to show that there are no significant differences in growth. Although this is not definitive proof that differences do not exist, this is a good first step in evaluating juvenile hatchery crabs for rehabilitation in Alaska. Before any rehabilitation efforts commence, research is needed first to better understand the early life history of juvenile *P. camtschaticus* such as larval behavior and settlement, habitat preferences, ontogenetic shifts in habitat and behavior. Secondly, comparative studies are needed to evaluate the behavior of hatchery crabs with respect to the behavior of wild

crabs. The more knowledge that can be learned prior to rehabilitation efforts, will make for a well planned and more successful rehabilitation effort.

## TABLES

Table 3.1. Mode, minimum and maximum molt stage for laboratory-reared hatchery-raised (n = 10) and wild-caught (n = 23) juvenile *P. camtschaticus* from May 2009 to August 2010.

		May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Wild crabs	Mode	-	C1	C1	C2	C4	C5	C6	C6	C7	C7	C8	C8	C8	C8	C9	C10
	Min	-	C1	C1	C1	C2	C4	C5	C5	C6	C6	C7	C7	C7	C8	C8	C8
	Max	-	C1	C2	C3	C4	C5	C7	C8	C8	C9	C10	C10	C10	C11	C11	C12
Hatchery Crabs	Mode	C1	C2	C3	C4	C5	C6	C6	C6	C6	C7	C7	C8	C8	C9	C9	C9
	Min	C1	C1	C2	C3	C4	C5	C5	C5	C4	C5	C5	C6	C6	C6	C6	C6
	Max	C1	C2	C3	C2	C5	C6	C6	C7	C8	C9	C9	C10	C10	C11	C11	C11

## FIGURES

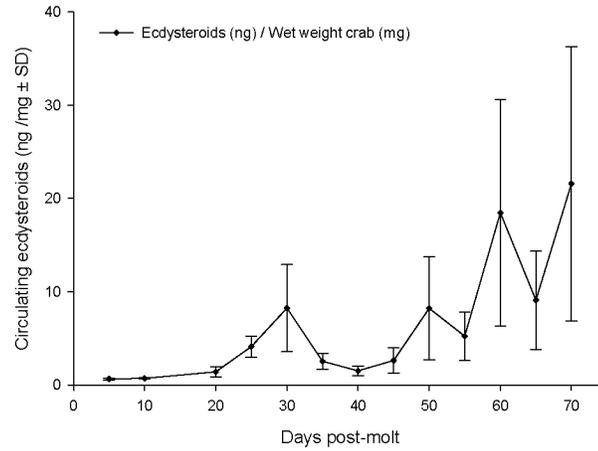


Figure 3.1. Ecdysteroids per wet weight crab (ng hemolymph/mg crab  $\pm$  SD) for homogenized juvenile *P. camtschaticus* (age-0). Three to five crabs were homogenized individually and ecdysteroid levels averaged for each point. Error bars represent standard deviation.