



EFFECTS OF TEMPERATURE ON GROWTH OF JUVENILE SNOW CRABS, *CHIONOECETES OPILIO*, IN THE LABORATORY

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ABSTRACT

The effect of water temperature on the growth of juvenile snow crabs *Chionoecetes opilio* (Fabricius, 1788) was investigated in laboratory culture experiments. Laboratory-born juveniles were cultured from instar I to VIII at four temperatures (approximately 1, 3, 5 and 8°C). The growth indices (size increments at moulting in mm and in % of premoult carapace width) were significantly higher in crabs reared at 5°C than in those reared at other temperatures. The relationship between the mean temperature (T) and intermoult period (D) of each instar was described by the heat summation theory equation: $D = K/(T - \alpha)$. The thermal constant K is the summation of the effective temperature for development (above the threshold temperature, α) up to a selected biological end point. The thermal constant tended to increase and the threshold temperature tended to decrease with increasing mean premoult carapace width of each instar, reaching asymptotes of 1573 day-degrees and -4.7°C , respectively. Size- and temperature-dependent growth models were developed for snow crab juveniles.

KEY WORDS: growth model, juvenile growth, juvenile intermoult period, rearing temperature, snow crab

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INTRODUCTION

Estimation of the age and growth of a commercially-harvested species provides information of life history traits that are important for fisheries management, e.g., lifespan, age at recruitment, age at first capture, age at maturity, and cohort identification. These parameters are important for modelling population dynamics for the development of an appropriate stock management strategy towards sustainable fisheries (Hoggarth et al., 2006; Chang et al., 2012). Age and growth of aquatic organisms are often estimated from body parts, such as the scales and otoliths of fishes and the shells of molluscs (Stevenson and Campana, 1992; Schöne et al., 2005; Dan et al., 2012), which show annual or even daily growth rings. However, crustaceans grow by moulting and they generally lack physical structures suitable for age estimation (Kilada et al., 2012). Therefore, estimation of age and growth of crustaceans has relied on other methods, e.g., captive rearing; mark and recapture experiments; length-frequency distribution analyses in wild populations; and assays of the age pigment, lipofuscin (Kurata, 1962; Hartnoll, 2001; Vogt, 2012).

The snow crab *Chionoecetes opilio* (Fabricius, 1788) (Brachyura: Majoidea) is widely distributed on muddy or sandy mud grounds at depths between 3 m and 1400 m in cold waters in the northern hemisphere (Squires, 1990; Yoshio and Hayashi, 1994; Lovrich et al., 1995; Dawe and Colbourne, 2002; Yanagimoto et al., 2004) and is an important fishery resource in the United States, Canada, Russia, Greenland, Japan, and Korea (Jadamec et al., 1999). Larvae of this species hatch in spring and metamorphose to the benthic crab stage after spending several months of pelagic life in the oceanic water column, as two zoeal stages and one megalopal stage (Yamamoto et al., 2014). After settlement on the sea bottom, snow crabs change their spatial distributions in relation to temperature and bottom substrate, and also seasonally according to reproductive and growth status (Kon, 1980; Lovrich et al., 1995; Comeau et al., 1998; Dawe et al., 2012). Snow crabs undergo a terminal moult to reach morphologically mature stages exhibiting secondary sexual characteristics: males with large chelae, and females with a broad abdomen (Ito, 1957; Conan and Comeau, 1986; Yamasaki and Kuwahara, 1991; Alunno-Bruscia and Sainte-Marie, 1998). Analysis of periodic changes of carapace size distributions in field collections has been used to estimate the

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approximate age of snow crabs from the sizes of the instars at the moult and the annual moulting frequency (Ito, 1970, 1984; Sainte-Marie et al., 1995; Alunno-Bruscia and Sainte-Marie, 1998; Comeau et al., 1998).

Among environmental factors, water temperature is the most important factor influencing moult increment and intermoult period, which determine crustacean growth (Kurata, 1962; Hartnoll, 1982; Anger, 2001). It has been suggested that the water temperature affects growth and survival of juvenile snow crabs in their natural habitat (Lovrich et al., 1995; Dionne et al., 2003; Boudreau et al., 2011). Captive rearing is an effective tool for elucidating the effect of temperature on moult increment and intermoult period in crustaceans (Kurata, 1962; Anger, 2001). However, the effect of temperature on growth of snow crabs has not been experimentally evaluated, except for a laboratory study on growth to maturity of laboratory-born juveniles at 3 and 8°C (Kobayashi, 1989). Under group culture conditions, the intermoult period was shorter in crabs reared at 8°C than in those reared at 3°C. Therefore, to improve our knowledge of the temperature-dependence of growth in snow crabs before the terminal moult, further laboratory studies covering a wider range of temperatures should be conducted, and more thoroughly analysed.

The present study aimed to elucidate the effects of water temperature on growth parameters of juvenile snow crabs (moult increment and intermoult period), through laboratory culture experiments at four temperatures (1, 3, 5, and 8°C). We compared the moult increment at different temperatures and applied the day-degree model for the relationship between body size and intermoult period among the different instars.

MATERIALS AND METHODS

Crab Source

Laboratory-born juvenile snow crabs were used in this study. Crabs were cultured from newly hatched larvae (first stage zoeae), which originated in broodstock females collected from the Sea of Japan, through second stage zoeae and megalopae to first-instar crabs in 2009–2011 at Obama Laboratory, Japan Sea National Fisheries Research Institute, Fisheries Research Agency, Fukui Prefecture, Japan. Zoeae were reared using 500- and 20,000-l tanks at 14°C according to the method of Kogane et al. (2007). Megalopae were stocked in 1000- and 6000-l tanks at a density of approx. 1 individual l⁻¹, and reared at 10°C until they moulted to first-instar crabs. Megalopae were fed *Artemia* (Utah strain) at a density of 3 individuals ml⁻¹ throughout the culture period. *Artemia*-nauplii were enriched with 1.5 ml l⁻¹ commercial emulsion of *n*-3 polyunsaturated fatty acids (Hyper Glos; Marineteck, Japan) at 22°C for 24 hours prior to feeding. Additionally, newly hatched snow-crab zoeae were given to megalopae at a density of 0.5 individuals l⁻¹ on the first day of culture.

Crab Culture Experiments

Juvenile snow crabs were cultured at nominally 1, 3, 5, and 8°C (see Table 1 for mean culture temperatures). We used three cohorts of juveniles born in 2009, 2010 and 2011. The initial numbers of first-instar crabs in the four culture temperatures were: 60 at 1°C (30 each from 2010 and 2011 cohorts), 30 at 3°C (2009 cohort), 30 at 5°C (2011 cohort), and 30 at 8°C (2011 cohort). In addition, 19 third-instar crabs from the 2009 cohort that were cultured at 3°C in another tank were also provided for the 1°C-group. The culture experiments were conducted from 29 August 2009, 9 May 2010, and 23 May 2011 until the juveniles reached instar VIII.

Crabs were individually housed and cultured using 1-l (10 × 10 × 10 cm), 5-l (15 × 26 × 13 cm), and 27-l (40 × 26 × 26 cm) box-shaped plastic mesh cages, and 100-l (46 × 78 × 28 cm) fibreglass-

reinforced plastic (FRP) tanks, according to the growth stage. The cages were placed in 600-l (2.0 × 1.0 × 0.3 m) rectangular FRP tanks in which water temperatures were controlled using a circulating cooling system. The water flow rate was regulated at 5 l min⁻¹ in 100-l tanks and 30 l min⁻¹ in 600-l tanks. Water temperatures were recorded every 2 hours using temperature-recording loggers (HOBO Water Temp Pro v2, Onset Computer Corp., MA, USA). Tanks were covered with Styrofoam boards to stabilise the water temperatures. Crabs were fed ad libitum three times per week with thawed North Pacific krill *Euphausia pacifica* Hansen, 1911 (body length approx. 15 mm) at 2–6 individuals per crab for instars I–V, thawed Antarctic krill *Euphausia superba* Dana, 1850 (body length approx. 50 mm) at 1 individual per crab for instars VI–VII, and artificial pellets for kuruma prawn *Marsupenaeus japonicus* (Bate, 1888) culture (Higashimaru, Kagoshima, Japan) at approx. 20–300 mg per crab for all instars. The given number of North Pacific krill and amount of artificial pellets were increased with crab growth. Before each feeding, uneaten foods, feces, and grime were removed from the culture cages and tanks by siphoning. Survival and moulting of cultured crabs were checked every 1–3 days, and the intermoult period of each crab was determined. If crabs had died during moulting, they were treated as the moulted individuals; however, the occurrence of these crabs was low (2.3% of all moulting events). Dead crabs and exuviae were collected and sexed according to their abdominal morphology. The carapace width (CW) of each intact animal was measured to the nearest 0.1 mm using a digital calliper (CD-S20C, Mitutoyo, Kanagawa, Japan) or with a digital photomicrographic camera (DS-Fi1-L2, Nikon, Tokyo, Japan) and stereomicroscope (SMZ1000, Nikon Corp., Tokyo, Japan). Measurements taken prior to moulting were termed premoult CW (PreCW), and those taken after moulting were termed postmoult CW (PostCW).

Data Analysis

Statistical analyses were performed with the R language (R3.1.1; R Development Core Team, 2014) with a 5% significance level.

Statistical differences between temperature groups of the survival rates at instars I–VII were evaluated with the χ^2 test and Tukey's post-hoc test. PostCW, moult increment (MI = PostCW – PreCW) in mm, and proportional growth rate (GR = MI × PreCW⁻¹) have been used as representative of the growth of crustaceans (Chang et al., 2012; Stevens, 2012). We used a general linear model (GLM) (McCullagh and Nelder, 1989; Everitt and Hothorn, 2009) to evaluate the effect of temperature on the growth of juvenile snow crabs. Three indices of crab growth were used as response variables. In these analyses, taking into account the effect of PreCW, the explanatory variables were PreCW (continuous variable) and temperature (categorical variable), as well as the interaction between PreCW and temperature. The GLM analysis was performed with the *lm* function and the significance of the explanatory variables was evaluated with an *F* test using the ANOVA function (type II) implemented in the car package (Fox and Weisberg, 2011) in R. Because the interaction term between PreCW and temperature in the GLM analysis was not significant, a multiple comparison test with the Tukey method was applied to assess the differences between temperature levels in the GLM analysis with the explanatory variables of PreCW and temperature using the *glht* function implemented in the multcomp package (Hothorn et al., 2008).

To express the intermoult period as day/degrees, the relationship between the number of days between the moults of individuals (intermoult period, *D*) and the mean culture temperature (*T*) was fitted to the following equation for each instar: $D = K/(T - \alpha)$. This equation, known as Réaumur's Law, is part of the theory of heat summation; the parameters *K* and α are the so-called 'thermal constant' and 'threshold temperature' for development, respectively (Hamasaki, 2003; Sudo, 2003; Hamasaki et al., 2009; Yamamoto et al., 2014). The thermal constant (day-degrees) is the summation of the effective temperature for development (above threshold temperature) up to a selected biological end point. An asymptotic relationship was found between the mean PreCW and estimates of the thermal constant and the threshold temperature of instars (see the Results section); therefore, these relationships were expressed as the following equation: K or $\alpha = a(1 - (1/\exp(b\text{PreCW})))$. The parameters *K*, α , *a* and *b* were estimated using a non-linear ordinary least squares method and evaluated with a *t*-test.

In the growth analyses, sex was not considered because our sample size was rather small; sex can be determined from instar V and similar growth at

Table 1. Mean culture temperature, number of crabs cultured, survival rate, and intermolt durations (number of days) of laboratory-born juveniles of the snow crab *Chionoecetes opilio* reared at four temperatures.

Instar	Temperature (°C)	Year cohort	N		Survival rate (%)	Number of days	
			Test	Moult		Mean ± SD	Range
I	0.99	2010	30	20	66.7	122 ± 17	72-149
	0.99	2011	30	18	60.0	120 ± 21	77-161
	3.48	2009	30	30	100.0	64 ± 13	43-97
	4.90	2011	30	30	100.0	37 ± 10	28-74
	8.02	2011	30	30	100.0	31 ± 7	21-43
II	0.95	2010	20	12	60.0	129 ± 7	119-145
	0.12	2011	17	16	94.1	112 ± 13	70-130
	3.96	2009	30	29	96.7	70 ± 11	56-100
	4.83	2011	30	29	96.7	55 ± 10	43-91
	7.94	2011	30	29	96.7	41 ± 12	16-67
III	0.96	2009	19	12	63.2	133 ± 33	76-222
	0.96	2010	12	7	58.3	134 ± 14	117-155
	1.21	2011	16	14	87.5	149 ± 23	119-183
	3.39	2009	29	26	89.7	86 ± 10	70-107
	4.90	2011	29	27	93.1	65 ± 11	52-106
	7.60	2011	29	24	82.8	51 ± 10	17-70
IV	0.92	2009	12	9	75.0	148 ± 13	133-165
	1.01	2010	6	5	83.3	149 ± 32	120-200
	1.15	2011	14	5	35.7	170 ± 19	151-201
	3.33	2009	26	13	50.0	123 ± 41	59-192
	4.93	2011	27	26	96.3	72 ± 16	30-108
	7.72	2011	24	21	87.5	68 ± 21	52-130
V	0.98	2009	9	5	55.6	189 ± 50	157-276
	1.15	2010	5	5	100.0	179 ± 27	143-203
	3.20	2009	13	11	84.6	141 ± 22	113-194
	4.98	2011	26	19	73.1	76 ± 14	34-100
	8.45	2011	21	13	61.9	87 ± 30	52-145
VI	0.95	2009	5	4	80.0	166 ± 9	155-174
	1.19	2010	3	2	66.7	168	154-181
	3.55	2009	11	7	63.6	125 ± 37	72-178
	5.05	2011	19	4	21.1	97 ± 16	75-113
	8.39	2011	13	10	76.9	73 ± 15	59-109
VII	1.06	2009	4	2	50.0	214	210-218
	3.17	2009	7	3	42.9	167 ± 30	134-193
	5.05	2011	4	1	25.0	124	
	8.14	2011	10	4	40.0	83 ± 2	81-85

the moult was reported in immature males and females (Ito, 1970; Alunno-Bruscia and Sainte-Marie, 1998; Comeau et al., 1998).

RESULTS

Data on the culture temperatures, survival rates, and intermolt periods of each instar are summarised in Table 1. The survival rates of instar-I and instar-II juvenile crabs were significantly higher at 3-8°C (97-100%) than at 1°C (63-74%) (Fig. 1). The survival rates of instar III crabs were higher at 3-8°C (83-93%) than at 1°C (70%) but the difference was not significant. The survival rates at instar IV were significantly higher at 5-8°C (88-96%) than at 1-3°C (50-58%). Thus, crabs at instars I-IV tended to show lower survival rates at 1°C. From instar V, survival rates did not differ among tem-

peratures, except for instars VI and VII crabs that showed lower survival rates at 5°C.

PreCW and temperature significantly affected all growth indices but the interaction term was not significant (Table 2); thus, the regression lines between PreCW and growth indices of crabs had similar slopes regardless of temperature (Fig. 2). A multiple comparison test showed that all growth indices were significantly higher in crabs reared at 5°C than in those reared at other temperatures ($P < 0.05$).

The intermolt periods (days) of crabs decreased with increasing temperature (Fig. 3); however, they tended to be longer in some individuals of instars IV and V reared at 8°C compared with crabs at 5°C. Estimates of thermal constants (K) and threshold temperatures (α) were statistically significant in all instars (Table 3). Thermal constants

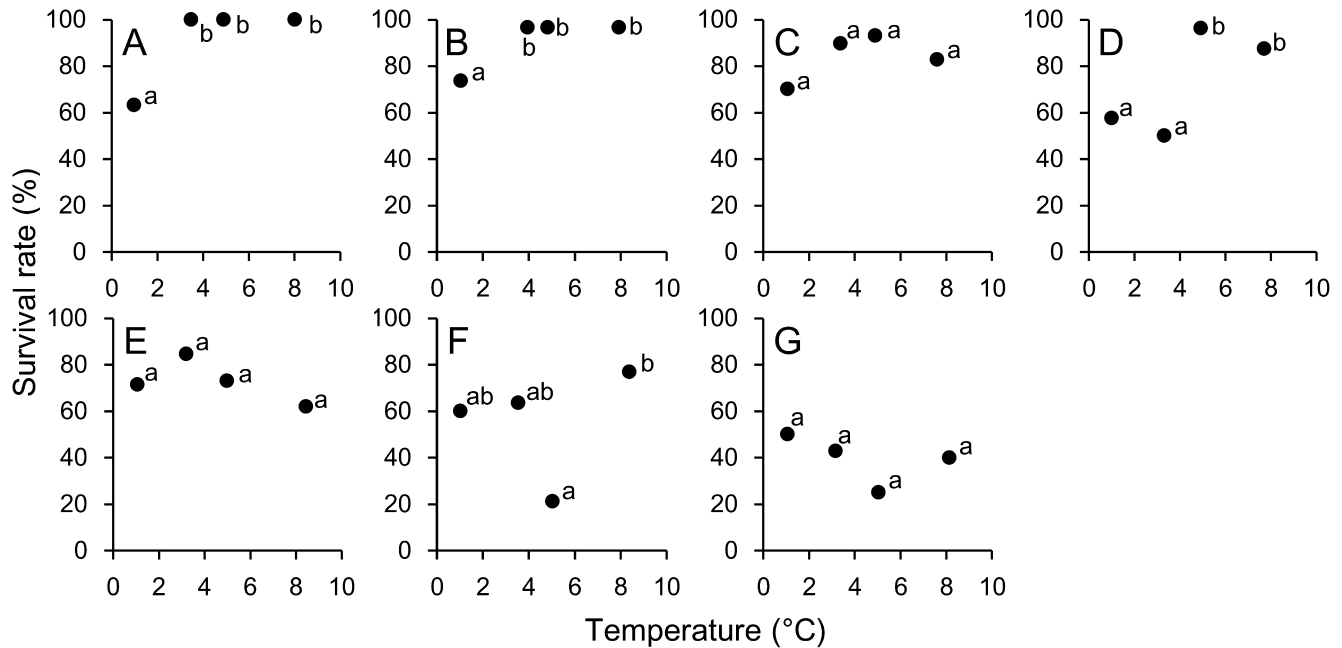


Fig. 1. Survival rates of juvenile snow crabs *Chionoecetes opilio* during each crab instar: A, instar I; B, instar II; C, instar III; D, instar IV; E, instar V; F, instar VI; G, instar VII. Different lower case letters within the same panel indicates statistically significant differences between temperatures (Tukey's HSD, $P < 0.05$).

tended to increase and threshold temperatures tended to decrease with increasing mean PreCW of each instar (Fig. 4). Their relationships were described by asymptotic regression curves with significant parameter estimates (Table 4). The asymptotes from these equations were 1573 day-degrees for the thermal constant and -4.7°C for the threshold temperature.

DISCUSSION

This is the first study of the growth of juvenile snow crabs in the laboratory over the relatively wide range of temperature of $1\text{--}8^{\circ}\text{C}$. We demonstrate that temperature greatly affects their growth and develop a growth model based on the number of day-degrees in the intermolt periods and the size of the animals.

Foyle et al. (1989) examined the bioenergetics of snow crabs in the laboratory by measuring oxygen uptake, activity, and food consumption in morphologically mature males (85–95 mm CW) at 3°C increments between 0°C and 18°C . They demonstrated that: 1) food consumption increased up to 6°C ; 2) metabolic costs increased with temperature and exceeded caloric intake above 7°C ; and 3) growth becomes slightly negative below 1°C . Furthermore, a comparison of the curves for digestible energy and total metabolic cost suggested that growth is optimum at around 4°C . This is consistent with the present observation that the growth indices were highest in snow-crab juveniles reared at 5°C and the intermolt periods of some individuals were extended at 8°C compared with crabs at 5°C .

Table 2. Results of the general linear model of the dependence of growth indices (PostCW, postmolt carapace width; GI, growth increment; GR, growth rate) of juvenile snow crabs *Chionoecetes opilio* on premolt carapace width (PreCW) and water temperature (WT).

Source of variation	PostCW				GI				GR			
	df	MS	F	p	df	MS	F	p	df	MS	F	p
Interaction model												
PreCW	1	10,718.3	31,659.59	0.000	1	439.2	1296.41	0.000	1	7919.0	109.62	0.000
WT	3	5.3	15.68	0.000	3	5.3	15.55	0.000	3	14.6	14.59	0.000
PreCW \times WT	3	0.9	2.55	0.055	3	0.9	2.52	0.057	3	0.1	0.13	0.942
Residuals	502	0.3			502	0.3			502	71.2		
No-interaction model												
PreCW	1	10,718.3	31,370.74	0.000	1	439.2	1284.77	0.000	1	7919.3	110.19	0.000
WT	3	5.3	15.53	0.000	3	5.3	15.41	0.000	3	1054.2	14.67	0.000
Residuals	505	0.3			505	0.3			505	71.9		

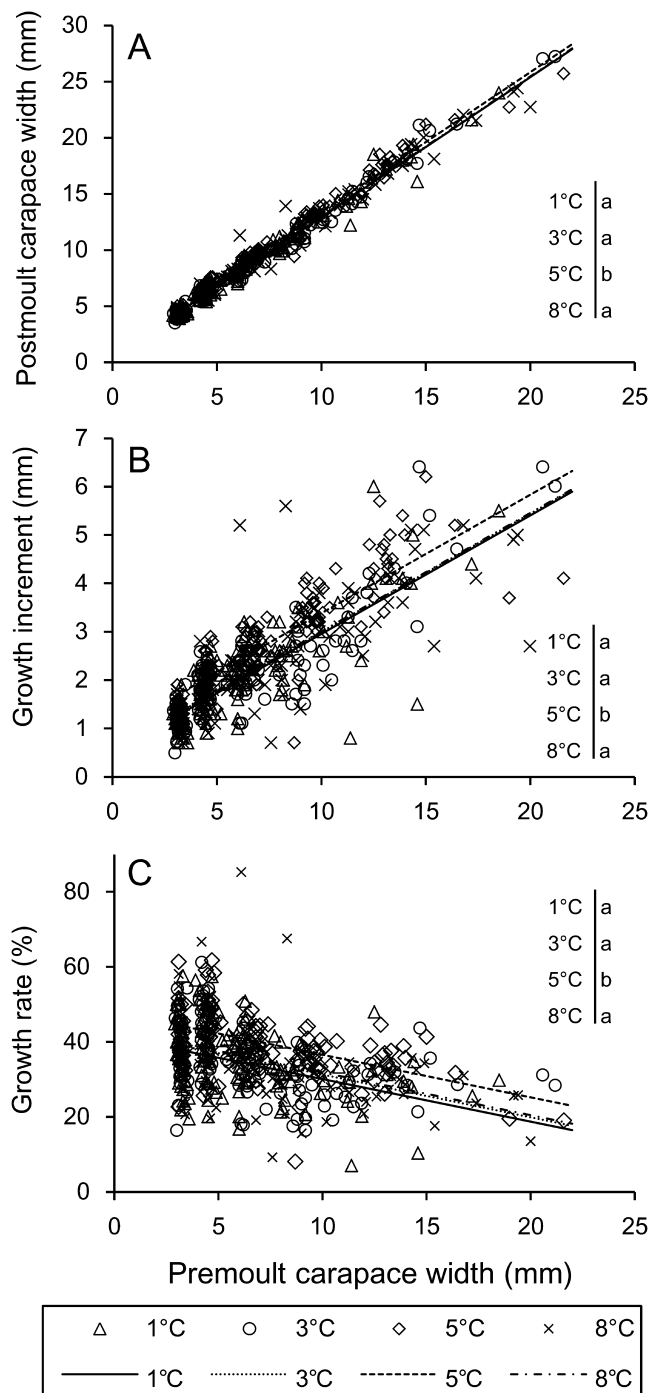


Fig. 2. Relationships between premoult carapace widths and postmoult carapace widths (A), growth increment (B), and growth rate (C) of juvenile snow crabs *Chionoecetes opilio*. The straight lines were drawn from regression analyses. Differences in growth indices between temperature groups ($P < 0.05$) are represented by a different lowercase letter following the temperature in the table.

Field studies have estimated the mean CW of each instar of juvenile snow crabs using size-frequency analysis and have demonstrated that CWs are similar in snow crab populations in the Gulf of St. Lawrence, the eastern Bering Sea, and the Sea of Japan (Table 5) (Orensanz et al., 2007;

Ernst et al., 2012). However, the mean CW values at each instar of laboratory-cultured snow crab juveniles were much smaller than those of wild crabs (Table 5) (Kobayashi, 1989; Sainte-Marie and Lafrance, 2002). This phenomenon has been reported in other decapod crustaceans (Kurata, 1962; Hartnoll, 1982) and may be a laboratory artefact that arises from a number of sources, such as diet, the limited size of the culture containers, and water quality (Stevens, 2012).

The intermoult periods of juvenile snow crabs increased with decreasing temperature, as previously reported for many decapod crustaceans (Kurata, 1962; Hartnoll, 1982). Heat summation theory equations were used to fit the relationship between temperature and intermoult period and estimated the thermal constants and threshold temperatures for snow crab juveniles. The estimates of the threshold temperature for development decreased asymptotically from -1.29°C at instar I to -4.87°C at instar VI and -4.02°C at instar VII. Yamamoto et al. (2014) estimated the threshold temperatures for larval development at 0.63°C from the first to second zoeal stages, -0.02°C from the first zoeal to megalopal stages, and -2.24°C during the megalopal stage. Therefore, the threshold temperatures for development of the snow crab decrease from the pelagic zoeal stages through megalopal and early benthic crab stages, suggesting that snow crabs strengthen their lower temperature tolerance towards their cold-water benthic life. This may explain the relatively higher survival rates of instar-V crabs reared at 1°C . However, it should be noted that some threshold temperature estimates fell below the sea-water freezing temperature (-1.8°C). Therefore, threshold temperature estimates for larvae and juveniles of the snow crab might be considered the relevant indices to represent thermal adaptation. This hypothesis should be evaluated by future physiological investigations of low-temperature adaptation by snow crab juveniles.

It has been documented that the size at terminal moult is positively correlated with habitat temperature in snow-crab populations (Somerton, 1981; Alunno-Bruscia and Sainte-Marie, 1998; Zheng et al., 2001; Orensanz et al., 2007; Burmeister and Sainte-Marie, 2010; Dawe et al., 2012). It was hypothesised that this relationship depends on the assumptions that: 1) moult increment is largely temperature independent (Burmeister and Sainte-Marie, 2010; Sainte-Marie et al., 2010); 2) intermoult period decreases with increasing temperature during the immature phase (Orensanz et al., 2007; Burmeister and Sainte-Marie, 2010); and 3) there exists an age-related trigger for the pre-pubertal and terminal moults (Orensanz et al., 2007; Burmeister and Sainte-Marie, 2010), coupled with the variable frequency of skip-moulting, which is directly related to size and inversely related to temperature (Dawe et al., 2012). Our experimental results support assumption #1 for the temperature-independent moult increment within 1–3°C and assumption #2 for the temperature-dependent intermoult period.

Our asymptotic equations between PreCW, and thermal constant and threshold temperature allow calculation of the intermoult period at each instar at designated temperatures. Thermal constant ($\text{day} \cdot ^{\circ}\text{C}$) and threshold temperature ($^{\circ}\text{C}$)

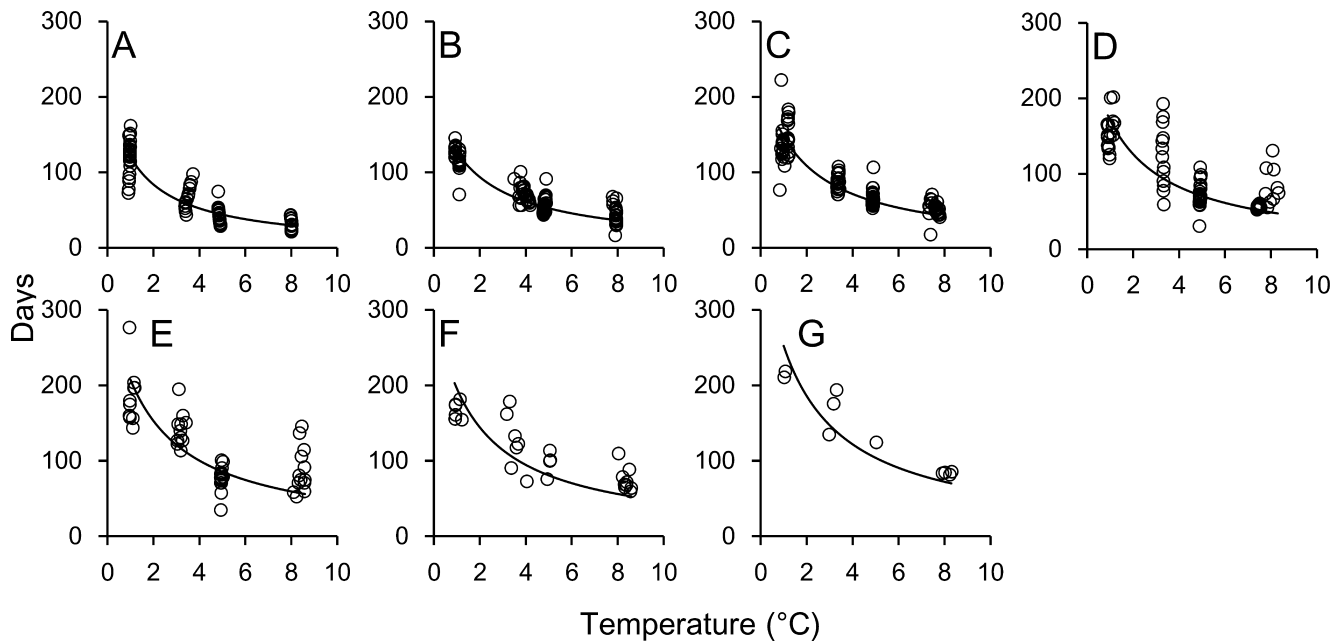


Fig. 3. Relationships between mean water temperature and the intermolt period of each instar of juvenile snow crabs *Chionoecetes opilio*: A, instar I; B, instar II; C, instar III; D, instar IV; E, instar V; F, instar VI; G, instar VII. The curves were drawn from the equation of heat summation theory (see Table 3) applied to the relationship between the two variables.

Table 3. Estimates of parameters (with SEs) of the heat summation theory equation ($D = K/(T - \alpha)$) expressing the relationship between mean temperature (T) and the intermolt period in each crab instar (number of days, D) of juvenile snow crabs *Chionoecetes opilio*. K and α are the 'thermal constant' and 'threshold temperature constant' for development, respectively.

Instar	n	Estimate (SE)	
		K	α
I	128	276.07 (15.93)*	-1.29 (0.14)*
II	115	429.06 (18.99)*	-2.54 (0.19)*
III	110	511.44 (33.06)*	-2.63 (0.28)*
IV	79	726.62 (74.77)*	-3.68 (0.58)*
V	53	801.73 (98.33)*	-3.28 (0.67)*
VI	27	996.53 (133.14)*	-4.87 (0.95)*
VII	10	1115.32 (177.68)*	-4.02 (0.10)*

H_0 , K or $\alpha = 0$; * $P < 0.05$.

Table 4. Estimates of parameters (with SEs) of asymptotic equations (K or $\alpha = a(1 - (1/\exp(b\text{PreCW})))$) between mean premolt carapace widths (PreCW) and thermal constants (K) or threshold temperatures (α) from the theory of heat summation equations (see Table 3) for juvenile snow crabs *Chionoecetes opilio*.

Response variable	Estimate (SE)	
	a	b
K	1573.00 (154.50)*	0.07 (0.01)*
α	-4.71 (0.70)*	0.14 (0.05)*

H_0 , a or $b = 0$; * $P < 0.05$.

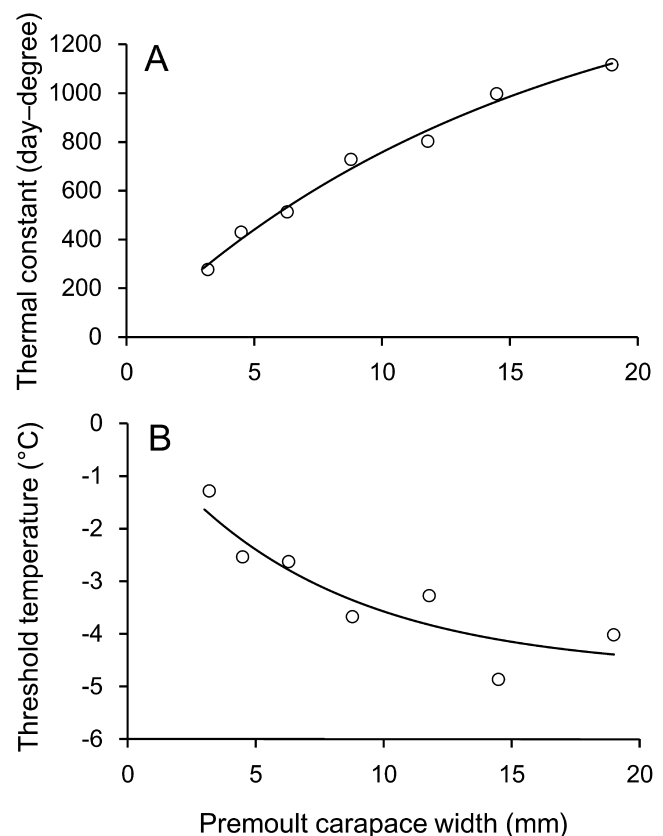


Fig. 4. Relationships between mean premolt carapace width and the thermal constant (A) and threshold temperature (B) in the heat summation equations (see Table 3) estimated for juvenile snow crabs *Chionoecetes opilio*. The curves were drawn from the asymptotic equations (see Table 4) applied to the relationship between the two variables.

can be calculated by substituting the CW value of the instar into the equations; then, intermolt period (days) of the instar can be calculated by dividing the thermal constant by the effective temperature for development as the value obtained by subtracting the threshold temperature from the designated temperature. Using these calculated intermolt periods at the mean CW of instars I–VIII of juvenile female snow crabs in the north-western Gulf of Saint Lawrence reported by Alunno-Bruscia and Sainte-Marie (1998), we obtained contour lines expressing the relationship between PreCW and the intermolt period in months at temperatures of -1°C to 4°C , as shown in Fig. 5A. An inverse relationship between mean CW and intermolt period was observed during instars I–IV at -1°C , suggesting that early benthic juvenile snow crabs could achieve faster growth rates at habitats with temperatures above 0°C . Our results are in agreement with field observations showing that juvenile snow crabs of instars I–IV are scarce in the core of the cold intermediate layer with temperatures below 0°C but are present immediately above and below this layer with

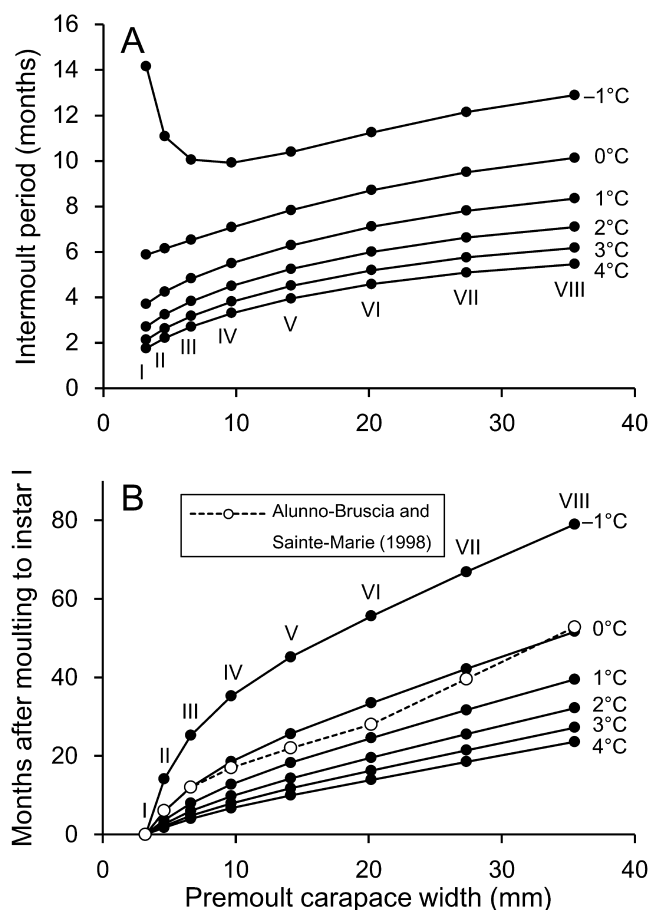


Fig. 5. Relationships between premolt carapace width and intermolt period (A) and age represented by cumulative months after moulting to instar I (B) at -1°C to 4°C calculated from the asymptotic equations between premolt carapace width and thermal constant or threshold temperature for juvenile snow crabs *Chionoecetes opilio* (see Table 4). Roman numerals (I–VIII) indicate instar nos. Ages at instars I–VIII estimated for wild populations in the north-western Gulf of Saint Lawrence (Alunno-Bruscia and Sainte-Marie, 1998) are also shown.

temperatures of 0 – 1°C in the north-western Gulf of Saint Lawrence (Dionne et al., 2003).

In the Gulf of Saint Lawrence, the eastern Bering Sea, and the Sea of Japan, the ages of snow crab populations have been estimated using size-frequency distributions from periodic field sampling (Ito, 1970, 1984; Sainte-Marie et al., 1995; Alunno-Bruscia and Sainte-Marie, 1998; Comeau et al., 1998; Ernst et al., 2012). In the Gulf of Saint Lawrence and the eastern Bering Sea, the intermolt period of snow crabs was estimated at 5–7 months for instars I–V and 1 year from instar VI (Sainte-Marie et al., 1995; Alunno-Bruscia and Sainte-Marie, 1998; Comeau et al., 1998; Ernst et al., 2012). Our calculated intermolt periods of instars I–V at 0 – 1°C (4–8 months) and from instar VI at -1 – 0°C (9–13 months) approximate the estimates of intermolt period in the wild populations. For example, the ages at instars I–VIII estimated in the north-western Gulf of Saint Lawrence population (Alunno-Bruscia and Sainte-Marie, 1998) were similar to our age estimates of crabs grown at 0 – 1°C (Fig. 5B). Dionne et al. (2003) reported that snow crabs of instars I–VIII were associated with temperatures between 0 – 2.0°C and never occurred on bottoms warmer than 3.3°C in the north-western Gulf of Saint Lawrence. In the Sea of Japan, Ito (1970, 1984) estimated intermolt periods of 1–2 months and 2–3 months for instars I and II–III, respectively, a total duration of 1 year from instar I to IV, a 6-month intermolt for instar V, and an annual moult from instar VI. Ishikawa Prefectural Fisheries Experimental Station (1981, 1982) investigated the distribution of snow-crab juveniles in the central Sea of Japan and documented that instars I and II were associated with larger temperature ranges of 0 – 10°C and 0 – 8°C , respectively, and that instars III–IV were mainly found within a narrower temperature range of 0 – 2°C . Moreover, Yoshio and Hayashi (1994) reported that juvenile snow crabs > 10 mm carapace length, i.e., instar III > 8 mm CW; calculated from Ito (1984), lives at a temperature range of 0.3 – 0.9°C . The intermolt periods of instar I at 0 – 10°C and instar II at 0 – 8°C were estimated as 1–6 months (mean, 2 months) and 1–6 months (mean, 3 months), respectively and for instars III–VIII reared at 0 – 1°C , they tended to increase from about 5–6 to 8–10 months. Thus, the intermolt periods estimated by Ito (1970, 1984) for the wild population in the Sea of Japan also correspond to the estimates of intermolt duration inferred from their habitat temperatures based on our growth model. Consequently, our day-degree-based growth model approximates the growth trend of wild snow-crab populations in the north-western Gulf of Saint Lawrence and the Sea of Japan.

Age estimations of the snow crabs at individual and population levels have been performed using lipofuscin assays (Allain et al., 2011a, b), radiometry of the exoskeleton (Nevissi et al., 1996), numbers of growth bands in the eyestalks (Kilada et al., 2012), and size-frequency distributions with a periodic sampling from wild populations. Further information on habitat temperatures of snow crabs, and changes with growth, could be used with our day/degree-based growth model to evaluate the feasibility of those methods of age estimation.

Table 5. Carapace width (mm) at instars I–VIII of cultured and wild populations of the snow crab *Chionoecetes opilio*.

Instar	Cultured crab (mean value)								Wild population (mean or modal value)					
	This study				Kobayashi (1989)		Sainte-Marie and Lafrance (2002)		North-western Gulf of Saint Lawrence		Bonne Bay, Gulf of Saint Lawrence		Eastern Bering Sea	Sea of Japan
	1°C	3°C	5°C	8°C	3°C	8°C	Crab	Exuviae	Female	Male	Female	Male		
I	3.2	3.2	3.2	3.2	2.7	2.8		3.03	3.22	3.19	3.1			2.9–3.0
II	4.4	4.3	4.6	4.5	3.6	4.1	3.94	4.11	4.63	4.63	5.0			4.3–4.6
III	5.9	6.3	6.8	6.2	5.2	5.7	5.48		6.62	6.60	7.0	7.9		6.3–6.6
IV	8.1	8.5	9.6	8.6	6.9	8.3			9.67	9.66	9.7	9.8	11.6	9–10
V	10.8	11.0	12.9	11.2	8.8	11.3			14.14	14.10	14.7	14.9	16.4	13–14
VI	13.9	13.9	17.5	14.6	11.9	13.6			20.20	19.96	20.9	21.6	21.4	19–20
VII	17.9	18.9	20.3	18.5	16.3	18.0			27.35	26.88	27.7	28.0	29.6	27–28
VIII	22.8	24.0	25.7	23.2	23.7	23.0			35.50	34.47	36.2	38.3		37–38

Sources of data: north-western Gulf of Saint Lawrence (mean value), Sainte-Marie et al. (1995), Alunno-Bruscia and Sainte-Marie (1998); Bonne Bay, Gulf of Saint Lawrence (mean value), Comeau et al. (1998); Eastern Bering Sea (mean value), Ernst et al. (2012); Sea of Japan (modal value), Ito (1970, 1984).

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