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EFFECTS OF TEMPERATURE ON GROWTH AND MOLTING IN BLUE CRABS (*CALLINECTES SAPIDUS*) AND LESSER BLUE CRABS (*CALLINECTES SIMILIS*)

by

Abigail Ann Kuhn

A Thesis
Submitted to the Graduate School,
the College of Science and Technology,
and the School of Ocean Science and Technology
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for the Degree of Master of Science

December 2017

EFFECTS OF TEMPERATURE ON GROWTH AND MOLTING IN BLUE CRABS (*CALLINECTES SAPIDUS*) AND LESSER BLUE CRABS (*CALLINECTES SIMILIS*)

by Abigail Ann Kuhn

December 2017

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ABSTRACT

EFFECTS OF TEMPERATURE ON GROWTH AND MOLTING IN BLUE CRABS (*CALLINECTES SAPIDUS*) AND LESSER BLUE CRABS (*CALLINECTES SIMILIS*)

by Abigail Ann Kuhn

December 2017

Temperature can exert impacts on many processes in ectotherms. With global temperatures rising due to climate change, many ectothermic species may exhibit changes in growth rates and size at maturity, and these changes can have population-level effects. Predicting responses of species to climate change will require not only knowledge of thermal tolerance limits, but also effects of temperature change on growth rates and other life history parameters. For arthropods that exhibit discontinuous growth (i.e., molting), this includes both intermolt period and growth per molt. Previous laboratory and field experiments suggest that temperature affects both intermolt period (IMP) and growth per molt (GPM) in many crustaceans, including blue crabs. Field surveys suggest that blue crabs reach maturity at larger sizes in cooler areas, and at smaller sizes in warm areas. In this study I investigate the effect of temperature on the growth process in blue crabs *Callinectes sapidus* and lesser blue crabs *Callinectes similis*, to examine differences in temperature sensitivity of growth rates across seasonal thermal regimes. Observed growth dynamics differed between species and were dependent on the time of collection and the temperature regime experienced by the crabs. Future research should examine the effect of temperature on growth in crabs from metamorphosis to maturity to determine if the response is consistent across all instars, and to directly assess effects of temperature on size at maturity.

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TABLE OF CONTENTS

ABSTRACT ii

ACKNOWLEDGMENTS iii

LIST OF TABLES v

LIST OF FIGURES vii

CHAPTER I - INTRODUCTION 1

CHAPTER II – MATERIALS AND METHODS 5

CHAPTER III - RESULTS 9

Callinectes similis 9

Callinectes sapidus 17

Growth under natural temperature fluctuations 17

Growth under constant experimental temperatures 24

CHAPTER IV – DISCUSSION 29

REFERENCES 34

LIST OF TABLES

Table 1 Sample sizes for each growth experiment 6

Table 2 Summary of mixed-model ANCOVA results, testing effects of increased temperature, instar, and the interaction of temperature and instar on IMP for C. similis crabs collected in February 2016 (a) and February 2017 (b). Significant P values are indicated in bold. 11

Table 3 Summary of mixed-model ANCOVA results, testing effects of increased temperature, premolt CW, and the interaction of temperature and premolt CW on GPM for C. similis crabs collected in February 2016 and February 2017. Significant P values are indicated in bold. 13

Table 4 Summary of mixed-model ANCOVA results, testing effects of increased temperature, instar, and the interaction of temperature and instar on postmolt CW for C. similis crabs collected in February 2016 and February 2017. Significant P values are indicated in bold. 15

Table 5 Summary of mixed-model ANCOVA results, testing effects of increased temperature, instar, and the interaction of temperature and instar on IMP for C. sapidus crabs collected in May 2016 and February 2017. Significant P values are indicated in bold. 19

Table 6 Summary of mixed-model ANCOVA results, testing effects of increased temperature, premolt CW, and the interaction of temperature and premolt CW on GPM for C. sapidus crabs collected in May 2016 and February 2017. Significant P values are indicated in bold. 21

Table 7 Summary of mixed-model ANCOVA results, testing effects of increased temperature, instar, and the interaction of temperature and instar on postmolt CW for C. sapidus crabs collected in May 2016 and February 2017. Significant P values are indicated in bold. 23
Table 8 Summary of mixed-model ANCOVA results, testing effects of increased temperature, premolt CW, and the interaction of temperature and premolt CW on IMP for C. sapidus crabs collected in July 2017. Significant P values are indicated in bold. 26
Table 9 Summary of mixed-model ANCOVA results, testing effects of increased temperature, premolt CW, and the interaction of temperature and premolt CW on GPM for C. sapidus crabs collected in July 2017. Significant P values are indicated in bold... 27
Table 10 Summary of mixed-model ANCOVA results, testing effects of increased temperature, instar, and the interaction of temperature and instar on postmolt CW for C. sapidus crabs collected in July 2017. Significant P values are indicated in bold. 28

LIST OF FIGURES

Figure 1. Seasonality of the temperatures experienced by crabs collected in February 2016, May 2016, and February 2017. Arrows indicate collection date. 6

Figure 2. Mean growth trajectories as a function of time since metamorphosis for each temperature treatment for *C. similis* crabs collected in February 2016 (a) and February 2017 (b). Line type indicates temperature treatment. Each data point represents a single postmolt CW measurement. 10

Figure 3. Mean (± SD) intermolt period at each juvenile instar for *C. similis* collected in February 2016 (a) and February 2017 (b) and reared at ambient temperatures (gray bars) and ambient + 5°C temperatures (white bars). n=40 crabs for February 2016 (a) and n=20 crabs for February 2017 (b). * indicates a significant difference between temperature treatments at $P < 0.05$. 12

Figure 4. Postmolt CW as a function of premolt CW for *C. similis* collected in February 2016 (a) and February 2017 (b) and reared at ambient temperatures (solid circles) and ambient + 5°C temperatures (open circles). Regression lines (ambient temperature treatment, solid line; ambient + 5°C, dashed line) indicate significant interaction effect of temperature and premolt CW on postmolt CW. 14

Figure 5. Mean (± SD) postmolt CW at each juvenile instar for *C. similis* collected in February 2016 (a) and February 2017 (b) and reared at ambient temperatures (gray bars) and ambient + 5°C temperatures (white bars). n=40 crabs for February 2016 (a) and n=20 crabs for February 2017 (b). * indicates a significant difference between temperature treatments at $P < 0.05$. 16

Figure 6. Mean growth trajectories as a function of time since metamorphosis for each temperature treatment for *C. sapidus* crabs collected in May 2016 (a) and February 2017 (b). Line type indicates temperature treatment (May 2016 shows the mean of both treatments, as there was no difference between treatments in IMP or GPM). Each data point represents a single postmolt CW measurement. 18

Figure 7. Mean (± SD) intermolt period at each juvenile instar for *C. sapidus* collected in May 2016 (a) and February 2017 (b) and reared at ambient temperatures (gray bars) and ambient + 5°C temperatures (white bars). 20

Figure 8. Postmolt CW as a function of premolt CW for *C. sapidus* crabs collected in May 2016 (a) and February 2017 (b). Regression lines indicate a significant effect of postmolt CW (mm) with increasing premolt CW. n=20 crabs in ambient treatment (solid circles), n=24 crabs in ambient + 5°C treatment (white circles). 22

Figure 9. Mean (± SD) postmolt CW at each juvenile instar for *C. sapidus* crabs collected in May 2016 (a) and February 2017 (b) and reared at ambient temperatures (gray bars) and ambient + 5°C temperatures (white bars). 24

Figure 10. Mean growth trajectories as a function of time since metamorphosis for each temperature treatment for *C. sapidus* crabs collected in July 2017. Line type and color indicates temperature. Each data point represents a single postmolt CW measurement. . 25

Figure 11. Intermolt period as a function of premolt CW for *C. sapidus* collected in July 2017 and reared at 20°C (solid circles), 25°C (gray circles) and 30°C (open circles). Regression lines (20°C, solid line; 25°C, dotted line; 30°C, dashed line) indicate significant interaction effect of temperature and premolt CW on IMP. 26

Figure 12. Postmolt CW as a function of premolt CW for *C. sapidus* crabs collected in July 2017 and reared at 20°C (solid circles), 25°C (gray circles) and 30°C (open circles). Regression lines (20°C, solid line; 25°C, dotted line; 30°C, dashed line) indicate larger postmolt CW at larger premolt CW. 27

Figure 13. Mean (± SD) postmolt CW at each juvenile instar for *C. sapidus* crabs reared at 20°C (black bars, n=34), 25°C (gray bars, n=31), and 30°C (white bars, n=35). Letters indicate significant differences based on post-hoc Tukey's HSD tests (levels connected by same letter are not significantly different). 28

CHAPTER I - INTRODUCTION

Global temperatures are changing and are projected to continue increasing (IPCC, 2014). Temperature can influence many life history characteristics, especially in ectotherms (Atkinson, 1994; Hartnoll, 1982), and growth rates in particular are quite sensitive to temperature variation. In general, organisms reared at a warmer temperature grow at a faster rate but ultimately reach a smaller body size (Atkinson, 1994). This trend (the Temperature-Size Rule) has been observed across taxa in animals, plants, bacteria, and protozoans (Angilletta and Dunham, 2003). Similarly, there is often a positive association between body size and latitude (i.e., James's Rule; Blackburn et al., 1999). With global temperatures rising (and substantial regional variation in rates of warming), species are likely to exhibit changes in growth rates and potentially size at maturity, and these changes can have population-level effects through altered predator-prey interactions (Araújo and Luoto, 2007; Sanford, 1999; Yang and Rudolf, 2010) and reproduction dynamics (Carson et al., 2010; Petes et al., 2008). Predicting species responses to climate change will require not only knowledge of thermal tolerance limits, but also effects of temperature change on growth rates and associated life history parameters. In commercially-harvested species, an understanding of thermal effects on growth rates will be necessary for successful stock assessment and management efforts, as effective fisheries management strategies rely on growth-related parameters including size at age and size at maturity (Froese et al., 2008).

Crustaceans exhibit discontinuous growth where growth occurs only in short periods associated with each molt as the animal sheds its exoskeleton. This pattern, combined with the absence of structures useful for determining age (e.g., otoliths, scales,

vertebrae), precludes accurate size at age determination in crustaceans and therefore traditional growth models such as the von Bertalanffy growth curve (von Bertalanffy 1938) cannot be used to estimate size at age. Instead, growth models for crustaceans include two parameters: (1) the change in size following a molt, known as the molt increment or growth per molt (GPM), and (2) the time interval between molts, called the intermolt period (IMP; Chang et al., 2012).

Studies on several crab species have shown both similar and contrasting patterns of thermal effects on intermolt period and growth per molt, respectively. Increased temperatures lead to shorter intermolt periods in many species including the red king crab *Paralithodes camtschaticus* (Stoner et al., 2010), the snow crab *Chionoectes opilio* (Yamamoto et al., 2015), and the Dungeness crab *Cancer magister* (Kondzela and Shirley, 1993). The effect of temperature on growth per molt, however, is variable among species (Hartnoll, 1982). Red king crabs exhibit a positive and linear relationship between growth per molt and temperature (Stoner et al., 2010), while blue king crabs show no relationship (Stoner et al., 2013). Yet other species show more complex relationships, with GPM initially increasing with increasing temperature before decreasing at higher temperatures (Anger, 1984). These varying relationships between temperature and growth per molt may be due to trade-offs between the increase in size at each molt and the amount of time between molts. The inconsistency of GPM responses to temperature underlies the importance of further investigations of the effect of temperature on growth, specifically over multiple molts and over a range of temperatures.

Even studies on the same species show consistent IMP responses and inconsistent GPM responses to temperatures. Field surveys suggest that blue crabs reach maturity at

larger sizes in cooler areas, and at smaller sizes in warm areas (Fisher, 1999; Darnell et al., 2009; Hines et al., 2010). Laboratory studies suggest that temperature strongly affects IMP and may also affect GPM in blue crabs. Previous laboratory experiments have found that IMP decreases at higher temperature (Brylawski and Miller, 2006; Leffler, 1972; Tagatz, 1968). Leffler (1972) and Cunningham and Darnell (2015) observed a decrease in GPM at warmer temperatures but Tagatz (1968) and Brylawski and Miller (2006) did not observe any effect of increased temperature on GPM.

Blue crabs, *Callinectes sapidus* Rathbun, are an ecologically and economically important species throughout their range, which spans from Nova Scotia to Argentina, including the Chesapeake Bay, Gulf of Mexico, and the Caribbean Sea. In 2015, blue crab landings in the United States totaled 159.6 million pounds for a dockside value of $237.6 million (NMFS, 2016). Stocks and landings of blue crabs have declined in recent years in areas with commercial fishing pressure, and decreases in size at maturity have been observed in some areas (Eggleston et al., 2004; Lipcius and Stockhausen, 2002). Decreases in size at maturity are especially concerning for female blue crabs because they undergo a terminal molt. The size at which female blue crabs reach maturity is the size they will remain throughout reproduction. Decreases in size at maturity could be the result of temperature shifts related to climate change, but additional research is needed to understand the factors that regulate growth and size at maturity in blue crabs. Temperature-driven shifts in growth could lead to changes in the age of entry into the fishery, reproductive output, size at maturity, and age at maturity.

Lesser blue crabs, *Callinectes similis* Williams, commonly co-occur with *C. sapidus* but do not support a commercial fishery due to their smaller size and offshore

distribution. Hsueh et al. (1993) reported the maximum size of *C. similis* to be two-thirds the maximum size of *C. sapidus*. While several studies have focused on the effects of increased temperature on growth in *C. sapidus*, the influence of temperature on growth in *C. similis* has not been investigated.

The goal of this study was to investigate the temperature-dependence of growth processes for blue crabs and lesser blue crabs. Specific objectives were to (1) determine effects of increased temperature on intermolt period, (2) examine the effects of increased temperature on growth per molt, and (3) determine if temperature affects size at a specific instar.

CHAPTER II – MATERIALS AND METHODS

Crabs for all experiments (*C. sapidus* and *C. similis*) were collected as megalopae using a 500-μm mesh plankton net deployed during nighttime flood tides in the Aransas Pass inlet in Port Aransas, Texas (27.83813° N, 97.05027° W), then transported to the Gulf Coast Research Laboratory in Ocean Springs, MS. Individual megalopae were placed into six-well plates with 15-mL of artificial seawater (~30 ppt) in each well. Well plates were then placed in temperature-controlled incubators under various temperature treatments. There were two types of temperature treatments: 1) natural, seasonal temperature fluctuations, and 2) constant, stable temperatures.

Two temperature treatments were used to examine growth rates under natural temperature fluctuations: ambient water temperature (relative to the collection site) and ambient + 5°C. Temperatures in the incubators were adjusted weekly to track current ambient water temperatures in Port Aransas, Texas, using data from the nearby NOAA/NOS/CO-OPS station 8775237 (2.27 km from the collection site) and/or National Data Buoy Center (NDBC) station PTAT2 (1.35 km from the collection site). The ambient + 5°C treatment was capped at a maximum of 35°C to avoid mortality at higher temperatures. Multiple collections occurred to examine differences in temperature sensitivity of growth rates across seasonal thermal regimes (Table 1, Figure 1). Three temperature treatments were used to examine growth rates under constant temperatures, using only *C. sapidus*: 20°C, 25°C, and 30°C. Crabs were collected on July 21, 2017 using the methods described above and reared at the constant experimental temperatures until the fifth instar.

Table 1 *Sample sizes for each growth experiment*

Experiment #	Collection Date	Ambient		Ambient +5°C		Target molt stage for conclusion of experiment
		C. sapidus	***C. similis***	***C. sapidus***	***C. similis***	
1	Feb. 20, 2016	–	40	–	40	12
2	May 8, 2016	20	–	20	–	10
3	Feb. 25, 2017	10	20	10	20	5

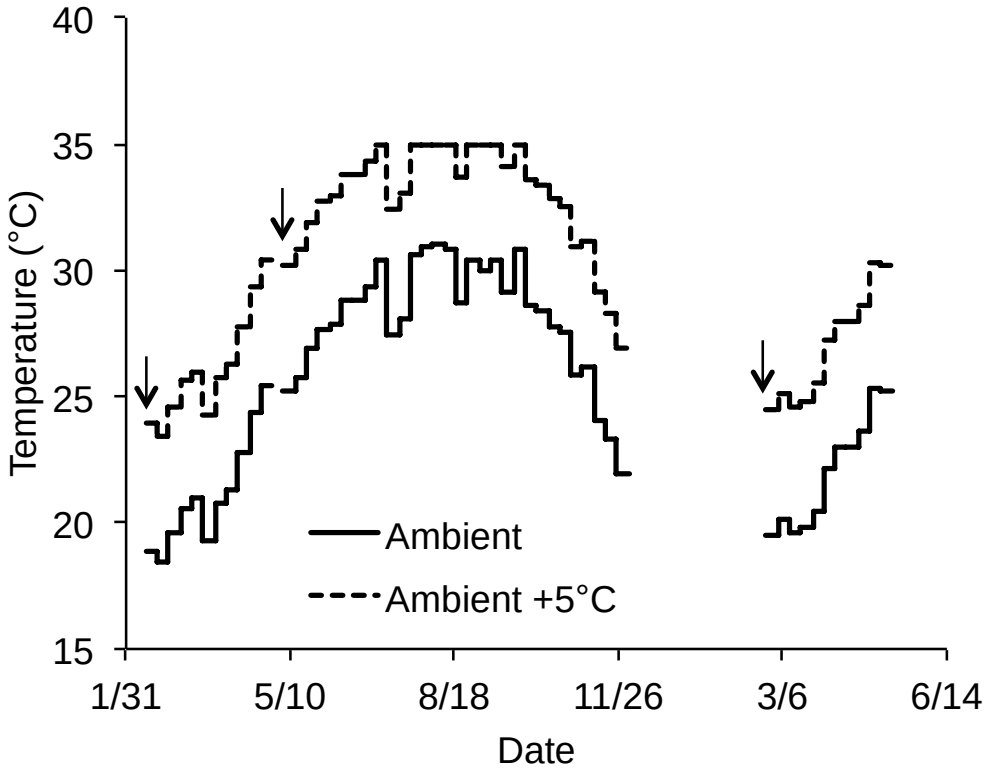

Figure 1. Seasonality of the temperatures experienced by crabs collected in February 2016, May 2016, and February 2017. Arrows indicate collection date.

Incubators were checked daily to ensure water temperature was consistent within treatments during each experiment. The light cycles in the incubators were adjusted weekly based on the timing of sunrise and sunset at the collection site (to the nearest five

minutes). After metamorphosis from megalopa to the first juvenile instar, the crabs were checked daily for molting and fed an appropriate food type based on their size. Carapace width in millimeters was measured following each molt. The crabs were fed *ad libitum* to minimize any effects of possible starvation on growth. For the first six instars, the crabs were fed a mixture of *Artemia* spp. nauplii one- and two-day post-hatch (~50/50 mix). The two-day post-hatch *Artemia* was enriched with algae paste (*Isochrysis* spp.) to supplement the low nutritional value of the nauplii. From the seventh instar to the twelfth instar, crabs were weaned off *Artemia* and offered initially two sizes of commercial shrimp pellets (Ziegler shrimp diet) and later small pieces of frozen shrimp. When molting occurred, the old exoskeleton was removed and carapace width measured. The carapace width of each crab was measured within one day after each molt and the crabs were measured with a stereoscopic microscope with an ocular micrometer, digital calipers, or a ruler throughout the course of the experiment, depending on crab size.

Crabs were moved to larger containers as they increased in size to ensure growth was not limited by the size of the container. Once the carapace width of the crab reached one-third of the diameter of the container, the crab was placed in a larger container. Crabs were moved from well plates (15.5 mL capacity, 3.48 cm diameter) to 237 mL jars (6.03 cm diameter) to one gallon (16.2 cm diameter) and two gallon (20.6 cm diameter) buckets as needed. Water in the containers was changed daily in order to prevent death due to buildup of waste products.

Data were analyzed primarily using linear mixed effects models, with individual crab ID as a random effect (to account for repeated measurements on individual crabs) using JMP v. 13.0.0 (SAS Institute, Inc., Cary, NC). *Callinectes sapidus* and *C. similis*

were analyzed separately and each experiment was analyzed independently since megalopae were collected during different seasons. The timing of molting was analyzed using a linear mixed-effects model with IMP (calculated as the length of time in days between one molt and the subsequent molt) as the response variable with temperature treatment, instar, and the treatment × instar interaction as fixed effects, and crab ID as a random effect. Growth per molt was analyzed using postmolt CW as the response variable with temperature treatment, premolt CW, and the treatment × premolt CW interaction as fixed effects, and crab ID as a random effect. Size-at-stage was analyzed using a linear mixed-effects model with postmolt carapace width (CW) as the response variable, with temperature treatment, instar, and the treatment × instar interaction as fixed effects, and crab ID as a random effect.

Experiments conducted under naturally fluctuating temperatures (Table 1) were analyzed using the methods described above. For the experiment conducted under constant temperatures, size-at-stage and growth per molt were analyzed as above. The timing of molting was analyzed using a linear mixed-effects model with IMP as the response variable with temperature treatment, premolt CW, and the treatment × premolt CW interaction as fixed effects, and crab ID as a random effect.

CHAPTER III - RESULTS

Callinectes similis

Growth trajectories for both experiments differed between the two temperature treatments due to differences in both IMP and GPM (Figure 2). *C. similis* collected in February 2016 were reared to the J12 instar and *C. similis* collected in February 2017 were reared to the J5 instar. 18 individuals survived to the 12th juvenile instar, and reached J12 after 179.8 ± 22.13 days (mean ± SD) when raised under ambient temperatures and 12 individuals survived to the 12th juvenile instar after 133.9 ± 15.70 days when reared under ambient +5°C temperatures in the February 2016 experiment (Figure 2a). 19 individuals survived to the 5th juvenile instar, and reached J5 after 39.58 ± 2.292 days (mean ± SD) when raised under ambient temperatures and all 20 individuals survived to the 5th juvenile instar after 30.9 ± 4.19 days when reared under ambient +5°C temperatures in the February 2017 experiment (Figure 2b).

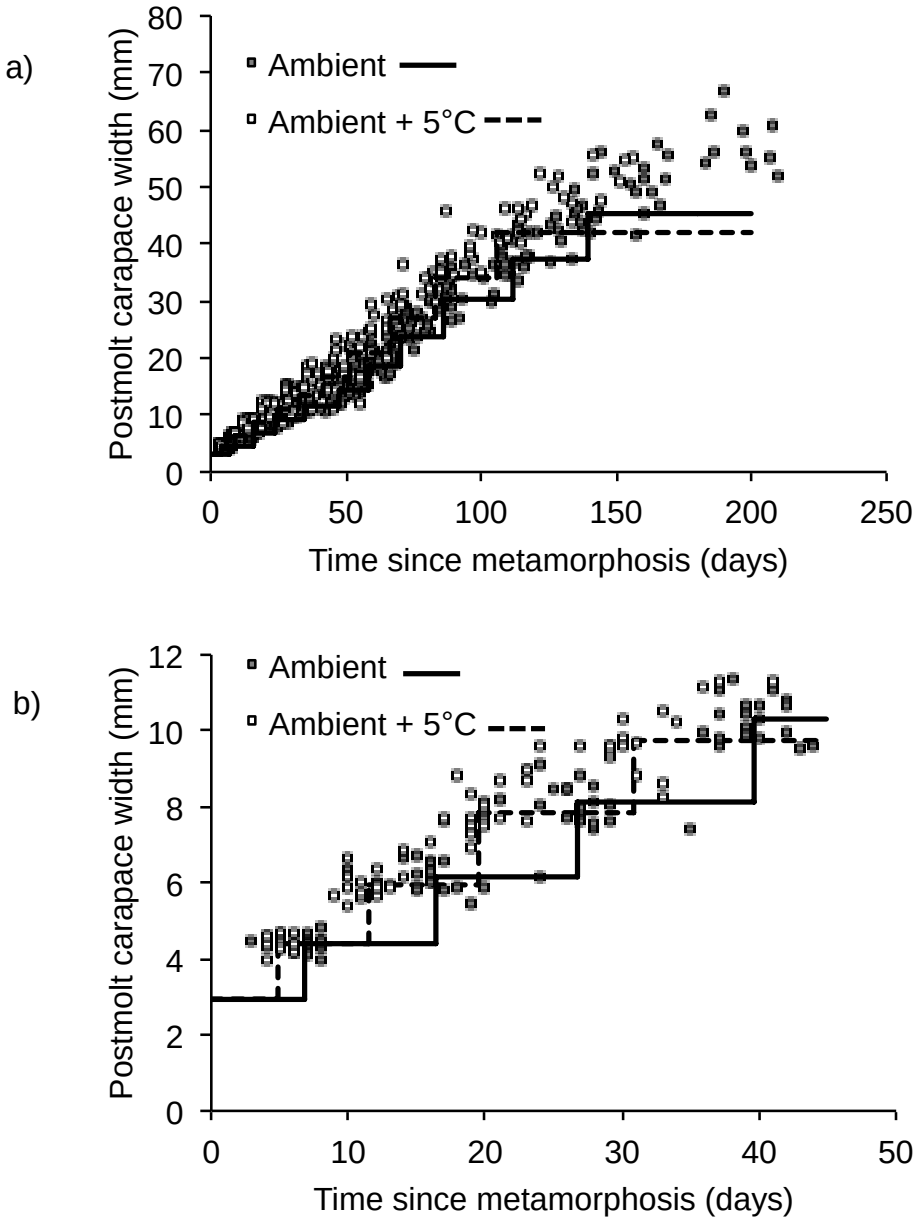

Figure 2. Mean growth trajectories as a function of time since metamorphosis for each temperature treatment for *C. similis* crabs collected in February 2016 (a) and February 2017 (b). Line type indicates temperature treatment. Each data point represents a single postmolt CW measurement.

Intermolt period for *C. similis* collected in February 2016 ranged from 3–42 days. Temperature, instar number, and the temperature × instar interaction all had a significant effect on IMP (Table 2, Figure 3a). In general, IMP increased with each subsequent molt

and was longer for crabs reared at ambient temperatures than for crabs reared at ambient + 5°C temperatures. There was also a significant temperature × instar interaction, as the difference between the two temperature treatments was not constant across instars. Yet, in all instars where IMP differed between the temperature treatments, IMP was always greater in the ambient treatment than the ambient +5°C treatment. Intermolt period for the February 2017 *C. similis* experiment ranged from 4–13 days, with a significant effect of temperature treatment and instar (Figure 3b); IMP was significantly longer for crabs reared at ambient temperatures than for crabs reared at ambient + 5°C temperatures.

Table 2 *Summary of mixed-model ANCOVA results, testing effects of increased temperature, instar, and the interaction of temperature and instar on IMP for C. similis crabs collected in February 2016 (a) and February 2017 (b). Significant P values are indicated in bold.*

	a) February 2016			b) February 2017		
Source	DF	*F*	*P*	DF	*F*	*P*
Temperature	1, 95.29	117.20	**<0.0001**	1, 35.7	61.79	**<0.0001**
Instar	10, 569.3	248.26	**<0.0001**	3, 111	101.7	**<0.0001**
Temperature × Instar	10, 569.3	8.3621	**<0.0001**	3, 111	0.3183	0.8122

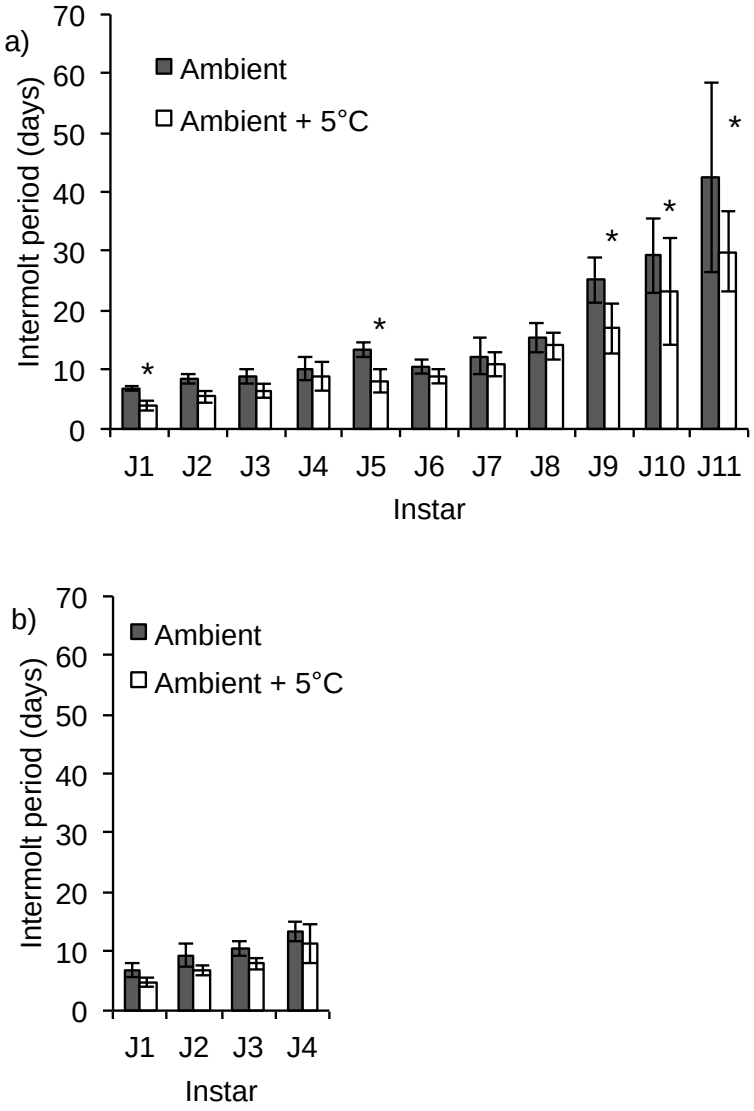

Figure 3. Mean (± SD) intermolt period at each juvenile instar for *C. similis* collected in February 2016 (a) and February 2017 (b) and reared at ambient temperatures (gray bars) and ambient + 5°C temperatures (white bars). n=40 crabs for February 2016 (a) and n=20 crabs for February 2017 (b). * indicates a significant difference between temperature treatments at $P < 0.05$.

Postmolt CW was strongly related to premolt CW, and was also dependent on temperature treatment (Table 3), with greater postmolt CW (relative to premolt CW; i.e., greater growth per molt) in the ambient treatment for crabs in the February 2016 experiment. The significant temperature × premolt CW interaction indicates a difference

in the slopes of the postmolt CW vs. premolt CW regressions (Figure 4a). Postmolt CW relative to premolt CW is larger for crabs in the ambient temperature treatment. Postmolt CW was strongly related to premolt CW, and was also dependent on temperature treatment for crabs in the February 2017 experiment as well. Postmolt CW increased with increasing premolt CW but the non-significant temperature × premolt CW interaction indicates no difference in the slopes of the postmolt CW vs. premolt CW regressions (Figure 4b).

Table 3 *Summary of mixed-model ANCOVA results, testing effects of increased temperature, premolt CW, and the interaction of temperature and premolt CW on GPM for C. similis crabs collected in February 2016 and February 2017. Significant P values are indicated in bold.*

	a) February 2016			b) February 2017		
Source	DF	*F*	*P*	DF	*F*	*P*
Temperature	1, 77.89	9.7674	**0.0025**	1, 36.4	5.0010	**0.0314**
Premolt CW	1, 616.8	86906	**<0.0001**	1, 118	11640	**<0.0001**
Temperature × Premolt CW	1, 616.8	4.6517	**0.0314**	1, 118	0.3295	0.5670

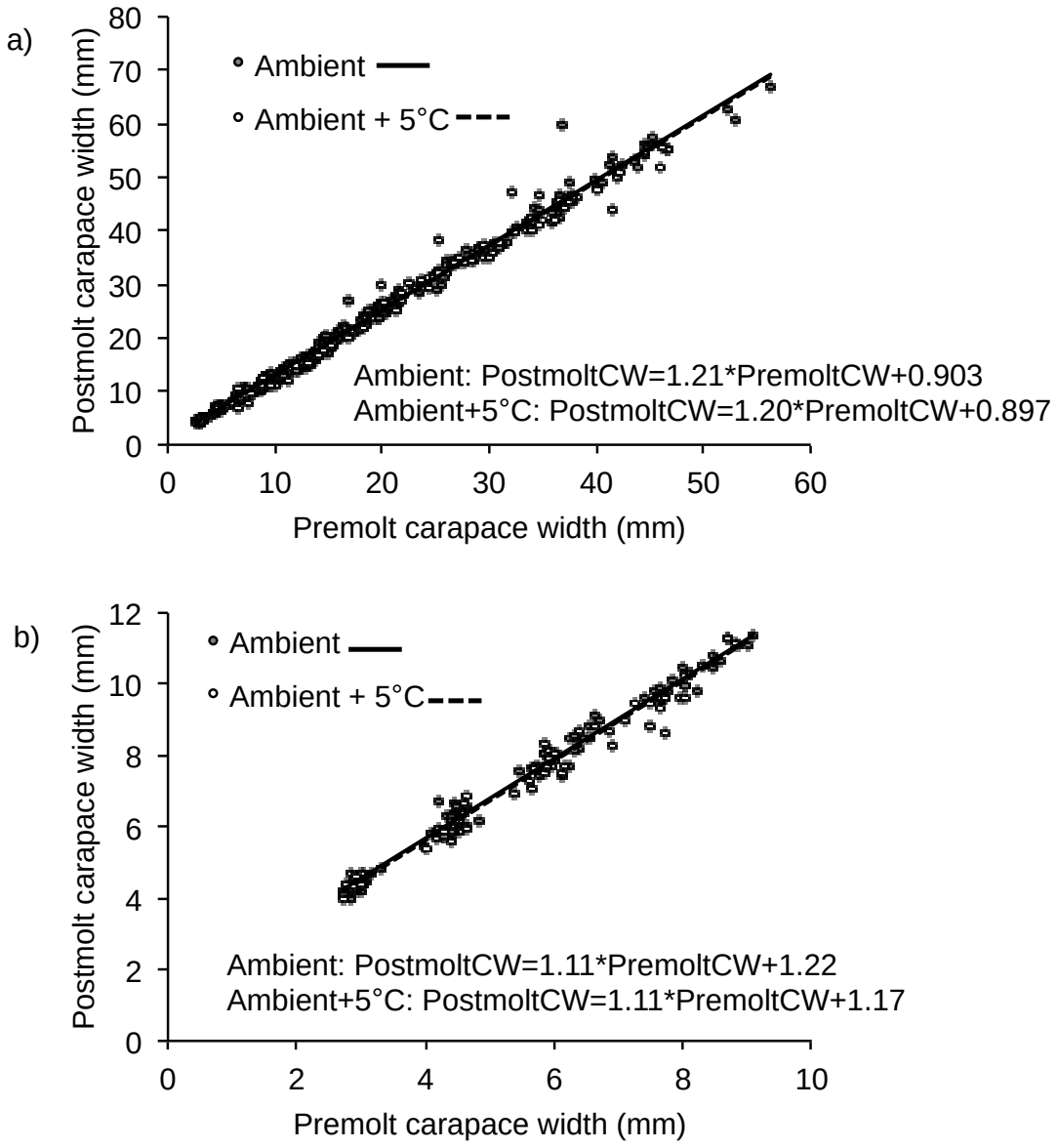

Figure 4. Postmolt CW as a function of premolt CW for *C. similis* collected in February 2016 (a) and February 2017 (b) and reared at ambient temperatures (solid circles) and ambient + 5°C temperatures (open circles). Regression lines (ambient temperature treatment, solid line; ambient + 5°C, dashed line) indicate significant interaction effect of temperature and premolt CW on postmolt CW.

The negative response of growth per molt to increased temperature resulted in crabs reaching each instar at a smaller size when reared at the warmer ambient + 5°C temperatures compared to crabs reared at ambient temperatures in the February 2016

experiment. Significant differences in postmolt CW between the two temperature treatments were observed in instars J8–J12 (Table 4, Figure 5a). Postmolt CW was not significantly different between the temperature treatments during the first seven instars, although there was a consistent trend of larger size in the ambient treatment. The increasing difference in mean postmolt CW between the two temperature treatments as the crabs progressed through the juvenile instars is reflected in the significant temperature × instar interaction. There was a marginally-significant difference ($P = 0.0515$) in size at age between the temperature treatments for crabs in the February 2017 experiment (Table 4, Figure 5b), with a trend of larger size in the ambient treatment. The increasing difference in mean postmolt CW between the two temperature treatments as the crabs progressed through the juvenile instars is reflected in the significant temperature × instar interaction.

Table 4 *Summary of mixed-model ANCOVA results, testing effects of increased temperature, instar, and the interaction of temperature and instar on postmolt CW for C. similis crabs collected in February 2016 and February 2017. Significant P values are indicated in bold.*

	a) February 2016			b) February 2017		
Source	DF	*F*	*P*	DF	*F*	*P*
Temperature	1, 92.0	34.780	**<0.0001**	1, 38.08	4.0437	0.0515
Instar	11, 628	3228.4	**<0.0001**	4, 151.2	3152.8	**<0.0001**
Temperature × Instar	11, 628	9.4582	**<0.0001**	4, 151.2	5.7183	**0.0003**

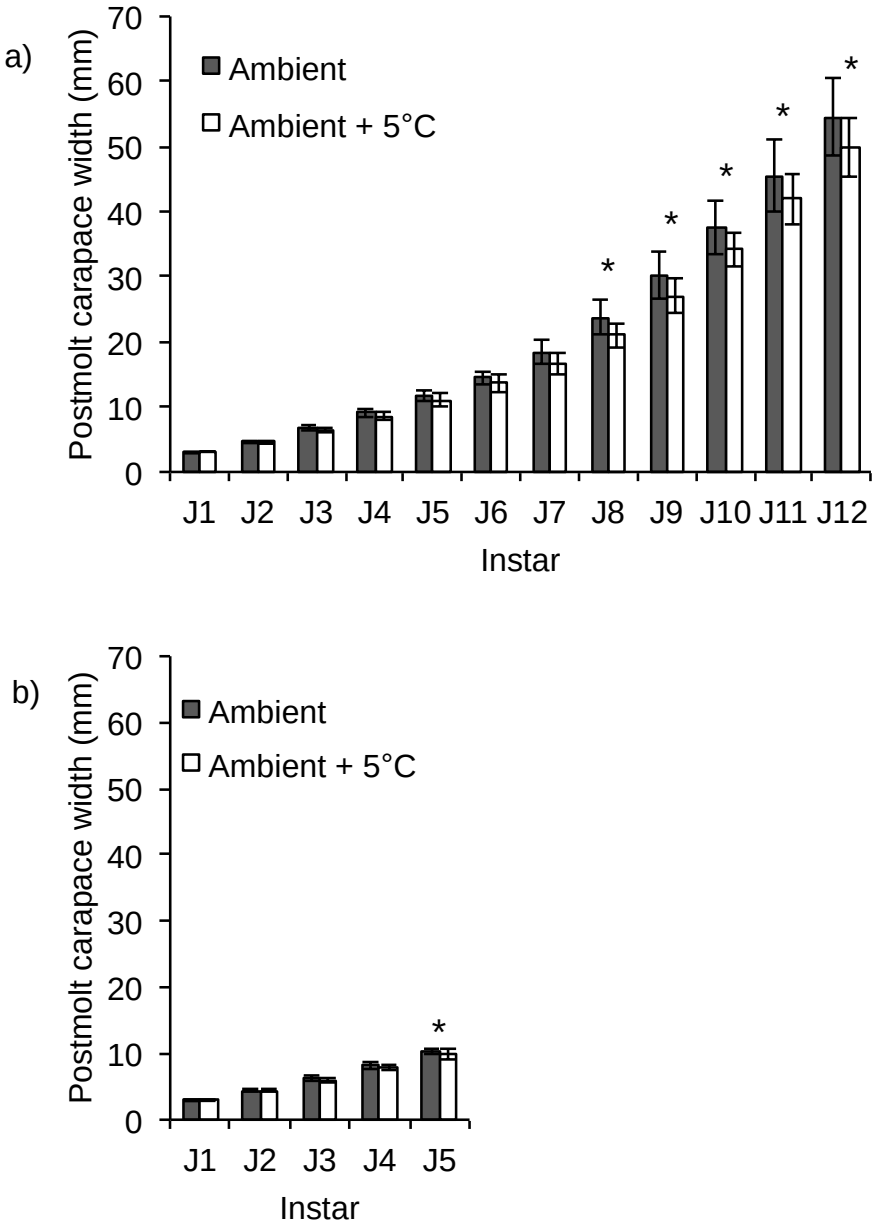

Figure 5. Mean (± SD) postmolt CW at each juvenile instar for *C. similis* collected in February 2016 (a) and February 2017 (b) and reared at ambient temperatures (gray bars) and ambient + 5°C temperatures (white bars). n=40 crabs for February 2016 (a) and n=20 crabs for February 2017 (b). * indicates a significant difference between temperature treatments at $P < 0.05$.

Callinectes sapidus

Growth under natural temperature fluctuations

Increased temperatures did not significantly alter IMP or GPM for crabs collected in May 2016 (Figure 6a) but increased temperatures significantly shortened IMP for crabs reared in ambient +5°C temperatures in the February 2017 experiment (Figure 6b). *C. sapidus* collected in February 2017 were reared to the J5 instar. All ten individuals survived to the 5th juvenile instar, and reached J5 after 42.1 ± 5.20 days (mean ± SD) when raised under ambient temperatures and all ten individuals survived to the 5th juvenile instar after 29.3 ± 2.63 days when reared under ambient +5°C temperatures.

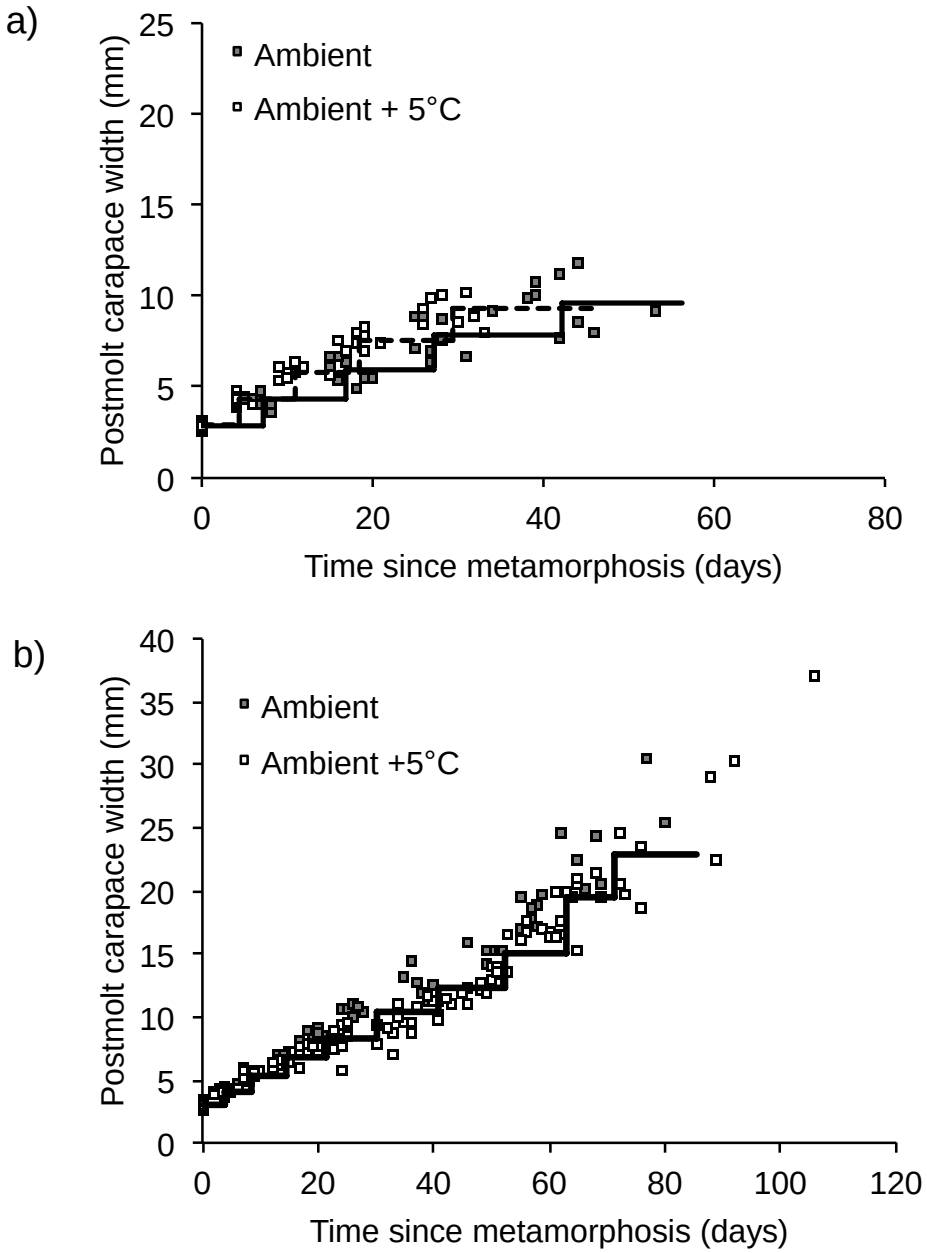

Figure 6. Mean growth trajectories as a function of time since metamorphosis for each temperature treatment for *C. sapidus* crabs collected in May 2016 (a) and February 2017 (b). Line type indicates temperature treatment (May 2016 shows the mean of both treatments, as there was no difference between treatments in IMP or GPM). Each data point represents a single postmolt CW measurement.

The effect of increased temperature on IMP for *C. sapidus* crabs reared under natural temperature fluctuations differed between the two collections (Table 5). Intermolt period for the May 2016 experiment ranged from 2–35 days, with no significant effect of

temperature treatment (Figure 7a). Intermolt period for the February 2017 experiment ranged from 4–25 days, with a significant effect of temperature treatment (Figure 7b); IMP was significantly longer for crabs reared at ambient temperatures than for crabs reared at ambient + 5°C temperatures.

Table 5 *Summary of mixed-model ANCOVA results, testing effects of increased temperature, instar, and the interaction of temperature and instar on IMP for C. sapidus crabs collected in May 2016 and February 2017. Significant P values are indicated in bold.*

	a) May 2016			b) February 2017		
Source	DF	*F*	*P*	DF	*F*	*P*
Temperature	1, 18.86	0.6386	0.4342	1, 18	48.35	**<0.0001**
Instar	8, 149.1	9.764	**<0.0001**	3, 54	45.33	**<0.0001**
Temperature × Instar	8, 149.1	0.7642	0.6348	3, 54	0.5094	0.6775

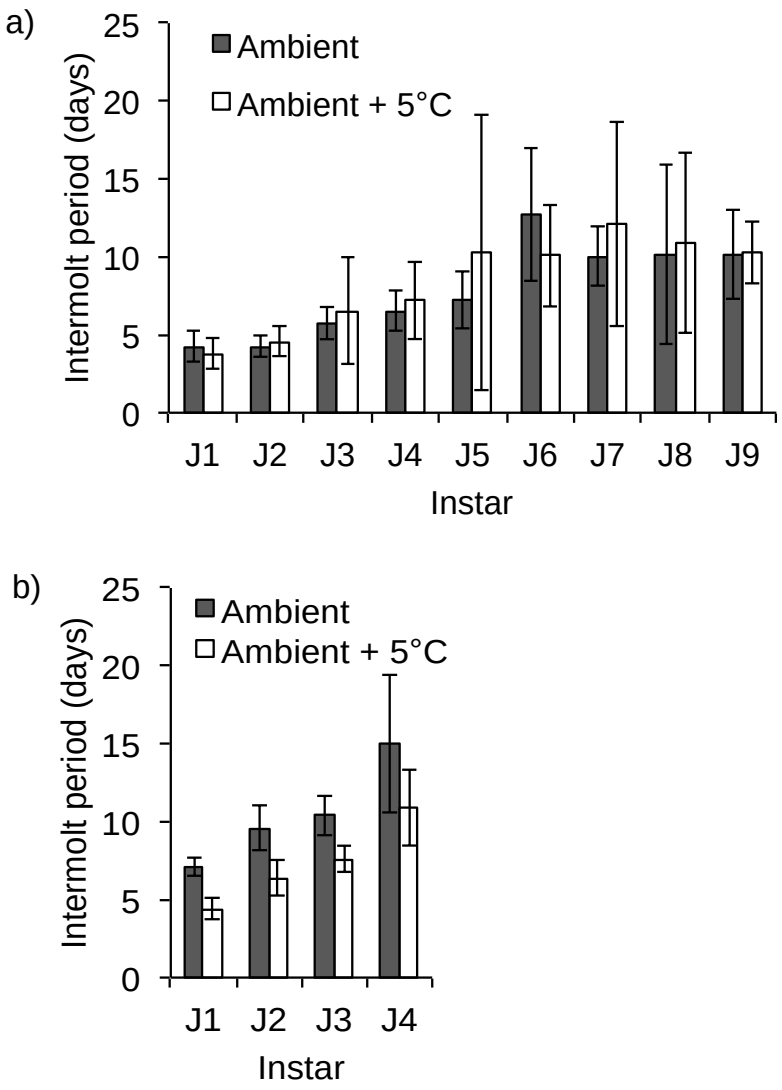

Figure 7. Mean (± SD) intermolt period at each juvenile instar for *C. sapidus* collected in May 2016 (a) and February 2017 (b) and reared at ambient temperatures (gray bars) and ambient + 5°C temperatures (white bars).

In both experiments under natural temperature fluctuations (Table 6), there was no effect of increased temperature on growth per molt. Although there was a strong effect of premolt CW on postmolt CW, there was no effect of temperature treatment or temperature × premolt CW interaction. Growth per molt based on postmolt CW was dependent only on premolt CW, and thus postmolt CW significantly increased with increasing premolt CW (Figure 8).

Table 6 *Summary of mixed-model ANCOVA results, testing effects of increased temperature, premolt CW, and the interaction of temperature and premolt CW on GPM for C. sapidus crabs collected in May 2016 and February 2017. Significant P values are indicated in bold.*

	a) May 2016			b) February 2017		
Source	DF	*F*	*P*	DF	*F*	*P*
Temperature	1, 19.63	0.1292	0.7231	1, 17.54	1.4083	0.2512
Premolt CW	1, 177.8	5805	**<0.0001**	1, 54.24	4866.7	**<0.0001**
Temperature × Premolt CW	1, 177.8	0.02270	0.8803	1, 54.24	1.7665	0.1889

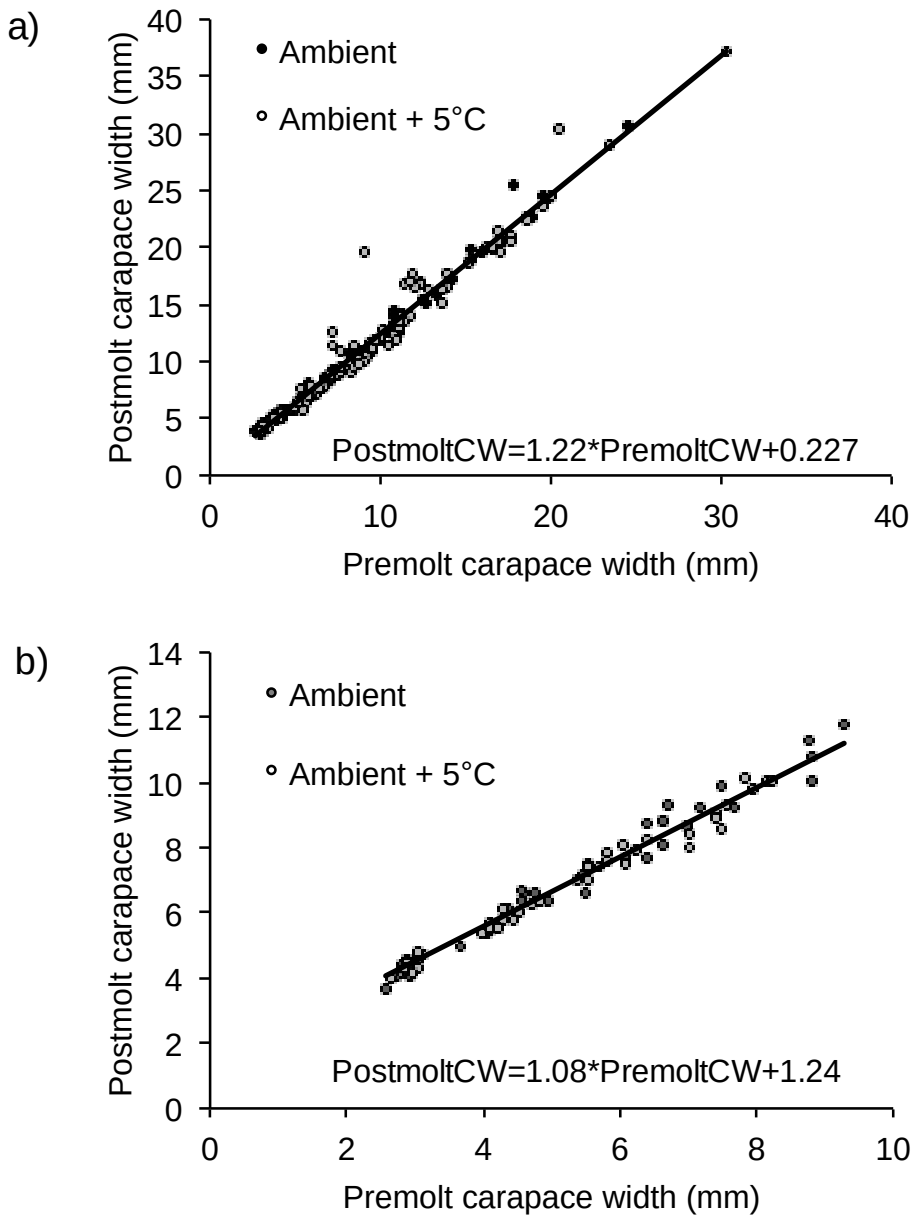

Figure 8. Postmolt CW as a function of premolt CW for *C. sapidus* crabs collected in May 2016 (a) and February 2017 (b). Regression lines indicate a significant effect of postmolt CW (mm) with increasing premolt CW. n=20 crabs in ambient treatment (solid circles), n=24 crabs in ambient + 5°C treatment (white circles).

The lack of an effect of temperature on GPM and postmolt CW resulted in similar CWs between the two treatments at each stage. Although carapace width increased at each instar, there was no significant effect of temperature treatment (Table 7, Figure 9).

Table 7 *Summary of mixed-model ANCOVA results, testing effects of increased temperature, instar, and the interaction of temperature and instar on postmolt CW for C. sapidus crabs collected in May 2016 and February 2017. Significant P values are indicated in bold.*

	a) May 2016			b) February 2017		
Source	DF	*F*	*P*	DF	*F*	*P*
Temperature	1, 22.24	2.283	0.1449	1, 18	0.2077	0.6540
Instar	9, 171.1	215.8	**<0.0001**	4, 72	732.3	**<0.0001**
Temperature × Instar	9, 171.1	0.4675	0.8948	4, 72	1.082	0.3719

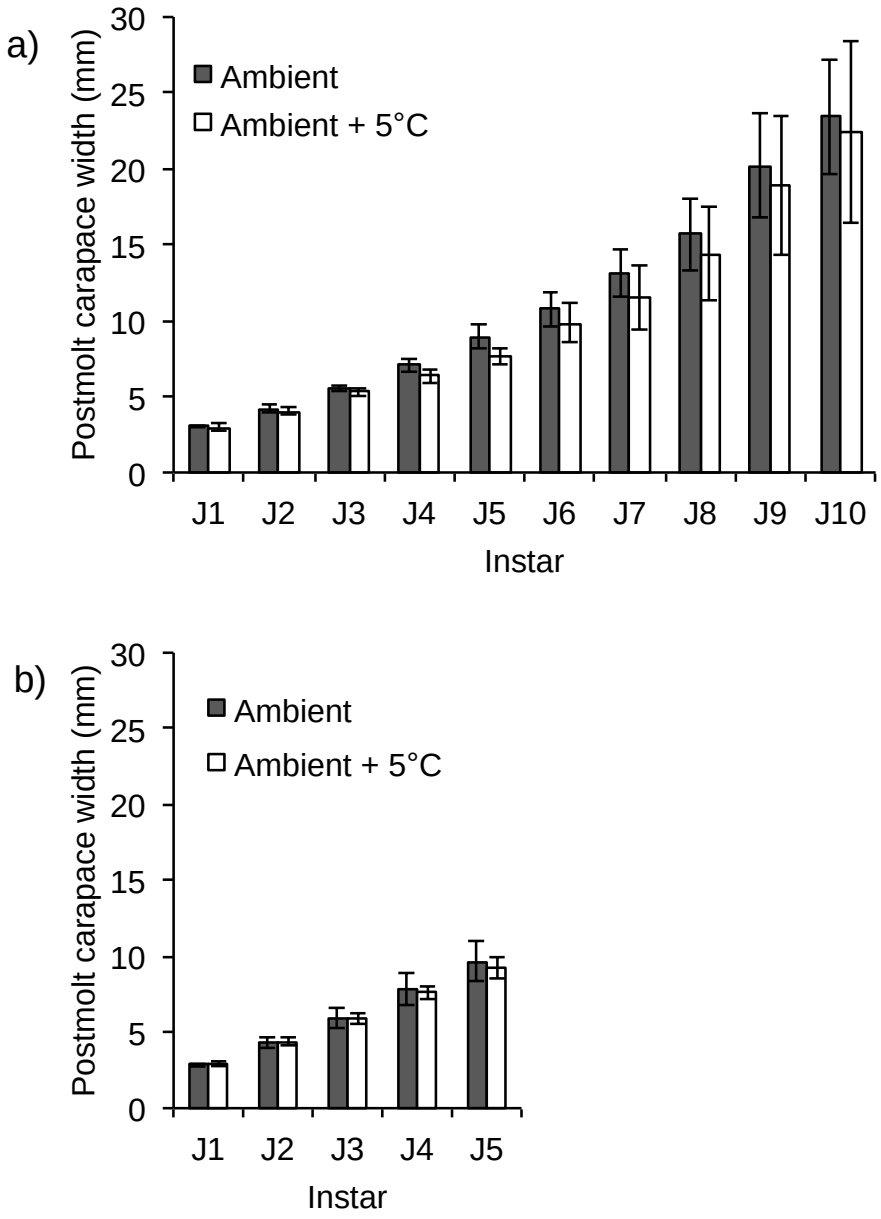

Figure 9. Mean (± SD) postmolt CW at each juvenile instar for *C. sapidus* crabs collected in May 2016 (a) and February 2017 (b) and reared at ambient temperatures (gray bars) and ambient + 5°C temperatures (white bars).

Growth under constant experimental temperatures

Growth trajectories differed between the three temperature treatments due to differences in both IMP and GPM (Figure 10). *C. sapidus* collected in July 2017 were

reared to the J5 instar. Crabs reared at 20°C (n=34) reached J5 at an average of 38.85 ± 2.11 days (mean ± SD) and crabs reared at 25°C (n=31) reached J5 at an average of 26.6 ± 2.22 days, while crabs reared at 30°C (n=35) reached J5 at an average of 24.71 ± 2.76 days.

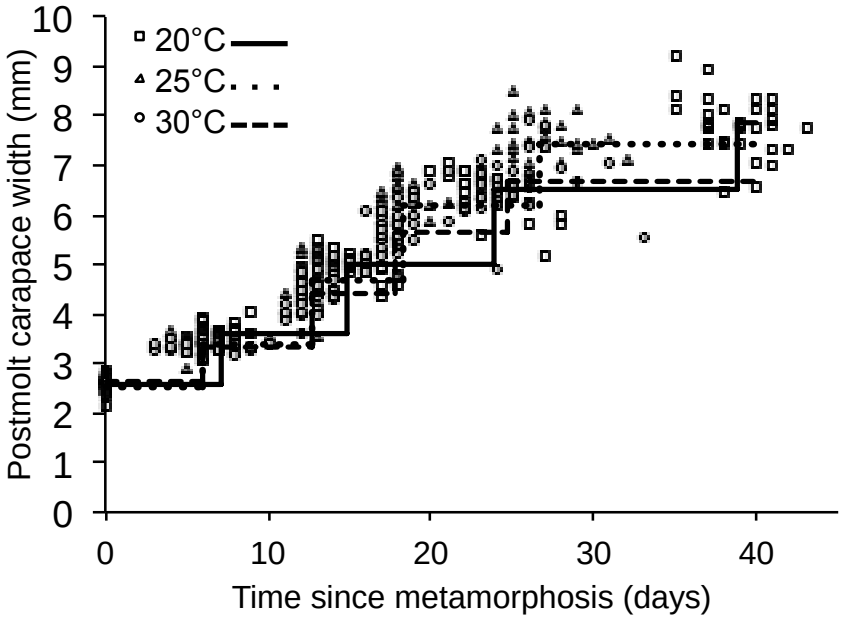

Figure 10. Mean growth trajectories as a function of time since metamorphosis for each temperature treatment for *C. sapidus* crabs collected in July 2017. Line type and color indicates temperature. Each data point represents a single postmolt CW measurement.

Temperature, premolt CW, and the temperature × premolt CW interaction all had a significant effect on IMP (Table 8). In general, IMP increased with increasing premolt CW and was longer for crabs reared at 20°C than for crabs reared at 25°C or 30°C. The significant temperature × premolt CW interaction indicates a difference in the slopes of the IMP vs. premolt CW regressions. As temperature increased, the effect of premolt CW on IMP was reduced (i.e., decreasing slope with increasing temperature in Figure 11). This resulted in a greater effect of temperature on IMP at larger sizes.

Table 8 *Summary of mixed-model ANCOVA results, testing effects of increased temperature, premolt CW, and the interaction of temperature and premolt CW on IMP for C. sapidus crabs collected in July 2017. Significant P values are indicated in bold.*

Source	DF	*F*	*P*
Temperature	2, 90.93	194.7942	**<0.0001**
Premolt CW	1, 324.7	147.9610	**<0.0001**
Temperature × Premolt CW	2, 324.5	50.5502	**<0.0001**

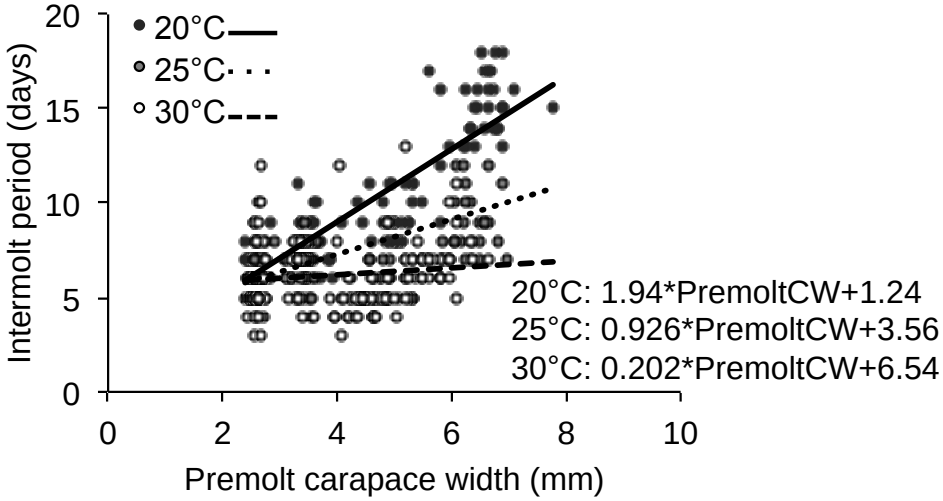

Figure 11. Intermolt period as a function of premolt CW for *C. sapidus* collected in July 2017 and reared at 20°C (solid circles), 25°C (gray circles) and 30°C (open circles). Regression lines (20°C, solid line; 25°C, dotted line; 30°C, dashed line) indicate significant interaction effect of temperature and premolt CW on IMP.

Postmolt CW was strongly related to premolt CW, and was also dependent on temperature treatment (Table 9), with greater postmolt CW (relative to premolt CW; i.e., greater GPM) in the 20°C treatment (Figure 12).

Table 9 *Summary of mixed-model ANCOVA results, testing effects of increased temperature, premolt CW, and the interaction of temperature and premolt CW on GPM for C. sapidus crabs collected in July 2017. Significant P values are indicated in bold.*

Source	DF	*F*	*P*
Temperature	2, 100.1	59.9134	**<0.0001**
Premolt CW	1, 320.3	197.9192	**<0.0001**
Temperature × Premolt CW	2, 320.3	2.6028	0.0756

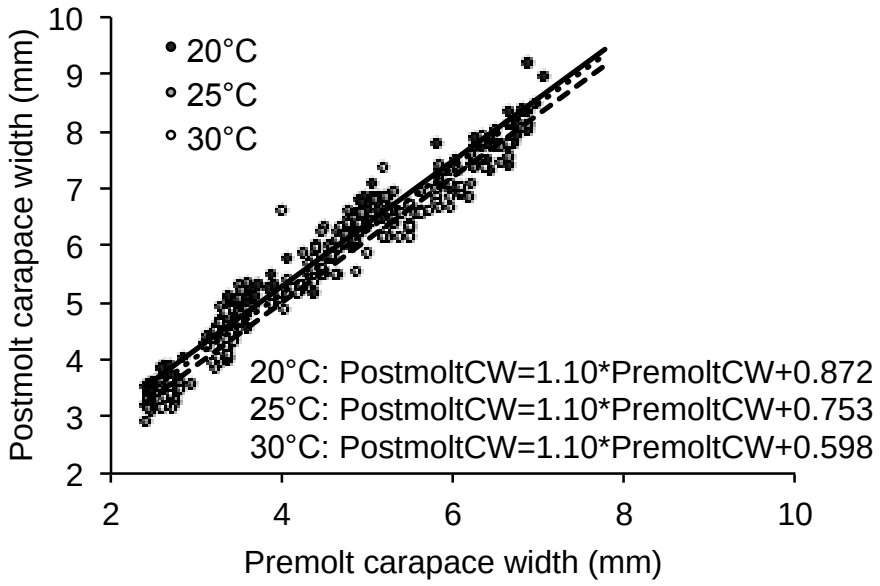

Figure 12. Postmolt CW as a function of premolt CW for *C. sapidus* crabs collected in July 2017 and reared at 20°C (solid circles), 25°C (gray circles) and 30°C (open circles). Regression lines (20°C, solid line; 25°C, dotted line; 30°C, dashed line) indicate larger postmolt CW at larger premolt CW.

The negative effect of increased temperature on GPM resulted in crabs reaching a smaller size at each stage when reared at 30°C compared to crabs reared at 20°C and 25°C (Table 10). There were significant differences in postmolt CW between the two temperature treatments in instars J3–J5 (Figure 13). Postmolt CW was not significantly

different between the temperature treatments at the first or second instars, although there was a consistent trend of larger size in the 20°C treatment. The increasing difference in mean postmolt CW between the two temperature treatments as the crabs progressed through the juvenile instars is reflected in the significant temperature × instar interaction.

Table 10 *Summary of mixed-model ANCOVA results, testing effects of increased temperature, instar, and the interaction of temperature and instar on postmolt CW for C. sapidus crabs collected in July 2017. Significant P values are indicated in bold.*

Source	DF	*F*	*P*
Temperature	2, 84.55	28.2355	**<0.0001**
Instar	4, 373.9	5177.811	**<0.0001**
Temperature × Instar	8, 373.9	31.9543	**<0.0001**

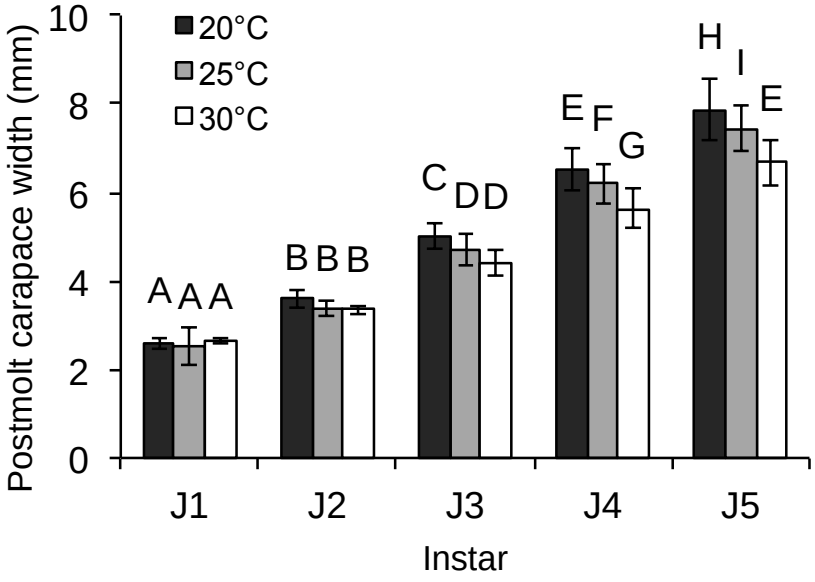

Figure 13. Mean (± SD) postmolt CW at each juvenile instar for *C. sapidus* crabs reared at 20°C (black bars, n=34), 25°C (gray bars, n=31), and 30°C (white bars, n=35). Letters indicate significant differences based on post-hoc Tukey's HSD tests (levels connected by same letter are not significantly different).

CHAPTER IV – DISCUSSION

This study investigated the effect of temperature on the growth process in blue crabs *Callinectes sapidus* and lesser blue crabs *Callinectes similis*. Results differed between species and were also dependent on the timing of collection and the temperature regime experienced by the crabs.

Both *C. similis* and *C. sapidus* both exhibited a shorter IMP under increased temperatures, consistent with previous studies on *C. sapidus* (Brylawski and Miller, 2006; Cadman and Weinstein, 1988; Cunningham and Darnell, 2015; Leffler, 1972; Tagatz, 1968) and other brachyuran crabs (Anger, 1984; Kondzela et al., 2015). Increased temperature almost universally increases the rates of physiological processes (Gillooly et al., 2001), including those associated with molting, thus shortening the period between molts. In experiments rearing crabs under natural temperature fluctuations, the warmer ambient +5°C treatment only had a significant effect on IMP for *C. sapidus* crabs collected in February 2017, with no effect of increased temperature on IMP for *C. sapidus* crabs collected in May 2016. This is likely the result of the non-linearity of temperature dependence of most physiological processes. Typically, the rate of physiological processes increases with increasing temperature up to some optimum temperature, before dropping off rapidly as the organism approaches its upper thermal limit (Huey and Kingsolver, 1989; Huey and Stevenson, 1979). We would thus expect IMP to decrease with increasing temperature up to a maximum before increasing at higher temperatures due to thermal stress. It is possible that, during the May 2016 experiment, our ambient and ambient +5°C treatments straddled the optimum temperature (i.e., where IMP would be at a minimum), resulting in similar IMPs in the

two treatments. Further experiments across a range of temperatures to clarify the form of the temperature-IMP relationship are needed to fully test this hypothesis.

Intermolt period generally increased with increasing premolt size. As crabs grew, the molts became less frequent. This was observed in all experiments, with IMPs as short as 2–3 d in the early juvenile (J1–J3) stages, or as long as 35 d in the later juvenile stages (>J10). Interestingly, when *C. sapidus* was reared under constant experimental temperatures, the effect of premolt size on IMP was reduced as temperatures were increased (Figure 9). At the highest temperature tested (30°C), IMP was constant across the range of sizes tested (J1–J5, 2.39–7.77 mm CW).

Effects of increased temperature on growth per molt (GPM) were less consistent. *C. similis* collected in February 2016 and February 2017 showed a significant decrease in GPM at warmer temperatures while *C. sapidus* collected at the same time showed no effect of temperature on GPM. This suggests that *C. similis* is more sensitive to increases in temperature than *C. sapidus*, which is consistent with their offshore distribution and thus more stable thermal regime relative to the estuarine distribution of *C. sapidus*. A negative effect of increased temperature on GPM was only observed in *C. sapidus* when held under constant experimental temperature treatments; *C. sapidus* reared under natural temperature fluctuations showed no effect of temperature on GPM, which may be a result of increased variation in GPM due to varying temperatures, as crabs were molting to each stage at different temperatures, even within a single treatment. When reared under constant experimental temperatures, GPM decreased as temperature increased. Cunningham and Darnell (2015) and Leffler (1972) also observed decreasing GPM with increasing temperature in *C. sapidus*. This pattern, and the resulting effects on body size

at each stage, is consistent with the Temperature-Size Rule (Atkinson, 1994), which states that organisms reared at a warmer temperature grow faster, but ultimately reach a smaller body size. Other studies, however, did not observe any effect of temperature on GPM in *C. sapidus* (Brylawski and Miller, 2006; Tagatz, 1968). The inconsistency in GPM results among these studies may be due to differences in temperature sensitivity of GPM as a function of juvenile instar, premolt size, or the range of temperatures tested (as observed for IMP, above). Decreasing GPM at increasing temperature may be due to differences in the temperature sensitivity of the molt process (controlling IMP) and the resource accumulation process (controlling GPM). If the molt process is more sensitive to temperature increases, IMP may decrease but resource accumulation may not increase at a sufficient rate to maintain a constant GPM. Physiological demands also increase with increasing temperatures (Gillooly et al., 2001) and physiological stress may also affect growth per molt.

Although temperature clearly has an impact on the molting process in brachyuran crabs, results differed between species, collection dates, and experimental protocols (i.e., constant vs. fluctuating temperatures). Differences between species are to be expected, reflecting adaptations to their particular environment. *C. similis* has an offshore distribution and would thus experience more stable temperatures than would *C. sapidus*, which has an estuarine distribution during the juvenile and adult phases of the life cycle. Among the experiments conducted with *C. sapidus*, discrepancies in the results may be due to the timing of collection or the range of temperatures tested. Crabs collected in early spring (February) experienced temperature fluctuations beginning at a much cooler temperature than crabs collected in early summer (May). Temperature at metamorphosis

for February-collected crabs was as low as 18.8°C whereas the lowest temperature at metamorphosis for May-collected crabs was 25.2°C. Effects of increased temperature on the molting process are likely non-linear, as discussed above. Additionally, effects of increased temperature on GPM were much clearer when crabs were reared at constant experimental temperatures. Rearing crabs under naturally fluctuating temperatures (ambient and ambient +5°C) introduced another source of variation due to seasonal temperature changes that may have masked the effects of increased temperature on GPM observed under constant temperatures.

Although results were not consistent across all experiments, crabs reared at warmer temperatures generally molted more frequently due to the negative effect of increased temperature on IMP and, in some experiments, were smaller at each instar due to the negative effect of increased temperature on GPM. If this pattern remained consistent throughout the juvenile period, it could result in crabs reaching maturity at a smaller size, and potentially earlier age during warmer years or as ocean temperatures increase due to climate change. However, snow crabs possess the ability to delay maturity to a later molt and therefore increased temperatures actually lead to snow crabs reaching maturity at a larger size (Burmeister and Sainte-Marie, 2010; Orensanz et al., 2007). It is unknown if the number of molts to maturity is fixed or plastic for blue crabs. Smaller size at maturity in female crabs may lead to decreases in individual reproductive output, as female body size is a primary factor limiting single-clutch fecundity in brachyuran crabs (Hines, 1982). Additionally, smaller size at maturity (and thus smaller maximum size for females, who undergo a terminal molt to maturity) may alter predator-prey interactions and result in increased mortality rates due to predation. Future research

should examine the effect of temperature on growth in crabs from metamorphosis to maturity to determine if the response is consistent across all molts, and to directly assess effects of increased temperature on size at maturity.

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