Study on early life history characteristics of the snow crab Chionoecetes opilio under laboratory conditions

Doctoral Dissertation

STUDY ON EARLY LIFE HISTORY CHARACTERISTICS OF THE SNOW CRAB *Chionoecetes opilio* UNDER LABORATORY CONDITIONS

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Abstract

The snow crab, *Chionoecetes opilio*, is distributed in cold waters off Alaska, Canada, Russia, Greenland, Japan, and Korea and is an important fishery resource in these regions. In Japan, most snow crabs have been caught in the eastern part of the Sea of Japan since the late 1990s; thus, this is the most important snow crab fishery in the region. Annual stock density of snow crabs peaked in 1970 and then declined greatly during the 1970s–1980s because of overfishing. Stock densities were restored after the 1990s to approximately one-third of the male and one-half of the female peaks, but the possible causes of the stock recovery have not been discussed. In general, the pelagic larval phase plays an important role in sustaining the population by facilitating larval dispersal and recruitment. Therefore, it is important to elucidate the effects of these factors on larval survival and development to understand the stock dynamics of the species. Moreover, estimating age and growth of a commercially harvested species provides life history trait information that is important for fisheries management, e.g., lifespan, age at recruitment, age at first capture, age at maturity, and cohort identification. A captive rearing experiment is an effective way to elucidate the life history of a crustacean. However, the biology and ecology during the early snow crab lifecycle is not well documented because of low larval survival under laboratory conditions. The objective of this study was to add to the basic knowledge of snow crab early life history characteristics under laboratory conditions.

We examined the effects of temperature and salinity on larval survival and development, as these factors regulate basal metabolic rate and feeding activities. The optimum temperature ranges for larval survival were 5–16°C for hatching to second zoeae, 5–14°C for hatching to megalopae, and 5–14°C for megalopae to the crab stage. The optimum salinity ranges for larval survival were 20–38 for hatching to second zoeae, 26–38 for hatching to megalopae, and 28–36 for megalopae to the crab stage. The relationships between temperature or salinity and larval period were elucidated. Moreover, the threshold temperatures, calculated from heat summation theory equations for larval development, were estimated to be −2.24 to 0.63°C; they decreased with larval development as an adaptation to deeper

vertical distribution during later larval stages.

Several studies have considered snow crab zoeae feeding ecology but not during the stages following megalopae. Therefore, the megalopae food consumption pattern was examined using *Artemia* nauplii. The megalopae food requirement was estimated to be ~190% of dry body weight of the first-instar crab. Two-segmented regressions provided good fits for the relationship between the number of days after metamorphosis and the cumulative number of *Artemia* consumed. The mean post-metamorphic breakpoint time in the rate of food consumption corresponded to intermediate of late premoult during the moulting cycle. A positive correlation was observed between crab size and the number of *Artemia* consumed during the entire megalopal period.

Body density is an important parameter when modeling larval vertical distribution in the water column. Thus, ontogenetic changes in larval body density were investigated in relation to moult stages, which were determined based on integumentary changes occurring during the snow crab larval moulting cycle. The moult-stage characteristics were based on a microscopic examination of changes in the integument, particularly the telson. The larval cuticle changed from a spongy structure to become conspicuously thicker and more solid in appearance during stages A–C. The epidermis retracted from the cuticle during stage D, and new setae and appendages formed. Body density of larval snow crabs was lowest just after moulting, increased significantly during stage C, and then increased gradually to reach a plateau at 1.0897–1.0931 g cm^{-3} during stage D. The larvae developed a density greater than that of seawater during the entire larval period.

Snow crabs change their spatial distribution in relation to temperature and the bottom substrate after settling on the sea bottom. They also change their distribution seasonally according to reproductive and growth status. Among environmental factors, water temperature is the most important factor influencing moulting and the intermoult period, which determine growth. Therefore, captive experiments were conducted using laboratory-born juvenile crabs during the early settlement phase (instar I–VII) to elucidate the effect of temperature on moulting and growth. Growth indices (size increases at moulting in mm and in percentage of premoult carapace width) were significantly higher in crabs

reared at 5°C than in those reared at other temperatures. The relationship between mean temperature and the intermoult period of each instar was described by a heat summation theory equation. The thermal constant tended to increase and the threshold temperature tended to decrease with the increase in mean premoult carapace width of each instar. Size- and temperature-dependent growth models were developed for juvenile snow crabs from these variables.

Wild-born immature crabs (carapace width, 16.2–42.9 mm) caught from the Sea of Japan were cultured at the natural habitat temperature (approximately 1°C). The growth indices and intermoult period were significantly affected by premoult carapace width, but sex did not affect these variables. Furthermore, premoult carapace width and days after moulting significantly affected the probability of moulting, and we developed a moulting probability model based on these variables. The model revealed that the number of days during the intermoult period when moulting occurred in 50% of instars VI, VII, and VIII was estimated to be 234, 284, and 346 days, respectively.

Finally, snow crab life history was estimated by referring to ecological and environmental information on their natural habitat. As a result, the durations from hatch to terminal moult instars were estimated to be 4–9 years in male crabs and 5–7 years in female crabs.

Publications

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Chapter 1

GENERAL INTRODUCTION

1.1 Status of the snow crab resource

The snow crab, *Chionoecetes opilio* (Fabricius, 1788), (Brachyura, Majoidea) is widely distributed on muddy and sandy-mud ground at depths of 3–1,400 m in the cold waters of the Northern Hemisphere (e.g., Squires, 1990; Yosho & Hayashi, 1994; Lovrich et al., 1995; Dawe & Colbourne, 2002). Snow crabs are an important fishery resource in Canada, Russia, Alaska, Japan, Greenland, and Korea, and the total number of landings in these countries was approximately 184,000 metric tons in 2009 (Jadamec et al., 1999; Higashimura, 2013) (Fig. 1.1). In Japan, most snow crabs have been caught in the eastern part of the Sea of Japan since the late 1990s (Fig. 1.2); thus, this region is the most important snow crab fishery area. Annual snow crab stock density in this region peaked in 1970 and then declined dramatically during the 1970s–1980s (Fig. 1.3) because of overfishing (Yamasaki, 1994). After the 1990s, stock densities were restored to approximately one-third of the male and one-half of the female peaks but the possible causes of the stock recovery have not been discussed. Many possible drivers that affect recruitment dynamics have been documented in the eastern Bering Sea and the Gulf of Sainte Lawrence, including gadid predation on juvenile instars (Orensanz et al., 2004; Burgos et al. 2013), cannibalism, and limited resources (space and/or food) at or soon after settlement (Sainte-Marie et al. 1996; Lovrich & Sainte-Marie, 1997), abundance of adult female snow crabs (Sainte-Marie et al. 1996; Parada et al., 2010), temperature during early post-settlement (Orensanz et al., 2004), cold conditions during early life history (Marcello et al., 2012), and regime shifts (Szuwalski & Punt, 2013).

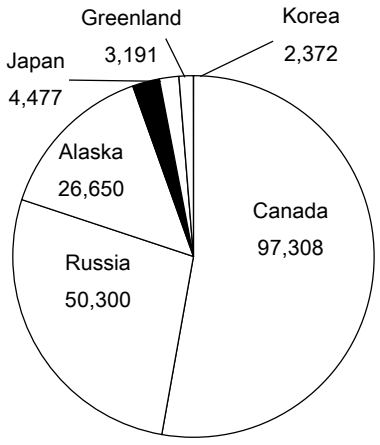

Figure 1.1. Amount of snow crab *Chionoecetes opilio* caught by country or region in 2009 (Higashimura, 2013).

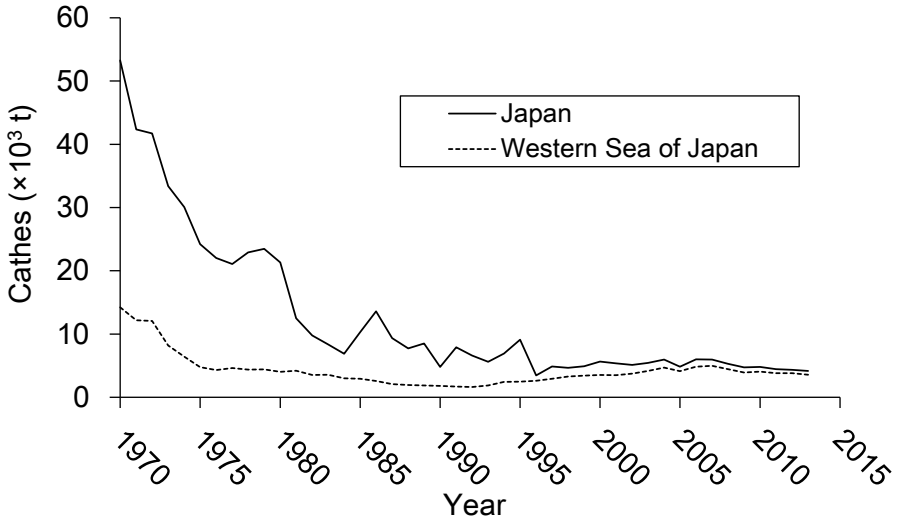

Figure 1.2. Amount of snow crab *Chionoecetes opilio* caught in Japan and the western Sea of Japan (http://www.maff.go.jp/j/tokei/kouhyou/kaimen_gyosei/index.html).

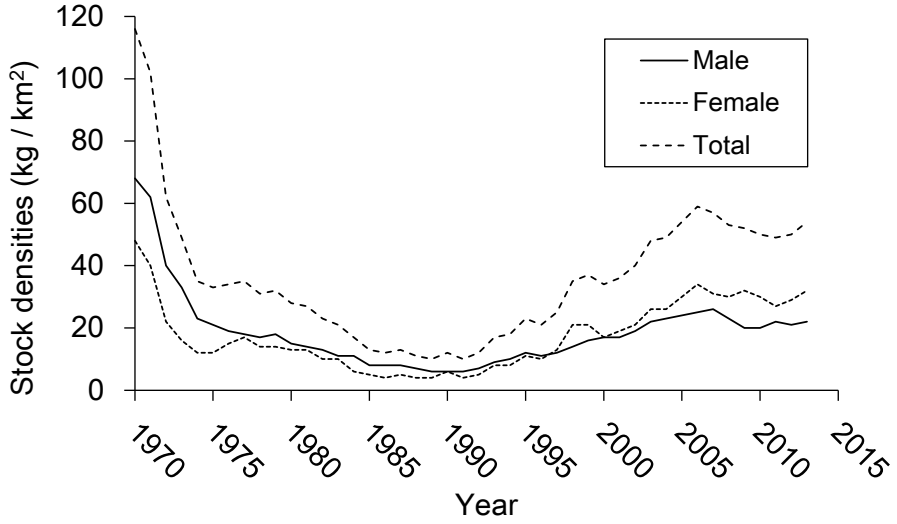

Figure 1.3. Snow crab *Chionoecetes opilio* stock densities in the western Sea of Japan (Ueda et al., 2015).

1.2 Problems studying the biology and ecology of snow crab early life stages

The pelagic larval phase generally plays an important role in sustaining the snow crab population by facilitating larval dispersal and recruitment (Sulkin, 1984; Anger, 2001, 2006). Survival and development of pelagic larvae are influenced by many environmental factors, such as water temperature,

salinity, food availability and quality, predation, and ocean currents (Sulkin, 1984; Anger, 2001; Forward, 2009). Therefore, it is important to elucidate the effects of these factors on larval survival and development to understand the stock dynamics of the species. Moreover, estimates of age and growth of a commercially harvested species provide life history trait information important for fisheries management, e.g., lifespan, age at recruitment, age at first capture, age at maturity, and cohort identification.

To elucidate the life history traits, including larval biology and ecology of the snow crab, field sampling of specimens from their natural habitat has been conducted in the Sea of Japan, the eastern Bering Sea, and the Gulf of Sainte Lawrence. Some groups have reported on larval biology and ecology, but they did not distinguish between snow crab and other *Chionoecetes* species (Fukataki, 1969; Ito & Ikehara, 1971; Incze, 1981; Incze et al., 1987; Kon et al., 2003); they did not collect a sufficient number of larvae owing to the limited sampling depth or period (Fukataki, 1969; Ito & Ikehara, 1971; Kon1980; Incze, 1981; Incze et al., 1987; Conan et al. 1996); or the larvae were collected by indirect sampling from the stomach contents of predatory fish (Konishi et al., 2012). Moreover, it is difficult to collect juvenile crabs during a periodic field sampling survey in the Sea of Japan because they inhabit the deep sea bottom (Ito, 1968, 1970, 1984; Kon, 1968, 1980)., and some crabs were collected from stomach contents of predator fishes (Ito, 1968; Kon, 1968; Konishi et al., 2012).

A captive rearing experiment is an effective way to elucidate the life history of a crustacean (Kurata, 1962; Anger, 2001). Many studies have been conducted on life history stages, including the embryo stage, development, and hatching (e.g. Kon, 1976, 1980; Moriyasu & Lanteigne, 1998; Kon & Adachi, 2005; Webb et al., 2007; Kuhn et al., 2011); larval stage, morphology (Kurata, 1963; Haynes, 1973; Motoh, 1973; Kon, 1967, 1980; Davidson & Chin, 1991), osmotic regulation (Charmantier & Charmantier-Daures, 1995); phototaxis and geotaxis (Kogane, 2007a; Konishi et al., 2011); and survival and development (Kon, 1970, 1973, 1979, 1980; Davidson & Chin, 1991; Lovrich & Ouellet, 1994); benthic stage, moulting, and growth (e.g. Kon, 1980; Moriyasu et al., 1987; Kobayashi, 1989; Sainte-Marie et al., 1995; Alunno-Bruscia & Sainte-Marie, 1998; Godbout et al., 2002; Hebert et al.,

2002; Sainte-Marie & Lafrance, 2002); age determination characteristics (Kilada et al., 2012); habitat selection (Dionne et al., 2003); cannibalism (Lovrich & Sainte-Marie, 1997; Sainte-Marie & Lafrance, 2002), osmotic regulation (Hardy et al., 1994; Charmantier & Charmantier-Daures, 1995), maturation (e.g., Sainte-Marie et al., 1995; Alunno-Bruscia & Sainte-Marie, 1998; Godbout et al., 2002); mating (e.g., Sainte-Marie & Lovrich, 1994; Urbani et al., 1998; Rondeau & Sainte-Marie, 2001; Sainte-Marie et al., 2008); and the reproductive cycle and fertile period (e.g., Moriyasu & Lanteigne, 1998; Webb et al., 2007; Sainte-Marie et al., 2010). However, little information is available on the biology and ecology of the snow crab early lifecycle because of poor larval survival under laboratory conditions. However, laboratory techniques for culturing larval snow crabs, including optimum water temperature, prey density, feeding schedule, dietary essential fatty acids, such as n-3 highly unsaturated fatty acids, and methods to prevent bacterial diseases have been improved by Kogane et al. (2005, 2007a, 2009, 2010). These techniques have helped achieve reliable larviculture, resulting in the production of juvenile crabs used in laboratory experiments (Kogane et al., 2007b).

1.3 Study objectives

The objective of this study was to improve basic knowledge of the early life history characteristics of the snow crab under laboratory conditions.

The effects of environmental factors, such as temperature and salinity, on larval survival and development of snow crab are examined in Chapter 2. Four groups have conducted snow crab larval rearing experiments at different temperatures (Kon, 1970; Davidson & Chin, 1991; Charmantier & Charmantier-Daures, 1995; Kogane et al., 2005). However, they did not report appropriate survival temperatures or the relationship between temperature and the developmental period because of the small number of cultured animals, poor survival, or a narrower temperature range compared with that of their natural habitat (Kon, 1970; Davidson & Chin, 1991; Charmantier & Charmantier-Daures, 1995; Kogane et al., 2005). Two studies have evaluated the effects of salinity on snow crab larval survival and development (Kon, 1973; Charmantier & Charmantier-Daures, 1995). Kon (1973) included

data from larvae that died during or immediately after moulting, thereby making it difficult to draw accurate conclusions concerning the effect of salinity on survival or on the duration of the developmental period. Additionally, Charmantier & Charmantier-Daures (1995) only determined lethal salinity for first-stage zoeae.

Food availability and quality are also important factors influencing larval survival and development. Several studies have considered effective diets, prey size, food consumption patterns, optimal prey density and feeding schedules, and the effects of varying dietary n-3 highly unsaturated fatty acid levels on snow crab zoeae (Paul et al., 1979; Harada & Yamamoto, 2000, 2006; Kogane et al., 2009; 2010). However, little is known about the feeding ecology of megalopae. Therefore, the food consumption pattern of megalopae was examined in Chapter 3.

Crustaceans grow by moulting. As a basis for understanding the behavioral, physiological, and biochemical changes that occur between successive moults (i.e., during the course of the moulting cycle; Chang 1995), staging techniques have been developed to characterize the morphologically distinct phases of the moulting cycle in decapods and other crustaceans. In general, marine benthic decapods do not migrate long distances, so dispersal and recruitment of their pelagic larvae generally play an important role sustaining their populations (Sulkin 1984; Anger 2001, 2006). Moreover, decapod larvae migrate in the sea by regulating their vertical distributions in relation to different water current directions and strengths at different depths and phases of the tidal cycle (Forward & Tankersley 2001). Body density is an important parameter for modeling larval vertical distribution in the water column (Konoshi et al., 2011). Therefore, ontogenetic changes in larval body density were investigated in relation to the moulting cycle stages in Chapter 4, which were determined based on changes in the integument that occur during the snow crab larval moulting cycle.

A growth and moulting model was developed for immature snow crabs in Chapter 5. After settling on the sea bottom, snow crabs change their spatial distribution in relation to temperature and the bottom substrate. They also change their spatial distribution seasonally according to reproductive and growth status (e.g., Kon, 1980; Lovrich et al., 1995; Comeau et al., 1998; Dawe et al., 2012). Among

environmental factors, water temperature is the most important influence on moulting and the inter-moult period, which determine growth of crustaceans (Kurata, 1962; Hartnoll, 1982; Anger, 2001). It has been suggested that 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). In contrast, Yosho & Hayashi (1994) reported that juvenile snow crabs with a carapace length > 10 mm, i.e., instar III > 8 mm carapace width (calculated from Ito (1984)), live at 0.3–0.9°C. Therefore, the captive experiments were conducted using laboratory-born juvenile crabs during the early settlement phase (instar I–VII) to elucidate the effect of temperature on moulting and growth and using wild-born immature crabs at the immature phase (instar VI–VIII) to understand growth and the intermoult period.

The results of this study are summarized in Chapter 6, and the snow crab life history is described using information on their natural habitat.

Chapter 2

EFFECT OF ENVIRONMENTAL FACTORS ON LARVAE

2.1 Effects of temperature on snow crab *Chionoecetes opilio* larval survival and development under laboratory conditions

2.1.1 Summary

To better understand the larval dispersal and settlement of snow crab *Chionoecetes opilio* in natural habitats, we tested the effects of temperatures ranging from ~1–20°C and ~1–18°C on the survival and developmental period of snow crab larvae in the zoeal and megalopal stages, respectively, through laboratory experiments. The survival rates of second zoeae and megalopae were significantly higher at 5–16°C and 5–14°C, respectively. There were no statistically significant differences among the survival rates of megalopae reared at 3–16°C, although higher survival rates were observed at 5–14°C. The mean numbers of days from hatching to second zoeae and megalopae and from megalopae to reach first crab instar were significantly shorter at higher temperatures. Moreover, the relationships between mean temperatures and larval periods were well described by the heat summation theory equations. The threshold temperatures for larval development were estimated to be −2.24–0.63°C; they decreased with the larval development as adaptation for deeper vertical distributions in later larval stages. On the basis of the larval distribution with respect to water temperature in natural habitats as well as the heat summation theory equations, the entire larval duration from hatching to first crab instar was estimated to be 74.4–123.4 days; this is similar to that in natural habitats inferred on the basis of the time lags in the occurrence of peak abundance between each larval stage.

2.1.2 Introduction

Many aquatic decapod crustaceans have a complex life cycle; these comprise embryonic, pelagic larval, and benthic juvenile–adult phases (Anger, 2001; 2006). The pelagic larval phase generally plays an important role in sustaining the population by facilitating larval dispersal and recruitment (Sulkin, 1984; Anger, 2001, 2006). The survival and development of pelagic larvae are influenced by many environmental factors such as water temperature, salinity, food availability and quality, preda-

tion, and ocean currents (Sulkin, 1984; Anger, 2001; Forward, 2009). Among these factors, water temperature most strongly affects the rates of larval survival, growth, and development by regulating basal metabolic rate and feeding activity (Wenner, 1985; Anger, 2001). Therefore, it is important to understand the effects of temperature on larval survival and development in order to understand the stock dynamics of a species.

Snow crab *Chionoecetes opilio* is widely distributed in cold waters in the northern hemisphere and is an important fishery resource in the United States, Canada, Russia, Greenland, Japan, and Korea (Jadamec et al., 1999). There are many published studies of the life history of snow crabs in the Sea of Japan, eastern Bering Sea, and Northwest Atlantic that aim to manage stocks or clarify the mechanisms of stock fluctuations (Adams, 1979; Kon, 1980; Comeau et al., 1991; Sainte-Marie et al., 1995; Conan et al., 1996; Ernst et al., 2012). The larval development of the snow crab comprises 2 zoeal stages and a megalopal stage (Kon, 1980). In the Sea of Japan, benthic mature snow crabs exist from depths of 200–600 m (Yosho & Hayashi, 1994), pelagic larvae (e.g., first and second zoeae) are mainly found at depths shallower than 150 m, and megalopae are found in deeper strata than zoeae (Kon et al., 2003). After settlement, crabs do not migrate long distances. Therefore, the survival and development of pelagic larvae are suggested to affect the strength of recruitment of snow crab populations (Zheng & Kruse, 2006; Kruse et al., 2007; Szuwalski & Punt, 2013).

Laboratory studies of the effects of temperature on the survival and development of snow crab larvae have been conducted; however, they did not cover the temperature range in the natural habitat and/or showed low survival rates of larvae (Kon, 1970; Davidson & Chin, 1991; Charmantier & Charmantier-Daures, 1995; Kogane et al., 2005). Therefore, the present study aimed to elucidate the effects of water temperature on the survival and development of snow crab larvae through laboratory rearing experiments covering the wide range of temperatures in which snow crab larvae may exist. The present study aims to provide a better understanding of larval dispersal and settlement of snow crab in natural habitats.

2.1.3 Material and Methods

2.1.3.1 Larva source

A total of 247 ovigerous females were caught in November 2011 in the Sea of Japan off Ishikawa Prefecture, Japan. The mean carapace width ± standard deviation of the females was 84.3 ± 3.9 mm (range, 79.0–93.0 mm). They were transferred to the Obama Laboratory, Japan Sea National Fisheries Research Institute, Fisheries Research Agency, Fukui Prefecture, and reared at 3°C in one 4-kL (1.3 × 3.9 × 0.85 m) rectangular tank with a recirculating system as described by Morita & Nogami (2003). The tank was not provided with sand as a substrate. Females were fed frozen Antarctic krill *Euphausia superba* twice a week. The main hatching season extended from February to March and several females hatched their larvae in the same day.

2.3.1.2 Larval rearing experiments

Larval rearing experiments were conducted during the zoeal stages (i.e., from first zoeae through second zoeae to metamorphosis to megalopae) and the megalopal stage (i.e., from megalopae to molt to first crab instar). First zoeae hatched on February 6, 2011 and megalopae metamorphosed on March 6, 2011 from zoeae that hatched on January 31, 2011 were used in experiments. One-liter plastic beakers were prepared to larval culture and placed in temperature-controlled baths. Larval rearing temperature levels were determined by taking the thermal distribution in the natural habitats where snow crab larvae are found (Fukataki, 1969; Ito & Ikehara, 1971; Kon, 1980; Kon et al., 2003). Zoeae were reared at 9 temperature levels (mean ± SD): 1.4 ± 0.1°C, 3.0 ± 0.1°C, 5.0 ± 0.1°C, 8.0 ± 0.1°C, 11.0 ± 0.2°C, 14.0 ± 0.1°C, 16.0 ± 0.1°C, 18.0°C ± 0.2, and 19.9 ± 0.1°C. Megalopae were reared at 10 temperature levels: 1.0 ± 0.1°C, 3.0 ± 0.2°C, 5.0 ± 0.1°C, 8.0 ± 0.1°C, 11.0 ± 0.2°C, 14.0 ± 0.2°C, 15.0 ± 0.1°C, 16.0 ± 0.1°C, 17.0 ± 0.1°C, and 18.1 ± 0.2°C. For each temperature level, 3 beakers (20 individuals/beaker) and 6 beakers (3 individuals/beaker) were set for the zoeal and megalopal stages, respectively. Larvae were cultured at 14°C to prepare test animals for the megalopal experiment.

Larval culture was based on the method of Kogane et al. (2009, 2010). Zoeae were fed rotifers *Brachionus plicatilis* at a density of 20 individuals/mL. Rotifers were enriched with 0.5 mL/L com-

mercial condensed marine phytoplankton (*Nannochloropsis* sp.; Mercian Co., Japan), 14 µL/L emulsified DHA 70G oil, and 28 µL/L EPA 28G oil (Hokkaido Fine Chemicals Co., Ltd., Japan) at 16°C for 18 h prior to feeding. *Artemia* nauplii (Utah strain), enriched with 1.5 mL/L of a commercial emulsion of n-3 polyunsaturated fatty acids (Hyper Glos; Marinetech Co., Ltd., Japan) at 22°C for 24 h, were given to megalopae at a density of 5 individuals/mL. Rearing water was not aerated. Baths were covered with styrofoam boards in order to stabilize water temperature. Each morning, larvae were transferred to newly prepared beakers with new seawater (salinity, ~33‰) and food using a large-mouthed pipette and the survivors were counted. Larval developmental stages were confirmed by unaided eye. Dead larvae were removed from the rearing beakers, and their larval stages were recorded. After transferring the larvae, 20 mg/L dihydrostreptomycin sulphate (Tamura-seiyaku Co., Japan) was added to the rearing water to prevent bacterial attachment to the larvae. Larval rearing was terminated when all surviving larvae molted to the megalopal or crab stages or died.

2.1.3.3 Statistical analyses

In the experiment from first zoeae to megalopae, differences in the mean values of survival rates ($n = 3$) were tested between temperature groups by one-way ANOVA and Tukey's post-hoc test. In the experiment from megalopae to first crab instar, differences in the total number of megalopae to reach crabs from 6 rearing beakers between temperature groups were tested by the χ^2 test and Tukey's post-hoc test.

The relationships between mean temperature and the mean number of days from hatching to reach second zoeae and megalopae, and that from megalopae to reach first crab instar were fitted with the following equation: $D = a / (T - b)$, where D is the time (in days) of each developmental stage, T is the mean value of temperatures in each developmental stage, and a and b are the so-called "thermal constant" and "threshold temperature constant" for development, respectively. This equation, known as Réaumur's Law, is part of the theory of heat summation (Kiritani, 1997, 2012; Hamasaki, 2003; Hamasaki et al., 2009). The thermal constant (day-degrees) is the summation of the effective temperature for development (>threshold temperature) up to a selected biological endpoint.

Table 2.1.1. Survival rates and number of days from hatching to reach second zoeae and megalopae of the snow crab *Chionoecetes opilio*, reared at 9 different temperatures.

Temperature (°C)	Survival rate (%)		Days			
	To second zoea	Megalopa	To second zoea		To megalopa	
1.4 ± 0.1	0.0[c]	0.0[c]				
3.0 ± 0.1	0.0[c]	0.0[c]				
5.0 ± 0.1	86.7 ± 10.4[a]	80.0 ± 8.7[a]	51.7 ± 3.5[a]	(46 – 59)	105.2 ± 4.9[a]	(96 – 120)
8.0 ± 0.1	90.0 ± 5.0[a]	78.3 ± 14.4[ab]	34.8 ± 2.9[b]	(30 – 41)	71.4 ± 4.1[b]	(65 – 85)
11.0 ± 0.2	91.7 ± 7.6[a]	83.3 ± 2.9[a]	21.0 ± 1.2[c]	(19 – 25)	48.6 ± 2.1[c]	(44 – 54)
14.0 ± 0.2	98.3 ± 2.9[a]	78.3 ± 10.4[ab]	15.3 ± 0.6[d]	(14 – 17)	34.1 ± 1.0[d]	(32 – 37)
16.0 ± 0.1	88.3 ± 10.4[a]	45.0 ± 25.0[b]	14.4 ± 0.7[d]	(13 – 15)	31.3 ± 1.2[e]	(30 – 34)
18.0 ± 0.2	38.3 ± 15.3[b]	0.0[c]	12.9 ± 0.5[e]	(12 – 14)		
19.9 ± 0.1	0.0[c]	0.0[c]				

Each value is the mean ± SD of 3 replicates (1 replicate is the mean value of all surviving zoeae per beaker). Values in parentheses are day ranges. Values with different superscript letters in the same column are significantly different ($P < 0.05$).

All data analyses were performed using R version 2.15.1. The level of significance was set at $P < 0.05$ for all statistical analysis.

2.1.4 Results

The first zoeae did not molt to second zoeae at 1.4°C, 3.0°C, or 19.9°C (Fig. 2.1.1, Table 2.1.1). The survival rates to second zoeae were significantly higher at 5.0–16.0°C (>86%) than at 18.0°C (38.3%). The second zoeae did not molt to megalopae at 18.0°C. The survival rate to megalopae reared at 16.0°C tended to be lower (45.0%) than those of larvae reared at 5.0–14.0°C (>78%). Megalopae molted to first crab instar at 1.0–17.0°C but not at 18.1°C (Fig. 2.1.2, Table 2.1.2). The survival rates of megalopae reared at 3.0–16.0°C did not differ significantly, but higher survival rates were observed at 5.0–14.0°C (>77%).

The mean number of days from hatching to reach second zoeae and megalopae, and from megalopae to reach first crab instar decreased significantly with increasing temperature (Tables 2.1.1, 2.1.2). Larvae took ~13–52 days to reach second zoeae at 5.0–18.0°C and 31–105 days to reach megalopae at

5.0–16.0°C. The duration of the megalopal stage ranged from 23–129 days at 1.0–17.0°C. Therefore,

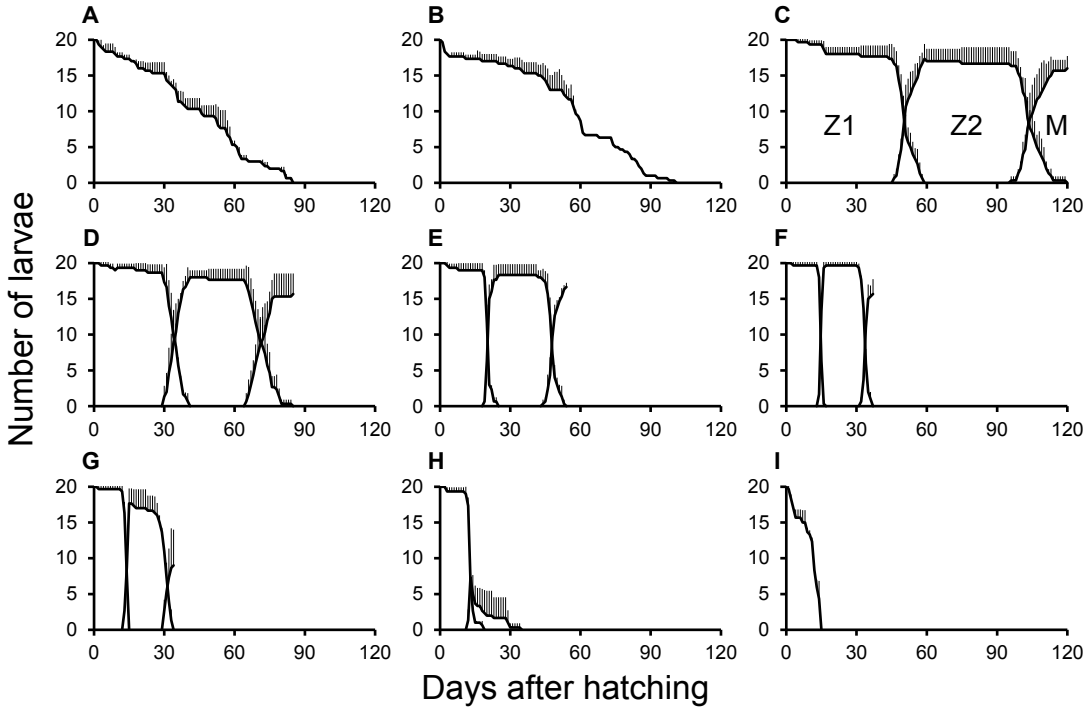

Figure 2.1.1. (A–I) Changes in the number of larvae survived from first zoeae to megalopae with respect to days after hatching of snow crab *Chionoecetes opilio* reared at 9 constant temperatures (n = 3): 1.4°C (A), 3.0°C (B), 5.0°C (C), 8.0°C (D), 11.0°C (E), 14.0°C (F), 16.0°C (G), 18.0°C (H), and 19.9°C (I). Z1 and Z2 are first and second zoeal stages, respectively. M, megalopal stage. Vertical bars indicate SDs.

the entire mean larval period extends from 68–213 days at 5–16°C. The heat summation theory equation was well fitted to the relationship between mean temperature and the mean number of days to reach each stage (Fig. 2.1.3). The threshold temperatures for larval development, i.e., parameter b in the equation, were estimated to be −2.24–0.63°C (Table 2.1.3) and decreased with the progression of larval development.

2.1.5 Discussion

Four studies have conducted rearing experiments of snow crab larvae at different temperatures (Kon, 1970; Davidson & Chin, 1991; Charmantier & Charmantier-Daures, 1995; Kogane et al., 2005).

Table 2.1.2. Survival rates and number of days from megalopae to reach first crab instar of the snow crab *Chionoecetes opilio*, reared at 10 different temperatures.

Temperature (°C)	Survival rate (%) to first crab instar	Days to first crab instar	
1.0 ± 0.1	22.2^{cd}	128.5 ± 11.1^{a}	(116 – 143)
3.0 ± 0.2	61.1^{abc}	77.3 ± 4.9^{b}	(66 – 84)
5.0 ± 0.1	94.4^{ab}	56.2 ± 4.2^{c}	(49 – 65)
8.0 ± 0.1	100.0^{a}	42.1 ± 3.4^{d}	(37 – 49)
11.0 ± 0.2	100.0^{a}	32.9 ± 2.3^{e}	(29 – 38)
14.0 ± 0.2	77.8^{ab}	25.9 ± 1.8^{f}	(23 – 29)
15.0 ± 0.1	66.7^{abc}	24.3 ± 2.2^{f}	(21 – 28)
16.0 ± 0.1	61.1^{abc}	23.1 ± 2.8^{f}	(19 – 27)
17.0 ± 0.1	50.0^{bc}	23.4 ± 1.8^{f}	(21 – 26)
18.1 ± 0.2	0.0^{d}		

Values in parentheses are day ranges.
Values with different superscript letters in the same column are significantly different ($P < 0.05$).

Table 2.1.3. Estimates of the parameters (with SEs) of the relationship between mean temperature (T) and the mean number of days required to reach each larval stage (D) of the snow crab *Chionoecetes opilio*.

Period	*n*	*a*	*b*
Z1–Z2	18	229.5 (11.05)*	0.63 (0.26)*
Z1–M	15	530.5 (22.38)*	–0.02 (0.26)^{NS}
M–C1	9	417.3 (0.09)*	–2.24 (0.09)*

Heat summation theory equation: $D = a / (T - b)$, where D is days required to reach each larval stage, T is mean temperature, and a and b are the so-called ‘‘thermal constant’’ and ‘‘threshold temperature constant’’ for development, respectively. H_0, $b = 0$; * $P < 0.05$; not significant (NS), $P > 0.05$. The SEs of each estimated parameter are shown in parentheses.

Kon (1970) reared snow crab zoeae at ~5–18°C and concluded the appropriate temperature for zoeal survival is ~7–15°C; he reared megalopae at ~10–15°C but could not evaluate the appropriate temperature for their survival because of a small number of cultured animals. Davidson & Chin (1991) and

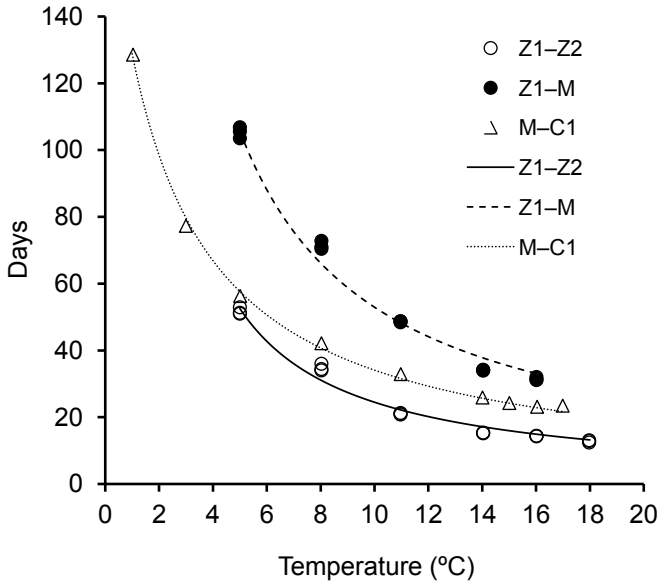

Figure 2.1.3. Relationships between mean water temperatures and the mean number of days of developmental period from first to second zoeae (Z1–Z2), first zoeae to megalopae (Z1–M), and megalopae to first crab instar (M–C1) of the snow crab *Chionoecetes opilio*. The curves were drawn from the equations of heat summation theory (see Table 2.1.3) applied to the relationship between the 2 variables.

Charmantier & Charmantier-Daures (1995) reared snow crab zoeae at ~8–14°C but do not mention the appropriate temperature for zoeal survival, because the survival rates to megalopal stage were 0–3%. Kogane et al. (2005) also tested the influence of temperature on the survival of snow crab larvae from zoeae through megalopae to first crab instar at ~10–16°C; they concluded the appropriate temperature for larval survival is ~10–14°C. Thus, although Kon (1970) and Kogane et al. (2005) provided the preliminary information about the appropriate temperature range for the survival of snow crab larvae, they did not investigate temperatures <5°C. The present study covered wide ranges of temperatures—~1–20°C and ~1–18°C—for culturing snow crab zoeae and megalopae, respectively. The present results demonstrate first zoeae are unable to molt to the next stage at ~1–3°C. Furthermore, the survival rates to second zoeae and megalopae are higher at ~5–16°C and ~5–14°C, respectively. Although megalopae were able to molt to first crab instar even at ~1–3°C, the temperature ranges in which survival rates were higher (~5–14°C) can be considered the appropriate temperature range for megalopae survival.

The developmental periods of snow crab larvae decreased with increasing temperatures, as is the case for many decapod crustaceans (Anger, 2001). Kogane et al. (2005) report the mean number of

days required to reach second zoeae from hatching at 10.1°C, 12.0°C, 13.9°C, and 15.9°C to be 22.5, 18.4, 15.3, and 13.3 days, respectively, and 48.7, 39.1, 32.4, and 27.3 days to reach megalopae, respectively. These values are generally concordant with the present results.

The Sea of Japan contains 2 snow crab species: *C. opilio* and *C. japonicus*. However, their larval distributions have been investigated without distinguishing species because of the morphologically similarity of the larvae. However, these records should represent the larval distribution of *C. opilio*, because the hatching seasons of both species overlap (Kon et al., 2003). The vertical distribution range of larvae of snow crabs tends to deepen with larval development, and water temperatures decrease with increasing depth in the Sea of Japan (Kon et al., 2003). Therefore, the decreasing threshold temperatures with the progression of larval development are considered to be an adaptation for shifting the vertical distribution of larvae in the water column.

Kon et al. (2003) intensively examined the vertical distribution of snow crab larvae using multiple opening/closing plankton nets and an environmental sensing system off Wakasa Bay in the Sea of Japan. They found that the first zoeae mainly occurred in strata from 0–100 m, where the temperature ranges from 8.4–12.4°C in March (average, 11.0°C); second zoeae occurred in strata 0–150 m deeper than the first zoeae, where the temperature ranges from 11.0–15.0°C in April (average, 12.5°C); and megalopae occurred in strata 50–200 m deeper than the second zoeae, where temperature ranges from 6.8–13.9°C in April (average, 10.8°C) and May (average, 9.8°C). Furthermore, some megalopae migrate downward for molting to 200–400 m, where the temperature decreases to 0.8–8.6°C (average, 3.3°C). Thus, snow crab zoeae and megalopae are generally distributed within the appropriate temperature ranges for survival (5–14°C) and molting (1–17°C). Kon et al. (2003) estimate the total duration of larval stages to be ~100 days (i.e., ~40 and ~60 days in the zoeal and megalopal stages, respectively) on the basis of the time lags in the occurrence of peak abundance between each larval stage. From the heat summation theory equations (Fig. 2.1.3, Table 2.1.3) and the average water temperatures experienced by zoeae (11.0–12.5°C) and megalopae (3.3–10.8°C) (Kon et al., 2003), the larval durations of snow crab were estimated to be ~42.4–48.1 and ~32.0–75.3 days for the zoeal and megalopal stages,

respectively. Therefore, the range of larval duration from hatching to the first crab instar was estimated to be 74.4–123.4 days (average 98.9 days), which is similar to that in nature.

The present study demonstrates water temperature greatly influences the survival and developmental rates of snow crab larvae. Appropriate temperatures for larval survival are important information for inferring larval distribution of snow crab in their natural habitats. Furthermore, the present results are important for understanding the potential effects of climate change on the snow crab population in the Sea of Japan, which is the southern limit of the distribution of this species.

2.2 Effects of salinity on snow crab *Chionoecetes opilio* larval survival and development under laboratory conditions

2.2.1 Summary

To better understand the factors influencing larval dispersal and settlement of the snow crab *Chionoecetes opilio* in its natural habitats, we tested the effects of salinities ranging from 18–38 and 20–38 on the survival and developmental duration of snow crab larvae in the zoeal and megalopal stages, respectively. Survivals to second-stage zoeae and to megalopae were highest at salinities of 20–38 and 26–38, respectively. There were no significant differences in survival among megalopae reared at salinities between 24 and 38, although survival tended to be higher at salinities range 28–36. The mean periods from hatching to the second zoeal and megalopal stages, and from the megalopal to first crab stage, were shortest at salinities of 30, 30, and 32, respectively, and progressively increased at salinities above and below these values.

2.2.2 Introduction

The pelagic phase of the crustacean life history plays an important role in sustaining populations by facilitating larval dispersal and recruitment (e.g., Sulkin, 1984; Anger, 2001, 2006). The larvae exhibit rapid growth and undergo morphogenetic changes in metamorphosis into the megalopal stage. This developmental process is influenced by environmental factors such as water temperature, salinity, food availability and quality, predation, and ocean currents (e.g., Sulkin, 1984; Anger, 2001; Forward, 2009).

Snow crab *Chionoecetes opilio* is one of the five species belonging to the genus *Chionoecetes* (Brachyura, Oregoniidae); it occurs on the continental shelf throughout the cold waters of the northern hemisphere and is an important fishery resource (e.g., Elner, 1982; Sinoda, 1982; Jadamec et al., 1999; Azuma et al., 2011). A number of researchers have studied the general biology of snow crab larvae with a focus on managing stocks or evaluating the mechanism underlying fluctuations in stock abun-

dance (e.g., Kon, 1980; Davidson & Chin, 1991; Incze et al., 1984; Lovrich & Ouellet, 1994). The entire duration of the larval period from hatching to first crab stage ranges from 74 d to 123 d (~2.5–4 months) and is dependent on water temperature (Yamamoto et al., 2014).

Other environmental factors may also influence survival and larval development in the snow crab. For example, salinity affects the duration of larval developmental and larval survival rates in many decapod crustaceans (Anger, 2003). Snow crab larvae are typically found in environments that do not experience large salinity fluctuations, including the Sea of Japan and the southeastern Bering Sea (Kon, 1980; Incze et al., 1987). Nevertheless, snow crab zoeae are also found at salinities ranging from 24–32 in the Gulf of Sainte Lawrence (Conan et al., 1996) and in very shallow water (2.5–3 m) (Saint-Marie & Dufour, 1988; Lovrich et al., 1995), where salinity might fluctuate. To date, two studies have evaluated the effect of salinity on larval survival and development in the snow crab (Kon, 1973; Charmantier & Charmantier-Daures, 1995). The analysis by Kon (1973) included data from larvae that died during or immediately after molting, thereby making it difficult to draw accurate conclusions concerning the effect of salinity on survival or on the duration of the developmental periods. Additionally, Charmantier & Charmantier-Daures (1995) only determined the lethal salinity for first-stage zoeae. Our objective was to evaluate the effects of salinity on the survival and development of snow crab larvae during the entire period of larval development.

2.2.3 Material and Methods

2.2.3.1 Source of larvae

A total of 185 ovigerous females were caught in November 2011 in the Sea of Japan off Ishikawa Prefecture, Japan. They were transferred to the Obama Laboratory at the Japan Sea National Fisheries Research Institute, Fisheries Research Agency, Fukui Prefecture, and reared at 3°C in one 4-kL (1.3 × 3.9 × 0.85 m) rectangular tank with recirculating seawater (salinity ~33), as described by Morita & Nogami (2003). Females were fed frozen Antarctic krill *Euphausia superba* twice weekly. The main

hatching season was from February to March 2012; often several females released their larvae on the same day.

2.2.3.2 Larval rearing experiments

Larval rearing experiments were conducted during the zoeal stages (i.e. from hatching to metamorphosis to the megalopa) and during the megalopal stage (i.e. from metamorphosis to the molt to the first crab stage). The first zoeae hatched on February 9, 2012. Megalopae that metamorphosed on March 16, 2012 from zoeae that hatched on February 9 and 10, 2012 were used in experiments. Larval culture was carried out in 1-L plastic beakers that were placed in temperature-controlled baths. The water temperature was maintained at 11°C during the zoeal stage and 8°C during the megalopal stage. These temperatures were chosen based on the thermal distribution in the natural habitat (Kon et al., 2003) and the optimum temperatures for larval survival (Yamamoto et al., 2014). Zoeae were reared at 11 experimental salinities: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, and 38 (three beakers per concentration, 20 individuals per beaker). Megalopae were reared at 10 salinities: 20, 22, 24, 26, 28, 30, 32, 34, 36, and 38 (five beakers per concentration, four individuals per beaker). Diluted seawater was prepared by adding freshwater to seawater, and high-salinity water was prepared by adding synthetic seawater salt (Instant Ocean, Napqo Ltd., Tokyo, Japan) to seawater. Salinity was measured using a multiparameter water-quality meter (556MPS, YSI Inc., Yellow Springs, OH, USA). Larvae were transferred directly from the holding tank (salinity ~33) to the experimental salinity at the beginning of the experiment. Larvae were cultured at 14°C and salinity 33 to prepare test animals for the megalopal experiment.

Larval culture was based on the method of Yamamoto et al. (2014). Zoeae were fed rotifers *Brachionus plicatilis* at a density of 20 individuals/mL. Rotifers were enriched with 0.5 mL/L commercial condensed marine phytoplankton (*Nannochloropsis* sp., Mercian Co., Ltd., Tokyo, Japan), 14 µL/L emulsified DHA 70G oil, and 28 µL/L EPA 28G oil (Hokkaido Fine Chemicals Co., Ltd., Hokkaido, Japan) at 16°C for 18 h prior to feeding. *Artemia* nauplii (Utah strain) were enriched with 1.5 mL/L of a commercial emulsion of n-3 polyunsaturated fatty acids (Hyper Glos, Marinetech Co., Ltd., Aichi,

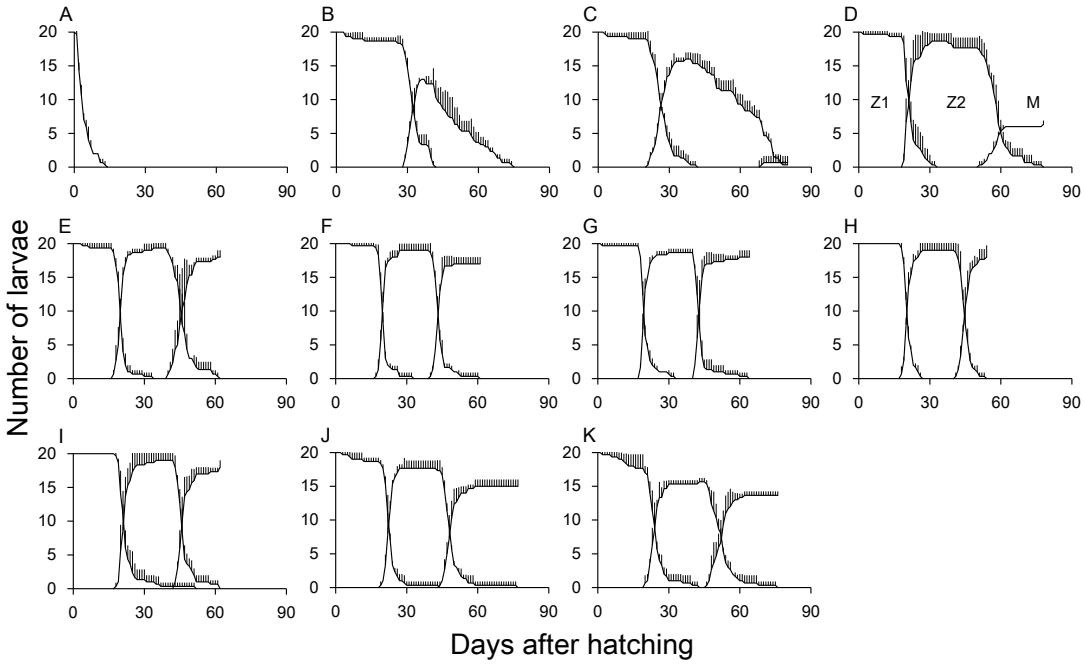

Figure 2.2.1 (A–K) Changes in the number of larvae surviving from the first zoeal to megalopal stage with respect to the number of days after hatching of the snow crab *Chionoecetes opilio*, reared at 11 constant salinities: 18.0 (A), 20.0 (B), 22.0 (C), 24.0 (D), 26.0 (E), 28.0 (F), 30.0 (G), 32.0 (H), 34.0 (I), 36.0 (J), and 38.0 (K). Z1 and Z2 represent the first and second zoeal stages, respectively. M is the megalopal stage. Vertical bars indicate SD (n = 3).

Japan) at 22°C for 24 h then given to megalopae at a density of five individuals/mL. The rearing water was not aerated. Baths were covered with Styrofoam boards to stabilize the water temperature. Each morning, larvae were transferred to newly prepared beakers with fresh experimental seawater and food, using a large-mouthed pipette, and the survivors were counted. Larval developmental stages were confirmed by visual examination with the unaided eye. Dead larvae were removed from the rearing beakers, and their developmental stage was recorded. After each transfer of larvae, 20 mg/L dihydrostreptomycin sulfate (Tamura-seiyaku Co., Ltd., Tokyo, Japan) was added to the rearing water to prevent bacterial attachment to the larvae. Larval rearing was terminated when all larvae had molted to the megalopal or crab stage, or had died.

2.2.3.3 Statistical analyses

Table 2.2.1. Survival rates and number of days from hatching to the second zoeal stage and the megalopal stage in snow crab *Chionoecetes opilio* larvae reared at 11 salinities.

	Survival rate (%)		Days			
Salinity	To second zoea	To megalopa	To second zoea		To megalopa	
18.0	0^{a}					
20.0	76.7 ± 2.9^{b}	0^{a}	33.0 ± 3.1^{f}	29 – 41		
22.0	85.0 ± 5.0^{b}	3.3 ± 2.9^{a}	27.2 ± 3.5^{e}	21 – 37	69.5^{f}	69 – 70
24.0	93.3 ± 5.8^{b}	31.7 ± 2.9^{b}	22.2 ± 2.8^{bc}	19 – 31	58.4 ± 5.5^{e}	51 – 78
26.0	95.0 ± 5.0^{b}	93.3 ± 7.6^{cde}	20.5 ± 2.0^{a}	17 – 30	47.0 ± 4.9^{bc}	40 – 62
28.0	93.3 ± 2.9^{b}	88.3 ± 2.9^{cde}	20.4 ± 1.8^{a}	17 – 27	44.3 ± 3.7^{ab}	40 – 61
30.0	93.3 ± 2.9^{b}	88.3 ± 2.9^{cde}	20.3 ± 2.1^{a}	18 – 30	43.3 ± 2.1^{a}	41 – 54
32.0	95.0 ± 8.7^{b}	90.0 ± 8.7^{cde}	21.1 ± 1.9^{ab}	18 – 27	45.4 ± 2.5^{ab}	41 – 54
34.0	95.0 ± 8.7^{b}	86.7 ± 2.9^{cde}	22.2 ± 2.8^{bc}	18 – 35	46.7 ± 2.8^{bc}	43 – 58
36.0	91.7 ± 2.9^{b}	75.0 ± 5.0^{cd}	23.1 ± 2.1^{cd}	19 – 30	48.9 ± 3.0^{cd}	44 – 59
38.0	81.7 ± 7.6^{b}	68.3 ± 2.9^{c}	24.7 ± 3.5^{d}	20 – 43	52.2 ± 3.6^{d}	46 – 62

Each value is the mean ±SD of three replicates (one replicate is the mean value of all surviving zoeae per beaker). Values in parentheses represent the range. Values with different superscript letters in the same column are significantly different ($P < 0.05$).

We tested for differences between the salinity treatments in the mean survival rate and the number of days ($n = 3$) from hatching to the second zoeal stage and to the megalopal stage, using one-way ANOVA and Tukey's post-hoc test. In the calculations of survival rates and the mean number of days from megalopal to crab stage, data for the individual larvae in each treatment group (five beakers) were pooled because of the small number of larvae reared in each beaker. We tested for differences between salinity treatments in the total number of individuals developing from megalopal to crab stage using a χ^2 test and Tukey's test. One-way ANOVA and Tukey's test were employed to test for differences in the mean period from the megalopa to the crab stage. All statistical analyses were performed in R (R3.1.1; R Core Team, 2014) with a 5% significance level.

2.2.4 Results

The first-stage zoeae reared at salinity 18 did not molt to second-stage zoeae (Fig. 2.2.1, Table 2.2.1). Survival to the second zoeal stage was significantly higher at salinities 20–38 (>77%) than at salinity 18 (0%). Second-stage zoeae did not molt to megalopae at salinity 20. The survival to mega-

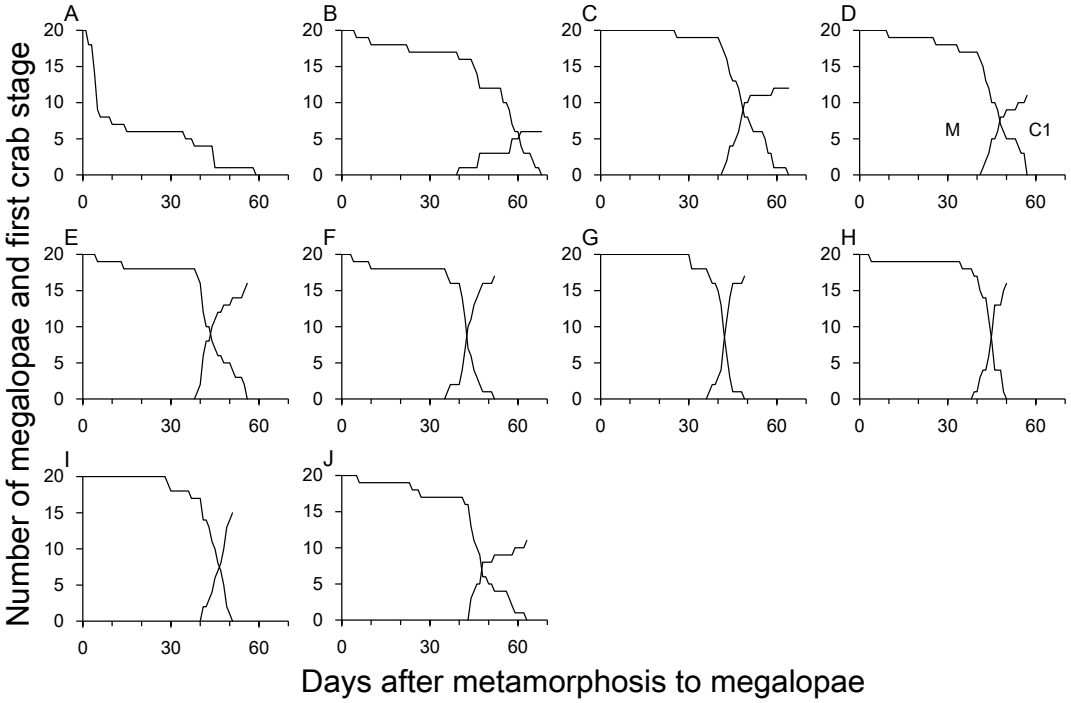

Figure 2.2.2 (A–J) Changes in the number of larvae surviving from the megalopal to first crab stage with respect to the number of days after metamorphosis to megalopa in the snow crab *Chionoecetes opilio*. Larvae were reared at 10 constant salinities: 20.0 (A), 22.0 (B), 24.0 (C), 26.0 (D), 28.0 (E), 30.0 (F), 32.0 (G), 34.0 (H), 36.0 (I), and 38.0 (J). M and C1 refer to the megalopal stage and first crab stage, respectively.

lopae tended to be lower at salinities 20–24 (<32%) than for larvae reared at salinities 26–38 (>68%). Megalopae molted to the first crab stage at salinities 22–38 but not at salinity 20 (Fig. 2.2.2, Table 2.2.2). The survival rates of megalopae reared at salinities 24–38 did not differ significantly among treatments, although survival tended to be higher at salinities 28–36 (>75%).

The mean duration of the periods from hatching to the second zoeal stage, and from hatching to the megalopal stage, were both shortest at salinity 30. The period from the megalopa to the first crab stage was shortest at salinity 32 (Tables 2.2.1, 2.2.2). Larvae took 20–33 d to reach the second zoeal stage at salinities 20–38 and 43–70 d to reach the megalopal stage at salinities 22–38. The duration of the megalopal stage ranged from 43–52 d at salinities 22–38. Thus, the total duration of the larval period ranged from 86–122 d at salinities 22–38.

Table 2.2.2. Survival rates and number of days to develop from the megalopal to first crab stage in the snow crab *Chionoecetes opilio* reared at 10 salinities.

Salinity	Survival rate (%)	Days	
20.0	0.0^{a}		
22.0	30.0^{b}	51.8 ± 8.3^{c}	40 – 61
24.0	60.0^{bc}	47.5 ± 4.5^{abc}	42 – 59
26.0	55.0^{bc}	47.5 ± 4.6^{abc}	42 – 57
28.0	80.0^{c}	44.8 ± 5.2^{ab}	39 – 56
30.0	85.0^{c}	43.4 ± 3.8^{a}	36 – 52
32.0	85.0^{c}	42.6 ± 2.8^{a}	37 – 49
34.0	75.0^{bc}	44.6 ± 2.9^{ab}	39 – 50
36.0	75.0^{bc}	46.5 ± 3.1^{abc}	41 – 51
38.0	55.0^{bc}	49.2 ± 6.4^{bc}	44 – 63

Each value for number of days is the mean ± SD of individual larvae in each salinity group. Values in parentheses represent the range. Values with different superscript letters in the same column are significantly different ($P < 0.05$).

2.2.5 Discussion

We tested the effect of a wide range of salinities (18–38 for zoeae and 20–38 for megalopae) and demonstrated that salinity influenced the survival and developmental rates of snow crab larvae. Kon (1973) reared snow crab zoeae in seawater with specific gravities ranging from 1.015–1.030 (salinities 19.8–39.1, calculated from Millero et al., 1980) and concluded that the optimal salinities for molting from the first to second zoeal stage and from the second zoeal to megalopal stage were 1.022–1.027 (salinities 28.9–35.3) and 1.019–1.026 (salinities 25.0–34.0), respectively. This author also reared megalopae at specific gravities ranging from 1.016–1.029 (salinities 21.1–37.9) and noted that the molting rate from megalopa to first crab stage increased with decreasing salinity. Nevertheless, the analysis in this early study was confounded by including data from larvae that died during or immediately after molting. Despite this, the results are generally consistent with our observations. Charman-

tier & Charmantier-Daures (1995) evaluated the salinity tolerance of snow crab first-stage zoeae and concluded that the lower and upper median lethal salinities were ~10 and ~42 at 24 h, 18 and ~41 at 48 h, and 22–25 and ~38 at 96 h, respectively. In the current study, we did not test the effect of salinity levels below 10 or above 38. Nevertheless, the survival rates at the lower and upper median lethal salinities reported by Charmantier & Charmantier-Daures (1995) were lower than in our study (i.e. survival rates for first-stage zoeae were 98.5% at salinity 18 after 48 h, 96.5–98.5% at salinities 22–26, and 98.5% at salinity 38 after 96 h). In the current study, larvae were transferred directly from seawater (salinity ~33) to the appropriate test concentration at the beginning of the rearing experiments. Generally, survival rates are lower and development times are longer for larvae that are directly transferred compared with larvae that are gradually acclimated to test conditions (e.g., Baylon & Suzuki, 2007; Baylon, 2010). Thus, our results should be interpreted with this in mind.

Zoeae and megalopae were unable to molt to the next stage when reared at salinities 18 and 20. Furthermore, the range of salinities associated with high survival became narrower during larval development; i.e. the rates of survival to the second zoeal, megalopal, and first crab stages were high at salinities of 20–38, 26–38, and 28–36, respectively. This phenomenon is consistent with observations in other decapod crustacean species, including the mud crab *Rhithropanopeus harrisii* (Costlow et al., 1966), the red king crab *Paralithodes camtschaticus* (Kurata, 1960), the fiddler crab *Uca subcylindrica* (Rabalalis & Cameron, 1985), the Chinese mitten crab *Eriocheir sinensis* (Anger, 1991), the red frog crab *Ranina ranina* (Minagawa, 1992), the grapsid crab *Armases miersii* (Anger, 1996), the mangrove crab *Sesarma curacaoense* (Anger & Charmantier, 2000), the crucifix crab *Charybdis feriatus* (Baylon & Suzuki, 2007), the mud crab *Scylla serrata* (Baylon, 2010; Dan et al., 2011), and the horsehair crab *Erimacrus isenbeckii* (Jinbo et al., 2012).

The Sea of Japan contains two *Chionoecetes* species, *C. opilio* and *C. japonicus*, whereas the southeastern Bering Sea primarily contains *C. opilio* and *C. bairdi* (*C. angulatus* and *C. tanneri* are also found but are rare). The distribution of *Chionoecetes* larvae has been documented without distinguishing between these species because of their morphological similarity (Incze, 1981; Incze et al.,

1987; Kon et al., 2003). Snow crab zoeae occur at salinities 24–32 in the Gulf of St. Lawrence (Conan et al., 1996). In the Sea of Japan, snow crab larvae (including *C. opilio* and *C. japonicus*) are found from early spring to early summer at 0–400-m depths (Kon et al., 2003). In this season, the salinities range from ~33–34.5 at these depths (Naganuma, 2000). In the southeastern Bering Sea, zoeae (only *C. opilio*) are found primarily from April to June in the upper 40 m and megalopae (*C. opilio* and *C. bairdi*) are present from July to September in the upper 60 m (Incze, 1981; Incze et al., 1987). The surface salinity is ~32 in April (Incze et al., 1987). Thus, the salinity in these regions matches the optimum salinity range for survival of snow crab larvae in our study. Moreover, the lower limit of the vertical distribution of *Chionoecetes* larvae becomes deeper during development (Incze, 1981; Conan et al., 1996; Kon et al. 2003). Thus, the decrease in the optimum salinity range for survival with the progression of larval development may be an adaptation to the shift in vertical distribution of larvae in the water column.

The periods from hatching to the second zoeal stage, from hatching to the megalopal stage, and from the megalopal to the first crab stage were shortest at salinities 30, 30, and 32, respectively, and increased outside these salinities. This appears to be a general phenomenon in decapod crustaceans and has been reported in several species, including *Rhithropanopeus harrisii* (Costlow et al., 1966), the southern king crab *Lithodes antarcticus* (Vinuesa et al., 1985), *Eriocheir sinensis* (Anger, 1991), *Ranina ranina* (Minagawa, 1992), the giant spider crab, *Macrocheira kaempferi* (Okamoto, 1995), *A. miersii* (Anger, 1996), *Sesarma curacaoense* (Anger & Charmantier, 2000), and *Erimacrus isenbeckii* (Jinbo et al., 2012). Kon (1973) reported that the minimum intermolt periods for the first and second zoeal stages and the megalopal stage were 210 day-degrees (~19 d at 11°C) at specific gravities of 1.022–1.024 (salinities 29–31), 210 day-degrees (~19 d at 11°C) at 1.020–1.022 (salinities 26–29), and 390 day-degrees (~35 d at 11°C), respectively. These values are consistent with our observations in the current study.

The effect of salinity on larval survival is important information for inferring larval distribution in natural habitats. Such information could also be incorporated into biophysical modeling (Parada et al.,

2010; Szuwalski & Punt, 2013; Mullowney et al., 2014) to more accurately infer the larval distribution and transport of snow crabs in their natural habitat.

Chapter 3

FOOD CONSUMPTION PATTERN IN MEGALOPAE

3.1 Food consumption pattern in snow crab *Chionoecetes opilio* (Decapoda, Majoidea) megalopae under laboratory conditions

3.1.1 Summary

The food consumption pattern in megalopae of the snow crab *Chionoecetes opilio* was investigated in the laboratory. Ten megalopae were individually cultured and given an excess of *Artemia* nauplii each day. All megalopae moulted into first-instar crabs 28–34 days after metamorphosis. The mean total number and total weight of *Artemia* consumed during the megalopal stage were 1920 individuals and 5.2 mg, respectively. Hence, the food requirement of snow crab megalopae was estimated as ~190% of the dry body weight of the first-instar crab. Initially, the number of *Artemia* consumed was nearly constant or decreased only slightly but, later, *Artemia* consumption decreased with development days. Two-segmented regressions provided good fits to the relationship between the number of days after metamorphosis and the cumulative number of *Artemia* consumed by individual megalopae. The mean value of the time after metamorphosis of the breakpoint in the rate of the food consumption was estimated as 69% of the stage duration, which corresponds to the intermediate of late premoult. Crab size (carapace width, wet and dry body weight) were not significantly dependent on the number of *Artemia* consumed during the entire megalopal period although a positive correlation between these variables was observed. These results provide useful information on the appropriate feeding schedule and management practice for culturing snow crab megalopae and contribute to understanding of megalopal growth efficiencies to the first-instar crab in their natural habitat.

3.1.2 Introduction

The snow crab *Chionoecetes opilio* (Fabricius, 1788) (Brachyura, Majoidea) is widely distributed on muddy and sandy-mud grounds at depths between 3 m and 1400 m in cold waters in the Northern Hemisphere (e.g., Squires, 1990; Yosho & Hayashi, 1994; Lovrich et al., 1995; Dawe & Colbourne, 2002) 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).

The pelagic larval phase generally plays an important role in sustaining the population by facilitating larval dispersal and recruitment (Sulkin, 1984; Anger, 2001, 2006). The survival and development of pelagic larvae are influenced by many environmental factors, including water temperature, salinity, food availability and quality, predation, and ocean currents (Sulkin, 1984; Anger, 2001; Forward, 2009). Among these factors, the effects of water temperature and salinity on survival and development of snow crab larvae have been studied (Yamamoto et al., 2014, 2015b). Food availability and quality are also considered to be important factors influencing snow crab larval survival and development. The food consumption of larval snow crabs has been studied in the laboratory (Paul et al., 1979; Harada & Yamamoto, 2000, 2006; Kogane et al., 2009, 2010).

Paul et al. (1979) examined the relationship between prey concentration and the food consumption of first-stage zoeae of snow crab, using copepods. Harada & Yamamoto (2000) examined the relationship between prey size and the food consumption of first- and second-stage zoeae and megalopae, using rotifers, newly hatched *Artemia* nauplii and cultured *Artemia*. However, these authors examined megalopal food consumption for only 52–59 days after hatching. Harada & Yamamoto (2006) studied effective diets for second-stage zoeae using rotifers, *Artemia* nauplii and cultured *Artemia*. Kogane et al. (2009, 2010) examined the optimal prey density and feeding schedule, and the effects of varying levels of dietary n-3 highly unsaturated fatty acids and the ratios of docosahexaenoic acid / eicosapentaenoic acid, throughout the whole zoeal stage.

Food consumption, however, has not been studied for the entire period of the snow crab megalopal stage. Therefore, the present study aimed to elucidate the pattern of food consumption of snow crab megalopae by examining daily *Artemia* intake before moulting to first-instar crabs in the laboratory.

3.1.3 Material and methods

3.1.3.1 Larval source

Laboratory-born snow crab megalopae used in this study were obtained as newly hatched first-stage zoeae from broodstock females collected from the Sea of Japan in 2009. They were cultured from zoeae to megalopae at the Obama Laboratory, Japan Sea National Fisheries Research Institute, Fisheries Research Agency, Fukui Prefecture, Japan. Zoeae were reared using a 20-kL tank at 14°C according to the method of Kogane et al. (2007a). Second-stage zoeae just prior to metamorphosing into megalopae were collected from the 20-kL tank and stocked in 1-L plastic beakers without feeding. Beakers were placed in a temperature-controlled bath at 10°C.

3.1.3.2 Megalopa culture experiments

Megalopae that metamorphosed on April 10, 2009 from zoeae that hatched on March 3, 2009 were used in experiments. The culture experiments were conducted from April 10, 2009 until the megalopae moulted to first-instar crabs. Immediately after moulting from zoeae to megalopae, 10 megalopae were individually stocked in 200-mL screw-top polystyrene bottles containing 60 mL of filtered sea water. The bottles were placed in an incubator controlled at 10°C (Cool-Incubator A5501; Ikuta-Sangyo Co., Ltd., Japan). This rearing temperature was selected based on the thermal distribution in the natural habitat (Kon et al., 2003) and the optimum temperatures for megalopal survival (Yamamoto et al., 2014). Larval culture was based on the method of Yamamoto et al. (2014). Each megalopa was given 200 *Artemia* nauplii (Utah strain) each day. *Artemia* were hatched over 24 h at 28°C and they were enriched with 1.5 mL L–1 of a commercial emulsion of n-3 polyunsaturated fatty acids (Hyper Glos, Marinetech Co., Ltd., Aichi, Japan) at 22°C for 24 h prior to feeding. The larval rearing water was not aerated. Illumination from light-emitting diodes was adjusted to a 12:12 h light-dark cycle with lights on at 06:00. Each morning, megalopae were transferred to newly prepared bottles with fresh experimental seawater and food, using a large-mouthed pipette. After each transfer of larva, 20 mg L–1 dihydrostreptomycin sulphate (Tamura-seiyaku Co., Ltd., Tokyo, Japan) was added to the rearing water to prevent bacterial attachment to the larvae. Numbers of *Artemia* consumed were de-

Table 3.1.1. The total numbers of *Artemia* consumed by individual megalopae and the body sizes of the first-instar crabs of the snow crab *Chionoecetes opilio* (Fabricius, 1788).

	Total *Artemia* consumption			Crab measurements			
No.	Individuals	Dry weight (mg)	Days to crab	Carapace width (mm)	Wet body weight (mg)	Dry body weight (mg)	Dry weight ratio (*Artemia* /crab)
1	2242	6.0	33	2.97	14.5	2.5	2.4
2	2060	5.6	30	3.19	17.2	3.7	1.5
3	1855	5.0	31	3.17	14.3	2.4	2.1
4	1898	5.1	29	2.88	14.0	2.2	2.3
5	1990	5.4	31	2.95	15.3	3.0	1.8
6	2053	5.5	34	2.92	14.4	2.3	2.4
7	1913	5.2	28	2.99	14.5	2.9	1.8
8	1976	5.3	30	2.96	15.3	3.2	1.7
9	1742	4.7	28	3.02	14.1	2.5	1.9
10	1470	4.0	31	2.89	14.3	2.3	1.7
Mean (SD)	1920 (208)	5.2 (0.6)	30.5 (2.0)	2.99 (0.11)	14.8 (1.0)	2.7 (0.5)	1.9 (0.3)

Mean dry weight of *Artemia* per individual: 0.0027 mg.

termined by counting the *Artemia* remaining in each experimental bottle using a stereomicroscope (SMZ1000, Nikon Corp., Tokyo, Japan). After moulting to first-instar crabs, the carapace width (CW) of each animal was measured to the nearest 0.01 mm using the stereomicroscope equipped with a digital photomicrographic camera (DS-Fi1-L2, Nikon Corp., Tokyo, Japan). Each crab was gently blotted on filter paper for wet body weight measurements. After drying for 24 h at 60°C, dry body weight was determined. Wet and dry body weights were measured on a digital analytical balance (model AE163, Mettler Toledo, Greifensee, Switzerland) to the nearest 0.1 mg. To estimate the quantity of food ingested during the megalopal stage on a dry weight basis, the mean dry weight of individual *Artemia* was determined. Three groups of about 500 *Artemia* were dried as described for first-instar crabs.

3.1.3.3 Data analysis

Statistical analyses were performed with the R language (R3.1.3; R Core Team, 2015) at a 5% significance level. Two-segment linear regressions were fitted to the relationship between the number of days after metamorphosis and the cumulative number of *Artemia* consumed (see the Results section) for each larva. To estimate the slopes and the possible breakpoint in the regressions, the 'segmented' package (Muggeo, 2003) was used. The relationship between *Artemia* consumption and crab size was also analyzed by linear regression.

Table 3.1.2. Estimated two-segmented linear equations and breakpoints in the relationships between the cumulative number of Artemia consumed by megalopae (y) and the days after metamorphosing into megalopae (x) of the snow crab *Chionoecetes opilio* (Fabricius, 1788).

No.	Breakpoint (SE)	< Break point	> Break point	Adjusted R square
1	18.6 (0.2)	$y = 97.5\,x + 58.1$	$y = 26.5\,x + 1380.8$	0.9991
2	20.7 (0.3)	$y = 86.8\,x + 78.3$	$y = 22.7\,x + 1407.9$	0.9985
3	22.4 (0.6)	$y = 73.8\,x + 109.7$	$y = 9.3\,x + 1556.2$	0.9932
4	21.5 (0.2)	$y = 78.6\,x + 84.6$	$y = 17.4\,x + 1400.7$	0.9993
5	22.4 (0.3)	$y = 76.2\,x + 138.1$	$y = 18.4\,x + 1432.6$	0.9986
6	23.7 (0.3)	$y = 73.3\,x + 159.9$	$y = 16.3\,x + 1511.6$	0.9989
7	20.6 (0.7)	$y = 75.0\,x + 165.4$	$y = 31.1\,x + 1070.2$	0.9963
8	19.6 (0.4)	$y = 82.3\,x + 89.8$	$y = 30.4\,x + 1107.2$	0.9983
9	18.5 (0.4)	$y = 72.3\,x + 139.1$	$y = 31.1\,x + 900.3$	0.9987
10	7.2 (0.3)	$y = 91.6\,x + 87.3$	$y = 31.8\,x + 520.4$	0.9981

3.1.4 Results

All megalopae moulted to first-instar crabs 28–34 days after metamorphosis (Table 3.1.1, Fig. 3.1.1). The mean (± SD) total number of *Artemia* consumed during the megalopal stage was 1920 (±208). Initially, the daily rate of consumption of *Artemia* varied but was essentially constant or decreased slightly. Later, the *Artemia* consumption tended to decrease with development days. Two-segmented regressions provided good fits to the relationship between the number of days after metamorphosis and the cumulative number of *Artemia* consumed in individuals (Table 3.1.2). The breakpoints in the food consumption rates of the larvae were estimated as 18.5–23.7 days after metamorphosis, which represented 56–74% (mean 69%) of the entire megalopal period, except for one larva with a breakpoint estimate of 7.2 days (~23% of the megalopal period) (Table 3.1.2, Fig. 3.1.2). The crab sizes (carapace width, wet body weight, and dry body weight) were not significantly dependent on the numbers of *Artemia* consumed during the entire megalopal period although positive correlations between these variables were observed (Fig. 3.1.3). The mean dry weight of individual *Artemia* was 0.0027 mg; thus, the mean total weight of *Artemia* consumed during the megalopal stage

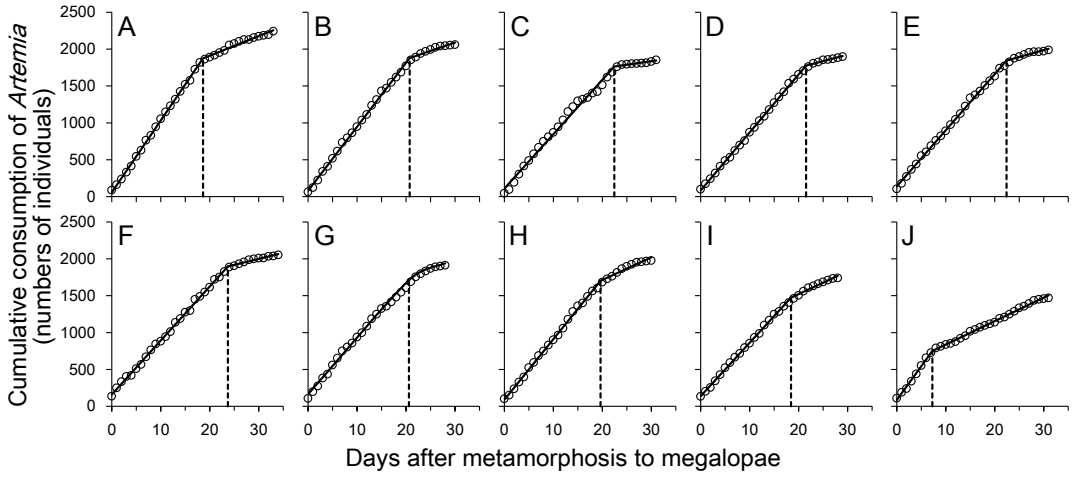

Figure 3.1.2. Changes in the cumulative number of *Artemia* nauplii consumed by ten individual megalopae of the snow crab *Chionoecetes opilio* (Fabricius, 1788). A–J correspond to numbers 1-10 in tables I and II. The segmented solid lines were drawn from segmented regression analysis of the relationship between cumulative consumption and time. The vertical dotted lines indicate the breakpoints (see Table 3.1.2).

was calculated as 5.2 (±0.6) mg, and the mean ratio of the total dry weight of *Artemia* consumed to the crab dry weight was 1.9 (±0.3).

3.1.5 Discussion

This was the first study to examine the food intake of snow crabs over the entire megalopal period in the laboratory. We demonstrated that snow crab megalopae feed predominantly during the early to middle phase of this stage, after which feeding rates decrease towards the moult to the first-instar crab. Food consumption during the entire megalopal period has been reported for Florida stone crabs *Menippe mercenaria* (Say, 1818) (Mootz & Epifanio, 1974), great spider crabs *Hyas araneus* (Linnaeus, 1758) (Anger & Dietrich, 1984; Harms et al., 1991), and European shore-crabs *Carcinus maenas* Linnaeus, 1758 (Dawirs & Dietrich, 1986), using newly hatched *Artemia* nauplii that were not enriched with fatty acids. The food consumption of these megalopae also decreased in the period immediately before moulting to crabs. Therefore, this phenomenon might be general in decapod megalopae. In these studies, *Artemia* consumption was expressed as numbers of individuals, except in that

of Harms et al. (1991) who represented consumption on a weight basis. The mean daily consumptions were ~69 individuals in *M. mercenaria* (~590 individuals during 8.5 days; Mootz & Epifanio, 1974), 12.7 individuals in *H. araneus* (546 individuals during 42.9 days; Anger & Dietrich, 1984), and 7.1–27.9 individuals in *C. maenas* at 12–25°C (170–229 individuals during 7.5–23.9 days; increasing with temperature; Dawirs & Dietrich, 1986). Snow crab megalopae consumed 1920 individuals during the entire megalopal period (30.5 days), and the mean daily *Artemia* consumption was 63 individuals. Despite the use of 24-hour-old *Artemia* nauplii that were enriched with fatty acids, in the present study, the daily consumption of snow crab megalopae was relatively high, and higher than in those other species. The carapace width of the megalopa of the snow crab is larger than that of other species: 1.6 mm in *M. mercenaria* (Johnson & Allen, 2012), 1.5 mm in *H. araneus* (Roff et al., 1984), 0.9 mm in *C. maenas* (Mohamedeen & Hartnoll, 1989), and 2.2 mm in snow crabs (Kurata, 1963). In addition, the intermoult period in snow crab megalopae (30.5 days) was longer than in the other species, except for *H. araneus*. Therefore it seems that the daily and total *Artemia* consumptions are related to megalopal size and the length of the megalopal period.

High growth rates associated with the intake of large quantities of food appear to be general features of decapod crustaceans. Higher growth rates under conditions of higher food density have been reported in several species, including *Penaeus indicus* Milne-Edwards, 1837 (Emmerson, 1980), red frog crabs *Ranina ranina* (Linnaeus, 1758) (Minagawa & Murano, 1993), Atlantic mud crabs *Panopeus herbstii* Milne-Edwards, 1834 (Welch & Epifanio, 1995), rock lobsters *Jasus edwardsii* (Hutton, 1875) (Tong et al., 1997), horsehair crabs *Erimacrus isenbeckii* (Brandt, 1848) (Jinbo et al., 2005), snow crabs (Kogane et al., 2010), and exotic freshwater prawns *Macrobrachium equidens* (Dana, 1852) (Gomes et al., 2014). In the present study, the carapace widths and body weights of first-instar crab stages of the snow crab tended to increase with the total number of *Artemia* consumed during the megalopal stage although there was no statistically significant relationship between these variables. We estimated the food requirement of snow crab megalopae as ~190% of the dry body weight after successful moulting to the first-instar crab.

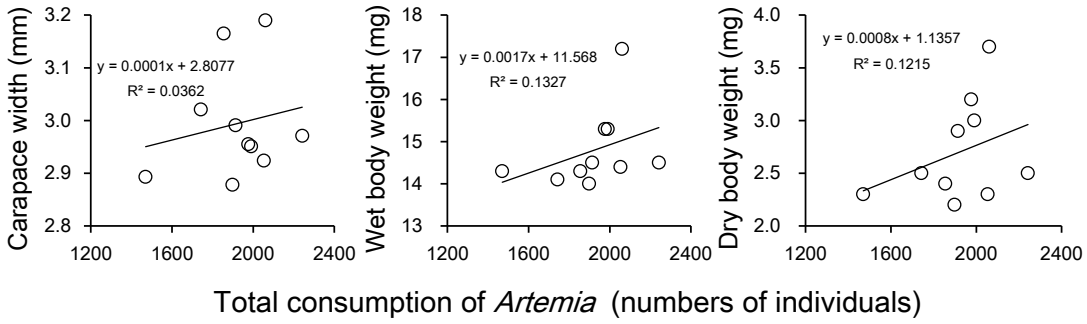

Figure 3.1.3. Relationships between the total number of *Artemia* consumed by megalopae and the body sizes of the first-instar crabs of the snow crab, *Chionoecetes opilio* (Fabricius, 1788): A, carapace width; B, wet body weight; and, C, dry body weight.

A breakpoint in the rate of food consumption by snow crab megalopae was observed at a mean of 69% of the elapsed time from the beginning of the megalopal stage to the juvenile moult. This timing corresponds to the intermediate of the late premoult stage of snow crab megalopae (Yamamoto et al., 2015c). The decreased feeding rates before ecdysis are generally known for decapod crustaceans (Anger, 2001). Moreover, the "point of reserve saturation" when animals become independent from food for the rest of the moulting cycle is generally found in the transition between stages C (intermoult) and D_0 (early premoult) in first stage zoeae of several decapod species (Anger, 1987). Similarly in megalopae of some decapod crustaceans, starvation during the late phase of the moulting cycle did not affect the survival or moult into crabs (e.g., Farrelly & Sulkin, 1988; Figueiredo et al., 2008). In the natural habitat, the vertical distribution range of larval snow crabs tends to become deeper with larval development in the southeastern Bering Sea (Incze, 1981; Incze et al., 1987) and in the Sea of Japan (Kon et al., 2003). In particular, megalopae occur within a layer 50–200 m deep in April to May but, in May in the Sea of Japan, some megalopae migrate downward and moult at 200–400 m (Kon et al., 2003). Ingesting food and accumulating energy until the intermediate premoult stage may allow them to concentrate on selecting appropriate settlement habitats for moulting into crabs.

In conclusion, we have revealed the food consumption pattern and requirements of the megalopal stage of the snow crab. Our results provide useful information for developing a feeding schedule and

management plan for the culture of snow crab megalopae. Szuwalski & Punt (2013) hypothesized that larval survival is influenced by food availability and advection to suitable nursery grounds and that these are the mechanisms driving the recruitment dynamics of snow crabs. Therefore, these data could be also used to more accurately infer megalopal survival and growth efficiencies to the first-instar crab stage of snow crabs in their natural habitat.

Chapter 4

CHANGES IN MOULTING CYCLE AND BODY DENSITY OF LARVAE

4.1 The moulting cycle and changes in body density in larvae of the snow crab *Chionoecetes opilio* (Brachyura: Majoidea) under laboratory conditions

4.1.1 Summary

The moulting cycle and the time course of changes in body density from hatching to the end of the megalopal stage in the snow crab (*Chionoecetes opilio*) larvae were investigated in laboratory-reared specimens. Morphological changes in the epidermis and cuticle were photographically documented to characterize the moult-cycle stages: A–B (postmoult), C (intermoult), D (premoult) and E (ecdysis). Moult-stage characteristics were based on a microscopical examination of integumental modifications, particularly of the telson. During stages A–C, the larval cuticle changed from a spongy structure to become conspicuously thicker and more solid in appearance. In stage D, the epidermis retracted from the cuticle and new setae and appendages were formed. The body densities of larval snow crabs were lowest just after moulting; they increased greatly during the stage C; and then gradually increased to reach a plateau at 1.0897–1.0931 g cm^{-3} during the stage D. Over the whole larval period, they have a density greater than that of seawater. These observations will assist in understanding of larval distribution and transport in snow crabs in their natural habitat, and provide a useful tool to determine the developmental stages of larvae sampled from the plankton and from larval cultures.

4.1.2 Introduction

Crustaceans grow by moulting. As a basis for understanding the behavioural, physiological and biochemical changes that take place between successive moults (i.e., during the course of the moulting cycle; Chang, 1995), staging techniques have been developed that characterize the morphologically distinct phases of the moulting cycle in adult decapods and other crustaceans. The moulting cycle is generally divided into five principal stages (A–E) with numerous substages (Drach, 1939; Skinner, 1962; Drach & Tchernigovtzeff, 1967). Moult-staging techniques have also been applied to the larvae of several species of decapod crustaceans in the laboratory (Freeman & Costlow, 1980; McNamara et

al., 1980; Anger, 1983, 1987; González-Gordillo et al., 2004; Hayd et al., 2008; Guerao et al., 2010).

In general, marine benthic decapods do not migrate long distances so that dispersal and recruitment of their pelagic larvae play an important role in sustaining their populations (Sulkin, 1984; Anger, 2001, 2006). Zoeae and megalopae of decapods migrate in the sea by regulating their vertical distributions in relation to the different directions and strengths of water currents at different depths and phases of the tidal cycle (Forward & Tankersley, 2001). The depth distribution of larvae in the water column is influenced by their swimming activity (Sulkin, 1984). Laboratory experiments have shown that larval behaviour changes in response to abiotic and biotic factors, including light intensity and wavelength, barometric pressure, gravity, temperature, salinity and chemical cues from predators (Forward, 1988, 2009; Sulkin, 1984). Moreover, the direction of larval swimming can be modelled in terms of drag forces, the downward gravitational force and the upward buoyant force (Konishi et al., 2011); body density is an important dimension for calculating these parameters. However, larval body density has been examined for only a few decapod crustacean species (Hamasaki et al., 2012, 2013; Ichikawa et al., 2014), and no study has investigated the ontogeny of larval body density in relation to the moulting cycle.

The snow crab *Chionoecetes opilio* (Fabricius, 1788) (Brachyura: Majoidea) is widely distributed throughout the cold waters of the northern hemisphere and is an important fishery resource (Elner, 1982; Sinoda, 1982). Snow crab larvae hatch as prezoeae; after a brief developmental period of less than one hour, they have a long developmental period while passing through first and second zoeae and megalopae before metamorphosing into benthic crabs (Kon, 1980). The duration of the entire larval period, from hatching until moulting to the first crab stage, is estimated to be 74–123 days (~2.5–4 months) based on larval culture experiments at different temperature levels, coupled with information of larval distribution and water temperature in natural habitats (Yamamoto et al., 2014). The estimated entire larval duration is similar to that in natural habitats inferred on the basis of the time lags in the occurrence of peak abundance between each larval stage; e.g., ~100 days in the Sea of Japan (Kon et al., 2003), over 90 days in the southeastern Bering Sea (Incze et al., 1982), and over 4 months in the

Gulf of Sainte Lawrence (Lovrich et al., 1995). The average depth of larvae increases with development (Incze, 1981; Conan et al., 1996; Kon et al., 2003); e.g., in the Sea of Japan, pelagic larvae (first and second zoeae) are found mainly in waters shallower than 150 m, whereas megalopae are found in deeper layers (Kon et al., 2003). A number of studies have elucidated the general biology of snow crab larvae, focused on managing stocks or on the mechanisms of fluctuations in stock abundance (e.g., Kon, 1980; Incze et al., 1984; Davidson & Chin, 1991; Lovrich & Ouellet, 1994). Their phototactic and geotactic behaviours have been studied to better understand the mechanisms of their spatiotemporal distribution (Kogane et al., 2007b; Konishi et al., 2011). However, their body densities were measured using specimens preserved in formalin solutions (Konishi et al., 2011) and might not reflect values in living animals. Moreover, moult-cycle stages have not been fully defined for snow crab larvae; Incze et al. (1984) described the epidermal retraction process in first and second zoeae caught from the plankton.

This study describes the integumentary changes occurring during the course of the moulting cycle in snow crab zoeae and megalopae, and examines the ontogenetic changes of larval body density in relation to the moulting cycle.

4.1.3 Material and methods

4.1.3.1 Source of larvae

A total of 185 ovigerous females were caught in November 2011 in the Sea of Japan off Ishikawa Prefecture, Japan. They were transferred to the Obama Laboratory, Japan Sea National Fisheries Research Institute, Fisheries Research Agency, Fukui Prefecture, and reared at 3°C in one 4-kL (1.3 × 3.9 × 0.85 m) rectangular tank with a recirculating system. The crabs were fed with frozen Antarctic krill *Euphausia superba* twice weekly. The main hatching season extended from February to March in 2012 and several females hatched their larvae on the same day.

The following larval stages were used in the study: prezoeae and first zoeae that hatched on February 20, 2012; second zoeae that moulted on February 20, 2012 from first zoeae that hatched on Feb-

ruary 1, 2012; and megalopae that metamorphosed on March 16, 2012 from second zoeae that hatched on February 1, 2012. One-litre plastic beakers were prepared for larval culture and placed in temperature-controlled baths. Water temperatures were regulated at 11°C for zoeae and 8°C for megalopae, reflecting their thermal distributions in their major natural habitats (Kon et al., 2003) and the optimum temperatures for larval survival (Yamamoto et al., 2014). Prezoeae were stocked in one beaker (10 individuals) for measuring the body density. Six beakers (100 individuals per beaker), eight beakers (50 individuals per beaker) and 25 beakers (20 individuals per beaker) were prepared for culturing first zoeae, second zoeae and megalopae, respectively to measure larval body density and to observe moult-cycle stages. In addition, one beaker (20 individuals per beaker) was established for each culture series to examine the time course of moulting success (the cumulative percentage that moulted completely relative to the number of larvae initially stocked).

Larval culture was based on the method of Yamamoto et al. (2014). Zoeae were fed rotifers of the *Brachionus plicatilis* species complex at a density of 20 individuals mL^{-1}. Rotifers were enriched with 0.5 mL L^{-1} of commercial condensed marine phytoplankton (*Nannochloropsis* sp., Mercian Co., Ltd., Tokyo, Japan), 14 μL L^{-1} emulsified DHA 70G oil and 28 μL L^{-1} EPA 28G oil (Hokkaido Fine Chemicals Co., Ltd., Hokkaido, Japan) at 16°C for 18 h prior to feeding. *Artemia* nauplii (Utah strain), enriched with 1.5 mL L^{-1} of a commercial emulsion of n-3 polyunsaturated fatty acids (Hyper Glos, Marinetech Co., Ltd., Aichi, Japan) at 22°C for 24 h, were given to megalopae at a density of 5 individuals mL^{-1}. The rearing water was not aerated. Baths were covered with Styrofoam boards to stabilize water temperature. Each morning, larvae were transferred to newly prepared beakers with new water and food using a large-mouthed pipette. After transferring the larvae, 20 mg L^{-1} dihydrostreptomycin sulphate (Tamura-seiyaku Co., Ltd., Tokyo, Japan) was added to the rearing water to prevent bacterial attachment to the larvae.

4.1.3.2 Moult-stage analysis

Moult-cycle stages were observed in groups of five larvae at intervals of 1–3 days, from 0–24 days after moulting to first and second zoeae, and at intervals of 1–2 days, from 0–43 days after moulting to

megalopae.

Larval morphological details were observed using a microscope (Eclipse 80i, Nikon Corp., Tokyo, Japan) equipped with a digital photo-micrographic camera (DS-Fi1-L2, Nikon Corp., Tokyo, Japan). The moult-cycle stages followed those determined for common spider crab *Maja brachydactyla* larvae by Guerao et al. (2010), based on Drach's classification system (Drach, 1939; Drach & Tchernigovtzeff, 1967): moult stage A–B (early and late postmoult combined), C (intermoult), D (premoult) with substages D_0, D_1 and D_2 (early, intermediate and late premoult), and E (ecdysis). The analysis of moult-cycle stages concentrated mainly on the telson because this part of the larval body is easy to examine without requiring dissection (Anger, 1983, 2001; Guerao et al., 2010). In addition, to examine synchronization of the moulting cycle in different structures, we also considered changes in: following the dorsal spine of the carapace, the endites of the maxillule and the endopod of the maxilla in second zoeae; and the rostrum, the endites of the maxillule and the endopod dactyl of the second maxilliped in megalopae. The distal tips of the epidermis in the dorsal spine of second zoeae and the rostrum of megalopae separate from the old cuticle and gradually retract and degenerate (Hamasaki, 1996; Guerao et al., 2010). Just before moulting, the epidermis of the dorsal spine of second zoeae completely disappeared, and in megalopae, the ratio of new cuticle length to old cuticle length in the rostrum decreased to ~1/2 (see the Results section). Therefore, the ratios of new cuticle length to old cuticle length in the dorsal spine of second zoeae and in the rostrum of megalopae were categorized as >3/4, 1/2–3/4, 1/4–1/2 and ≤1/4 in second zoeae and >3/4, 1/2–3/4 and ≤1/2 in megalopae, respectively.

4.1.3.3 Body density measurement

Larval body density was measured for 10 individual prezoeae. For first and second zoeae, groups of ten larvae were used for body density measurements at intervals of 1–3 days from 0–24 days after moulting; for megalopae, groups of five larvae were used for body density measurements at intervals of 1–3 days from 0–45 days after moulting. The body densities of individual larvae were determined by measuring their specific gravity using the method of Tsukamoto et al. (2009). We prepared a series

of solutions of sucrose in 500 mL distilled water adding 46–170 g sucrose at 2–10-g intervals. The temperatures of the sucrose solutions were regulated at 11°C for zoeae and 8°C for megalopae. The specific gravities of these solutions were measured using a standard hydrometer (Wakairo Keiki Seisakusho Co. Ltd., Tokyo, Japan). Individual larvae were anaesthetized with ethyl carbamate (0.5 M for 10 min), washed with a sucrose solution of the designated specific gravity, and then transferred into a glass test beaker filled with the same solution (50 mL). This procedure was repeated with different sucrose solutions. The vertical position of the larva was observed, and when the larva maintained neutral buoyancy at the mid-depth of a beaker, the specific gravity of the solution was deemed to be equal to the body density of the larva. The test larvae were not returned to the culture beakers.

An asymptotic relationship was observed between the number of days after moulting and the body density of larvae (see the Results section); these relationships were expressed by the asymptotic regression model: $y = ab^x + c$, and by the Gompertz model (Gompertz, 1825): $y = ab^{\exp(-cx)}$, where y is the body density of the larva, x is the time (in days), and c in the asymptotic regression model and a in the Gompertz model are numeric parameter representing the asymptote. The parameters a, b and c were estimated using a non-linear ordinary least squares method and evaluated with a t-test. Akaike's information criteria (AIC) were calculated and the model having the minimum AIC value was selected as the optimum model. Statistical analyses were performed with R language (R3.1.1; R Core Team 2014) with a 5% significance level.

4.1.4 Results

4.1.4.1 Moult-cycle stages

First zoea

Stages A–B: (Fig. 4.1.1A). Immediately after moulting from prezoeae, the epidermal tissues had a spongy structure with numerous interconnected lacunar spaces (stage A; Fig. 4.1.1A). Subsequently, most of the endocuticle was secreted (stage B). The epidermal tissues began to concentrate along the inner cuticle surface, becoming gradually denser in appearance. Together, stages A and B lasted for 1

Table 4.1.1. Morphological changes in the maxillule, maxilla and maxilliped, and their duration in larval *Chionoecetes opilio*.

Larval stage	Region	Morphological observation	Range (days)
Second zoea	Endites of maxillule	Before apolysis	0–4
		Apolysis	6–15
		Epidermal invaginated new setae	15–18
		Completely formed new setae	20–24
	Endopod of maxilla	Before apolysis	0–4
		Apolysis	4–13
		Fibrous structured in retracted setae	12–24
		Completely formed megalopal shape	20–24
Megalopa	Endites of maxillule	Before apolysis	0–15
		Apolysis	13–37
		Epidermal invaginated new setae	30–42
		Completely formed new setae	37–45
	Endopod dactyl of the second maxilliped	Before apolysis	0–9
		Apolysis	11–37
		Epidermal invaginated new setae	28–38
		Completely formed new setae	36–45

day (Fig. 4.1.2A).

Stage C: (Fig. 1B). The epidermal lacunae were absent and the cuticle became conspicuously thicker and more solid in appearance. Throughout this stage, the epidermal tissues continued to grow, gradually increasing in density and extent (Fig. 4.1.1B). The stage was characterized by a lack of any major morphological change, by the completion of the endocuticle and by an apparent accumulation of biomass (tissue growth) in the entire larval body. Stage C was observed after 1–9 days after moulting (Fig. 4.1.2A).

Substage D_0: (Fig. 4.1.1C). The beginning of early premoult was indicated by an incipient separation of the epidermis from the cuticle (apolysis). This process was observed near the base of the setae of the telson (Fig. 4.1.1C). Substage D_0 was observed 6–13 days after moulting (Fig. 4.1.2A).

Substage D_1: (Fig. 4.1.1D). The beginning of the intermediate premoult substage was characterized by the appearance of a circular epidermal invagination at the base of the retracted setae (Fig. 4.1.1D). Deep epidermal folds are a prerequisite for the enlargement of the size of setae as well as for formation of new setae in the following second zoeal stage. Substage D_1 was observed 12–15 days after moulting (Fig. 4.1.2A).

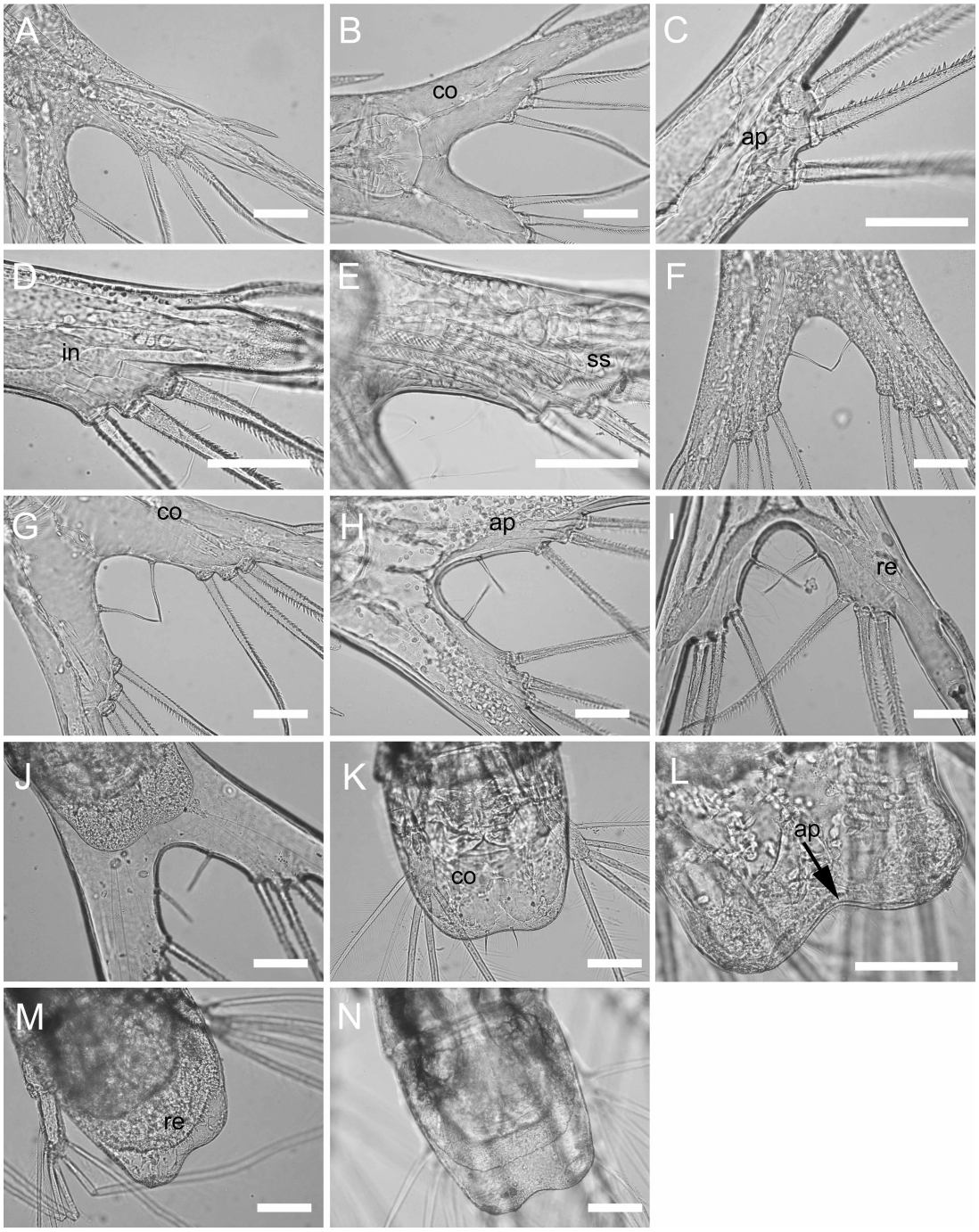

Figure 4.1.1. Larval telson of *Chionoecetes opilio*: (A)–(E): first-stage zoea; (F)–(J): second-stage zoea; (K)–(N): megalopa; (A), (F): moult-stage B (postmoult); (B), (G), (K): moult-stage C (intermoult); (C), (H): moult-stage D_0 (early premoult); (L): moult stage $D_{0–1}$; (D), (I), moult-stage D_1 (intermediate premoult); (E), (J), (M), (N): moult-stage D_2 (late premoult). (A) 0 days; (B) 1 day; (C) 6 days; (D) 14 days; (E) 17 days; (F) 0 days; (G) 1 day; (H) 15 days; (I) 18 days; (J) 24 days; (K) 3 days; (L) 9 days; (M) 24 days; (N) 42 days. ap, apolysis; co, epidermal condensation; in, epidermal invagination; re, retraction of tissues. Scale bar = 0.1 mm.

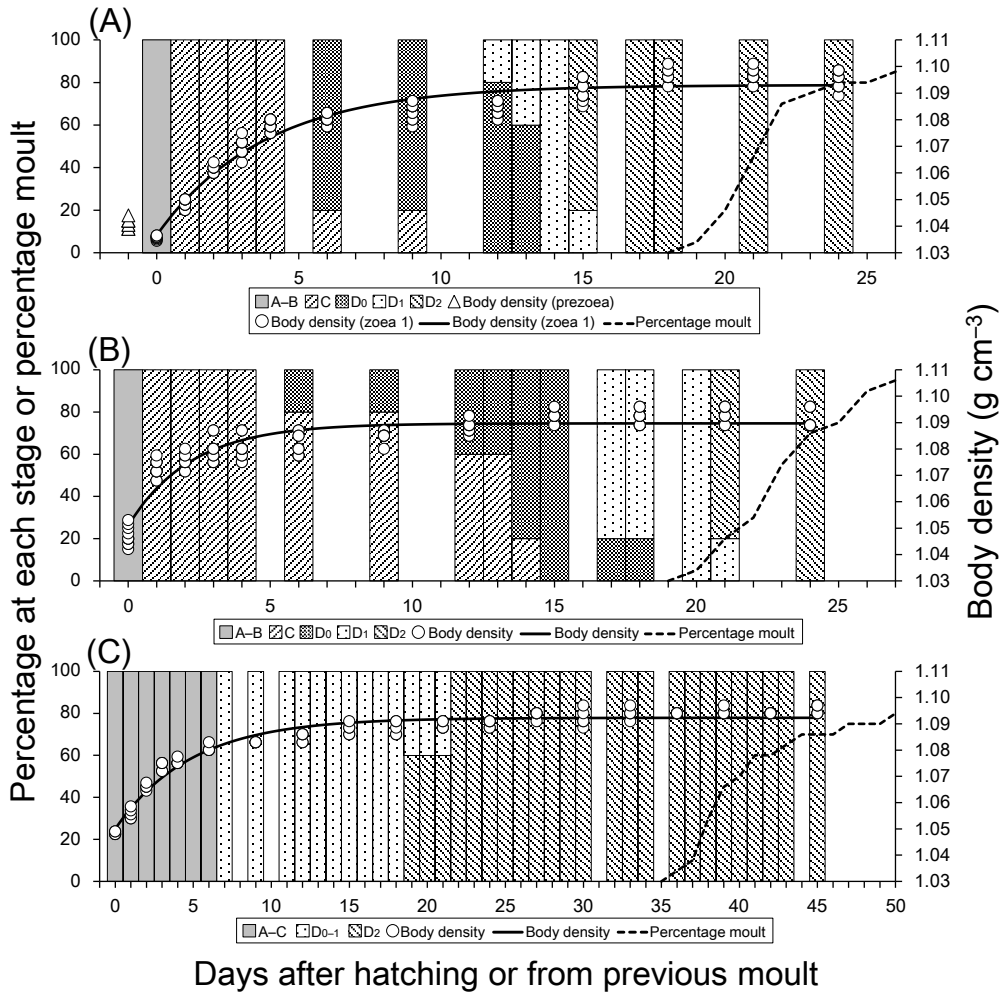

Figure 4.1.2. Changes in the relative proportions (%) of *Chionoecetes opilio* larvae exhibiting different moulting substages and larval body density with respect to days after hatching, or after the previous moult: (A) first-stage zoea; (B) second-stage zoea; (C) megalopa. The dashed line shows the cumulative percentage of the initial number of larvae stocked that successfully moulted. The solid line curves were drawn from the asymptotic equations (see Table 4.1.2) applied to the relationship between the number of days and the body density of larvae.

Substage D_2: (Fig. 4.1.1E). The beginning of late premoult was characterized by the appearance of new cuticle on the epidermal surface of the setae and spines, which reached their greatest retraction from the old cuticle sheath. The new setae of the telson were completely formed, and their secondary spinules were clearly visible because of a distinct lining with a thin microscopically conspicuous new

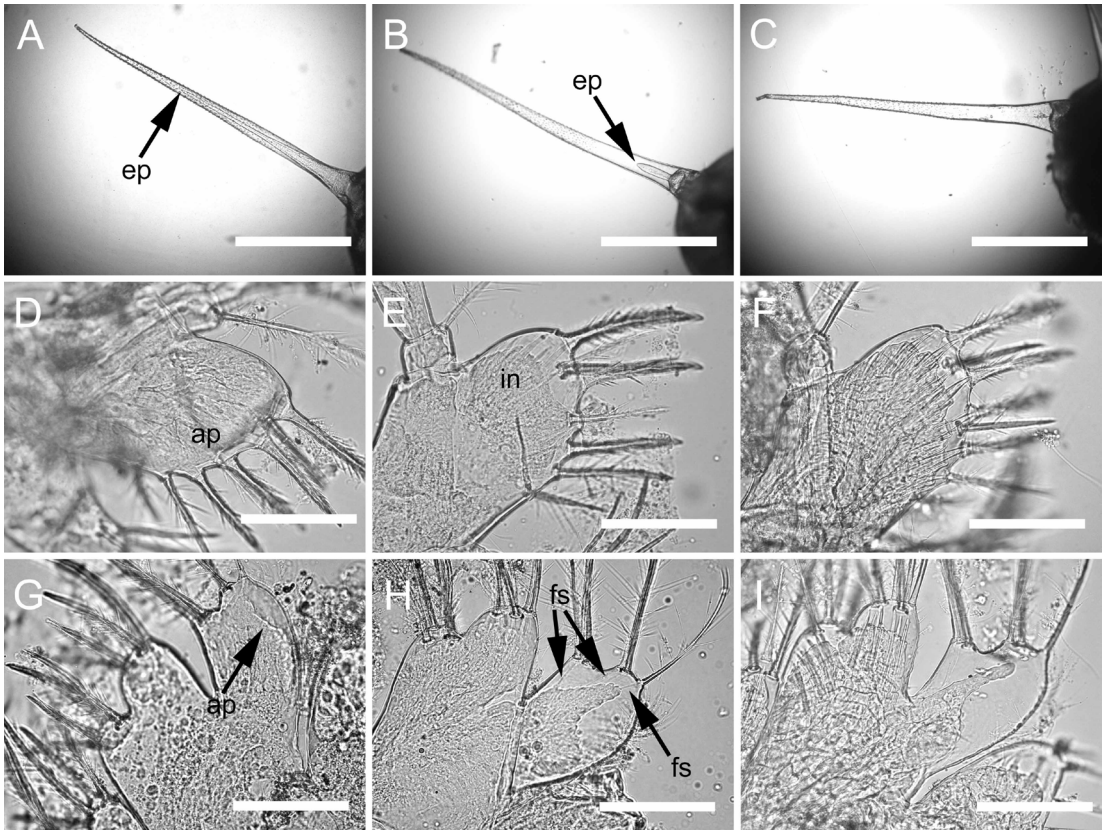

Figure 4.1.3. Second zoeae of *Chionoecetes opilio*. (A)–(C): dorsal spine, (D)–(F): basal endite of the maxillule, (G)–(I): endopod of the maxilla. (A) 14 days; (B) 20 days; (C) 24 days; (D) 13 days; (E) 15 days; (F) 21 days; (G) 13 days; (H) 15 days; (I) 24 days. ap, apolysis; ep, epidermis; fs, fibrous structure; in, epidermal invagination. Scale bar = 1 mm (A)–(C) and 0.1 mm (D)–(I).

cuticle on their surface (Fig. 4.1.1E). Therefore, the main characteristics of substage D_2 were the termination of morphogenesis and the protection of the newly formed structures with a new exoskeleton. The thickness and visibility of the new cuticle increased considerably throughout this substage. Substage D_2 was observed at 15–24 days and ecdysis (stage E) occurred 19–26 days after moulting (Fig. 4.1.2A).

Second zoea

Stages A–C: (Fig. 4.1.1F, G). Postmoult and intermoult stages were morphologically very similar to those in the first zoea. Moult stages A–B (Fig. 4.1.1F) lasted for 1 day and stage C (Fig. 4.1.1G)

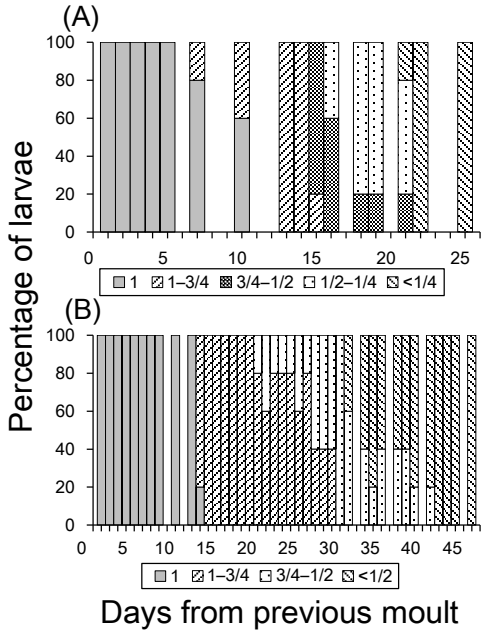

Figure 4.1.4. Changes in the relative proportions (%) of larvae exhibiting different ratios of new cuticle length to old cuticle length in the dorsal spine of second zoeae (A) and the rostrum of megalopae (B) with respect to days after moult in *Chionoecetes opilio*.

was observed 1–14 days after moulting (Fig. 4.1.2B).

Substage D_0: (Figs. 4.1.1H and 4.1.3A, D, G). As in the first zoea, apolysis was observed at the base of the setae of the telson (Fig. 4.1.1 H). Substage D_0 was characterized by setal degeneration and was observed 6–18 days after moulting (Fig. 4.1.2B). At the distal tip of the dorsal spine, retraction of epidermis from its sheath began 6 days after moulting (Fig. 4.1.4A). From 14 days on, retraction of the spines was much advanced, and the ratio of new cuticle length to old cuticle length in the dorsal spine decreased to 3/4–1/4 (Fig. 4.1.4A). Apolysis was also observed at the bases of the setae of the endites of the maxillule (Fig. 4.1.3D) during the period of 6–15 days and at the margin of the endopod of the maxilla (Fig. 4.1.3G) 4–13 days after moulting (Table 4.1.1).

Substage D_1: (Figs. 4.1.1I and 4.1.3B, E, H). The epidermal matrix degenerated, beginning at the distal tips of the furca and setae, so that gradually the typical round or slightly bilobed shape of the megalopal telson formed (Fig. 4.1.1I). Remains of the setae became fibrous in structure. Substage D_1 was observed 17–21 days after moulting (Fig. 4.1.2B). The ratio of new cuticle length to old cuticle length in the dorsal spine decreased to less than 1/2 in most larvae (Figs. 4.1.3B and 4.1.4A). Deeply

Table 4.1.2. Estimates of parameters (with SEs) of the asymptotic equation ($y = abx + c$) and the Gompertz model ($y = ab^{\exp(-cx)}$) relating the number of days after moulting to each stage to the body density of larval *Chionoecetes opilio*.

Period	Model	*n*	*a*		*b*		*c*		AIC
Z1–Z2	Asymptotic regression model	120	–0.0563	(0.0012)*	0.7683	(0.0104)*	1.0931	(0.0007)*	–966.5
	Gompertz model	120	1.0930	(0.0007)*	0.9486	(0.0011)*	0.2686	(0.0137)*	–965.5
Z2–M	Asymptotic regression model	120	–0.0386	(0.0015)*	0.6376	(0.0235)*	1.0897	(0.0006)*	–932.8
	Gompertz model	120	1.0897	(0.0006)*	0.9646	(0.0014)*	0.4550	(0.0373)*	–932.2
M–C1	Asymptotic regression model	95	–0.0423	(0.0010)*	0.8135	(0.0092)*	1.0923	(0.0004)*	–844.7
	Gompertz model	95	1.0923	(0.0004)*	0.9613	(0.0009)*	0.2091	(0.0116)*	–843.9

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

folded epidermal invaginations were observed at the bases of the setae of the endites of the maxillule (Fig. 4.1.3E) 15–18 days after moulting (Table 4.1.1). The retracted epidermis of the setae of the endopod of the maxilla became fibrous in structure (Fig. 4.1.3H) and this phase was observed 12–24 days after moulting (Table 4.1.1).

Substage D_2: (Figs. 4.1.1J and 4.1.3C, F, I). The epidermal matrix had completely retracted from the zoeal furca; the posterior margin of the megalopal telson became clearly visible and was now covered by a new cuticle (Fig. 4.1.1J). This phase was observed 21–24 days after moulting (Fig. 4.1.2B). The new cuticle of the dorsal spine completely disappeared 21–24 days after moulting (Figs. 4.1.4A and 4.1.3C). New setae appeared in the endites of the maxillule (Fig. 4.1.3F) and were observed 20–24 days after moulting (Table 4.1.1). The megalopal shape of the endopod of the maxilla was complete (Fig. 4.1.3I) and the fibrous structure of the setae almost disappeared. This structure was observed 20–24 days after moulting (Table 4.1.1). After this phase, ecdysis (stage E) was observed 20–27 days after moulting (Fig. 4.1.2B).

Megalopa

Stages A–C: (Fig. 4.1.1K). The morphological characters were similar to those of these stages in the zoea (Fig. 4.1.1K). These moult stages were observed for 0–6 days after moulting (Fig. 4.1.2C). Unlike the first and second zoeae, stage C was not clearly distinguished from stages A–B.

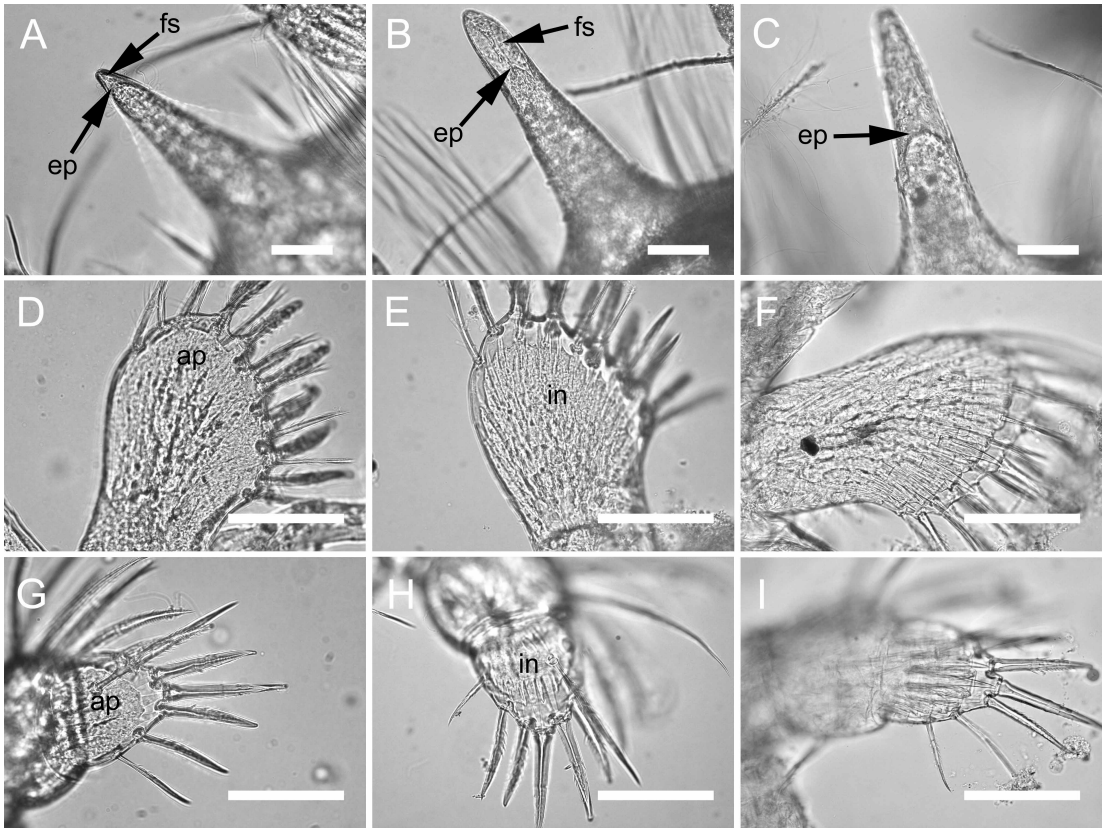

Figure 4.1.5. Megalopae of *Chionoecetes opilio*. (A)–(C): rostrum, (D)–(F): basal endite of the maxillule, (G)–(I): endopod dactyl of the second maxilliped. (A) 12 days; (B) 23 days; (C) 42 days; (D) 26 days; (E) 33 days; (F) 41 days; (G) 26 days; (H) 32 days; (I) 41 days. ap, apolysis; ep, epidermis; fs, fibrous structure; in, epidermal invagination. Scale bar = 1 mm (A)–(C) and 0.1 mm (D)–(I).

Substage D_{0-1}: (Figs. 4.1.1L and 4.1.5A, D, G). The beginning of substage D_0 was observed in the terminal margin of the telson when the epidermis detached from the cuticle (Fig. 4.1.1L). Unlike the second zoea, substage D_1 could not be clearly distinguished from substage D_0. This phase (D_{0-1}) was observed 7–21 days after moulting (Fig. 4.1.2C). Apolysis started at the tip of rostrum (Fig. 4.1.5A) 12 days after moulting (Fig. 4.1.4B). The ratio of new cuticle length to old cuticle length in the rostrum was mostly greater than 3/4 (Fig. 4.1.4B). Likewise, apolysis commenced in the endites of the maxillule (Fig. 4.1.5D) and in the endopod dactyl of the second maxilliped (Fig. 4.1.5G) 13 and 11 days after moulting, respectively (Table 4.1.1).

Substage D_2: (Figs. 4.1.1M, N and 4.1.5B, C, E, F, H, I). Substage D_2 was characterized by the

appearance of a new cuticle. The epidermis in the posterior margin of the megalopal telson appeared strongly retracted and the fibrous structure gradually disappeared, so that the characteristic margin of the crab telson was formed (Fig. 4.1.1M, N). This moult stage was observed 19–45 days after moulting (Fig. 4.1.2C). The ratio of new cuticle length to old cuticle length in the rostrum gradually decreased from more than 3/4 to less than 3/4 (Figs. 4.1.4B, 4.1.5B, C). In this substage, new setae gradually formed in the endites of the maxillule (Fig. 4.1.5E, F) and in the endopod dactyl of the second maxilliped (Fig. 4.1.5H, I). These new setae were completely formed by 36 or 37 days after moulting (Table 4.1.1). Megalopae underwent ecdysis (stage 4.1.E) 36–50 days after moulting (Fig. 4.1.2C).

4.1.4.2 Body density

The body densities of larvae decreased immediately after moulting from prezoeae to first zoeae (Fig. 4.1.2). The same phenomenon was observed at subsequent moults. Later after ecdysis, during stage C (intermoult), the body densities of zoeae and megalopae greatly increased and then gradually increased to a plateau during stage D (premoult) (Fig. 4.1.2). Estimates of all parameters in the asymptotic regression models and in the Gompertz models were statistically significant (Table 4.1.2). The AIC values were lower in the asymptotic regression models than the Gompertz models. The numeric parameters representing the asymptote were 1.0931 g cm^{-3} (asymptotic regression model) and 1.0930 g cm^{-3} (Gompertz model) in first zoeae, 1.0897 g cm^{-3} in second zoeae (both models) and 1.0923 g cm^{-3} in megalopae (both models), respectively (Table 4.1.2).

4.1.5 Discussion

A problem preventing the precise staging of moulting of crustacean larvae is that the larval integument is thin and relatively unstructured and morphological changes are often indefinite (Anger, 2001; Hayd et al., 2008; Guerao et al., 2010). Thus, transitions between moult stages and substages could not be identified with the same accuracy and timing precision as in Drach's classification for adult crabs (Drach, 1939; Drach & Tchernigovtzeff, 1967). As in studies of the larvae of the Amazon

River prawn *Macrobrachium amazonicum* (Hayd et al., 2008) and *M. brachydactyla* (Guerao et al., 2010), for snow crab larvae we combined all postmoult stages (A–B) as well as Drach's substages $D_{2–4}$ within the premoult period (stage D); also, substages $D_{0–1}$ in megalopae could not be separated because of the absence of setagenesis in the telson.

The course of the moulting cycle of larval snow crabs determined by morphological analysis of the telson was similar to that previously described in larval Majoidea by Anger (1983) for great spider crab *Hyas araneus* and Guerao et al. (2010) for *M. brachydactyla*. Additionally, a lack of synchrony of developmental speed within different parts of the larval body was recognized in snow crab larvae as described for *H. araneus* and *M. brachydactyla* (Anger, 1983; Guerao et al., 2010).

Anger (1987) studied the moulting cycle of first zoeae of nine decapod species and concluded that, at a constant culture temperature, stages A–C and substage D_0 comprise 35–50% and 17–20%, respectively of the moulting cycle in most species. Most of the interspecific variation was observed after substage D_1. Observations on other decapod species (McNamara et al., 1980; Hamasaki, 1996; Hayd et al., 2008; Guerao et al., 2010) generally agree with those on first zoeae reported by Anger (1987). In snow crab first zoeae, stages A–C lasted for 10 days (0–9 days after hatching but usually less than 7 days: 0–6 days) and substage D_0 lasted for 8 days (6–13 days after hatching). First zoeae moulted to second zoeae 19–26 days after hatching (mean 21.6 days). Therefore, the percentages of stages A–C and substage D_0 of the mean duration of the first zoeal development were 46% (mostly less than 32%) and 37%, respectively. Although the relative duration of stages A–C in snow crab first zoeae was similar to that in decapod first zoeae examined in previous studies (McNamara et al., 1980; Anger, 1987; Hamasaki, 1996; Hayd et al., 2008; Guerao et al., 2010), the relative duration of substage D_0 in snow crabs was much longer than in these first zoeae. The developmental periods of first zoeae examined by these authors lasted for ~1–13 days after hatching, much shorter than that of snow crabs (mean 21.6 days in the present study). Therefore, the longer substage D_0 of first zoeae in snow crabs compared to other decapods might be related to the longer development period.

Anger (1983) studied changes in the schedule of stages of the moulting cycle of *H. araneus* larvae

reared at constant temperature, during successive moults from first zoea to megalopa. The percentage of the moult cycle occupied by stage B slightly increased, stage C markedly increased, substage D_0 became shorter, substage D_1 remained fairly constant and substages $D_{2–4}$ became slightly shorter. Thus, the relative interval of the premoult stage (substages $D_{0–4}$) became shorter with larval development. However, this tendency was not observed in the swimming crab *Portunus trituberculatus* (Hamasaki, 1996) or *M. brachydactyla* (Guerao et al., 2010). However, in Hamasaki (1996), Guerao et al. (2010), and this study, some stages or substages were combined. Hamasaki (1996) reported that the combined stages A–C, stage D, substage D_0 and substage D_1 remained fairly constant relative to larval development in *P. trituberculatus*. Guerao et al. (2010) showed that, in *M. brachydactyla*, the relative lengths of combined stages A–B, stage C and stage D remained fairly constant at 15°C whereas at 18°C stages A–B and stage C became slightly shorter and stage D became slightly longer with larval development. In the snow crab, we observed that stages A–C were longest and stage D was shortest in the second zoeae, which is different from that observed in *H. araneus* larvae.

The body densities of larval snow crabs were lowest just after moulting; they increased greatly during the intermoult phase (stage C); and then gradually increased to reach a plateau at 1.0897–1.0931 g cm^{-3} during the premoult phase (stage D). Physiological and biochemical changes occur during the moulting cycle of crustaceans and tissue growth is mainly achieved during the intermoult period (Skinner, 1962; Stevenson, 1985; Chan et al., 1988). Therefore, tissue growth might be related to the rapid increase in the body density during the premoult period in snow crab larvae. Konishi et al. (2011) measured the body densities of snow crab larvae using formalin-preserved specimens and reported that their body densities increased with larval development. These observations differ from values obtained using living animals in the present study, which presumably are more reliable indicators of the natural state.

To live planktotrophically within a certain depth range in the ocean, snow crab larvae must have a means of adjusting their vertical distribution. The specific gravities of seawater in which genus *Chionoecetes* larvae occurs are ~1.019–1.026 in the Gulf of Saint Lawrence (calculated based on Millero

et al., 1980 and Conan et al., 1996) and 1.025–1.030 in the Sea of Japan (based on Naganuma, 2000 and Kon et al., 2003). Therefore snow crab larvae have a density greater than that of seawater and they continuously sink in the water column if they are inactive. The vertical distribution range of larval snow crabs tends to become deeper with larval development in the natural habitat. Kon et al. (2003) intensively examined the vertical distribution of snow crab larvae (including *C. opilio* and *C. japonicus*) off Wakasa Bay in the Sea of Japan. They found that the first zoeae mainly occurred at a depth of 0–100 m, second zoeae occurred in a layer 0–150 m deeper than that of first zoeae and megalopae occurred in a layer 50–200 m deeper than the second zoeae. Furthermore, some megalopae migrate downward and moult at 200–400 m. Similarly, in the southeastern Bering Sea, zoeae (only *C. opilio*) are found in the upper 40 m and megalopae (including *C. opilio* and *C. bairdi*) are present in the upper 60 m (Incze, 1981; Incze et al., 1987). Kogane et al. (2007b) examined snow crab larval phototaxis and geotaxis in the laboratory and reported that first zoeae exhibited positive phototaxis and negative geotaxis, second zoeae showed slight phototaxis and positive geotaxis, while megalopae are negatively phototactic and positively geotactic. Kogane et al. (2007b) also observed that first zoeae floated in the upper layer of the rearing water, but almost all of the later larvae sank to the bottom of the rearing tank. Moreover, Konishi et al. (2011) reported that zoeae swam more frequently than megalopae. Snow crab larvae might sink with larval development because of decreasing upward swimming activity in the natural habitat. The passive sinking velocities of snow crab larvae are considered to increase even though their body density remains the same because their body volumes increase with larval development (Konishi et al., 2011) and this may help their downward migration.

Body densities were measured in laboratory-reared larvae of the Japanese spiny lobster *Panulirus japonicus* (Hamasaki et al., 2012), the coconut crab *Birgus latro* (Hamasaki et al., 2013) and the horsehair crab *Erimacrus isenbeckii* (Ichikawa et al., 2014). The mean body density of Japanese spiny lobster larvae was 1.097 g cm^{-3} and did not change with growth and development during the phyllosomal period (Hamasaki et al., 2012). The mean body densities of the coconut crab larvae were 1.086–1.089 g cm^{-3} in first to third zoeae and decreased to 1.072–1.075 g cm^{-3} in fourth zoeae and megalo-

pae (Hamasaki et al., 2013). Hamasaki et al. (2013) speculated that the reduction in body density after the fourth zoeae in the coconut crab might be related to the emigration behaviour of the megalopae, which acquire shells and migrate from the sea to the land. In contrast, the body density of horsehair crab larvae significantly increased from 1.080 g cm^{-3} in zoeae to 1.148 g cm^{-3} in megalopae (Ichikawa et al., 2014). Ichikawa et al. (2014) inferred that the increased body density of the horsehair-crab megalopae might be adaptive to shallow coastal environments with cyclic tidal currents, where megalopal settlement occurs (Abe, 1977). Unlike the horsehair crab megalopae, the asymptotic larval body densities were similar in zoeae and megalopae of the snow crab. Kon et al. (2003) reported that snow-crab megalopae migrate downward from 50–200 m to 200–400 m and settle on the bottom in the weak water currents of the Sea of Japan. Therefore, the difference in the body densities of megalopae between horsehair crabs and snow crabs may be attributed to the different physical conditions in their settlement environments. This inference could be tested in further studies of the ontogeny of body density in decapod crustaceans having different settlement habitats.

In conclusion, we have characterized and have provided a time scale for the moult-cycle stages of snow crab larvae. The ontogeny of body density in relation to the moulting cycle is described in zoeae and megalopae. These data could be incorporated into a biophysical model (Parada et al., 2010, Konishi et al., 2011, Szuwalski & Punt, 2013, Mullowney et al., 2014) to more accurately infer the larval distribution and transport of snow crabs in their natural habitat. Furthermore, the time scale data and the moult-staging technique could provide a useful tool for evaluating the developmental process of larvae sampled from the plankton and from larval cultures.

Chapter 5

GROWTH AND DEVELOPMENT OF IMMATURE CRABS

5.1 Effects of temperature on growth of juvenile snow crabs, *Chionoecetes opilio*, in the laboratory

5.1.1 Summary

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.

5.1.2 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 & 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 crusta-

ceans 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 (Yosho & Hayashi, 1994; Squires, 1990; Lovrich et al., 1995; Dawe & 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 & Comeau, 1986; Yamasaki & Kuwahara, 1991; Alunno-Bruscia & Saint-Marie, 1998). Analysis of periodic changes of carapace size distributions in field collections has been used to estimate the approximate age of snow crabs from the sizes of the instars at the moult and the annual moulting frequency (Ito, 1970, 1984; Saint-Marie et al., 1995; Alunno-Bruscia & 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°C 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.

5.1.3 Material and Methods

5.1.3.1 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 0.5 kL and 20 kL tanks at 14°C according to the method of Kogane et al. (2007a). Megalopae were stocked in 1 kL and 6 kL tanks at a density of ~1 individuals 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; Marinetech Co., Ltd., 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.

5.1.3.2 Crab culture experiments

Juvenile snow crabs were cultured at nominally 1°C, 3°C, 5°C, and 8°C (see Table 5.1.1 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 August 29, 2009, May 9, 2010, and May 23, 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) fibre-glass-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, ~15 mm) at 2–6 individuals per crab for instars I–V, thawed Antarctic krill *Euphausia superba* Dana, 1850 (Body length, ~50 mm) at 1 individual per crab for instars VI–VII, and artificial pellets for kuruma prawn *Marsupenaeus japonicus* (Bate 1888) culture (Higashimaru Co., Ltd, Kagoshima, Japan) at ~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 Corp., Kanagawa, Japan) or with a digital photomicrographic camera

(DS-Fi1-L2, Nikon Corp., 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).

5.1.3.3 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 & Nelder, 1989; Everitt & 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 & 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; Ha-

masaki 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 Result section); therefore, these relationships were expressed as the following equation: K or $\alpha = a(1 - (1/\exp(bPreCW)))$. 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 the moult was reported in immature males and females (Ito, 1970; Comeau et al., 1998; Alunno-Bruscia & Sainte-Marie, 1998).

5.1.4 Results

Data on the culture temperatures, survival rates, and intermoult periods of each instar are summarised in Table 5.1.1.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. 5.1.1). 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 temperatures, 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 5.1.2); thus, the regression lines between PreCW and growth indices of crabs had similar slopes regardless of temperature (Fig. 5.1.2). 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 ($P < 0.05$).

Table 5.1.1. Mean culture temperature, number of crabs cultured, survival rate, and intermoult durations (number of days) of laboratory-born juveniles of the snow crab *Chionoecetes opilio* reared at four temperatures.

Temperature			N		Survival rate	Number of days	
Instar	(ºC)	Year cohort	Tested	Molted	(%)	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
	1.12	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

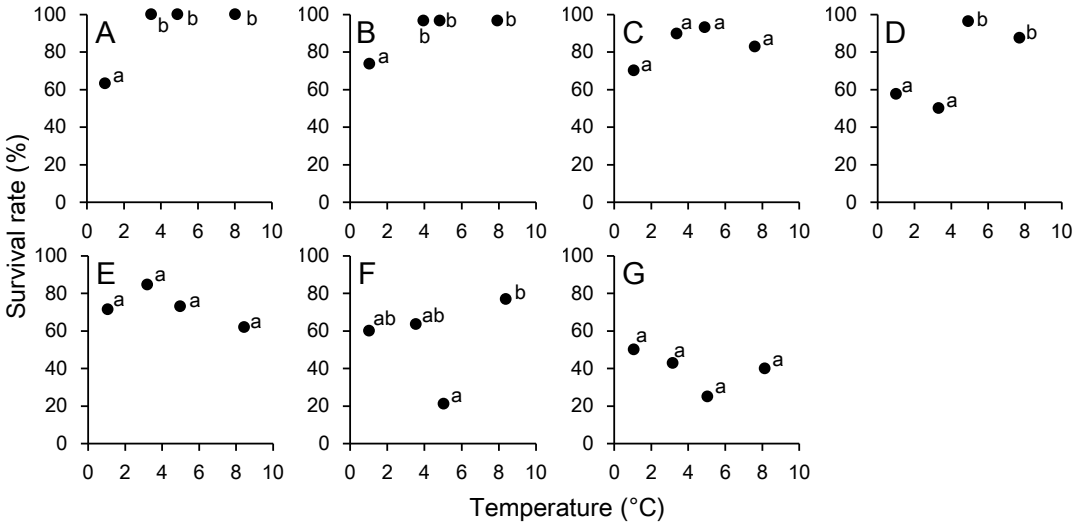

Figure 5.1.1. Survival rates of juvenile snow crabs *Chionoecetes opilio* during each crab instar: A, instar I; B, instar II; C, instar III; D, instar IV; E, instar V; F, instar VI; G, instar VII. Different lower case letters within the same panel indicates statistically significant differences between temperatures (Tukey's HSD, $P < 0.05$).

Table 5.1.2. Results of the general linear model of the dependence of growth indices (PostCW, postmoult carapace width; GI, growth increment; GR, growth rate) of juvenile snow crabs *Chionoecetes opilio* on premoult carapace width (PreCW) and water temperature (WT).

	PoCW				GI				GR			
Source of variation	df	MS	*F*	*p*	df	MS	*F*	*p*	df	MS	*F*	*p*
Interaction model												
PrCW	1	10718.3	31659.59	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
PrCW × 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	72.2		
No interaction model												
PrCW	1	10718.3	31370.74	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		

The intermoult periods (days) of crabs decreased with increasing temperature (Fig. 5.1.3); 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 5.1.3). Thermal constants tended to increase and threshold temperatures tended to decrease with increasing mean PreCW of each instar (Fig. 5.1.4). Their relationships were described by asymptotic regression curves with significant parameter estimates (Table 5.1.4). The as-

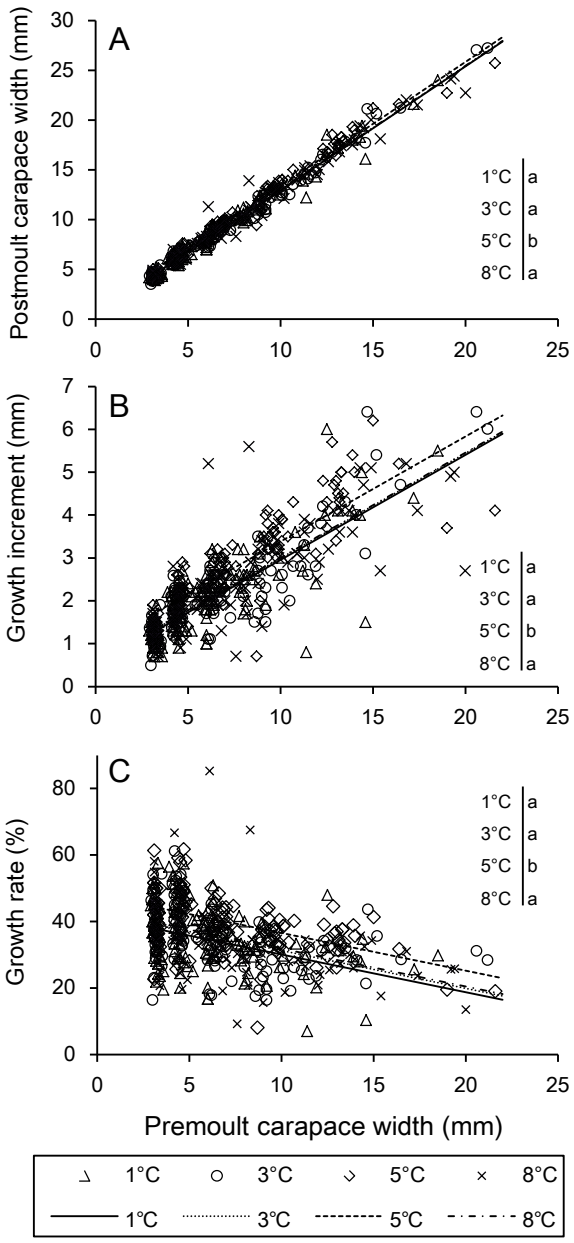

Figure 5.1.2. Relationships between premoult carapace widths and postmoult carapace widths (A), growth increment (B), and growth rate (C) of juvenile snow crabs *Chionoecetes opilio*. The straight lines were drawn from regression analyses. Differences in growth indices between temperature groups ($P < 0.05$) are represented by a different lowercase letter following the temperature in the table.

ymptotes from these equations were 1573 day-degrees for the thermal constant and −4.7°C for the threshold temperature.

5.1.5 Discussion

This is the first study of the growth of juvenile snow crabs in the laboratory over the relatively

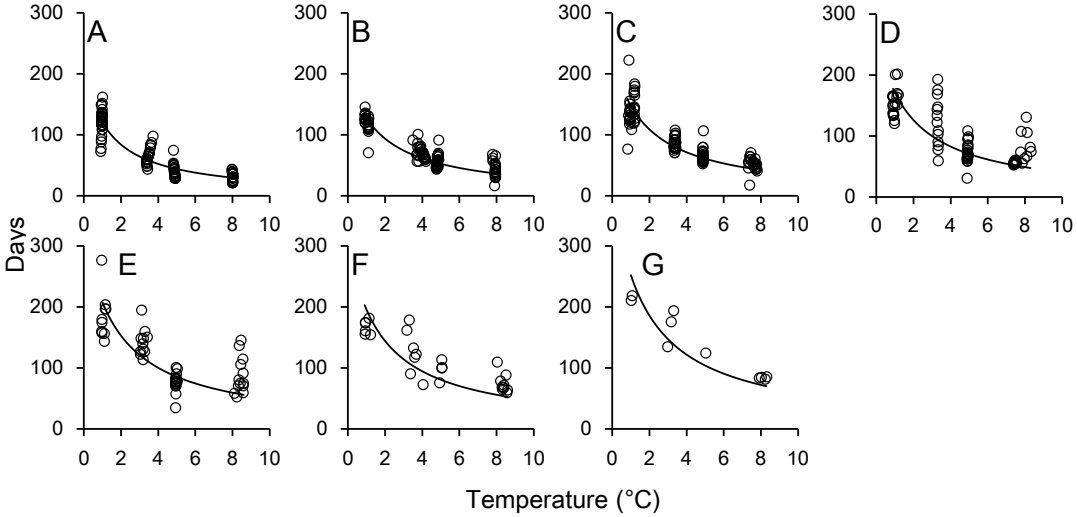

Figure 5.1.3. Relationships between mean water temperature and the intermoult period of each instar of juvenile snow crabs *Chionoecetes opilio*: A, instar I; B, instar II; C, instar III; D, instar IV; E, instar V; F, instar VI; G, instar VII. The curves were drawn from the equation of heat summation theory (see Table 5.1.3) applied to the relationship between the two variables.

wide range of temperature of 1–8°C. We demonstrate that temperature greatly affects their growth and develop a growth model based on the number of day-degrees in the intermoult 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 intermoult periods of some individuals were extended at 8°C compared with crabs at 5°C.

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

Table 5.1.3. Estimates of parameters (with SEs) of the heat summation theoryequation ($D = K / (T - \alpha)$) expressing the relationship between mean temperature (T) and the intermoult 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.

		Estimate (SE)	
Instar	*n*	*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 5.1.4. Estimates of parameters (with SEs) of asymptotic equations (K or $\alpha = a(1 - (1 / \exp(b\text{PreCW})))$) between mean premoult carapace widths (PreCW) and thermal constants (K) or threshold temperatures (α) from the theory of heat summation equations (see Table 5.1.3) for juvenile snow crabs *Chionoecetes opilio*.

Response variable	Estimate (SE)	
	a	*b*
K	1573.00 (154.5)*	0.07 (0.01)*
α	–4.71 (0.70)*	0.14 (0.05)*

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

Gulf of St. Lawrence, the eastern Bering Sea, and the Sea of Japan (Table 5.1.5) (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 5.1.5) (Kobayashi, 1989; Saint-Marie &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

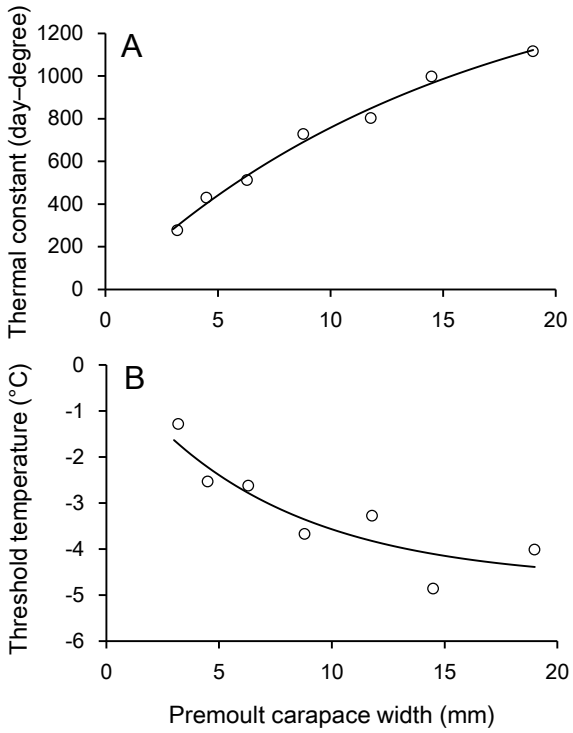

Figure 5.1.4. Relationships between mean premoult carapace width and the thermal constant (A) and threshold temperature (B) in the heat summation equations (see Table 5.1.3) estimated for juvenile snow crabs *Chionoecetes opilio*. The curves were drawn from the asymptotic equations (see Table 5.1.4) applied to the relationship between the two variables.

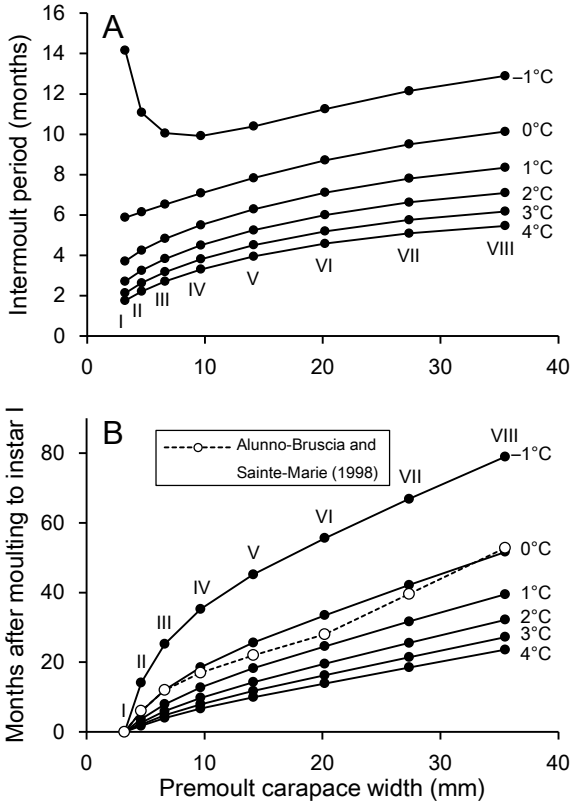

Figure 5.1.5. Relationships between premoult carapace width and intermoult period (A) and age represented by cumulative months after moulting to instar I (B) at −1°C to 4°C calculated from the asymptotic equations between premoult carapace width and thermal constant or threshold temperature for juvenile snow crabs *Chionoecetes opilio* (see Table 5.1.4). 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.

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; Alno-Bruscia & Sainte-Marie, 1998; Zheng et al., 2001; Orensanz et al., 2007; Burmeister & 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 & Sainte-Marie, 2010; Sainte-Marie et al. 2010); 2) intermoult period decreases with increasing temperature during the immature phase (Orensanz et al., 2007; Burmeister & Sainte-Marie, 2010); and 3) there exists an age-related trigger for the pre-pubertal and terminal moults (Orensanz et al., 2007; Burmeister & 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 (day-°C) and threshold temperature (°C) can be calculated by substituting the CW value of the instar into the equations; then, intermoult 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 intermoult 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 & Sainte-Marie (1998), we obtained contour lines expressing the relationship between PreCW and the intermoult period in months at temperatures of −1°C to 4°C, as

shown in Fig. 5.1. 5A. An inverse relationship between mean CW and intermoult 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 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; Saint-Marie et al., 1995; Alunno-Bruscia & Sainte-Marie, 1998; Comeau et al., 1998; Ernst et al., 2012). In the Gulf of Saint Lawrence and the eastern Bering Sea, the intermoult period of snow crabs was estimated at 5–7 months for instars I-V and 1 year from instar VI (Saint-Marie et al., 1995; Alunno-Bruscia & Sainte-Marie, 1998; Comeau et al., 1998; Ernst et al., 2012). Our calculated intermoult 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 intermoult 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 & Sainte-Marie, 1998) were similar to our age estimates of crabs grown at 0–1°C (Fig. 5.1. 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 intermoult 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 intermoult 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, Yosho & 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 in-

termoult 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 intermoult periods estimated by Ito (1970, 1984) for the wild population in the Sea of Japan also correspond to the estimates of intermoult 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 5.1.5. Carapace width (mm) at instars I-VIII of cultured and wild populations of the snow crab *Chionoecetes opilio*.

	Cultured crab (mean value)								Wild population (mean or modal value)					
	This study				Kobayashi (1989)		Saint-Marie and Lafrance (2002)		Gulf of St. Lawrence		Newfoundland		Eastern	Sea of
Instar	1°C	3°C	5°C	8°C	3°C	8°C	crab	exuvium	male	female	female	male	Bering Sea	Japan
I	3.2	3.2	3.2	3.2	2.7	2.8		3.03	3.19	3.22	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			4.3–4.4
III	5.9	6.3	6.8	6.2	5.2	5.7	5.48		6.60	6.62	7.0		7.9	6–7
IV	8.1	8.5	9.6	8.6	6.9	8.3			9.66	9.67	9.7	9.8	11.6	9–10
V	10.8	11.0	12.9	11.2	8.8	11.3			14.10	14.14	14.7	14.9	16.4	13–14
VI	13.9	13.9	17.5	14.6	11.9	13.6			19.96	20.20	20.9	21.6	21.4	19–20
VII	17.9	18.9	20.3	18.5	16.3	18.0			26.88	27.35	27.7	28.0	29.6	27–28
VIII	22.8	24.0	25.7	23.2	23.7	23.0			34.47	35.50	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).

5.2 Growth and moulting of wild-born immature snow crabs, *Chionoecetes opilio*, in the laboratory

5.2.1 Summary

Growth and moulting of wild-born immature snow crabs *Chionoecetes opilio* were investigated by laboratory culture experiments. Crabs with 16.2–42.9 mm carapace width caught from the Sea of Japan were cultured at a temperature of their natural habitat (approximately 1°C). The growth indices (size increments at moulting in mm and in % of premoult carapace width) and intermoult period were significantly affected by premoult carapace width, but sex did not affect these variables. Furthermore, we demonstrated that premoult carapace width and days after moulting significantly affected moulting probability and we developed a moulting probability model based on these variables. From this model, the number of days of intermoult periods when moults occurred in 50% of crabs of instars VI, VII, and VIII was estimated at 234, 284, and 346 days, respectively.

5.2.2 Introduction

The snow crab *Chionoecetes opilio* Fabricius, 1788 (Brachyura, Majoidea) is widely distributed on muddy and sandy mud at depths between 3 m and 1400 m in cold waters off Alaska, Canada, Russia, Greenland, Japan, and Korea and is an important fishery resource in these regions (Yosho & Hayashi, 1994; Lovrich et al., 1995; Jadamec et al., 1999; Dawe & Colbourne, 2002; Burmeister & Sainte-Marie, 2010). 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 bed, snow crabs change their spatial distribution 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: male snow crabs with large chelae, and female snow crabs with a broad abdomen (Ito, 1957; Conan & Comeau, 1986; Yamasaki &

Kuwahara, 1991; Alunno-Bruscia & Saint-Marie, 1998). Analysis of periodic changes of carapace size distributions in field collections have been used to estimate the approximate age of snow crabs from the sizes of the instars at the moult, and the annual moulting frequency (e.g., Ito, 1970, 1984; Saint-Marie et al., 1995; Alunno-Bruscia & Sainte-Marie, 1998; Comeau et al., 1998).

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 toward sustainable fisheries (Hoggarth et al., 2006; Chang et al., 2012). However, crustaceans grow by moulting and they generally lack physical structures suitable for age estimation (but see Kilada et al., 2012). Therefore, captive rearing is an effective method for estimation of age and growth of crustaceans. The growth and intermoult period of the snow crab were studied by Kobayashi (1989) and Yamamoto et al. (2015a) using laboratory-born juvenile crabs. However, the mean carapace width (CW) values of each instar of laboratory-cultured snow crab juveniles were much smaller than those of wild crabs (Kobayashi, 1989; Yamamoto et al., 2015a). Moreover, Kobayashi (1989) reared crabs from instar I to instar XI (mature) at 3 and 8 °C, temperatures that are higher than that of the natural habitat in the Sea of Japan (Yosho & Hayashi, 1994). Yamamoto et al. (2015a) reared crabs from instar I to instar VIII at 1–8°C and estimated the effect of temperature on the snow crab intermoult period, but the intermoult periods after instar VII remained unclear. Growth with moulting of laboratory-reared snow crab was also studied by Kon (1980), Moriyasu et al. (1987), Sainte-Marie et al. (1995), Alunno-Bruscia & Sainte-Marie (1998), and Hebert et al. (2002), using crabs captured from the wild, but the intermoult period was studied by Kon (1980) only. Kon (1980) attempted to elucidate the intermoult period of snow crabs, but was unsuccessful because crabs did not moult more than twice.

This study aimed to elucidate the growth and intermoult period of immature snow crabs through laboratory culture experiments, using wild captured crabs. Moreover, we applied a generalized (binomial) linear mixed-effect model (GLMM) to generate the relationship between carapace widths and

moulting probability.

5.2.3 Material and methods

5.2.3.1 Crab source and rearing experiments

A total of 61 immature snow crabs were caught with a bottom otter trawl on June 2–11 and 30, 2011 in the Sea of Japan off the coasts from Ishikawa to Kyoto Prefectures, Japan (Table 5.2.1). The numbers of female and male snow crabs were 30 (16.2–42.9 mm CW) and 31 (16.9–36.3 mm CW), respectively. On the vessel, crabs were kept in cooled boxes where temperatures were maintained at approximately 1°C by immersing frozen seawater and plastic bottles with frozen freshwater in the boxes. Each box was weakly aerated. Crabs were transferred to the Obama Laboratory, Japan Sea National Fisheries Research Institute, Fisheries Research Agency, Fukui Prefecture on June 13 and 30, 2011. The culture experiments were conducted from June 13 and 30, 2011 until December 28, 2012.

Crabs were individually housed and cultured using 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 tanks, according to the growth stage. The cages were placed in 600-L (2.0 × 1.0 × 0.3 m) rectangular fibreglass-reinforced plastic tanks in which water temperatures were controlled at 1°C using a circulating cooling system. The mean temperature (± standard deviation (SD)) during the culture experiment was 1.1 (±0.1) °C. Rearing temperature was selected based on the crab's thermal distribution in the Sea of Japan (Yosho & Hayashi, 1994). 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 h using temperature-recording loggers (HOBO Water Temp Pro v2, Onset Computer Corp., Bourne, MA, USA). Tanks were covered with Styrofoam boards to stabilise the water temperatures. Crabs were fed ad libitum three times per week with thawed Antarctic krill *Euphausia superba* Dana, 1850 (body length approximately 50 mm) at one to four individuals per crab and thawed Japanese littleneck *Ruditapes philippinarum* Adams & Reeve, 1850 (wet weight approximately 5 g) at one individual per crab. The given number of Antarctic krill was increased with crab growth. Before each feeding, uneaten foods, faeces, and grime were re-

Table 5.2.1. Number of crabs cultured, initial carapace width and survival number in successive moult times of wild-captured immature snow crab *Chionoecetes opilio* (Fabricius, 1788).

		CW (mm)		Survival number in successive moult times			
Sex	N	Mean±SD	Range	1	2	3	4
Male	31	24.1 ± 6.3	16.9 – 36.3	27	10	2	0
Female	30	24.1 ± 6.2	16.2 – 42.9	29	17	4	0

CW, carapace width; SD standard deviation.

moved 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 (the occurrence of these crabs was 4.5% of all moulting events). The CW of each intact crab was measured to the nearest 0.1 mm using a digital calliper (CD-S20C, Mitutoyo Corp., Kanagawa, Japan). Measurements taken prior to moulting were termed premoult CW (PreCW), and those taken after moulting were termed postmoult CW (PostCW).

5.2.3.2 Data analysis

Statistical analyses were performed with the R language (R3.1.3; R Core Team, 2015) with a 5% significance level.

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 & Nelder, 1989; Everitt & Hothorn, 2009) to evaluate the effect of PreCW and sex on the growth or intermoult period of immature snow crabs. In these analyses, three indices of crab growth or intermoult period were used as response variables, and PreCW (continuous variable) and sex (categorical variable), as well as the interaction between PreCW and sex were explanatory variables. The GLM analysis was performed with the lm function in R.

The probability of moulting was modelled according to the method of Durán et al. (2013) using a GLMM with a logit link function implemented in the lme4 package (Bates et al., 2014) in R. Succes-

sive measurements from the same crab were considered non-independent repeated measurements, and the effect of the individual crab was considered a random factor. GLMM was specified as follows:

$$\text{Moult/non}_{ij} \sim \text{binomial}\left(p_{ijk},\ 1\right)$$

$$\text{Logit}\left(p_{ijk}\right) = \beta_0 + \beta_1 \text{DAM}_{ijk} + \beta_2 \text{PreCW}_{ij} + \text{Crab Effect}_j$$

$$\text{Crab Effect}_j \sim \text{normal}(0,\ \sigma),$$

where p_{ijk} is the probability of moulting after k days from the last moult. The ij ranges from 1 to 33 intermoult period values (j = 27 crabs, i = 1 to 2 intermoult periods). DAM denotes the days after last moult. Crab Effect is assumed to be normally distributed with zero mean and standard deviation σ. In GLMM analysis, sex was not considered because there was no statistically significant difference between sexes in the intermoult period analysis (see Results section).

5.2.4 Results

Survival numbers in successive moult times of each sex are summarised in Table 5.2.1. Most crabs moulted once and nearly half the crabs twice, but only 10% of crabs moulted three times, and no crabs moulted four times. At the end of the experiment, 10 male crabs and 12 female crabs were alive.

PreCW significantly affected all growth indices but sex did not affect growth (Table 5.2.2). Moreover, the interaction between PreCW and sex was not significant (Table 5.2.2); thus, the regression lines between PreCW and growth indices of crabs had similar slopes regardless of sex (Fig. 5.2.1).

The intermoult period (days) of crabs was highly variable, ranging from 129 days to 392 days. The intermoult periods increased significantly with increasing PreCW in both sexes (Fig. 5.2.1), and there was no statistically significant difference between sexes (Table 5.2.2). Estimates of the moultingprobability as a function of the DAM experienced by a crab and the PreCW in the GLMM showed that both effects are significant in the sense that the 95% credibility intervals do not include zero (Table 5.2.3). In this model, between-crab variability was important, as indicated by the highest value of

σ.

Table 5.2.1. Number of crabs cultured, initial carapace width and survival number in successive moult times of wild-captured immature snow crab *Chionoecetes opilio* (Fabricius, 1788).

Source of variation	PostCW				GI				GR				Intermoult period			
	df	MS	*F*	*p*	df	MS	*F*	*p*	df	MS	*F*	*p*	df	MS	*F*	*p*
PreCW	1	4338.0	2108.22	0.000	1	74.6	36.27	0.000	1	484.3	18.89	0.000	1	57350.0	24.40	0.000
SEX	1	2.1	1.03	0.313	1	2.1	1.03	0.313	1	25.4	0.99	0.323	1	16.0	0.01	0.934
PreCW × SEX	1	0.0	0.00	0.991	1	0.0	0.00	0.991	1	0.0	0.00	0.995	1	395.0	0.17	0.685
Residuals	68	2.1			68	2.1			68	25.6			29	2350.7		

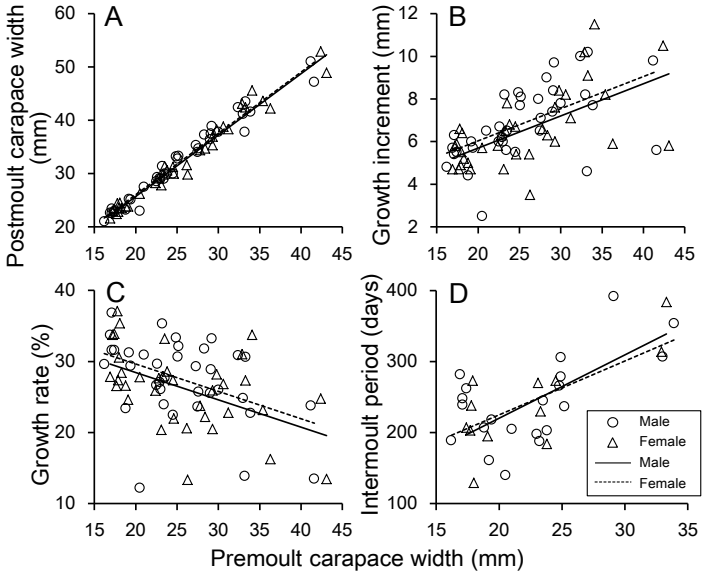

Figure 5.2.1. Relationships between premoult and postmoult carapace widths (A), growth increment (B), growth rate (C), and intermoult period (D) of immature *Chionoecetes opilio* (Fabricius, 1788). The straight lines were drawn from regression analyses.

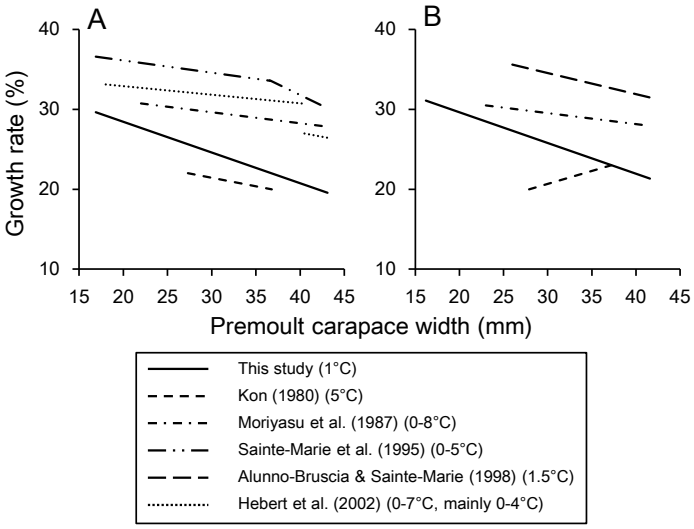

Figure 5.2.2. Changes in the growth rate with premoult carapace width in male (A) and female (B) of the snow crab *Chionoecetes opilio* (Fabricius, 1788) in the current and previous studies. Culturing crabs were captured from the Sea of Japan (this study; Kon, 1980), Baie des Chaleurs in the Gulf of Saint Lawrence (Moriyasu et al., 1987; Hebert et al., 2002), and Baie Sainte-Marguerite in the Gulf of Saint Lawrence (Sainte-Marie et al., 1995; Alunno-Bruscia & Sainte-Marie, 1998). The culturing temperatures are shown in the legend. Sainte-Marie et al. (1995) showed a breakpoint in the regression. Hebert et al. (2002) showed the regressions for immature and adolescent crabs.

Table 5.2.3. Parameter estimates (median and 95% credibility intervals) of the moulting probability model of immature snow crab *Chionoecetes opilio* (Fabricius, 1788).

Parameter	2.5%	Median	97.5%	*p*
β0	−23.479	−21.211	−16.765	<0.000
β1 (DAM)	0.180	0.189	0.205	<0.000
β2 (PreCW)	−1.248	−1.174	−1.028	<0.000
σ Crab effect	70.418	73.340	76.262	

PreCW, premoult carapace width; DAM, days after last moult

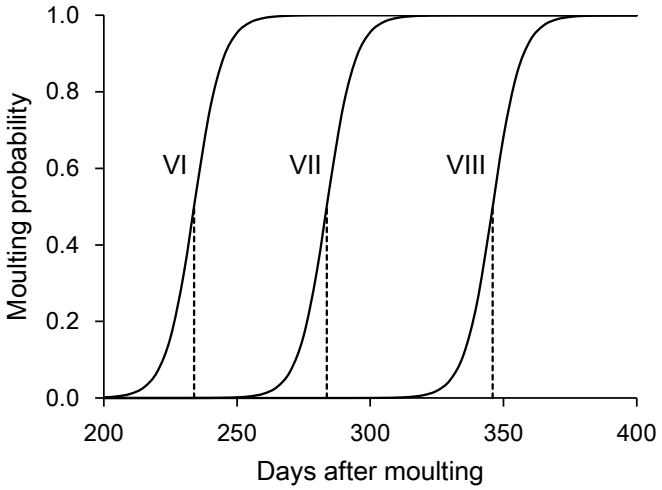

Figure 5.2.3. Moulting probability for progressively larger *Chionoecetes opilio* (Fabricius, 1788), in instar VI (carapace width 19.5 mm), instar VII (carapace width 27.5 mm), and instar VIII (carapace width 37.5 mm), drawn from the generalized (binomial) linear mixed-effect model (see Table 5.2.3). Carapace widths of each instar are from wild snow crabs reported by Ito (1970). The vertical dotted lines indicate the days for half moulting probability.

5.2.5 Discussion

In this study, snow crabs with 16.2–42.9 mm CW were reared and the growth of crabs with 16.2–43.1 mm PreCW and the intermoult period of crabs with 16.2–33.9 mm PreCW were determined. We found that growth and intermoult period were significantly affected by PreCW, but sex was not affected by these variables. Furthermore, we demonstrated that PreCW and DAM significantly affected

moulting probability, and we developed a moulting probability model based on these variables.

There have been several studies that conducted captive rearing experiments of immature snow crabs to reveal their growth, using crabs captured from the natural habitat: off Wakasa Bay in the Sea of Japan (Kon, 1980), Baie des Chaleurs in the Gulf of Saint Lawrence (Moriyasu et al., 1987; Hebert et al., 2002), and Baie Sainte-Marguerite in the Gulf of Saint Lawrence (Sainte-Marie et al., 1995; Alunno-Bruscia & Sainte-Marie, 1998). Moreover, laboratory-born juvenile snow crabs were used for captive rearing experiments to determine the growth and intermoult period (Kobayashi, 1989; Yamamoto et al., 2015a). Like the current study, previous studies suggested that CW, growth rate (GR), and intermoult period of immature snow crabs are similar between the sexes under laboratory conditions (Kon, 1980; Moriyasu et al., 1987; Kobayashi, 1989; Alunno-Bruscia & Sainte-Marie, 1998). Here, we compared the relationships between PreCW and GR of captive wild-born snow crabs from different studies (Fig. 5.2.2). GR of the immature crabs in the Gulf of Saint Lawrence were relatively higher than those of the Sea of Japan, and this tendency was observed in both sexes. However, this difference might not be attributed to the geographical variation, because, field studies have estimated the mean CW of each juvenile snow crab instar using size-frequency analysis and have demonstrated that CWs are similar in snow crab populations in the Gulf of Saint Lawrence, the eastern Bering Sea, and the Sea of Japan (Comeau et al., 1998; Orensanz et al., 2007; Ernst et al., 2012). On the other hand, it has been reported that the mean CW values of each laboratory-cultured snow crab instar (Kobayashi, 1989; Sainte-Marie & Lafrance, 2002; Yamamoto et al., 2015a) and also other decapod crustaceans (Kurata, 1962; Hartnoll, 1982) were smaller than those of wild crabs. Stevens (2012) suggested that this phenomenon 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. Therefore, the lower GR in the current result might be affected by these factors.

The primary subjects in previous studies (Moriyasu et al., 1987; Sainte-Marie et al., 1995; Alunno-Bruscia & Sainte-Marie, 1998; Hebert et al., 2002) that collected crabs from their natural habitat and reared them in the laboratory were to mainly understand the moulting season and the growth at

moulting. Kon (1980), however, reared 341 immature crabs to determine the intermoult period, but crabs did not moult more than twice. Therefore, the intermoult period of captive wild immature snow crabs has not been determined. However, Kobayashi (1989) and Yamamoto et al. (2015a) reared laboratory-born snow crabs from instar I to XI and instar I to VIII, and showed intermoult periods from instar I to X and instar I to VII, respectively. Kobayashi (1989) reared crabs at 3 and 8°C, but these temperatures are higher than that of the natural habitat in the Sea of Japan. Yosho & Hayashi (1994) reported that juvenile snow crabs of >10 mm carapace length, i.e. instar III >8 mm CW (calculated from Ito (1984)), live at a temperature range of 0.3–0.9°C. Yamamoto et al. (2015a) reared crabs at 1–8°C and estimated the effect of temperature on snow crab intermoult period, but the intermoult periods after instar VII were unclear. Ito (1970) estimated the modal value of CW in each instar as follows: instar VI, 19–20 mm; instar VII, 27–28 mm; and instar VIII, 37–38 mm. These values of CWs were assigned as 19.5 mm, 27.5 mm, and 37.5 mm to the moulting probability growth model in the current study (Table 5.2.3); then, the number of days of intermoult periods when moults occur in 50% of crabs in each instar increases with growth; 234 days (instar VI), 284 days (instar VII), and 346 days (instar VIII) (Fig. 5.2.3). These values of intermoult periods of the instars VI (PreCW 19.5 mm) and VII (PreCW 27.5 mm) crabs were relatively longer than those of the instars VI (PreCW 13.9 mm) and VII (PreCW 17.9 mm) laboratory-born crabs cultured at ~1°C by Yamamoto et al. (2015a). Yamamoto et al. (2015a) developed the growth model of the snow crab based on the relationships between PreCW (<19 mm) and thermal constant or threshold temperature in the heat summation theory equations. From this growth model, the intermoult period at PreCW 19.5 mm (instar VI in the current study) could be estimated at 258 days. This calculated value is similar to the estimated intermoult period (234 days) for instar VI crabs in current study. Thus, the intermoult period of the snow crab principally depends on the PreCW.

The intermoult periods of snow crab after instar VI were estimated as once per year by periodic field sampling in the Sea of Japan (Ito, 1970) and Gulf of Saint Lawrence (Sainte-Marie et al., 1995; Alunno-Bruscia & Sainte-Marie, 1998; Hebert et al., 2002). To clarify the difference in the intermoult

period between wild population and cultured crabs, further investigation of the environment of their habitat and further study of the effect of various factors on the intermoult period of snow crabs is needed. Understanding the factors influencing gaps of growth between wild and captive crabs would provide useful biological and environmental information for better understanding the causes of fluctuations of snow crab populations in the wild.

Chapter 6

CONCLUDING REMARKS

6.1 Conclusion

It is important to understand the life history of a species to understand stock dynamics and manage stocks. Captive rearing experiments were conducted under laboratory conditions to better understand early snow crab life history. The following results were shown in the current study.

The effects of water temperature on snow crab larval survival and development were investigated in Chapter 2.1. The results show that higher survival rates were observed at 5–14°C from hatching to second-stage zoeae, 5–11°C from hatching to megalopae, and 5–11°C from megalopae to first-stage crabs. Water temperature greatly influenced snow crab larval developmental rate. The threshold temperatures estimated from the heat summation theory equations for larval development were −2.24 to 0.63°C; they decreased as the larvae developed and adapted to deeper vertical distributions in the water column at later larval stages.

The effects of salinity on snow crab larval survival and development were investigated in Chapter 2.2. As results, higher survival rates were observed at 20–38 from hatching to second-stage zoeae, 26–38 from hatching to megalopae, and 28–36 from megalopae to first-stage crabs. The mean durations from hatching to the second zoeal and megalopal stages and from the megalopal to the first-stage crab were shortest at salinities of 30, 30, and 32, respectively, and increased progressively at salinities above and below these values.

Food consumption patterns of snow crab megalopae were examined using *Artemia* nauplii in Chapter 3. The results show that the mean total number and total weight of *Artemia* consumed during the megalopal stage were 1920 individuals and 5.2 mg, respectively, and the food consumption rate decreased after the beginning of the late premoult stage. The food requirement of snow crab megalopae was estimated to be 190% of dry body weight of the first instar crab, and a positive correlation was detected between the number of *Artemia* consumed and crab size.

The moulting cycle and time course changes in snow crab larval body density were examined in laboratory-reared specimens in Chapter 4. The moulting cycle was documented photographically to characterize the stages: A–B (postmoult), C (intermoult), D (premoult), and E (ecdysis). The body

density of larval snow crabs was lowest just after moulting, increased dramatically during stage C, and then increased gradually to reach a plateau at 1.0897–1.0931 g cm^{-3} during stage D. The snow crab larvae had a density greater than that of seawater during the entire larval period.

The effects of water temperature on growth of instar I–VII snow crabs were investigated in Chapter 5.1. The results show that the growth indices, including postmoult carapace width, increased during the moult stage, and growth rate was significantly higher in crabs reared at 5°C than in those reared at other temperatures. The thermal constant and threshold temperature estimated from the heat summation theory equations for crab development tended to increase and decrease with increasing mean premoult carapace width of each instar, reaching asymptotes of 1,573 day-degrees and −4.7°C, respectively.

Growth and moulting of wild-born immature snow crabs (carapace width, 16.2–42.9 mm) were assessed in the laboratory in Chapter 5.2. As results, growth and the intermoult period were significantly affected by premoult carapace width, but not sex. Premoult carapace width and days after moulting significantly affected the probability of moulting, and a moulting probability model based on these variables was developed. The model revealed that the numbers of days during the intermoult periods when moults occurred in 50% of instar VI, VII, and VIII crabs were 234, 284, and 346 days, respectively.

These results provide important information for inferring snow crab larval distribution, transport, and survival and for estimating growth and the intermoult periods in the benthic stage in their natural habitat. Furthermore, these results will help in understanding the potential effects of climate change on the snow crab population. These results could be applied to snow crab larval and juvenile culture under optimal conditions.

6.2 Snow crab life history cycle in the Sea of Japan

The lifecycle of the snow crab in the Sea of Japan is discussed in this sub-chapter with reference to snow crab ecological and environmental information on their natural habitat, such as temperature, salinity, and water density.

The snow crab hatching season occurs during February–April (Ito, 1963; Fukataki, 1969; Kon et al., 2003). Seasonal and horizontal larval distributions in the Sea of Japan have been reported by Fukataki (1969) off Honshu, but he only investigated surface water. Seasonal and vertical larval zoeae and megalopae distributions have been reported by Kon et al. (2003) from mid-March to early June in waters off Fukui Prefecture, and Honda (2013) reported those for megalopae from the end of May to early July in the waters off Niigata and Shimane Prefectures. Fukataki (1969) and Honda (2013) mainly found snow crab larvae east of 133°E and south of 38°N in the middle and eastern parts of the Sea of Japan. Data on snow crab vertical distributions based on temperature and salinity at 132, 135.5, and 138.5°E were published on the website after 2003 as the "Japan Sea Data Assimilation Experiment ver. 2" (JADE 2, http://jade2.dc.affrc.go.jp/jade2/). Temperature, salinity, and specific gravity data for 36, 37, and 38°N at 135.5°E from January to July 2014 were referred to as a representative location to estimate the general larval development trend in the Sea of Japan and are illustrated in Fig. 6.2.1, 6.2.2, and 6.2.3. The specific gravities were calculated from Millero et al. (1980).

Kon et al. (2003) found that first zoeae, second zoeae, and megalopae occur mainly in the 0–100, 0–150, and 50–200 m strata, respectively. Honda (2013) reported that megalopae occur mainly in the 100–300 m stratum. Newly hatched first-stage zoeae might be exposed to a temperature range of 8–12°C in the 0–100 m stratum in the middle of the hatching season in March (Fig. 6.2.1). Second zoeae are also exposed to 8–12°C in the 0–150 m stratum. The duration of the zoeal stage was estimated to be 37–66 days, based on the heat summation theory equation (Table 2.1.3). Thus, zoeae might metamorphose to megalopae during April and May. Megalopae could be exposed to a wider range of temperatures than those of zoeae, such as 2–14°C in the 50–300 m stratum (Fig. 6.2.1). Thus, the duration of the megalopal period was estimated to be 26–98 days based on the heat summation theory equation, and megalopal settlement and moulting into crabs on the bottom of the sea would occur 2–5 months

after hatching. Salinity is maintained at a fairly constant 34.0–34.5 at this time, regardless of season and depth (Fig. 6.2.2). Thus, snow crab larval survival and development were not affected by salinity (Tables 2.2.1 and 2.2.2). The specific gravity of seawater decreased over time in the upper strata (~100 m) but increased with increasing depth (Fig. 6.2.3). However, the specific gravity was always less than larval body density (Fig. 4.1.2). Therefore, the specific gravity of seawater may not affect larval vertical distribution. The snow crab larval depth distribution increases during development (Incze, 1981; Incze et al., 1987; Kon et al., 2003), but the timing of the megalopal settlement phase is unknown. The megalopal feeding rate decreased significantly after the intermediate premoult stage (Fig. 3.1.3). Thus, megalopae may settle to the bottom during this phase, and then moult into crabs.

Several studies have collected immature snow crabs from various depths of their natural habitat in the Sea of Japan (Kon, 1969; Ito, 1970, 1984; Ishikawa Prefectural Fisheries Experimental Station, 1981, 1982; Yosho & Hayashi, 1994; Kanemaru, 1994). Here, the relationships between the collected depth, month, and snow crab instars from different studies were examined (Tables 6.2.1 and 6.2.2). As a result, crab instars I–II, III–VII, and VIII are collected mainly from 200–500 m, 250–500 m, and 200–400 m, respectively, but the depth distributions after instar IX were ambiguous owing to differences in survey depth data (Kon, 1969; Ito, 1970; Ishikawa Prefectural Fisheries Experimental Station, 1981, 1982), and the small number of crabs caught (Yosho & Hayashi, 1994). Crab instars I, II, III, and after IV are caught mainly during May/June–October (Ishikawa Prefectural Fisheries Experimental Station, 1981, 1982; Ito, 1984), August/September–January (Ishikawa Prefectural Fisheries Experimental Station, 1981, 1982; Ito, 1984), September–May (Ito, 1984), and year round (Ito 1970; Ishikawa Prefectural Fisheries Experimental Station, 1981, 1982), respectively. Water temperature on the continental slope at 135.5°E in 2014 was referred from JADE 2 as a representative location to estimate the general moulting pattern of immature snow crab in this sub-chapter (Fig. 6.2.4). According to the crab depth distribution data and temporal-spatial temperature distribution, instar I–II crabs are exposed to 0.8–7°C, instar III–VII are exposed to 0.8–4°C, and instar VIII crabs are exposed to 0.9–7°C. A snow crab growth model was developed in Chapter 5.1, based on the relationships between

PreCW (<19 mm) and the thermal constant or threshold temperature from the heat summation theory equations (Table 5.1.4). The value of PreCW 19 mm corresponds to the modal value of instar VI CW reported by Ito (1970). Moreover, the snow crab intermoult period depends on PreCW (Chapter 5.2). Therefore, the instar I–VI intermoult periods were estimated at these temperature ranges by using this growth model (Table 6.2.3). Ito (1970, 1984) estimated the modal value of CW in each instar as follows: instar I, 2.9–3.0 mm; instar II, 4.3–4.6 mm; instar III, 6.3–6.6 mm; instar IV, 9–10 mm; instar V, 13–14 mm; instar VI, 19–20 mm. Thus, the values of 2.95 mm, 4.45 mm, 6.45 mm, 9.5 mm, 13.5 mm, and 19.5 mm were applied in this growth model. The relationship between temperature and PreCW >19 mm was not investigated in this study; therefore, the instar VII and VIII intermoult period of wild-born crabs reared at 1°C was used for calculating the age at terminal moult (Table 6.2.3). Furthermore, the intermoult periods after instar IX could not be investigated in this study. Pubescent crabs reportedly undergo an annual moult in the Sea of Japan (Ito, 1970), except for some that skip moulting (Ueda et al., 2012). The instars of the terminal moult are assumed to be instars X–XIII in male crabs and XI in female crabs for managing the snow crab population in the Sea of Japan (Ueda et al., 2012). Therefore, the durations from hatching to terminal moult instars were estimated to be 4–9 years in male crabs and 5–7 years in female crabs (Table 6.2.3).

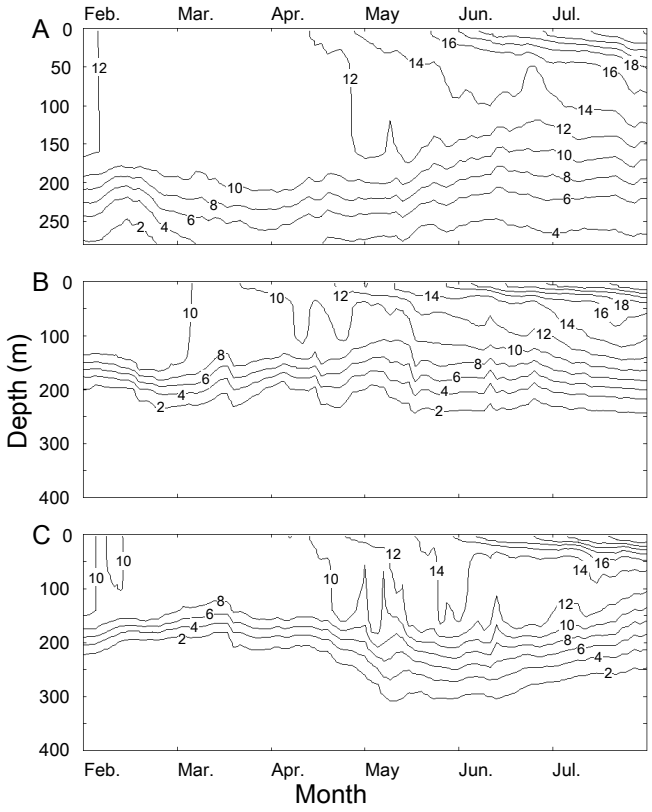

Figure 6.2.1. Vertical and seasonal temperature distributions in the Sea of Japan, in 2014 (from JADE 2): A, 36°N, 135.5°E; B, 37°N at 135.5°E; C, 38°N at 135.5°E.

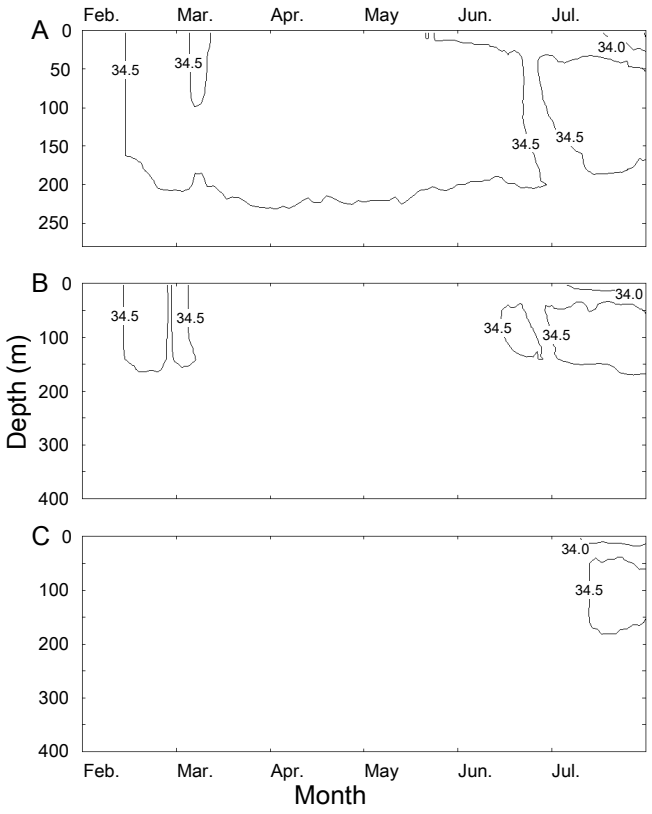

Figure 6.2.2. Vertical and seasonal salinity distributions in the Sea of Japan, in 2014 (from JADE 2): A, 36°N, 135.5°E; B, 37°N at 135.5°E; C, 38°N at 135.5°E.

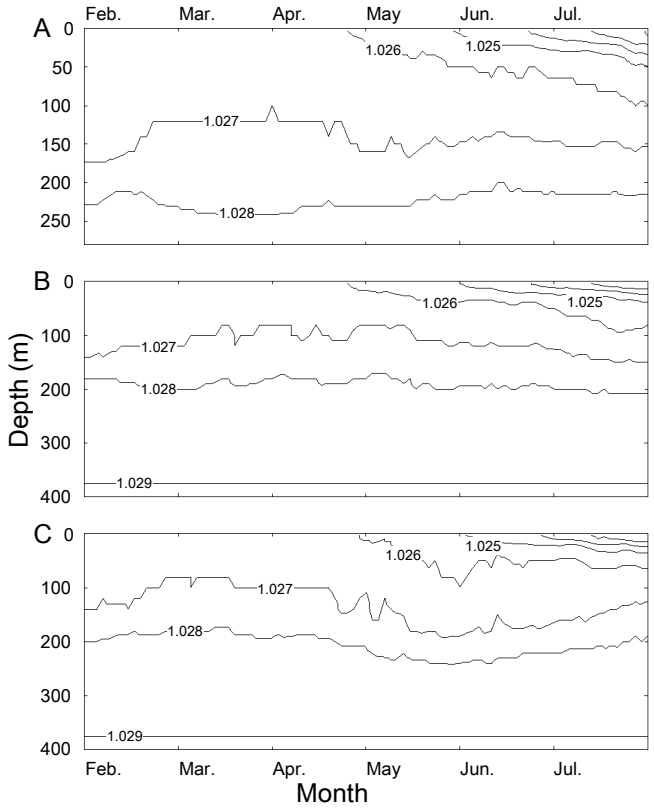

Figure 6.2.3. Vertical and seasonal water density distributions in the Sea of Japan, in 2014 (temperature and salinity, JADE 2; water density, calculated from Millero, 1980): A, 36°N, 135.5°E; B, 37°N at 135.5°E; C, 38°N at 135.5°E.

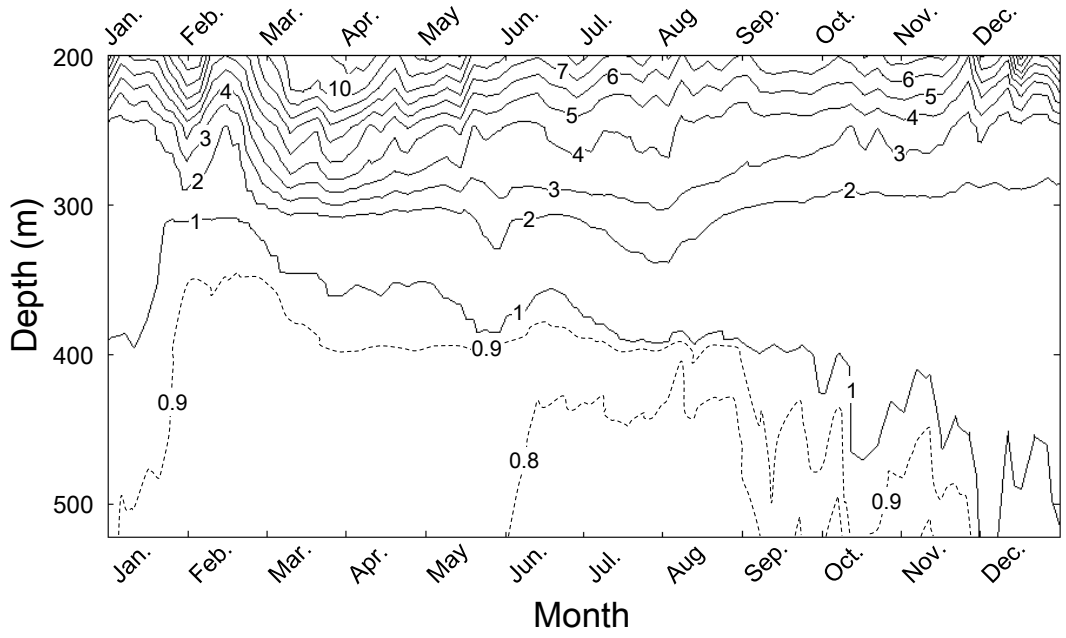

Figure 6.2.4. Changes in water temperature on the continental shelf at 135.5°E off Honshu, Sea of Japan (from JADE 2).

Table 6.2.1. Sea of Japan sampling depth for immature snow crab *Chionoecetes opilio* from previous studies.

		Collected depth in each instar (m)									
Literature	Survey depth (m)	I	II	III	IV	V	VI	VII	VIII	IX	X
Kon (1969)	175–375						≥250	≥250	All depth	≤250	≤250
Ito (1970)	175–350						≥200	≥200	≥200	≥200	≥200
Ito (1984)	200–300	All depth	All depth	All depth							
Ishikawa Pref. (1981, 1982)	200–300	All depth	All depth	≥250	≥250	≥250	≥250	≥250	≥250	≥250	≥250
Yosho & Hayashi (1994)[*1]	60–1500			280–520	280–520	280–520	280–520	280–520	250–280	250–280	250–280
Kanemaru (1996)[*1]	200–700	200–500		250–500			300–400	300[*2]			

*1: Relationship between carapace width, carapace length, and instar was estimated by Ito (1970, 1984) and Oh et al. (2011).

*2: One crab was caught.

Table 6.2.2. Sampling months for collecting immature snow crabs *Chionoecetes opilio* from the Sea of Japan in previous studies.

		Occurrence month in each instar (m)									
Literature	Survey month	I	II	III	IV	V	VI	VII	VIII	IX	X
Kon (1969)	Nov.–Jun.						No data	No data	No data	No data	No data
Ito (1970)	whole year				year round	year round	year round	year round	year round	year round	year round
Ito (1984)	Jun.–Jan.	Jun.–Oct.	Aug.–Jan.	Sep. –May							
Ishikawa Pref. (1981, 1982)	Jan.–Feb.	May–Oct.	Sep.–Jan.								
Yosho & Hayashi (1994)	Oct.			Oct.	Oct.	Oct.	Oct.	Oct.	Oct.	Oct.	Oct.
Kanemaru (1996)	Sep.	Sep.		Sep.			Sep.	Sep.			

Table 6.2.3. Estimated snow crab *Chionoecetes opilio* intermoult periods for each stage/instar and durations from hatching to each stage/instar.

Stage/Instar		Intermoult period (months)	Duration reaching to each stage/instar
Larval stage	Zoea	1 – 2	
	Megalopa	1 – 3	1 month – 2 months
Benthic stage	I	1 – 4	2 months – 5 months
	II	1 – 4	3 months – 9 months
	III	3 – 5	4 months – 1 year 1 month
	IV	3 – 6	7 months – 1 year 6 months
	V	4 – 6	10 months – 2 years 0 month
	VI	4 – 7	1 year 2 months – 2 years 6 months
	VII	9	1 year 6 months – 3 years 1 month
	VIII	11	2 years 3 months – 3 years 10 months
	IX	12	3 years 2 months – 4 years 9 months
	X	12	4 years 2 months – 5 years 9 months
	XI	12	5 years 2 months – 6 years 9 months
	XII	12	6 years 2 months – 7 years 9 months
	XIII		7 years 2 months – 8 years 9 months

Instars I–VI and VII–VIII intermoult periods were calculated by the growth models in Table 5.1.4 and Table 5.2.3, respectively, applying a value of PreCW in each instar estimated by Ito (1970, 1984).
Instar IX–XIII intermoult periods are from Ito (1970).
Terminal moult instars of male crabs (X–XIII) and female crabs (XI) were assumed values for managing the snow crab population in the Sea of Japan (Ueda et al., 2012).

6.3 Future directions

Although a number of studies have elucidated the biology and ecology of mature-stage snow crabs, relatively few studies have been published on larval and early post-settlement stages. This study adds early snow crab life-history data; however, some important information is lacking, including behavioral characteristics, such as upward/downward swimming activities, responses to tidal currents, temperature and light preferences, preferred wavelengths of light, the benthic stage, and temperature and substrate preferences. In particular, the temperature and substrate preferences of benthic juveniles are important to understand the distribution of snow crabs in their natural habitat (e.g., Coulombe et al., 1985; Brêthes et al., 1987; Robichaud et al., 1989; Dionne et al., 2003; Choi, 2010). These characteristics must be described to develop a snow crab stock dynamics model.

Skip moulters are known in both sexes of adolescent snow crabs (e.g., Sainte-Marie et al., 1995; Comeau et al., 1998; Hebert et al., 2002; Ernst et al., 2012). Skip moulting is reportedly caused by temperature and food (Dutil et al., 2010). Adolescent male snow crab skip moulters occur in the Sea of Japan (Ueda et al., 2012), but the factor(s) affecting their occurrence have not been investigated. Moreover, four main diseases affect wild *Chionoecetes* crabs, including Bitter Crab Syndrome (BCS), caused by *Hematodinium* parasitic dinoflagellates (Meyers et al., 1987); Black Mat Syndrome (BMS), caused by the *Trichomaris invadens* ascomycete fungus on the exterior of the carapace (Van Hyning and Scarborough, 1973); hell disease, which is known as black spot caused by a chitinolytic bacterium (Benhalima et al., 1998); and Milky Hemolymph Syndrome (MHS), caused by a bacilliform virus (Kon et al., 2011). The prevalences of BCS, BMS, and MHS may affect snow crab and tanner crab, *Chionoecetes bairdi,* population dynamics (Sparks and Hibbits, 1979; Hicks, 1982; Dawe et al., 2010; Siddeek et al., 2010; Kon et al., 2011; Mullowney et al., 2011; Klinushkin and Ryazanova, 2014). Therefore, research on the pathogenicity and virulence of these diseases under laboratory conditions is needed to understand their impact on snow crab populations. Further studies on the biological and ecological characteristics of snow crab larval and benthic stages and pathogenic organisms would improve the stock management strategy towards a sustainable snow crab fishery by modeling the dispersal and recruitment processes and interactions between pathogens and snow crab populations.

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