Life History of the Galatheid Crab *Munida subrugosa* in Subantarctic Waters of the Beagle Channel, Argentina

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Abstract

Galatheid crabs of the genus *Munida* are the most abundant decapods in coastal waters off Tierra del Fuego, including the Beagle Channel (55ºS, 68ºW). Other galatheids off New Zealand, the North Pacific, and Central America, have proved to be of potential commercial interest, but the only fishery currently exploited is that for *Pleuroncodes monodon*, off central Chile (ca. 35ºS).

Monthly benthic samples were taken in the Beagle Channel starting in November 1997 in order to investigate distribution, reproduction, and feeding habits of *Munida subrugosa*. Two galatheid species were found, *M. subrugosa* being significantly more abundant than *M. gregaria.* The reproductive cycle of *Munida subrugosa* started in June, and was reflected by the maximum size of oocytes, the maximum value of gonadosomatic index in both females and males, and by the proportion of ovigerous females. The embryonic development lasted 90-120 days. Fecundity (~100-11,000 eggs) correlated with female size. Females attained their gonadal maturity size at ~11 mm carapace length (CL). Males reached morphometric maturity at ~24 mm CL, although males ~10 mm CL presented spermatophores. *Munida subrugosa* has two different feeding habits, as a predator (feeding on crustaceans and macroalgae) and/or as a deposit feeder (ingesting sediment, foraminiferans, diatoms, and particulate organic matter). The proportion of inorganic matter was higher at depths >40 m, suggesting that the condition of deposit feeder increases with depth.

Introduction

The galatheid crabs *Munida subrugosa* and *M. gregaria* (Fig. 1) are anomurans, 5-7 cm total length, that are very abundant in waters of the southwestern Atlantic. *Munida subrugosa* is morphologically similar to *M. gregaria,* and since they are sympatric in the southwestern Atlantic, the two species have been confused and misidentified. Moreover, Williams (1980) suggests that they are morphological variants of the same species, but this topic is still a matter of controversy.

In the Southern Hemisphere, *M. subrugosa* and *M. gregaria* occur in large benthic concentrations, mainly off New Zealand and South America (Rayner 1935, Williams 1980). Around the southern tip of South America, in Atlantic waters, *M. subrugosa* and *M. gregaria* occur on the continental shelf from Uruguay (35ºS) to Cape Horn (55ºS), including the Islas Malvinas (Falkland Islands). Off the Chilean Pacific coast of South America, their southern distribution reaches the island of Chiloé (41ºS; Retamal 1973). The bathymetric distribution of *M. subrugosa* is from the sublittoral down to 1,137 m depth (Gorny 1999).

The economic significance of *Munida* spp. was recognized by Rayner (1935) as early as 1935, but the interest in *M. subrugosa* has occurred only recently due to ecological concerns and the development of several commercial uses. For human consumption, decorticated tails are accepted as cocktail shrimp (Aurioles-Gamboa and Balart 1995, Lovrich et al. 1998; but see Kashkina and Kashkin 1993). Moreover, *P. monodon* proved to be a source of digestive proteases which serves in the manufacture of cheeses (García-Carreño and Fernández-Cortés 1995). Due to high levels of carotenoids, galatheids could be used as a source of pigments for tissue coloring in cultured salmon and trout, and for skin and egg coloring in chickens (Burd and Jamieson 1988, Kashkina and Kashkin 1993, Aurioles-Gamboa and Balart 1995, Carrillo-Domínguez et al. 1995). Finally, due to the amino and fatty acid balance of galatheids, either whole animals or waste portions provide a high quality source for the manufacture of balanced food (Burd and Jamieson 1988, Kashkina and Kashkin 1993, Zeldis 1989).

The only fishery for galatheids has developed off the Chilean Pacific coast (36ºS), and in the 1980s produced profits between $6.2 and $11.8 million per year (US) (Aurioles-Gamboa and Balart 1995). In 1977, the annual landing for this fishery peaked at ca. 50,000 t of *Pleuroncodes monodon* and *Cervimunida johni*. Thereafter, landings dramatically decreased to ca. 8,000 t (Aurioles-Gamboa and Balart 1995) and the fishery was closed for 3 years due to overfishing (Roa and Bahamonde 1993). Other scientific initiatives were conceived for the development of new fisheries for galatheids, off the Mexican Pacific coast (25ºN) for *P. planipes* (Aurioles-Gamboa and Balart 1995), off the North American Pacific coast at 48ºN for *Munida quadrispina* (Burd and Jamieson 1988), and off the New Zealand Pacific coast at 45ºS (Zeldis 1989). Nevertheless, none of these potential fisheries has developed yet. Currently, off the Atlantic coast of southern

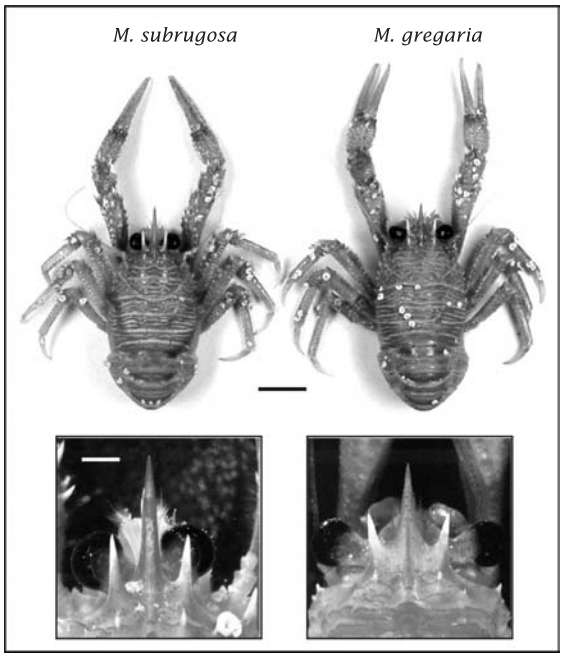

Figure 1. General aspect and morphology of the rostral spine, ocular peduncles, and cornea of Munida subrugosa and Munida gregaria collected in the Beagle Channel, Argentina. The black and white scale bars represent 1 cm (above) and 3 mm (below), respectively.

South America (ca. 40ºS) *M. subrugosa* is the main species in the bycatch of the trawling fisheries for the shrimp *Pleoticus muelleri* and for the king crab *Lithodes santolla* (J.H. Vinuesa, CADIC, and A. Pettovello, Univ. Patagonia Austral, Puerto Deseado, Argentina, pers. comm.).

Munida spp. are suspected to be key species in the coastal subantarctic ecosystem of the southern tip of South America. First, *Munida* spp. are the dominant decapod species in the area, and in terms of biomass they constitute 50% of the benthic community (Arntz and Gorny 1996). Both species are very abundant and video-recorded concentrations are as high as 3-27 individuals per m^2 (Gutt et al. 1999; M. Gorny and M.A. Retamal pers. comm.). Second, the relatively small size, the highly valuable chemical composition, and high densities of occurrence make *Munida* spp. the favorite prey of numerous top predators (e.g., octopuses, crustaceans, fishes, birds, whales, sea lions, seals, and otters) (partially reviewed by Rodríguez and Bahamonde 1986). Third, like their Pacific relative *P.*

monodon, Munida spp. are believed to prey on small herbivores and graze on algae. Hence, these species are suspected to constitute a direct intermediate between the lower and the higher levels of the trophic web. Therefore, fishing for *Munida* spp. could have a negative effect on the abundance of the top predators, unless they are carefully managed.

Due to the collapse of other crustacean fisheries such as *Lithodes santolla* (Lovrich 1997), *Munida subrugosa* of southern South America could reach commercial interest in the near future. However, the background knowledge about its biology is currently limited. In this study we document basic biological parameters such as the abundance, reproduction, and feeding habits of *M. subrugosa* to provide some basic commercial exploitation guidelines, in order to ensure the lowest possible impact on the ecosystem.

Material and Methods

Study Site and Sampling

The Beagle Channel is situated at the southern tip of South America (55ºS, 68ºW) and is delimited by the Isla Grande de Tierra del Fuego to the north, and Isla Hoste and Isla Navarino to the south. The channel has an irregular sea bottom, consisting of muddy or mud-sandy sediments (Brambatti 1991).

Sampling was conducted on a monthly basis in a sector of 45 km of the Beagle Channel Argentina, from Punta Segunda to Bahía Lapataia, between November 1997 and December 1999. Samples were obtained with an epibenthic trawl net of 10 mm mesh size and 1.7 m mouth width, specially designed to operate with a small boat. For each sampling period one sample was obtained at each of six randomly selected positions at each of three sampling locations (Punta Segunda, Ushuaia, and Lapataia) in each of two depth strata namely <40 m and >40 m. The 40 m depth is known for the lower bathimetric distribution of benthic macroalgae, e.g., *Macrocystis pyrifera* (Küneman 1970). Tows were performed at 0.65-0.80 m per second during 5-25 min. The initial and final positions of the tows were recorded with a geostationary satellite positioning system. The maximum depth of each haul was also recorded. All but 30 captured crabs were immediately fixed in 4% formalin in seawater. The other 30 crabs were frozen at –18ºC.

In the laboratory, both species (*Munida subrugosa* and *M. gregaria*) were determined to specific levels based on the following characters: (1) basis of rostral spine wider in *M. gregaria,* (2) ocular peduncles longer in *M. gregaria,* (3) cornea of *M. subrugosa* kidney-shaped (Fig. 1) and (4) meropodite of the third maxilliped in *M. subrugosa* bearing distal spine (Retamal 1981). Crabs were further sorted by species, sex, and ovigerous condition. The standard measure of body size, carapace length (CL), was determined to the nearest 0.1 mm on all collected crabs with a dial calliper. The carapace length was the midline distance between the posterior orbital margin, excluding rostral spine, to the posterior median margin.

Density and Biomass

Crab densities of *M. subrugosa* and *M. gregaria* and biomass of *M. subrugosa* were calculated using the tow distance and the trawl width, and are presented as number of crabs or wet weight of crabs per 100 m^2, respectively. Tow distance over the bottom was estimated as the difference between boat positions at the beginning and at the end of each tow. Wet weight used for biomass calculations was evaluated from a subsample of 369 *M. subrugosa,* which were measured and weighed to the nearest 0.01 g. A predictive regression (Sokal and Rohlf 1995) between CL and wet weight (ww) was used, as follows: log ww = 3.03 × logCL – 3.17 (Tapella, unpubl. results). Each 0.1 mm CL size class of size frequency distributions was transformed to its corresponding wet weight. Biomass of each size class >10 mm CL was calculated by multiplying the wet weight by the number of animals present in the size class interval. Biomass per tow was then computed as the sum of the biomasses per size class.

Maturity Size

We used two methods to determine the size at which 50% of individuals were mature: (1) Analysis of the reproductive features. This method adjusts a logistic function to the proportion of mature animals for each size class to provide 50% maturity. Both females and males were considered mature if they had eggs attached to the pleopods or spermatophores in their vas deferens, respectively. The proportion of mature females was calculated for each 1 mm CL size class interval. (2) Allometric growth of the right chela (males only). This method is based on the relative change in chela growth at sexual maturity (Hartnoll 1978). We used the routine MATURE1 (Somerton 1980) to determine the proportion of morphometrically mature individuals. Values of "juvenile" and "adult" required by the routine were chosen by judging the scatterplot of chela size versus carapace length and established at 18.0 and 26.5 mm CL, respectively.

Reproductive Cycle

Three standard methods for determination of the reproductive cycle in crustacean decapods were used: the temporal variation of (1) the gonadosomatic index (GSI) for females and males, (2) the oocyte diameter, and (3) the proportion of ovigerous females. Each month a subsample of 15-25 *M. subrugosa* crabs of both sexes >10.9 mm CL (gonadal maturity size) was randomly selected. Crabs were measured and dissected. Their gonads were removed and dried at 55ºC to constant weight. Gonad dry weight was recorded to the nearest 0.1 mg. A gonadosomatic index was calculated as the ratio between the ovary dry weight and size (CL), multiplied by 1,000. Each month, a subsample of 8-11 females >10.9 mm CL was randomly selected and dissected. The ovary was removed and preserved in 4% formalin seawater. In each female, 80-100 oocytes were randomly chosen, and their diameter measured to the nearest 0.02 mm using

an eyepiece micrometer on a compound microscope. The proportion of ovigerous females was calculated as the ratio between ovigerous and non-ovigerous females >10.9 mm CL.

Fecundity was defined as the number of eggs per clutch, and was estimated only in females with broods recently extruded (eggs with uniform yolk and no ocular pigment visible). Pleopods with eggs attached were removed from each female by cutting from their base, and preserved in buffered 4% formalin seawater. Later, eggs were detached from the pleopods and the clutch was blotted and weighed to the nearest 0.1 mg (*WC*). Three subsamples were then weighed to the nearest 0.1 mg (*ws*) and eggs in each subsample were counted (*ns*). Fecundity (*F*) was calculated as:

$$F = \sum_{i=1}^{3} (WC \times ns / ws) / 3$$

Natural Diet

For analysis of stomach contents, samples of *M. subrugosa* were collected once every 3 months. Immediately after trawling, one subsample of 10-30 individuals per stratum was randomly selected. To identify food items and quantify their relative abundances in the stomach contents, animals of one of the subsamples were injected with and submerged in 4% formalin seawater. Crabs were dissected, their stomachs excised, and the contents rinsed into a dish with 1 ml of 4% formalin seawater. Relatively large food items were identified to the lowest taxonomic level possible under a binocular microscope at 20× magnification. Two 0.4-ml subsamples of the stomach content were mounted on slides and examined under a binocular microscope at 100×. We recorded the occurrence of each item with an ocular grid marked with 25 intersection points. From each slide we examined 3 fields giving a total number of 150 intersection points for each animal. The relative abundance (*RA*) and the frequency of occurrence (*FO*) of each item were calculated as follows:

$$RA = \left(i_a / \sum i\right) \times 100$$

$$FO = \left(N_i / N_t\right) \times 100$$

where i_a is the number of intersection points for item a; $\sum_i$ is the number of intersection points for all items, N_i is the number of stomachs with item i and N_t is the total number of stomachs.

To quantify the organic matter present in the stomach contents, the other subsample was preserved frozen at –20ºC until the time of processing. Crabs were defrosted, dissected, the stomach incised, and contents rinsed into a ceramic dish with 1-3 ml of filtered water. The stomach contents were dried to constant weight at 56ºC, weighed, burned for 24 h at

450ºC, and the ashes were weighed. The quantity of organic matter of the stomach contents was the difference between the dry and ash weights.

Results

Density of Munida subrugosa and Munida gregaria and Biomass of M. subrugosa

In the study area, *Munida subrugosa* and *M. gregaria* simultaneously occurred in our trawl samples. In 1999, the overall average density of both species combined was 47.5 (±1S.D. = 92.8) individuals per 100 m^2 ($n = 64$). However, *Munida subrugosa* was the more abundant species, and had a density at most 8 times greater than *M. gregaria*. The density of both species was on average 2.5 times higher at <40 m depth (Fig. 2), and was only higher at >40 m depth in March and September 1999 (Fig. 2). Densities of both species differed among the three studied locations and between both depth strata (Table 1). At <40 m depth, *M. subrugosa* was always more abundant than *M. gregaria,* with both species occurring at similar densities only in Lapataia. At >40 m depth, *M. gregaria* was virtually absent and occurred at an average density of 0.02 (±0.05) individuals per 100 m^2. During 1999, the mean biomass of *M. subrugosa* was 3.4 g per m^2 (±6.2; $n = 58$). As expected, the biomass of *M. subrugosa* was significantly higher at <40 m (4.9 g per m^2; $n = 35$) than at >40 m of depth (1.3 g per m^2; $n = 27$) (Mann Whitney test, $U = 685$; $P = 0.003$). For the studied sector of the Beagle Channel, the estimated biomass of the stock of *M. subrugosa* was 558.9 t at <40 m and 121.3 t at >40 m depth.

Gonadal and Morphometric Maturity

In females 50% of gonadal maturity occurred at 10.9 mm CL (Fig. 3). The smallest female carrying eggs was 8.9 mm CL. A total of 54 males of 8.8-30.5 mm CL were examined and all had spermatophores in their vas deferens. Size at morphometric maturity was calculated for 428 males (8.2-29.8 mm CL). The slope of the regression of right chela length on the carapace length for morphometrically immature males (<18 mm CL) was significantly less ($F = 309.1$; $P < 0.001$) than that for morphometrically mature males (>26.5 mm CL). The estimated size of 50% morphometric maturity was 24.4 mm CL (95% confidence limits: 18.2-26.5 mm CL; Fig. 4).

Reproductive Cycle

In female *M. subrugosa*, GSI values peaked in May-June, followed by decreasing values (Fig. 5), suggesting the occurrence of oocyte extrusion. This process might extend until July-August, because GSI values were still widely distributed. From September to March, GSI values were practically constant. Hence, we propose that the accumulation of yolk, i.e., secondary vitellogenesis, began in March and extended through August. In males,

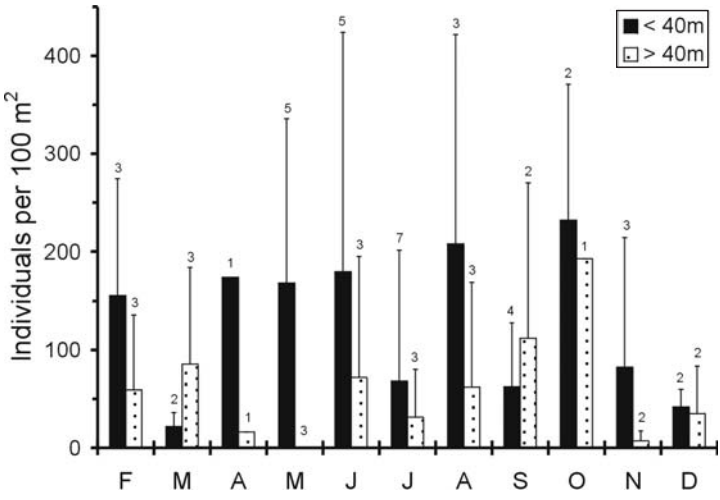

Figure 2. Average abundance of Munida subrugosa and Munida gregaria during 1999 at two depth strata in the Beagle Channel, Argentina. Lines above bars represent the standard deviations. Numbers above bars are sample sizes.

Table 1. Abundance of *Munida subrugosa* and *Munida gregaria* during 1999, at two depth strata, in three different locations in a sector of 45 km of the Beagle Channel, Argentina.

Beagle Channel locations		*M. subrugosa*		*M. gregaria*	
		<40 m	>40 m	<40 m	>40 m
Lapataia	Denstiy	56.75	0.03	38.77	0
	S.D.	78.18	0.05	55.57	0
	n	13	6	13	6
Ushuaia	Density	143.21	91.66	10.53	0.03
	S.D.	168.4	90.89	15.32	0.06
	n	16	14	16	14
Punta Segunda	Density	108.75	31.89	0	0.03
	S.D.	121.72	60.19	0	0.07
	n	8	7	8	7

Abundances are given as densities: individuals per 100 m^2. S.D. = standard deviation; *n* = number of trawls.

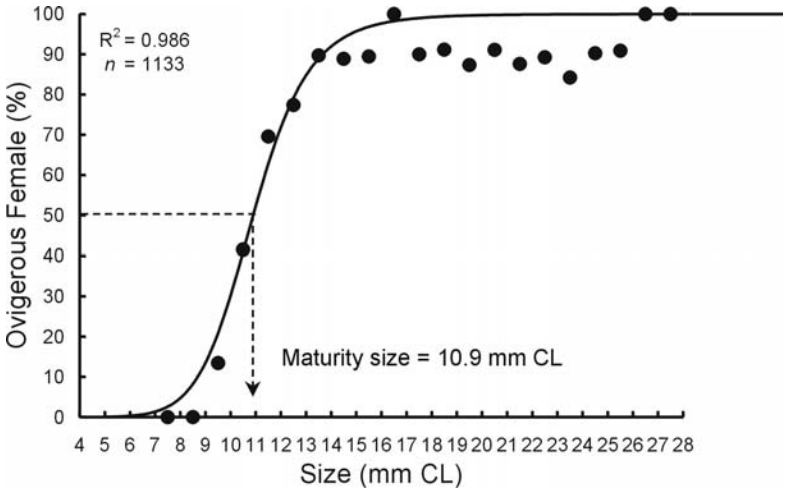

Figure 3. Gonadal maturity in female Munida subrugosa. Size at 50% maturity is given by the intersection between the calculated logistic function and the ordinate at 50%. R^2 is the determination coefficient of the logistic function; n is the sample size.

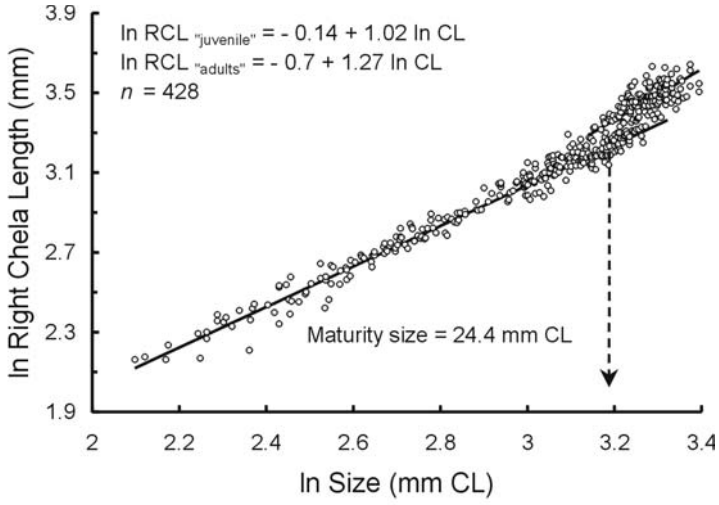

Figure 4. Relationship between right chela and carapace lengths in male Munida subrugosa. Dotted arrow shows the proportion of 50% of morphometric maturity. Loglinear functions for exclusive "juvenile" and "adults" are shown. RCL is the right chela length; CL is the carapace length; n is the sample size.

maximum values of GSI occurred in June, and thereafter GSI values decreased gradually (Fig. 5). From November to March the GSI values were relatively constant. Hence, mating might have extended from May to August and the accumulation of sperm material in the vas deferens likely occurred between April and June.

Two different types of oocytes occurred in the ovaries of *M. subrugosa*. In live animals, oocytes in pre-vitellogenesis or primary vitellogenesis were white or translucent and their size varied between 0.04 and 0.34 mm diameter. Oocytes in pre-vitellogenesis occurred permanently in ovaries, and were exclusively present between September and February. Oocytes in secondary vitellogenesis were green or yellow, in live or fixed animals, respectively. Sizes varied from 0.36 to 0.9 mm in diameter and occurred between March and August. Average oocyte size was clearly seasonal, peaked in June, gently decreased thereafter until September, and remained approximately constant between October and February (Fig. 6). Between June and August standard deviations of the average oocyte size were larger than in other months. Therefore, oocyte extrusion occurred between June and August.

Ovigerous females of *M. subrugosa* mainly occurred between May and October (Fig. 7). The monthly proportion of ovigerous females changed in the two sampling years: ovigerous females varied around 80% in 1998 and between 40% and 90% in 1999. In October and November proportions of ovigerous females decreased and were minimal. Hence, oocyte extrusion occurred in May.

Fecundity

Fecundity of *M. subrugosa* correlated positively with female size. The log of the number of eggs per female increased significantly with the log of female CL (log $F = -2.6 + 4.6$ log CL; $F = 495.5$; $P < 0.01$; $r^2 = 0.82$). The number of eggs per female varied from 124 eggs for a female of 11.15 mm CL to 10,750 eggs for a female of 25.65 mm CL.

Feeding Habits

A total of 582 stomachs of *M. subrugosa* were analyzed, of which only 3% were empty. Food items were identified in 260 stomachs (158 at <40 m and 102 at >40 m depth), and another 322 stomachs were used to quantify the organic matter of the stomach content (158 at <40 m and 164 at >40 m depth). Macroscopically, the stomach content was an amorphous mass, frequently green, in which no definite material could be recognized.

In the Beagle Channel, two food categories constituted the diet of *M. subrugosa*: benthic material and food items. Sediment and undetermined material were present in nearly all stomachs of analyzed individuals (Table 2), and accounted for 20-30% in relative abundance of food items in the stomach contents (Table 2). The undetermined material was particulate organic matter (POM). However, a fraction of POM could be food in a high degree of digestion. The diet of *M. subrugosa* was also composed of at

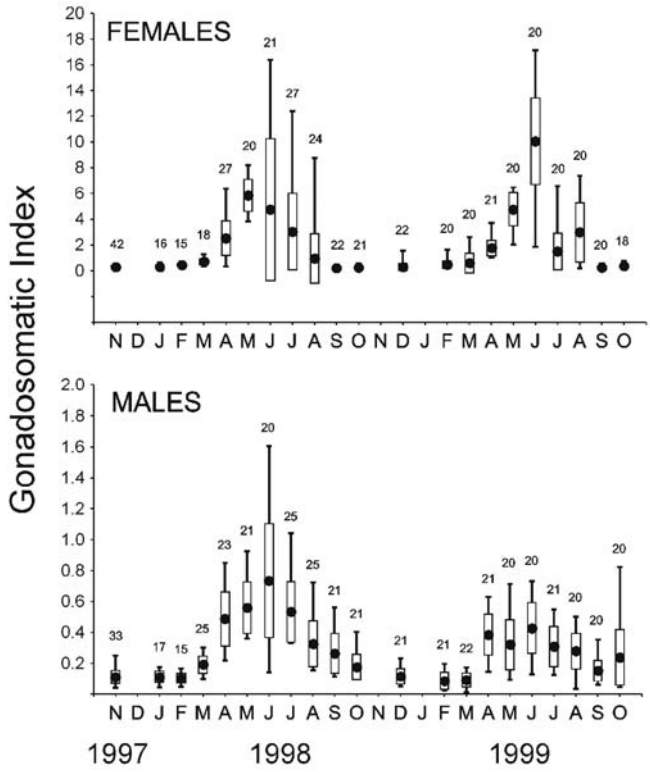

Figure 5. Monthly values of gonadosomatic index (dry gonadal weight/carapace length × 1,000) for female and male of Munida subrugosa from November 1997 to October 1999. Circles, empty rectangles, and vertical lines are averages, standard deviations, and range values of gonadosomatic index, respectively. Numbers above vertical lines are sample sizes.

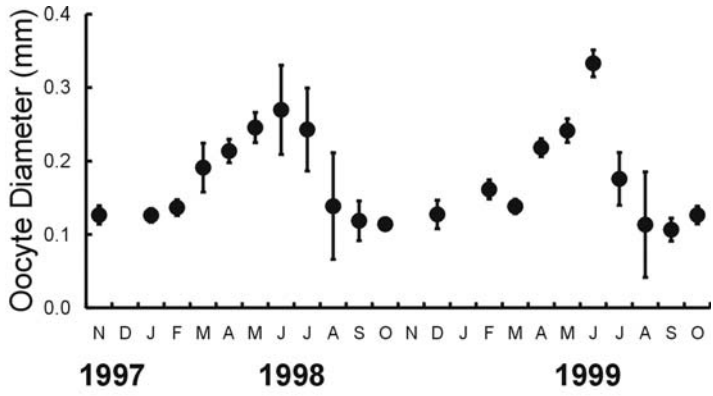

Figure 6. Monthly average oocyte diameters for female Munida subrugosa from November 1997 to October 1999. Values of average and standard deviation were calculated from individual averages obtained from 8-12 (mostly 10) females. To obtain the individual averages, 80-90 oocytes of each female were measured.

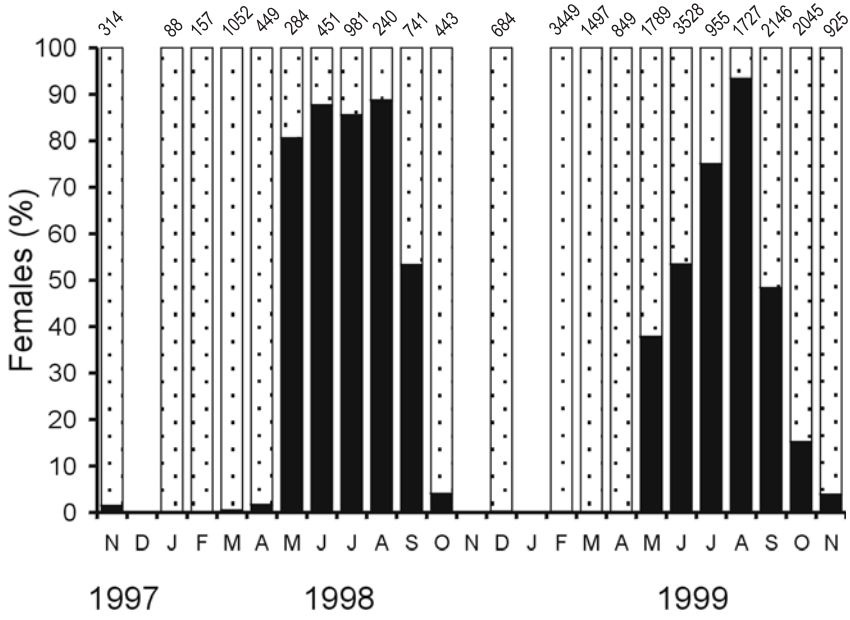

Figure 7. Percentage of ovigerous females (black bars) of Munida subrugosa >10.9 mm CL, between November 1997 and October 1999. Sample sizes are indicated above bars. Months with no samples are bare.

Table 2. Frequency of occurrence and relative abundance of food items of *Munida subrugosa* at two depth strata of the Beagle Channel.

		Frequency of occurrence (%)		Relative abundance (%)	
	Food items	<40 m	>40 m	<40 m	>40 m
Algae	Algae undetermined	34.2	33.3	6.2	5.5
	Ballia spp.	17.7	18.6	1.4	1.1
	Rhizoclonium spp.	5.7	0	0.4	0
	Cladophora spp.	10.8	8.8	0.8	0.7
	Monostroma spp.	0	3.9	0	2.0
	Hincksia spp.	0	8.8	0	0.8
	Desmarestia spp.	4.4	3.9	1.9	0
	Trailliella spp.	36.1	18.6	2.3	0.8
	Other algae	12.7	7.9	1.6	1.8
	Diatoms	42.4	22.5	1.6	0
Worms	Oligochaeta	20.2	14.7	1.8	1.0
	Nematoda	14.6	32.3	0.8	1.6
	Polychaeta	0	10.8	0	1.8
	Polychaete tubes	19.6	0	4.7	0
Crustacea	Crustacea undetermined	46.8	55.9	11.4	14.7
	Ostracoda	1.9	6.9	0.1	1.2
	Munida spp.	8.9	2.9	5.7	1.9
	Other Crustacea	5.7	7.8	0.3	0
Others	Plant leaves	7.0	0	1.4	0
	Parazoa	5.1	2.0	0.1	0.1
	Foraminifera	55.7	31.4	1.9	0
	Other protozoans	1.3	19.6	0.1	2.0
	Hydrozoa	7.0	2.9	0.1	0.1
	Bryozoa	0.6	0	0.1	0
	Mollusca	7.0	0	0.6	0
	Echinoidea	4.4	0	0.1	0
Sediment		95.6	100	21.8	30.8
Particulate organic matter		94.9	100	32.9	32.1

Other protozoans are rotiferans and undetermined cysts. Other algae include the following genera: *Sphacelaria, Hymenena, Ceramium, Griffithsia, Ulva, Chaetomorpha, Ectocarpus.* Other crustaceans include amphipods and copepods (mostly Harpacticoidea).

least 28 food items (Table 2). In terms of percentage of occurrence, Foraminifera, diatoms, crustaceans, nematodes, some small macroalgae such as *Ballia* spp. and *Trailliella* spp., and polychaete tubes appeared as the main food items in the diet of *M. subrugosa* (Table 2). However, in terms of relative abundance only crustaceans, algae, and polychaete tubes were dominant food items (Table 2). The occurrence of conspecific or congeneric preys was also recorded.

The proportion of inorganic matter in the stomach contents was higher at >40 m depth. Between April 1998 and December 1999, the proportion of organic matter in stomach contents was constant at 46.8 (±13.5)% and 36.1 (±9.0)% at <40 m and >40 m depth, respectively. Moreover, the proportion of organic matter at <40 m depth was significantly higher than at >40 m depth (Student's $t = 3.53$; $P < 0.001$).

Discussion

Results presented in this article constitute the first information on the biology of *M. subrugosa* in the Beagle Channel. Although we found that *M. subrugosa* and *M. gregaria* are more abundant at depths <40 m, they occur differentially in the Beagle Channel. We speculate that *M. gregaria* is likely excluded from soft bottoms with 3-dimensional structures (e.g., with algae or polychaete tubes of *Chaetopterus* spp., at sites Ushuaia and Punta Segunda). These types of bottoms could provide an adequate benthic habitat for the juvenile phase of *M. subrugosa* (unpubl. data), whereas the juvenile *M. gregaria* are pelagic and occur in the water column (Zeldis 1985). Therefore, juvenile *M. gregaria* are likely exported offshore from the Beagle Channel, explaining their lesser abundance inside the channel. Adults probably occupy marginal habitats with apparently higher quantities of inorganic material and terrigenous debris such as occurs at Lapataia site.

The results of abundance derived from our benthic trawls are useful for comparison with other quantitative methods. In the Strait of Magellan and the channels of Tierra del Fuego, visual methods, such as photographs or video recordings, provide average and maximum densities of *Munida* spp. between 3-12 and 7-27 individuals per m^2, respectively (Gutt et al. 1999; M. Gorny and M.A. Retamal, pers. comm.). These visual methods surveyed areas of 1,578 and 257 m^2 of seafloor (Gutt et al. 1999; M. Gorny and M.A. Retamal, pers. comm.) that are much smaller than the 57,454 m^2 trawled in our study during 1999. Methods of direct observations could be biased if, at the scale of the sampling units, the spatial distribution of crabs is patchy (Conan and Maynard 1987), as occurs with both *M. subrugosa* (M. Gorny and M.A. Retamal, pers. comm.) and *M. gregaria* (Zeldis 1985). We also realized that our trawl may underestimate abundances of *Munida* spp. A video recording of our trawl mouth let us determine that only 15% of crabs occurring in the trawl path were lost by escaping behavior (Tapella, unpubl. results). These two types of biases could explain the

differences between our average density of *Munida* spp. of 0.5 and the 8-28 individuals per m^2 in previous visual studies.

Our estimations of biomass are the first for galatheids in the Beagle Channel, and serve to evaluate the economical potential of the *Munida* fishery. The average biomass of 3.5 t per km^2 estimated for *M. subrugosa* >5 mm CL is relatively low compared with the reported biomass of other economically valuable species. Aurioles-Gamboa and Balart (1995) estimated the biomass of the benthic phases of *Pleuroncodes planipes* off Mexico to be between 4 and 176 t per km^2. After the fishery collapse and 3 years of closure, the biomass of *P. monodon* off central Chile varied between 20 and 47 t per km^2 (Roa and Bahamonde 1993). Our estimations of biomass could increase by about 10% if the potential abundance of *M. gregaria* is considered for biomass calculations.

In the Beagle Channel the reproductive cycle of *M. subrugosa* is annual and begins with mating, which mainly occurs in June (Figs. 5-7). However, mating may extend further, until August, since gonadosomatic indexes and oocyte diameter decreased slowly after June (Figs. 5 and 6). Females >10.9 mm CL are mature and keep their eggs in their abdomens over a period of 3 months, mainly between June and September, when larval hatching occurs (Lovrich 1999). Ovigerous females occur until November but at lower proportions (Fig. 7), a fact which agrees with the occurrence and abundance of recently hatched zoeae in the plankton until December (Lovrich 1999). This contrast with a limited number of samples from the Strait of Magellan, from which Rodríguez and Bahamonde (1986) speculated that the reproductive cycle of *M. subrugosa* begins in April, the embryogenesis lasts 8-9 months, larval hatching occurs between October and January, and females attain gonadal maturity at 13.5 mm CL.

In male *M. subrugosa,* size at morphometric maturity does not serve as a good indicator of the size at which the crabs reproduce as may occur in other anomuran crabs such as lithodids. Our results indicate morphometric maturity at 24.4 mm CL (Fig. 4) and we speculate that gonadal maturity (spermatophores in the vas deferens) is attained at about 10 mm CL. For males, attaining the size of morphometric maturity is not a requisite to mate successfully (Paul 1992, Sainte-Marie et al. 1999, and references therein). Moreover, extremely divergent sizes of males and females at coupling, e.g., a 25 mm CL male and a 10 mm CL female, may constrain the adequate fertilization of extruding eggs. Therefore, we hypothesize that male *M. subrugosa* attain functional or behavioral maturity (participating in mating couples) at an intermediate size between gonadal and morphometric maturity sizes. Moreover, we suggest that functional maturity could be attained immediately after gonadal maturity.

Munida subrugosa of the Beagle Channel has two different and complementary feeding habits. As a predator *M. subrugosa* grazes on small macroalgae or feeds on crustaceans. As a deposit feeder *M. subrugosa* uses POM as a source of energy. Such a bipartite feeding habit was also observed in other galatheid species (Nicol 1932, Cartés 1993, Kashkina

and Kashkin 1993, Aurioles-Gamboa and Pérez-Flores 1997). Moreover, POM could provide additional food not considered in this study, such as bacterial flora adhered to organic and inorganic particles (Petchen-Finenko 1987). Channels around Tierra del Fuego and particularly the Beagle Channel could serve as an environment for the accumulation of organic matter of terrigenous origin, which could serve as a direct or indirect food source for *Munida* spp. (see "plant leaves" in Table 2). This is supported by the fact that channels are oceanographically isolated because there is limited water exchange through the shallow connecting passages and the open ocean (Antezana 1999). Hence, the organic matter such as leaves of the deciduous forest of the southern beech *Nothofagus* spp. may be retained in the channels. This is supported by the fact that leaves occur in our trawl throughout all the year (M.C. Romero, unpubl. results). Our observations also suggest that feeding habits are likely related to crab distribution depth, since animals at >40 m depth showed greater quantities of sediment (Table 2) and inorganic matter in their stomachs, thus probably the condition of deposit feeder increases with depth. The condition of deposit feeders is also supported by high abundance of *Munida* spp. found associated with soft bottoms containing high concentration of phytodetritus and biogenic debris (Gutt et al. 1999; M. Gorny and M.A. Retamal, pers. comm.). Moreover, as deposit feeders, *M. subrugosa* crabs are probably responsible for bypassing the organic matter recycling by bacteria, and thus could make this organic matter available to top predators.

We hypothesize that *Munida subrugosa* plays a key role in the marine benthic ecosystem of the Beagle Channel for several reasons. First, this species is highly abundant (0.5 to 70 individuals per m^2, this study and Gutt et al. 1999). In terms of wet weight, *M. subrugosa* is one of the most dominant species, accounting for 50% to benthic captures (Arntz and Gorny 1996), and in terms of numbers, represents more than 90% of decapod fauna (Arntz et al. 1999, Pérez-Barros 2001). Second, larvae and adults of *M. subrugosa* are prey of several marine organisms, most of which are top predators and also of economic interest (partially reviewed by Rodriguez and Bahamonde 1986). Third, because of its feeding habits, *M. subrugosa* may constitute short trophic chains. As a deposit feeder *M. subrugosa* is able to incorporate rotting organic matter into the trophic web, otherwise only available to decomposers or to detritivores not frequently preyed upon. As a herbivore, *M. subrugosa* is the direct link between primary producers and terminal predators (Longhurst et al. 1967). Therefore, trophic chains in which *M. subrugosa* participate could be more efficient in the energetic transfer, because fewer trophic levels in a trophic chain imply less energetic losses.

High abundances of *Munida* spp. in southern South America suggest the potential that a fishery could be developed in the near future. The results of this study can serve as the initial basis for developing this fishery, until more biological information is available. Given the condition of

M. subrugosa as a key species in the ecosystem of the Beagle Channel, any fishery should be extremely conservative. The exploitation of intermediate trophic links at a large scale could undermine the food base of those species occupying higher trophic levels. Thus, the overall commercial marine stocks would decrease and the stability of the ecosystem would be threatened (Kashkina and Kashkin 1993). Furthermore, in contrast to open ocean areas in which other galatheid fisheries have been developed (c.f. Roa and Bahamonde 1993, Aurioles-Gamboa and Balart 1995), the relatively small area of the Beagle Channel constrains the development of the fishery to a small scale. Hence, we suggest that the management of the fishery for *Munida* spp. should consider:

1. Fishing should be banned during the period of egg-carrying, i.e., May-October. This could also be advantageous for the development of the fishery for *Munida* spp. as a complement to the king crab fishery, during its closed season, e.g., November-December (Lovrich 1997).

2. Landings should be limited by means of quotas as occurs in the Chilean fishery for *P. monodon*. Until population parameters that allow the forecast of the stock dynamics (e.g., growth and natural mortality rates, and generational time) can be calculated we recommend that quotas should be conservative and based on annual stock assessments.

3. A size limit could be imposed taking into consideration our calculations of female gonadal maturity and fecundity. Since larger animals yield more meat (Lovrich et al. 1998), a minimum legal size of 20 mm CL will maximize meat yields of landed animals, but still allow for reproductive opportunities for the sublegal crabs not retained. A size limit along with limited landings should ensure that the reproductive potential will not be significantly reduced.

4. Gear limitations should consider features like mesh size and gear design. The mesh size is used in the *P. monodon* fishery and will allow the control of legal size limits. The design of the gear should minimize sea-bottom disturbance, as is known to occur with standard beam trawls with tickler chains (c.f. Kaiser et al. 1994), and should maximize captures of the target species. Moreover, if authorized gear is small and operated by small-scale fishers, the fishery effort could be kept relatively controlled and the impact on the *Munida* spp. populations probably minimized.

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The Complete Larval Development of *Chionoecetes japonicus* under Laboratory Conditions

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Abstract

Complete larval development of the red snow crab, *Chionoecetes japonicus* Rathbun, from hatching to crab 1 stage, was observed under laboratory conditions for the first time. The newly hatched zoeae were reared in artificial seawater with a salinity of 35% and under light conditions of 10 light hours and 14 dark hours. The larvae were fed with decapsulated eggs and newly hatched nauplii of *Artemia*. Green microalgae, *Dunaliella teriolecta*, 100-500 cells per ml, were also added to cultures. Combinations of three kinds of antibiotics (chloramphenicol, 5 mg per liter; streptomycin, 50 mg per liter; and ampicilin, 50 mg per liter) were used for preventing bacterial infections. The crab 1 stage was obtained 118 days after hatching at 8.5-11.0ºC. The crab 1 of *C. japonicus* is different from the congener *C. opilio* (Fabricius) in carapace morphology and live color.

Introduction

The red snow crab, *Chionoecetes japonicus* Rathbun, is an important commercial species in the deep-sea fauna of Japan. The landings, however, have declined remarkably in recent years. Information on its life history, therefore, is required for basic resource management of the deep-sea crabs. The larval development of *C. japonicus*, from zoea to megalopa stage, has been documented by Motoh (1970, 1976, 1982). Suzuki et al. (1983) commented about the crab 1 stage in their report, but no detailed morphological

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description has been available. Thus the crab 1 stage remains unknown to date. This paper describes the complete larval development of *C. japonicus* under laboratory conditions, with emphasis on its first postlarval forms.

Materials and Methods

Ovigerous females were collected by R/V *Tateyama-maru* using crab pots off Toyama Bay (137º17′E, 37º00′N) at a depth of 1,000 m in January-February 1999. They were kept in an aquarium with running deep-sea water at 1-3ºC. One of the females released zoeae on 18 March 1999.

The newly hatched zoeae were reared in glass beakers with 1,500 ml of artificial seawater (ASW), Jamarin U® (Jamarin Laboratory, Osaka), of 35% salinity, and the water was gently aerated using a glass tube 5 mm in diameter. The rearing beaker contained 150-220 larvae during the zoeal stage. The megalopae were transferred to small beakers with 500 ml ASW each containing 3-5 larvae. The temperature was kept at 8.0-11.0 ± 0.5ºC and light schedule at 10 light hours and 14 dark hours throughout the larval culture. The larvae were fed with decapsulated eggs of *Artemia* for a week after hatching, then with newly hatched nauplii. Green microalgae, *Dunaliella teriolecta*, 100-500 cells per ml, were also added to cultures. Combinations of three kinds of antibiotics (chloramphenicol, 5 mg per liter; streptomycin, 50 mg per liter; and ampicilin, 50 mg per liter) were used for preventing bacterial infections. Nonfeeding series larvae reared without food were also observed. For morphological comparison, larval specimens of *C. opilio* were examined.

The appendages were dissected with fine insect pins under a Nikon SMZ-10 stereomicroscope and mounted on silicon-coated glass slides. Drawings and measurements were made with a drawing tube attached to an Olympus BH-2 microscope. All illustrations were made with Adobe Illustrator® 5.5J (Adobe Systems Inc.). Most of the terminology for setae follows that of Ingle (1992). All setal arrangements are listed from proximal to distal. Roman numerals "I" in setation denote dorsolateral setae. Carapace length (CL) was measured from the anterior border of the eye to the posterior border of the carapace in zoeae, and from the tip of the rostral spine to the posterior border of the carapace in postlarval forms. The distance between the tip of the rostral spine and the tip of the dorsal spine (RDL) was also measured in zoeae. These values are shown as mean ± S.D. and range in parentheses in the text. Voucher specimens from the present study will be deposited at the Zoological Institute, Faculty of Science, Hokkaido University, Japan.

Results

Figure 1 shows the survival rate of each larval stage during laboratory rearing. The zoeae in nonfeeding condition died about 20 days after hatching. Heavy mortality was observed in the megalopa stage due to abnormal molting. Only one crab 1 specimen was obtained 118 days after hatching. A morphological description of each developmental stage is given below.

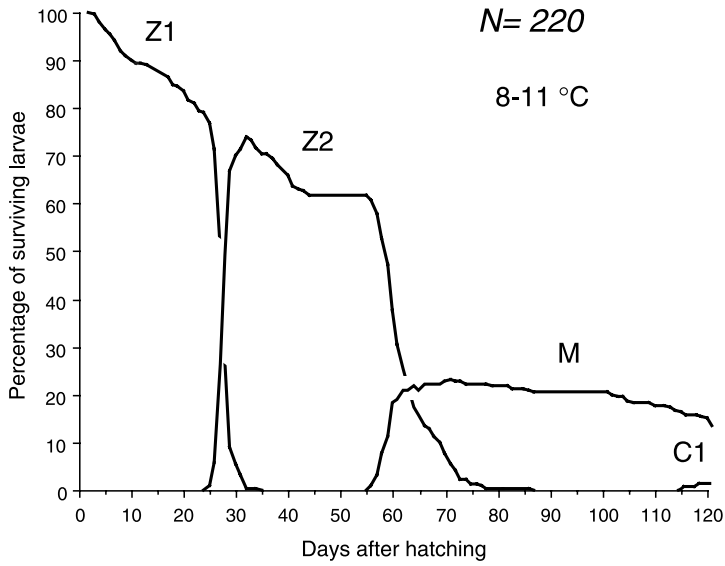

Figure 1. Percentage and duration of surviving larvae of Chionoecetes japonicus Rathbun reared under laboratory conditions.

Zoea 1

Dimensions: CL = 1.24 ± 0.07 mm (1.11-1.39 mm, 12 specimens), RDL = 5.33 ± 0.21 mm (5.04-5.78 mm, 12 specimens).

Carapace (Fig. 2A): Long rostral and dorsal spines, shorter lateral spines. All spines with spinules increasing in size distally. Lateral margin with a notch accommodating the maxilla. Five plumose setae on the posterolateral margin, the anterior seta the largest (= majid seta) (Fig. 2A', the arrow). Eyes sessile.

Antennule: Uniramous, conical projection with 2 long aesthetascs, and 1 simple seta.

Antenna: Biramous, protopodal process very long and pointed with 2 longitudinal rows of spinules. Exopod pointed with 2 subterminal spinous spines.

Mandibles: Molar and incisor processes developed. No rudiment of palp.

Maxillule: Coxal endite with 7 denticulate setae. Basal endite with 3 serrated cuspidate spines and 4 setae. Endopod two-segmented, with 1, 6 setae.

Maxilla: Bilobed coxal and basal endites with 4+4 and 5+6 plumodenticulate setae, respectively. Endopod unsegmented, with 3 subterminal and 3 terminal plumodenticulate setae. Scaphognathite with 12-13 soft plumose setae on its margin.

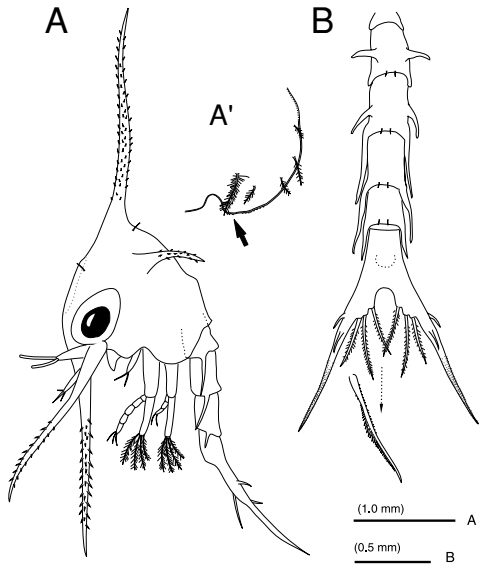

Figure 2. Zoea 1 of Chionoecetes japonicus Rathbun: A lateral view; A', inner side of anterolateral margin of carapace; B, abdomen in dorsal view.

Maxilliped 1: Protopod with 2+2+3+3 inner setae. Endopod five-segmented with 3, 2, 1, 2, 4+I setae. Exopod with 4 plumose natatory setae.

Maxilliped 2: Protopod with 1+1+1+1 inner setae. Endopod three-segmented with 1, 1, 4+I setae. Exopod as in maxilliped 1.

Abdomen (Fig. 2B): Five somites and telson. Somites 2 and 3 with pairs of dorsolateral spines. Somites 3-5 with long posterolateral spines. Telson bifurcated, each furca with 3 pairs of inner posterior setae. Each furcal shaft with lateral and dorsal spines proximally.

Coloration: Reddish chromatophores are conspicuous, but these are not recognizable just after hatching.

Zoea 2

Dimensions: CL = 1.82 ± 0.09 mm (1.69-1.93 mm, 7 specimens), RDL = 6.64 ± 0.24 mm (6.40-7.07 mm, 7 specimens).

Carapace (Fig. 3A): Eyes stalked.

Antennule: Larger in size, with 9-10 aesthetascs in two tiers. Rudiment of endopod emerged.

Antenna: Rudiment of endopod about a half length of the process.

Mandible: Rudiment of palp emerged.

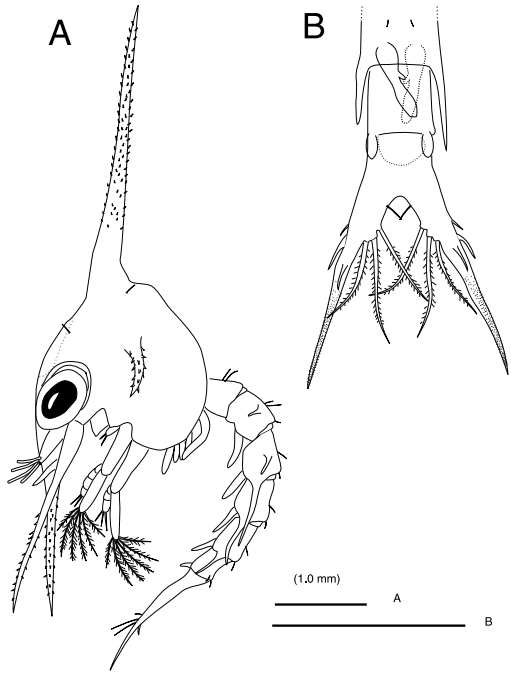

Figure 3. Zoea 2 of Chionoecetes japonicus Rathbun: A, lateral view; B, telson in ventral view.

Maxillule: Coxal endite with 7 denticulate setae. Basal endite with 5 serrated cuspidate spines and 4 setae. Endopod as in zoea 1. A plumose seta on dorsal side.

Maxilla: Coxal and basal endite with 4+4 and 5+5 setae, respectively. Endopod as in zoea 1. Scaphognathite with 21-23 marginal plumose setae.

Maxilliped 1: Exopod with 6 plumose natatory setae.

Maxilliped 2: Exopod as in maxilliped 1.

Maxilliped 3 and pereiopods: Enlarged, but no clear segmentation.

Abdomen (Fig. 3B): Biramous pleopod rudiments on abdominal somites 2-5, and uniramous uropod rudiment on somite 6 posteriorly. Each telsonal furca with additional inner posterior setae, and 1 small lateral spine, which is occasionally lacking.

Coloration: Reddish as in zoea 1 stage.

Megalopa

Dimensions: CL = 2.99 ± 0.11 mm (2.83-3.26 mm, 20 specimens).

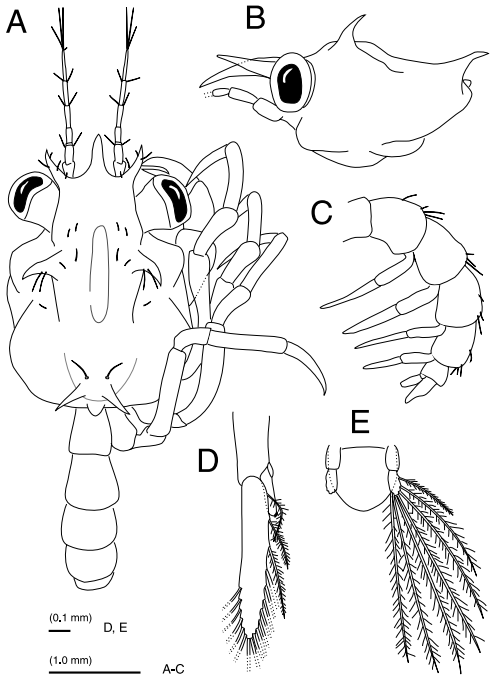

Figure 4. Megalopa of Chionoecetes japonicus Rathbun: A, dorsal view; B, carapace in lateral view; C, abdomen in lateral view; D, first pleopod; E, telson and uropod.

Carapace (Fig. 4A, B): Rostral spine, pair of supraorbital, gastric dorsal, and cardiac dorsolateral spines. Dorsal carina on gastric region.

Antennule (Fig. 5A): Peduncle three-segmented with 1 short simple seta on proximal, 2 on middle, and 2 on distal segment. Dorsal ramus two-segmented, with 8 and 7-9 aesthetascs in two tiers, and 1 long subterminal seta and 1 dorsal seta on distal segment. Ventral ramus two-segmented, with 0, 3+1 setae.

Antenna (Fig. 5B): Peduncle three-segmented, with 1, 2, 3 distal setae. Flagellum five-segmented, with 0, 3, 4, 0, 4 setae.

Mandible (Fig. 5C): Spoon-shaped, no molar process. Palp three-segmented, with 12-13 denticulate setae on distal segment.

Maxillule (Fig. 5D): Coxal endite with 7-9 plumodenticulate setae. Basal endite with 9 denticulate cuspidate setae and 9-11 setae. Endopod two-segmented, with 1 terminal seta on distal segment.

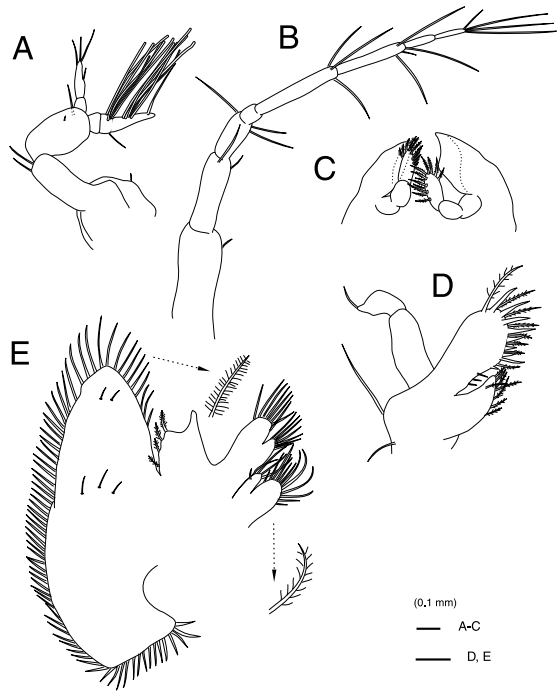

Figure 5. Megalopa of Chionoecetes japonicus Rathbun: A, antennule; B, antenna; C, mandibles; D, maxillule; E, maxilla.

Maxilla (Fig. 5E): Coxal and basal endite bilobed, with 10-12+4-5 and 6-8+9-11 plumodenticulate setae, respectively. Endopod single-lobed, with 4 plumose setae on its inner margin. Scaphognathite with 70-76 soft marginal plumose setae and with 4-6 simple setae.

Maxilliped 1 (Fig. 6A): Coxal and basal endite not distinctly separated, with 26-29 setae on its inner side. Endopod unsegmented, with 2 simple setae. Exopod two-segmented with 1 plumose seta on proximal segment and 4 long plumose setae on distal segment. Epipod triangular, with 19-22 long setae.

Maxilliped 2 (Fig. 6B): Endopod four-segmented with 2, 0-1, 5, 6-8 denticulate setae. Exopod two-segmented with 1 simple seta on proximal segment and 4 long plumose setae distal segment. Epipod with 8-9 long setae.

Maxilliped 3 (Fig. 6C): Endopod five-segmented, proximal segment (= ischium) large, broadening distally with teeth on its inner margin. Exopod two-segmented, with 4 long plumose setae on distal segment. Epipod with 13-15 long marginal setae.

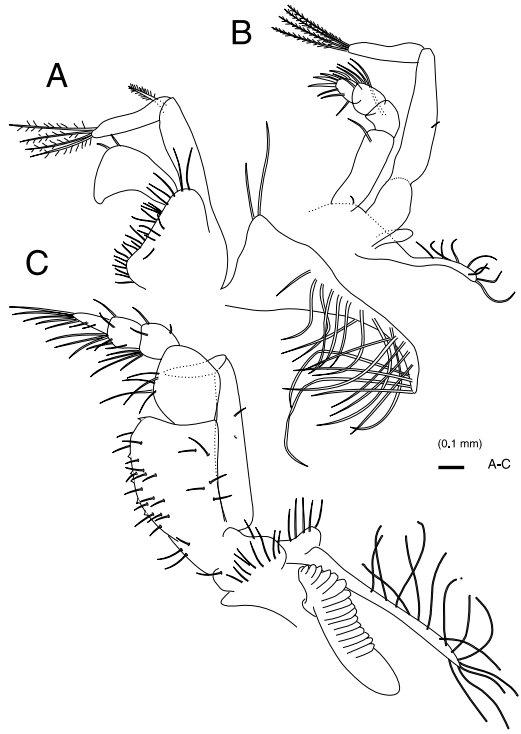

Figure 6. Megalopa of Chionoecetes japonicus Rathbun: A, maxilliped 1; B, maxilliped 2; C, maxilliped 3.

Pereiopods (Fig. 7A-E): Surface covered with serrulate or simple setae. Coxa and ischium of pereiopods 2-4 with 1 ventral spine. Dactyli of pereiopods 2-5 long and acute.

Abdomen (Fig. 4C): Biramous pleopods on somites 2-5, exopodal plumose setae progressing posteriorly 16-18, 17-19, 16-17, 14-16, endopods with 3-4 apical hooks on their inner side. Uropod (Fig. 4E) uniramous with 7 natatory plumose setae. Telson a rounded plate.

Coloration: Reddish as in zoeal stages.

Crab 1

Dimensions: CL = 3.91 mm, carapace width = 2.73 mm.

Carapace (Fig. 8A, B): Pyriform with pair of supraorbital and suborbital spines. Rostral spine, cardiac dorsolateral spines, and dorsal carina on gastric region absent. Dorsal surface with numerous tubercules and hooked-hairs. Posterior margin fringed with tubercules.

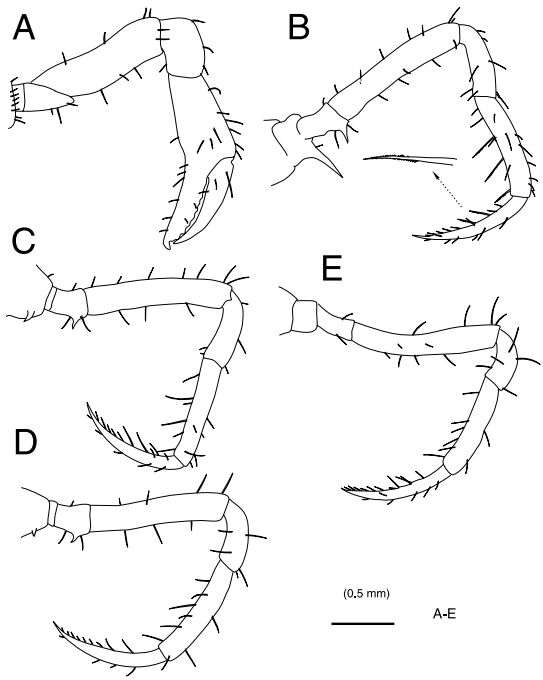

Figure 7. Megalopa of Chionoecetes japonicus Rathbun: A, pereiopod 1 (cheliped); B, pereiopod 2; C, pereiopod 3; D, pereiopod 4; E, pereiopod 5.

Pereiopods (Fig. 8C): Elongate, with more numerous setae and tubercules than in megalopa.

Abdomen (Fig. 8D): Pleopods on somites 2-5, but no sexual differentiation.

Coloration: Red chromatophores on carapace and appendages.

Discussion

The comparison of the larval forms of *Chionoecetes* spp. has been made in some previous studies (Sando 1968; Motoh 1976, 1982). The general morphology of the zoeae of *C. japonicus* in the present study agrees well with Motoh's (1976) description. In this study, our comparison focuses on the postlarval forms, i.e., megalopa and crab 1 stages. Motoh (1976) compared laboratory-reared megalopae of *C. japonicus* and *C. opilio* and stated that the spine on the ischium of pereiopod 1 (= cheliped) is not found in *C. japonicus.* In the present study, this ischial spine was also present as in *C. opilio*, but it is very small. Among the three prominent spines on the frontal

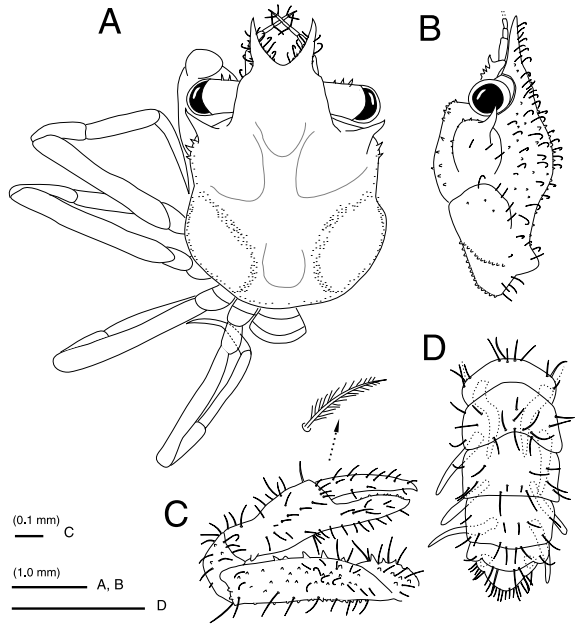

Figure 8. Crab 1 of Chionoecetes japonicus Rathbun: A, dorsal view; B, carapace in lateral view; C, pereiopod 1 (cheliped); D, abdomen in dorsal view.

region of the carapace, i.e., rostral spine (RS) and supraorbital spines (SOS), the RS of *C. japonicus* is shorter than that of *C. opilio* (Fig. 9). The mean ratio of RS/SOS is 1.37±0.14 in *C. japonicus* while it is 1.92±0.23 in *C. opilio*.

Suzuki et al. (1983) mentioned the laboratory-reared crab 1 stage of *C. japonicus* in their short report, but no detailed morphological description or illustration was given. Instead, photographs of specimens of juvenile crabs of *C. japonicus* and *C. opilio*, collected in the field, were presented. The size of these crabs (2.9 mm in carapace width) is nearly equal to that of the crab 1 stage of *C. japonicus* studied in the present study. They also listed the morphological differences of carapace armature between the crab 1 stages of these two species as follows: (1) spinules on branchial region, (2) number of spinules, (3) rows of spinules on the posterolateral margin, (4) direction of rostral spines, and (5) outline of posterior half region. In the present study, among their diagnostic characters, the rows of spinules on the posterolateral margin of *C. opilio*, at least in the crab 1 stage, are not completely parallel as they commented. On the other hand, it is confirmed that the RS of *C. opilio* are curved inward, while those of *C. japonicus* are rather straight (Fig. 9).

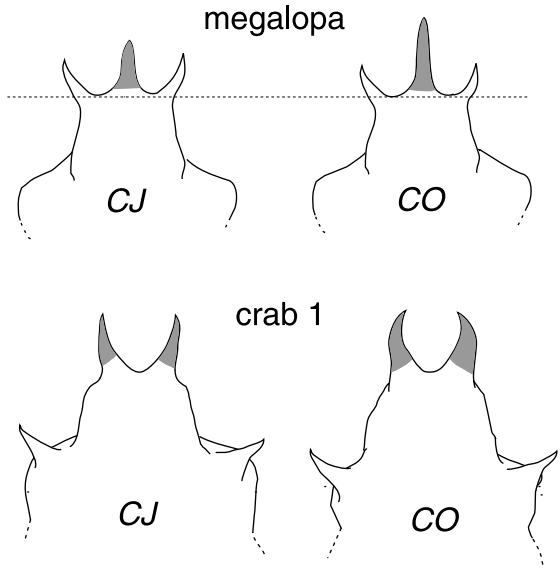

Figure 9. Comparison of frontal region of carapace in megalopa and crab 1 of Chionoecetes japonicus Rathbun (CJ) and C. opilio (Fabricius) (CO). Note the difference in rostral and supraorbital spines (shaded area) of the two species.

The postlarval forms of two *Chionoecetes* species are now known and they are distinguishable by their coloration in the live specimens and by the morphology of anterior spines on carapace. Knowledge of the larvae of *C. angulatus* Rathbun and *C. tanneri* Rathbun is still lacking and further studies will be necessary.

Acknowledgments

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Growth, Maturity, and Mating of Male Southern King Crab (*Lithodes santolla*) in the Beagle Channel, Argentina

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Abstract

Until the early 1990s, *Lithodes santolla* constituted the main target species of the mixed fisheries of the southern tip of South America. In the Beagle Channel, Argentina, the fishery is regulated mainly by a fishing season that extends between January and October, and by the exclusive extraction of males of a legal size >110 mm of carapace length (CL). It is essential to know whether animals protected by this regulation are able to reproduce, and thus guarantee the continuity of the fishery. Due to the lack of fishery controls, the fishery was overexploited and closed in 1994. As part of fishery rehabilitation, a growth model was also needed. We studied growth per molt of male *Lithodes santolla* crabs larger than gonadal maturity (ca. 70 mm CL) by a size frequency analysis jointly with molt increment data. Increment-at-molt was independent of the crab size and averaged 11.4 (±1.7) mm CL. The growth factor significantly decreased with premolt size, from 12.0-15.8% to 7.5-9.5% of premolt size in crabs ca. 75 and 130 mm CL, respectively. We found 8 molt instars that satisfactorily explain the size frequency distributions. Male crabs 73.5-105 mm CL molt twice a year in autumn and spring, whereas crabs >105 mm CL molt only once, each autumn. Hence, male *L. santolla* probably reach the legal size 2 years later than attaining the size at morphometric maturity (SMM), and enter into the fishery stock at their 7th -8th year. SMM calculated with the routine MATURE (Somerton 1980) strongly depended on the choice of

chela dimension and the inclusion of smaller crabs in calculations. The best estimation of SMM was 75.4 (±4.8) mm CL. To determine the functional (= behavioral) maturity size, we studied the constitution of mating pairs. In mating couples, most of males were larger than females, size of mating males and females correlated positively, and only males >94.2 mm CL participated in the pairs. Hence, the present legal size for the fishery could be interpreted as adequate.

Introduction

Among the four species of lithodids that are a target species for a commercial fishery in coastal waters off the southern tip of South America, the southern king crab *Lithodes santolla* (formerly *L. antarcticus* Jacquinot) has been the most valuable. The relatively easy access to lithodid crabs that occur between 2 and 50 m depth (Macpherson 1988, Boschi et al. 1992) and high densities, especially of *L. santolla*, encouraged the development of the fishery since the 1930s. Fisheries for the southern king crab have developed south to 40ºS (Fig. 1). In the Beagle Channel and in the Strait of Magellan, the southern king crab *Lithodes santolla* and the stone crab *Paralomis granulosa* constitute a mixed fishery (Lovrich 1997a). Annual landings of the southern king crab in the Chilean and Argentine fisheries of Tierra del Fuego reached a maximum of 2,877 t in 1984 (Vinuesa et al. 1996), as a consequence of increasing exports due to the collapse of the fishery for red king crab *Paralithodes camtschaticus* in the Bering Sea (Blau 1985, Otto 1990). Similarly, the decrease in yields of the southern king crab after 1985 has promoted the development of the fishery for the stone crab *P. granulosa*, and the maximum landing combined for Chilean and Argentine fisheries was 3,608 t in 1991 (Vinuesa et al. 1996). Likewise, the decrease in landings of *L. santolla* from Tierra del Fuego encouraged the development of more northerly fisheries, off the Pacific and Atlantic coasts (Vinuesa et al. 1996). Currently, more than 95% of Argentine landings of the southern king crab are recorded in the city of Comodoro Rivadavia from the fishery of the Golfo San Jorge (Fig. 1) .The fishery off the Pacific coast of the 10th and 11th Región of Chile (40-50ºS) currently contributes 50% of total Chilean landings.

In the Beagle Channel, the Argentine trap fishery for *Lithodes santolla* is managed by three main regulations: (1) the exclusive landing of males of legal size, >110 mm carapace length (CL), (2) a fishing season that extends from January to October, and (3) a maximum allowable effort of 1,000 traps, distributed in the 210 km^2 of the total surface of the Argentine sector of the Beagle Channel. Nevertheless, due to permanent and systematic transgressions of rules, and lack of controls (G.A. Lovrich and J.H. Vinuesa, pers. observations), the fishery collapsed. This collapse was evidenced in the decrease of (1) total yields of the fishery; (2) the relative abundance of males, females, and legal-sized crabs; (3) average size of males and females; and (4) the proportion of ovigerous females (Bertuche

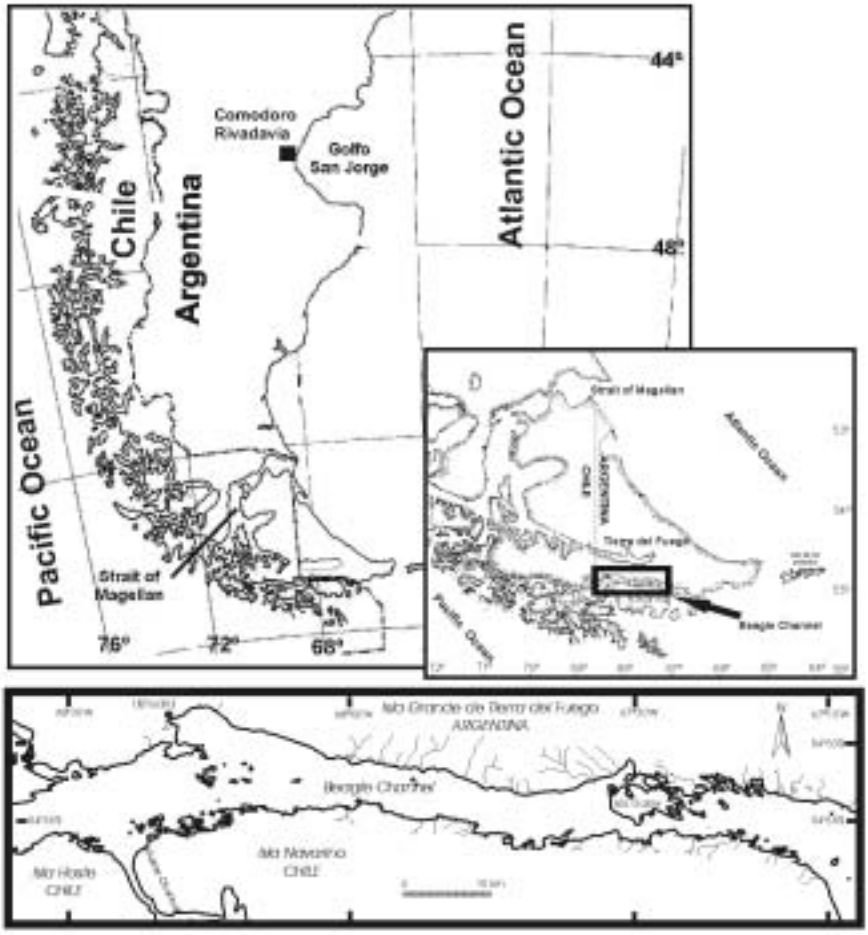

Figure 1. Southern tip of South America (top), location of Tierra del Fuego and Strait of Magellan (middle), and the Beagle Channel (bottom).

et al. 1990, Wyngaard and Iorio 1996, Lovrich 1997a). Consequently, the area of the Beagle Channel west to 68ºW has been closed to fishing since 1994 (Fig. 1). Furthermore, management problems have increased with the development of the fishery. The provincial government closes overexploited areas, but authorities have no basic biological information to judge when to reopen them. The Beagle Channel is shared by Chile and Argentina, and hence the fishery management has international concerns. The Chilean fishery is less well known than the Argentinean fishery, exploitation rates are higher, and so far there is no bi-national conservation policy.

In fisheries regulated by a legal size, knowledge of size at sexual maturity is usually employed because it provides the biological basis to establish regulations that allow a fraction of the population to reproduce before they are harvested (Donaldson and Donaldson 1992). In anomuran and brachyuran crabs, three steps of maturation are currently recognized: (1) gonadal maturity is defined as the size at which generative organs become functional, i.e., in males, vasa deferentia contain spermatophores, (2) morphometric maturity is the size of attaining sexual secondary characters, and (3) functional or behavioral maturity is the size at which mating and successful fertilization occurs (Conan and Comeau 1986, Conan et al. 1990, Sainte-Marie et al. 1997, Watters and Hobday 1998, Sampedro et al. 1999). Although gonadal and morphometric estimates of size at sexual maturity have been the most commonly reported, behavioral maturity is probably most critical in reproduction (Paul 1992), since the development of secondary characteristics may not confer behavioral maturity resulting in successful copulation (c.f. Hartnoll 1969, Sainte-Marie et al. 1995). In male *Lithodes santolla* of the Beagle Channel, the size at morphometric maturity is reportedly attained at 92.6 mm CL and hence, a legal size of 110 mm CL was enforced in 1983 (Boschi et al. 1984). At that time, the rationale for this was that the reproductive biology of *L. santolla* of the Beagle Channel was supposedly similar to that reported for the red king crab *Paralithodes camtschaticus* off Kodiak Island (c.f. Powell and Nickerson 1965, Powell et al. 1974). Hence, we consider that the legal size for male *L. santolla* in the Beagle Channel is somewhat arbitrary.

As for most commercially important marine species, knowledge of growth, maturity, and mating sizes are important for good management of the southern king crab. Growth is generally determined from the size of molt increments relative to the size of individuals, estimates of the intermolt periods, and size frequency analyses. Previous information on growth of *L. santolla* is fragmentary. Juvenile animals molt several times during their first years and supposedly reach gonadal maturity at the 18th instar, i.e., ca. 70 mm CL, at 4 years old (Vinuesa et al. 1990). Males that attain morphometric maturity (>92.6 mm CL) are supposed to molt once a year and grow about 10 mm CL at each molt (Geaghan 1973, Boschi et al. 1984). A growth model for male *L. santolla* was described from the integration of Geaghan's (1973) data from the Strait of Magellan with a few data obtained in the Beagle Channel (Boschi et al. 1984). Unfortunately

available information does not allow for the identification of size classes, their abundance or relative proportion, and hence forecasts of recruitment into the commercial stock are not available. Moreover, in the Strait of Magellan the Atlantic king crab *L. confundens* is seemingly sympatric with the king crab *L. santolla* (Macpherson 1988). *Lithodes confundens* was established as a new species in 1988, and was found to be the only species occurring at the Atlantic entrance of the Strait of Magellan (Lovrich et al. 2002). We suspect it has been misclassified as *L. santolla* in the study of Geaghan (1973), and hence this has presumably led to incorrect conclusions concerning *L. santolla*.

In this article we offer basic biological information on the growth and molting of male southern king crab *L. santolla* that allows managers to make decisions on the fishery. We calculated sizes of morphometric and behavioral maturity. We also provide a growth model that identified instars from size frequency data collected in the field, with the help of a Hiatt growth function that describes growth increments obtained from male crabs kept in captivity. We also determined the molting frequency of male *L. santolla* >70 mm CL.

Material and Methods

Study Site and Sampling

The Beagle Channel is located at the southern tip of South America (Fig. 1). The international boundary between Argentina and Chile is approximately at the middle of the channel, so that east to 68º36.6′ W the northern half of the channel belongs to Argentina. For management purposes, the Argentine fishery in the Beagle Channel is divided in two areas of 120 km^2 each. An area currently closed to fishing is near the city of Ushuaia and encompasses a section of ca. 40 km of the channel west to 68ºW. This area has been exploited since the beginning of the fishery, ca. 1965, but was closed in 1994 due to a stock collapse of *L. santolla* (Lovrich 1997a). Eastward, a contiguous area of ca. 70 km of the channel is currently open to crab fishing, where the target species is principally the stone crab *Paralomis granulosa.*

Specimens of *Lithodes santolla* were collected between June 1995 and July 1997 in both the areas closed and open for crab fishing. Captures were made with commercial conical traps deployed at 10-80 m depth (Boschi et al. 1984). Additional samples were taken in November and December 1995 and 1996, in which crabs were caught with six tangle nets of 40 mm stretch mesh and 25 m length, deployed at the bottom between 2 and 30 m depth, at 5 m depth intervals. During each survey, all crabs were sorted by sex and then the carapace length (CL), chela length (ChL), and height (ChH) of each sampled crab were measured with a dial caliper to the nearest 0.1 mm (Lovrich and Vinuesa 1993).

All crabs were sorted into one of the following categories of carapace age (adapted from Lovrich and Vinuesa 1993): (1) postmolt (POM; shell soft, bright red, and non-calcified, without epibionts), (2) early intermolt

(EIM; shell hard bright red, without epibionts), (3) intermolt (IM): shell hard, variably covered by epibionts, mainly serpulids), (4) intermolt with barnacles (CIM; shell hard, with at least five barnacles *Notobalanus flosculus* on the carapace), (5) premolt (MOL; new shell partially visible under the old one), and (6) exuviae (EX). Additional samples taken in September 1978 and March 1984 in the area closed to fishing after 1994 were used to fit the growth model to the observed size frequency distributions. In the latter samplings, male CLs were originally measured to the nearest 1 mm.

In November and December 1996 and 1997, sampling in a coastal area near the Bridges Islands (54°52′S; 68°12′W) was conducted to collect sexually paired *L. santolla*. Sampling was done by scuba diving and by using tangle nets placed along two transects separated by 0.6 km, oriented perpendicularly to isobaths. Bottoms from 2 to 30 m depth were surveyed. Once a week, and alternatively in each transect, tangle nets were deployed at intervals of 5 m depth for 96 h. All crabs from nets were sorted according to their degree of participation in mating couples and measured. The other transect was surveyed by scuba divers, and all couples in pre- and postcopulatory embrace (rostrum to rostrum, one individual holding the other), in copulatory embrace (sternum to sternum), and single individuals were collected. Depth of occurrence of couples was recorded and pairs were isolated in bags. For those crabs sampled by nets and scuba, we determined sex, molt stage, CL, ChL, and ChH for males. In females we also inspected presence of eggs or pleopod appearance. Gonad condition was graded as mature, immature, or spawned by simple visual inspection (Vinuesa 1984).

Growth

In the Beagle Channel, 50% of male *L. santolla* crabs attain gonadal maturity at 70 mm CL (Vinuesa 1984), and accordingly all crabs >70 mm CL were judged as mature and kept for the growth study.

In March 1996 and immediately after sampling, male southern king crabs >70 mm CL were measured and identified with modified numbered spaghetti tags (see Lovrich and Vinuesa 1995 for details). Crabs were kept in four 2.25 m^3 pens submerged at 5 m depth in the Beagle Channel. These pens had two shelves, so that each pen was divided into three compartments of 0.75 m^3. Pens were checked for molting or dead individuals every week by floating the cages using an air-lift system. Each time crabs were enumerated and excess food consisting of live mussels *Mytilus chilensis* was supplied. Once crabs molted, exuviae were removed and measured. Postmolt size was determined for crabs only after the carapace had hardened, usually 1 week after molting.

Male growth was described using the Hiatt function, size increment at molt, and growth factor (percentage increase in size over premolt CL). Model I regression (Sokal and Rohlf 1995) was used to fit the Hiatt model, size at molting, or growth factor to premolt CL and 95% confidence intervals were constructed to test whether slopes significantly differed from 1 (Hiatt

and growth factor) or 0 (size at molting). The modal (i.e., instar) composition, proportion of crabs in each instar, mean size-at-instar, and S.D.-at-instar, of each identifiable instar within our size frequency data were then calculated using the multiple size frequency analysis method initially described by Smith and Jamieson (1989a). This method was designed to allow the progression of instars identified from the frequency data to follow a growth pattern consistent with that described by the molt increment data, i.e., our Hiatt model. We know that a large number of crabs as small as 75 mm CL (about 35 mm smaller than the legal size) were taken by the fishery due to a lack of enforcement of fishery regulations (Lovrich, personal observations). Therefore we assumed, and our data indicated, that our sampled population was not significantly modified by knife-edged exploitation at a minimum size limit (Smith and Jamieson 1989a,b).

Morphometric Maturity

We followed Somerton (1980) and used the MATURE1 routine to estimate the size at 50% morphometric maturity (SMM) for male *L. santolla* crabs. For this purpose, we used two different chela dimensions, chela length and height, to evaluate SMM. Data sets were partitioned into 50 subsets of 200 pairs of CL and chela size by means of bootstrapping (Somerton and Otto 1986, Sokal and Rohlf 1995). For each subset we estimated the SMM and their fitting error, and then we calculated the average SMM and its variance according to Somerton (1980). Values of "juvenile" and "adult" required by the routine were chosen visually from the scatterplot of chela size versus carapace length (Fig. 2) and were established at 67.5 and 105.5 mm CL, respectively. If only relatively large crabs, such as those occurring in the fishing captures, are used for this analysis, then SMM will be biased because of the lack of crabs that clearly define a straight line for juveniles. Therefore, we used additional data (CL and chela size) from 107 juvenile male *L. santolla* 19-70 mm CL captured by scuba diving and tangle nets during August 1995, and from 6 crabs 1.6-3.0 mm CL captured inside the holdfast of the kelp *Macrocystis pyrifera* during November 1999 and March 2000. Measurements of partially regenerated chelae, i.e., remarkably small to visual inspection, were excluded from this analysis.

The linear functions for juveniles and adults generated with MATURE1 were used to evaluate the condition of morphometric maturity of males participating in sexual pairs. Male sizes were used to generate expected values of chela size with each regression line, which were contrasted with the observed values. The lesser absolute difference between the observed and expected values for juvenile and adult males was used as a criterion to determine the morphometric maturity of a given male.

Standard statistical analyses were performed according to Sokal and Rohlf (1995). Data subjected to parametric tests were first checked for normality and homogeneity of variance using Kolmogorov-Smirnov and Bartlett's tests, respectively. Orthogonal contrasts were used to compare mean SMM calculated for different crab size ranges and chela dimensions.

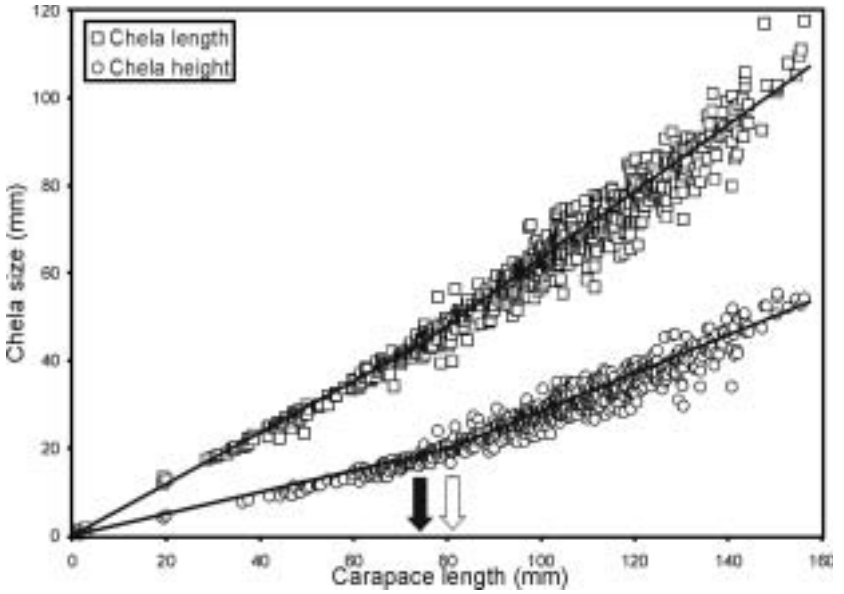

Figure 2. Scatterplot of chela size versus carapace length for male southern king crab Lithodes santolla collected in the Beagle Channel from July 1995 to June 1997. N for chela length and chela height was 721 and 681, respectively. Projections on the abscissas of the intersecting lines—interpreted at the size of morphometric maturity—are denoted by a black (chela height) or white (chela length) arrow. Parameters of linear regressions are presented in Table 2.

Results

Growth Increments

In April 1996, 66 males of 75.3-131.1 mm premolt CL molted in the submerged pen. A significant Hiatt model was obtained from crabs molted in pens (Table 1). Increment-at-molt for the studied size range was independent of premolt size (Table 1), varied between ca. 8.4-15.4 mm CL, and averaged 11.4 (±1.7) mm CL. Slopes for the regression of increment on premolt size and for the Hiatt function were not significantly different from 0 and 1, respectively (Table 1). The growth factor significantly decreased with premolt size, from 12.0-15.8% of premolt size in crabs ca. 75 mm CL to 7.5-9.5% of premolt size in crabs 110-130 mm CL (Table 1). Therefore, the average growth per molt (11.4 mm CL) is alone sufficient to model the growth of males 75.3 to 131.2 mm CL.

Overall, the size frequency distributions of male *L. santolla* were multimodal and biased to crabs <110 mm CL (= legal size) (Fig. 3). The size

Table 1. Functions that describe growth in male *Lithodes santolla* for 66 male crabs between 75.3 and 131.6 mm CL that molted in captivity in April 1996.

		r^2	F	P	95% CI slope
Hiatt function	$CL_{i+1} = 10.471 + 1.009\ CL_i$	0.992	4035.7	<0.001	0.98-1.04
Increment at molt	$I = 10.471 + 0.009\ CL_i$	0.074	0.36	0.553	–0.02-0.04
Growth factor	$G = 22.729 - 0.111\ CL_i$	0.412	44.8	<0.001	–0.14- –0.08

CL_i = Premolt carapace length; CL_{i+1} = postmolt carapace length; r^2 = coefficient of determination; F = lineal regression F-statistic; P = probability that H_0 is true (slope = 0); CI = confidence interval.

frequency analysis performed in these size frequency distributions required 8 instars for an adequate fit of the data (Fig. 3). In all cases the probability of chi-square (χ^2) goodness-of-fit demonstrated that the quality of the fit of the estimated to the observed size frequency distributions was good (all $P > 0.15$). However, the representation of modal groups in each of the four samples was uneven. Between July 1995 and June 1997 in the area of the Beagle Channel closed to fishing, crab instars of sublegal sizes, i.e., instars I, II, and part of IV, accounted for 90.7 % of crabs. Conversely, in the area open to fishing sublegal crabs of instars I and III accounted for 52.1% of crabs. Instars I through IV accounted for 83.5 and 99.2% of the samples from September and March 1984, respectively.

Molting Frequency of Male Lithodes santolla

We detected molting activity in male *L. santolla* >70 mm CL mainly in autumn (Fig. 4). The highest frequencies of crabs with carapaces in stages premolt, postmolt, or exuviae occurred from March to May. After molting, the different molt stages indicating carapace aging gradually changed, with carapace age clearly increasing toward summer (December-February). Molting stages that indicate the oldest carapaces (i.e., the advanced intermolt, CIM) clearly occurred between November, when barnacles *Notobalanus flosculus* settle on hard substrates (Lovrich and Calcagno 1999) and April, after which this molt stage disappeared due to molting. Moreover, the prevalence of barnacles onto all size classes of *L. santolla* was similar, because the null hypothesis of differential prevalence of *N. flosculus* by size class was rejected ($G = 9.56$; $P = 0.48$). In March-May, molting crabs were 80-145 mm CL (Fig. 5A). Molting also occurred in July-December, but less frequently, and only in crabs 70-105 mm CL (Fig. 5B).

Morphometric and Behavioral Maturity

The estimated SMM of *L. santolla* of the Beagle Channel calculated with the MATURE1 routine strongly depended on the choice of chela dimension and the inclusion of smaller crabs in calculations, resulting in significantly different values of SMM (Table 2; ANOVA $F = 16.24$; $P < 0.001$). In all cases,

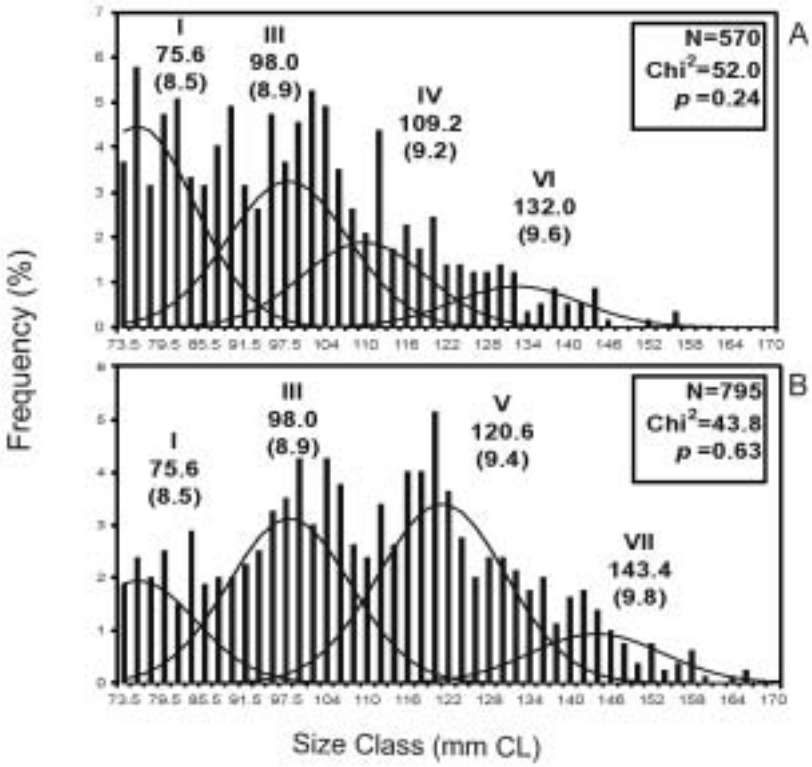

Figure 3. Size frequency distributions of male Lithodes santolla in the Beagle Channel in size classes of 2 mm CL. Samples were from July 1995-June 1997 in (A) a closed area, (B) an open area, (C) in March 1984, and (D) in September 1978 in the same area as (A) formerly open to fishing. Bars and lines indicate observed and estimated frequencies, respectively. N = sample size; Chi² = the value of chi-square, (χ^2) goodness-of-fit refers to quality of the fit of the estimated to the observed size frequency distributions. The higher the value of P, the better the fit. Instars I-VIII are identified, and also indicated are means and standard deviations (S.D.) for each modal group.

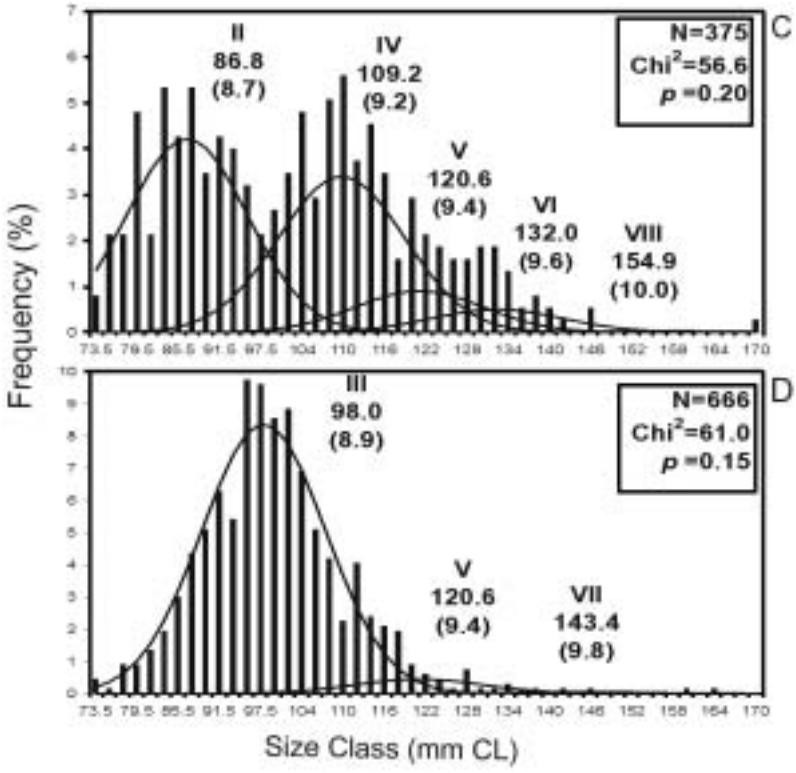

Figure 3. (Continued.)

the regression slope of chela size on carapace length for juvenile males (<67.5 mm CL) was significantly less than that for adult males (>105.5 mm CL) (Fig. 2). Better fits, i.e., less fitting error and larger *F*-values, were obtained when small crabs (1.6-3.0 mm CL) were included by bootstrapping. The estimated $SMM_{chela\ length}$ were not significantly different between samples with or without smaller crabs (Table 2; $F = 1.89$; $P = 0.17$). Once combined, $SMM_{chela\ length}$ was 80.8 (± S.D. 4.3) mm CL. Conversely, $SMM_{chela\ height}$ was 75.4 (± 4.8) mm CL and significantly lower in samples including smaller crabs ($F = 46.6$; $P < 0.001$), and also significantly lower than $SMM_{chela\ length}$ ($F = 19.86$; $P < 0.001$). Therefore, SMM is best estimated from the sample that includes small crabs. We judge that the SMM calculated with chela height is more appropriate because it has less fitting error, and is within the modal group corresponding to the instar I (75.6 mm CL) fitted to size frequency distributions obtained in the field (Fig. 3).

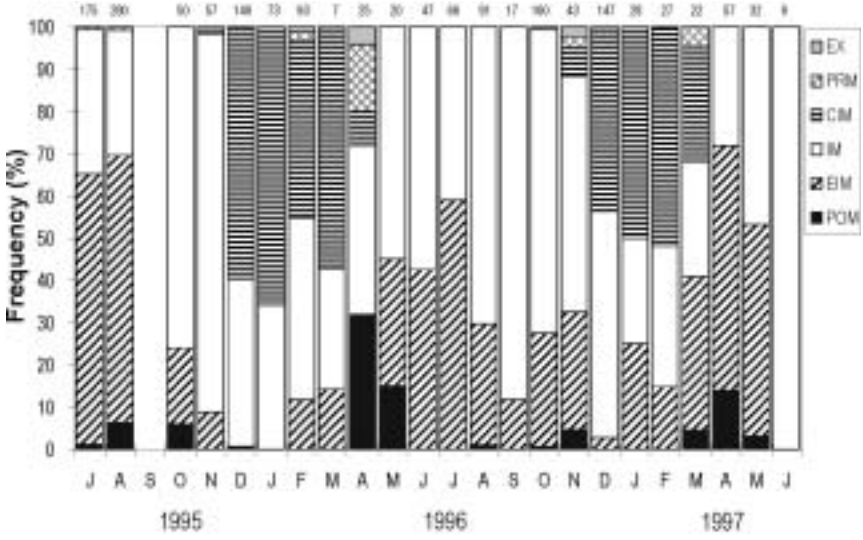

Figure 4. Monthly percent frequency of molt stages of male Lithodes santolla >70 mm CL, in the Beagle Channel. Numbers above each column represent sample sizes. POM = postmolt; EIM = early intermolt; IM = intermolt; CIM = intermolt with epizoic barnacles; PRM = premolt; EX = Exuviae.

In December 1995 and 1996, scuba divers and tangle nets collected 19 and 16 pairs of *Lithodes santolla* that were in precopulatory embrace or in copulatory position, respectively. Pairs were found between 2 and 10 m depth, always hidden, covered by or within a kelp forest of *Macrocystis pyrifera*. All couples of *L. santolla* were composed of males in intermolt or intermolt with barnacles stage with females being in a premolt, molting, or postmolt stage. Males in couples held females by firmly clasping the meropodite of the female's first pereiopods. All females in couples were judged potentially fecund because all had mature ovaries. Females in premolt stage had setae with remnants of egg-capsules or virgin setae, while females in postmolt stages had already extruded oocytes that were fixed on the pleopods. Four couples were also attended by a third male, which grasped the female by one of her walking pereiopods. The size of grasping males correlated with female size (Fig. 6A; $r = 0.65$; $P < 0.01$). Five of 35 females (14.3%) were larger than their mates, yet 3 of them were in postmolt stage and were probably smaller than the male prior to their molt.

Among the 270 males captured in November and December 1995 and 1996, only 39 (14%) were participating in mating couples (Fig. 6B), and uncoupled females were not found. Males participating in couples were >94.2 mm CL, and one of the males attending another pair was 91.7 mm

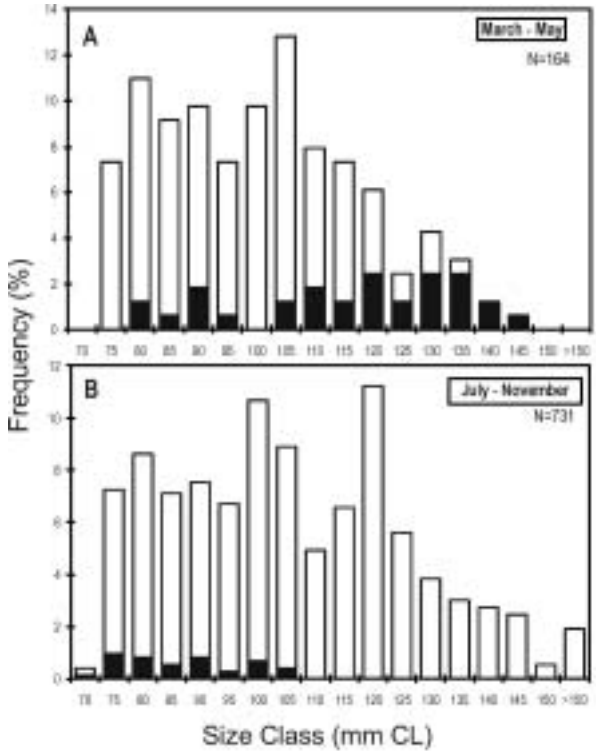

Figure 5. Frequency of male Lithodes santolla in a molting phase (black bars) or in intermolt (open bars) in the Beagle Channel, in 5 mm CL size classes. Crabs in stages postmolt, premolt, and exuviae were considered to be in a molting phase. A: March-May 1996 and 1997. B: July-December 1995 and 1996. Size classes are represented by their inferior limit, i.e., 60 mm CL corresponds to the size class of 60 to 64.9 mm CL and so on. N = sample size.

Table 2. Size at morphometric maturity (SMM) of male *Lithodes santolla* of the Beagle Channel calculated for two chela dimensions and two different crab size ranges, the latter resulting from including or excluding small crabs (1.6-3.0 mm CL) in bootstrap samples.

	Size range (mm CL)	
	1.6-156.2	19.3-156.2
Chela length (ChL)		
Mean SMM (mm)	80.0	82.1
S.D. (mm)	4.3	4.1
Mean fitting error[a]	1.5	2.0
N	31	19
F-range	61.5-173.9	19.9-61.3
Juvenile line: log ChL = –0.40 + 0.97 log CL		
Adult line: log ChL = –1.04 + 1.14 log CL		
Chela height (ChH)		
Mean SMM (mm)	75.4	85.4
S.D. (mm)	4.8	7.1
Mean fitting error[a]	1.4	1.6
N	25	25
F-range	48.5-253.1	23.3-61.2
Juvenile line: log ChL = –1.40 + 0.98 log CL		
Adult line: log ChL = –3.13 + 1.41 log CL		

[a]Somerton (1980).

S.D. = standard deviation; *N* = number of bootstrap samples containing crabs of the corresponding size range. *F*-range = range of values of *F* that test whether a 2 line model fits the data better than a single line.

CL. All but two males in couples (94.3%) were morphometrically mature (Fig. 6A). These two morphometrically immature males were >115 mm CL. Ten of 39 males (25.6%) participating in mating couples were <110 mm CL, the legal size of the fishery (Fig. 6B).

Discussion

Our results allow us to comprehend a model of growth and maturation of male southern king crab *L. santolla* >70 mm CL and its implications in fishery management. Male gonadal maturity is attained at 70 mm CL (Vinuesa 1984), at an instar with a mean size of ca. 69.7 mm CL during their fourth year (c.f. Vinuesa et al. 1990). Morphometric maturity (75.4 mm CL, Table 2) is attained one instar later (instar I in Fig. 3) than gonadal maturity. During the fifth year, male *L. santolla* molt twice (Fig. 5), to in-

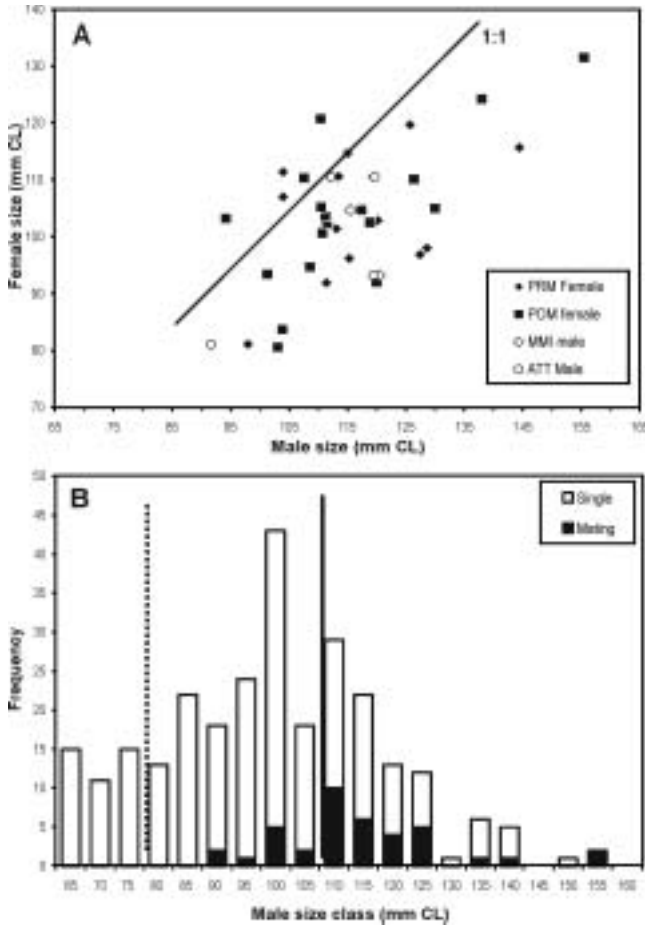

Figure 6. A: Carapace lengths of males and females in couples of Lithodes santolla collected by a scuba diver and by tangle nets at 2-10 m depth in the Beagle Channel in December 1995 and 1996. The line represents a 1:1 ratio of male to female size. PRM = premolt; POM = postmolt; ATT = attendant male, not participating in mating, with PRM female. All males were morphometrically mature except for those MMI that were morphometrically immature coupled with PRM females.

B: Frequency of occurrence of 270 male Lithodes santolla participating in or attending couples (mating) or without a partner (single), in 5 mm CL size classes, at 2-30 m depth in the Beagle Channel in November-December 1995 and 1996. The broken line represents estimated size at morphometric maturity and the continuous line represents legal size. Size classes are represented by their inferior limit, i.e., 60 mm CL corresponds to the size class of 60 to 64.9 mm CL and so on.

stars 75.6 and 86.8 mm CL. In the sixth year, between March and May, crabs molt only once into the instar 98.0 mm CL, and the male intermolt period probably lengthens, since they probably skip the molt that occurs in July-November. According to their size and mating pairs found in this study, crabs within this instar probably are able to mate. If they molted again in July-November, their exoskeletons probably would not be hard enough—if it is a prerequisite—to participate in mating (Powell et al. 1974, but see Powell and Nickerson 1965). Crabs >110 mm CL enter the fishable stock, and after 98 mm CL they continue to molt annually. Hence, we believe that a crab at the minimum legal size (110 mm CL) is probably 7 or 8 years old.

Our results corroborate the fragmentary information reported in previous studies on growth of male *L. santolla*. Methods of mark and recapture showed that growth per molt in male crabs >70 mm CL is 11.1 mm CL in the Strait of Magellan (Geaghan 1973) and 9.3 mm CL in the Beagle Channel (Boschi et al. 1984). Crabs were reported to molt mainly during March-May (Geaghan 1973, Boschi et al. 1984). However, our results show that crabs <105 mm CL also molt during July-November (Fig. 5). Likewise, Geaghan (1973) suggested that male crabs <105 mm CL molt biannually and larger crabs molt annually, because the 14% of crabs <105 mm CL recaptured within one year after release increased twice by the average increment per molt. From his data, we calculated that the frequency of biannual molters decreases from 83% to 4% at size classes of 65-69 and 100-104 mm CL, respectively. Conversely, we showed that the frequency of biannual molters was constant (Fig. 5). Our results may be biased since traps may exclude crabs near molting because they do not feed, and select for dominant larger crabs in intermolt which are more vulnerable to enter traps and thus better sampled (Miller 1990). Therefore, we hypothesize that the probability of the occurrence of a second molt within a year, and after attaining the morphometric maturity, decreases with crab size. Previous studies suggest that male *L. santolla* >130 mm CL may skip their annual molt (Geaghan 1973, Boschi et al. 1984), yet our results do not support such a hypothesis. All of our sampled crabs molted in March-May, as evidenced by the disappearance of crabs with the epizoic barnacle *Notobalanus flosculus* (Fig. 4) that had settled during the previous spring-summer, i.e., mainly in November-December (Lovrich and Calcagno 1999). If male *L. santolla* had skipped their annual molt, then *N. flosculus* should have been found throughout all the year and their prevalence should have been size-specific, as occurs in the sympatric lithodid, the stone crab *Paralomis granulosa* (Lovrich and Calcagno 1999).

Male southern king crabs may have the opportunity to mate once or twice before entering the fishery. We found that male crabs >94.2 mm CL participate in mating pairs (Fig. 6). Given the size distribution for mating males (Fig. 6B) these crabs probably belong to instars with means of 98.0 and 109.2 mm CL (Fig. 3). Therefore, male crabs mate at these instars before entering the fishery (110 mm CL), at most in two reproductive sea-

sons. This observation may lead to the conclusion that the main regulation of the fishery of *L. santolla*—by legal size for landing males—is adequate. This assertion may be false because our findings likely reflect an anomalous situation of the population, in which small crabs are capable of mating principally because of relaxed male competition for mates. This is supported by the observations done in several species of crabs, in which mating is hierarchical and generally the larger males are more successful in the competition for females (e.g., Christy 1987; Smith et al. 1994; Elner and Beninger 1995; Orensanz et al. 1995; Sainte-Marie et al. 1997, 1999 and references therein). Small male *Paralithodes camtschaticus* crabs may mate in the absence of large males when a surplus of pubescent females exists (Powell et al. 1973). Hence, we suppose that relatively small crabs may have occurred in sexual pairs since potential successful competitors, i.e., larger males, were scarce in the population which was biased to smaller crabs (Fig. 3A). Furthermore, the offspring production from breeding with relatively small males may be limited. Male *Paralithodes camtschaticus* crabs are polygynous and one male can mate with up to seven females that produce full egg clutches (Powell et al. 1974). However, smaller crabs are limited to copulate effectively with only one female and further matings seemingly do not produce clutches with 100% of fertilized eggs (Paul and Paul 1990). Hence, it is also probable that small male southern king crabs are not capable of mating with multiple females, and therefore the production of new offspring may have been limited. The low egg production probably reflects, in the closed area of the fishery in 1994, concomitant decreasing of the proportion of ovigerous females from 85% to 11%, the male average size (from 108.2 to 90.6 mm CL), the male relative abundance (from 9.3 to 1.6 male per trap), and the proportion of legal males (from 27% to 8%), from 1981 to 1994 respectively (Wyngaard and Iorio 1996).

Despite the extent of studies on lithodids, information on their mating system is still scarce. The size of participants in mating pairs of *Paralithodes camtschaticus* has been reportedly unrelated, except that females tend to be smaller than males (Powell and Nickerson 1965). We found that mating pairs of *L. santolla* (Fig. 5) and *L. confundens* (Lovrich et al. 2002) were constituted by males larger than females. The male-only fishery of the Beagle Channel has removed the largest males in the population (c.f. Fig. 3 and Wyngaard and Iorio 1996), and consequently the fishery could have contributed to the reduction of the number of mating opportunities (Smith and Jamieson 1991). In this scenario of males larger than females in mating couples and removal of legal males, the largest, and thus potentially more prolific, ovigerous females are expected to be less frequent, and the egg production of the population reduced (Smith and Jamieson 1994). However, in the closed area in 1994, and after two decades of high exploitation rates, the highest proportions of ovigerous females occurred among the largest females, so that the size at 50% of ovigerous females increased from ca. 70 mm CL to 103 mm CL from 1981

to 1994 (Wyngaard and Iorio 1996). This observation was also corroborated in the open area of the fishery in 1995-1996 and 1998 (Lovrich 1997b, Lovrich et al. 1999). We have no rationale to explain this fact.

Size at morphometric maturity estimated with least-squares techniques fitting linear or log-transform models may be affected when crabs with extremely small or large body sizes are included in a data set (Table 1) (Watters and Hobday 1998). These extreme values have strong leverage on the slopes of fitted lines for the juvenile and adult phases, and hence influenced the intersection point of the two lines that denotes the SMM. Furthermore, log-transform models detect only one change in the chela relative growth (Watters and Hobday 1998), in a size range that is generally delimited a priori by the researcher, i.e., the juvenile and adult phases (c.f. Somerton 1980). However, this single change in the chela relative growth has often been used to establish legal size limits. Notwithstanding the limitations of the least-squares method, we still consider our calculation of SMM useful to compare with previous studies. In 19 different locations of the coastal southeastern Pacific, Guzmán and Ríos (1986) calculated male SMM for the southern king crab using Somerton's model (1980), and the chela length as the claw dimension of their choice. The SMM ranged from 80.6 to 114.1 mm CL, and the authors originally attributed these differences to geographical variations in the SMM. Nevertheless, from their results (their Table 3) we detected that estimated SMM were biased, as are our estimates of SMM (Table 2), because they were positively correlated with both minimum ($r = 0.55$; $P < 0.05$) and maximum ($r = 0.46$; $P < 0.05$) sizes of crabs used for the calculations. Similarly, Boschi et al. (1984) reported SMMs of 91 and 99 mm CL, including or excluding in the calculations male *L. santolla* as small as 55 mm CL, respectively.

We are confident that the information here provided is useful to manage the fishery of *L. santolla* of the Beagle Channel. The method used here has satisfactorily identified different size classes from size frequency distributions. At any time, and with updated data of a fishery survey, this model can be used along with growth increments and molting frequency to forecast the evolution of the population and the commercial stock, so that authorities can decide the reopening of closed areas. Furthermore, we recommend the inclusion of observations of mating couples in regular fishery surveys to determine which fraction of male southern king crab *L. santolla* is participating in the reproductive process and assuring the reproductive output.

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Growth and Molting of Golden King Crabs (*Lithodes aequispinus*) in the Eastern Aleutian Islands, Alaska

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Abstract

The Alaska Department of Fish and Game (ADFG) tagged and released 7,621 male and 2,141 female golden king crabs, *Lithodes aequispinus,* during July-August 1997 in the eastern Aleutian Islands, Alaska. Tagged crabs were recovered in each of four subsequent commercial fisheries, from 1997 to 2000. We estimated inter-annual growth in carapace length and inter-annual molting probabilities of 1,576 recaptured males and explore growth, molting, and changes in the reproductive condition from 83 recaptured females. Estimated growth increments in carapace length in one molt (14.5 mm) and two molts (30.7 mm) for males in this study were consistent with each other, and indicate that males within the size range examined grow about 15 mm per molt. Growth of females is smaller than that for males and tends to decrease with size and maturity.

Introduction

Commercial fishing for golden king crabs began in the Aleutian Islands in 1981, and through 1999 yielded a total harvest of 66,000 t worth $389 million in ex-vessel value (Bowers 2000). Systematic surveys of golden king crabs in the Aleutian Islands were conducted by the Alaska Department of Fish and Game (ADFG) in 1991, 1997, and 2000 near Yunaska, Chagulak, and Amukta islands (Blau and Pengilly 1994, Blau et al. 1998, Tracy et al. 2000). A component of each survey included release of tagged crabs for recovery in subsequent commercial fisheries. A comprehensive tagged-crab recovery program in the commercial fishery provided data for estimating growth per molt and molting probabilities for male golden king crabs and gaining insights on growth, molting, and the reproductive cycle of female crabs.

Although estimates of growth and molting probability for golden king crabs in the Aleutians have not been published, data exist for other areas in Alaska. Molting increments of golden king crabs were determined in a mark-recapture experiment in southeastern Alaska by Koeneman and Buchanan (1985), who estimated that males grew an average of 16 mm in carapace length (CL) in a single molt and 31.3 mm in two molts. A laboratory study of the growth of Cook Inlet, Alaska, crabs by Paul and Paul (2000) showed that male growth averaged 10.2 mm and female growth averaged 6.6 mm. Additionally, Lovrich et al. (2002) noted that the growth increment for the southern king crab, *Lithodes santolla,* averaged 11.4 mm.

Methods

At 66 survey stations around Amukta, Chagulak, and Yunaska islands 7,621 male and 2,141 female golden king crabs were tagged and released during 25 July to 27 August 1997 (Blau et al. 1998). We refer to this area near 52º45′N, 170º30′W as the "Yunaska Island area" in this report. Crabs were captured using rectangular king crab pots. Tagging location, date, and fishing depth were recorded for each pot retrieved. All healthy uninjured male crabs ≥121 mm CL were tagged. Sublegal or legal size for males was determined by measuring the straight-line distance across the carapace width (CW) outside the spines using fixed measuring sticks set at 152.4 mm (6.0 inches), the established legal carapace width for the fishery. In terms of carapace length, male crabs generally recruit to legal size at 137 mm CL (Blau and Pengilly 1994). Sublegal size male crabs 90-120 mm CL and female crabs ≥90 mm CL, were tagged at rates of 20-30% per pot. All tagged crabs were measured, shell condition was assessed, and crabs were released on or adjacent to the capture location. The carapace length of each crab, measured from the posterior margin of the right eye orbit to the midpoint of the posterior margin of the carapace using Vernier calipers, was recorded to the nearest millimeter (Wallace et al. 1949). Shell condition of tagged crabs was categorized either as new shell or old shell, based primarily on the degree of scratching on the coxae. Coxae of new-shell crabs are either unscratched or only slightly scratched whereas the coxae of old-shell crabs are heavily scratched and visible epibionts are present. Female crabs were classified as mature when eggs, empty egg cases, or funiculi were present. External polyvinyl isthmus-loop tags were used to tag crabs as detailed in Gray (1965).

Tagged-crab recoveries during the 1997-2000 commercial fishing seasons were documented at sea by shellfish observers on all 13-16 fishing vessels that participated annually in the fishery and by ADFG dockside samplers at shore-based processing plants. Crab size, shell condition, legal status for male crabs, and reproductive condition of females were recorded at recovery. Recaptured crabs not retained for processing (e.g., females and sublegal males) were sampled and, if alive, re-released on or near the fishery capture location with tags still attached.

Only crabs with corresponding data for both release and recovery data were used in our analyses. Data clearly indicating recording errors were omitted, leaving data from 1,645 tagged-crab recoveries (1,576 from males and 69 from females) for analysis. The recovered males ranged in size at release from 91 to 183 mm CL; recovered females ranged in size from 93 to 143 mm CL at release. Crabs were grouped by fishery season of recovery to provide four samples identified by months of liberty between release and recapture: 0-4 months (1997 season), 12-15 months (1998 season), 24-27 months (1999 season), and 36-38 months (2000 season). Fishery seasons were from mid-August or early September to late October or late November.

The difference between carapace length at release and carapace length at recovery was used as an estimate of growth. Scatter plots of estimated growth on carapace length at release and histograms of the distribution of growth for males (Fig. 1) indicated three growth modes centered at roughly 0 mm, 15 mm, and 30 mm. We attributed those three modes to measurement error at release or recovery of animals that had not molted, to growth in a single molt, and to growth in two molts, respectively. Attributing estimated growth of males to measurement error, a single molt, or to two molts is problematic for growth increments with low frequencies falling between peaks of modes; i.e., for estimated growths of 3-6 mm and of 21-25 mm. That difficulty is compounded by the broadening of frequency distributions with successive modes and by the fact that growth for animals that have molted once or twice will also have a component of measurement error in addition to true growth. Nonetheless, given the low relative frequency of those problematic estimated growths (37 of 1,576 samples), any errors in attributing them to measurement error, a single molt, or to two molts has little influence on our analysis. Although two males were recorded with estimated growth of –6 mm, we attributed estimated growth for males of only ≤5 mm to measurement error without true growth. Males with estimated growth of 6-23 mm were assumed to have molted once, whereas males with estimated growth of more than 23 mm were assumed to have molted twice. The estimated growth of 528 recovered males was attributed to growth from a single molt and growth from 85 recovered males was attributed to growth from two molts. Growth of males was also analyzed by shell condition at release and by legal-size status. We estimated probability of molting before recovery as a function of carapace length at release using logistic regression applied to new-shell males released and recovered within 12-15 months. Probability of sublegal males molting to legal size within a year as a function of carapace length at release was estimated using a logistic regression applied to releases of sublegal, new-shell males recovered within 12-15 months.

Given possible effects of maturity status at release and apparent effects of size at release on growth per molt, data from tag recoveries of females were too sparse to allow for estimation of growth per molt parameters. Instead, we provide a descriptive summary of the data on female growth, molting, and change in reproductive status as a function of maturity status at release and by recovery period.

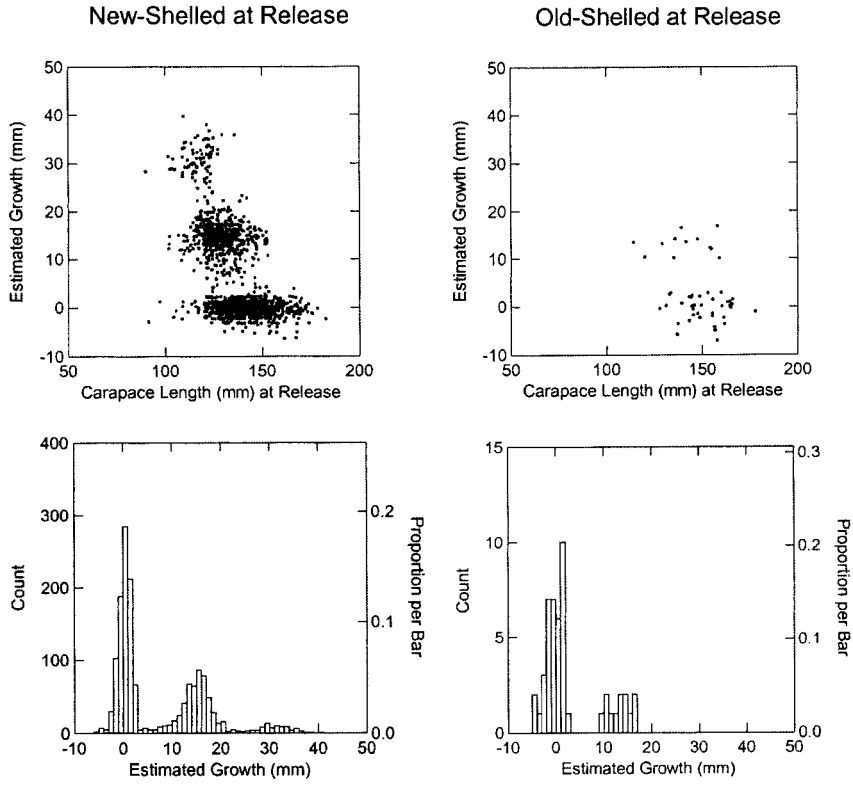

Figure 1. Estimated growth in carapace length as related to carapace length at release from 1,527 recoveries of male golden king crabs tagged and released in new-shell condition (top left panel) and 49 recoveries of male golden king crabs tagged and released in old-shell condition (top right panel) with histograms of estimated growth for the 1,527 males in new-shell condition (lower left panel) and for the 49 males in old-shell condition (lower right panel). A slight random jitter has been applied to the scatter plots to clarify densities obscured by overlapping data values. All crabs were tagged and released in the Yunaska Island area, Alaska, July-August 1997 and recovered during the commercial fishery 0-4, 12-15, 24-27, and 36-38 months after release.

Results

Growth and Molting Probability of Males

The release size of males that were estimated to have grown one or two molts before recovery ranged from 91 to 183 mm CL. Scatter plots of growth by carapace length at release indicated that only new-shell crabs molted twice before being recovered (Fig. 1). Estimated growth per molt of males showed no strong relationship with release size over the range of carapace lengths examined. Linear correlation between growth from two molts and carapace length at release was not significant ($r = 0.153$, N = 85, $P = 0.163$ for a two-tailed test). For males released in new-shell condition, a weak, but significant, negative linear correlation existed between estimated growth from one molt and release size ($r = –0.094$, N = 528, $P = 0.031$ for a two-tailed test), but the correlation was not significant if data from those crabs recovered 0-4 months after release were excluded ($r = –0.058$, N = 517, $P = 0.190$ for a two-tailed test). Likewise, the linear correlation between estimated growth from one molt and release size was not significant for males released in old-shell condition ($r = –0.002$, N = 12, $P = 0.996$ for a two-tailed test).

Estimates of mean growth for a single molt by shell condition and legal status at release were similar (13.9-15.8 mm for new-shell males and 12.5-13.2 mm for old-shell males) for recoveries obtained 12-15 months, 24-27 months, and 36-38 months after release (Table 1). Growth for a single molt of crabs recovered 0-4 months after release varied, however, with sublegal crabs averaging 19.7 mm in growth and legal crabs averaging 10-11 mm estimated growth. Consequently, we pooled single-molt growth of crabs recovered 12-38 months after release for a mean growth of 14.5 mm. Mean growth in a single molt for males recovered 12-38 months after release did not differ significantly by legal status for either new-shell males ($t = 0.23$, d.f. = 515, $P = 0.8$) or old-shell males ($t = –0.40$, d.f. = 9, $P = 0.7$). Mean growth in a single molt for new-shell males released and recovered 12-38 months later (14.5 mm) was significantly greater ($t = 1.757$, d.f. = 526, $P = 0.039$ for a one-tailed test) than for old-shell males (12.9 mm). Estimated two-molt growth for all 85 recoveries averaged 30.7 mm (Table 2). The mean growth for males released as sublegals and recovered 24-27 months later (30.1 mm) was comparable to that for those recovered 36-38 months after release (31.3 mm).

The percentage of males that molted before recovery increased with time between release and recovery: 1.5% of those recovered 0-4 months after release, 64% of those recovered 12-15 months after release, and 95% or more of those recovered 24-27 or 36-38 months after release (Table 3). Few males recovered either 0-4 months or 12-15 months after release molted twice before recovery. Of those males recovered 36-38 months after release, a majority (59%) had molted twice. Males released as old-shells had a higher incidence of molting than those released as new-shells when recovered 12-15 months and 24-27 months after release. Sublegal new-shell males molted at a higher rate (80%) than legal new-shell males (19%) before being recovered 12-15 months and 24-27 months after release.

Table 1. Mean and standard deviation (S.D.) of estimated growth in carapace length (mm) from a single molt by shell condition and legal status at release for male golden king crabs tagged and released in the Unaska Island area, Alaska, July-August 1997 and recovered during subsequent commercial fishery seasons 0-4, 12-15, 24-27, and 36-38 months after release.

		Shell condition at release								
		New shell			Old shell			All shell conditions		
Months after release	Statistic	Sublegal	Legal	All	Sublegal	Legal	All	Sublegal	Legal	All
0-4	N	3	8	11	0	1	1	3	9	12
	Mean	19.7	10.0	12.6	—	11	11	19.7	10.1	12.5
	S.D.	3.51	3.63	5.66	—	—	—	3.51	3.41	5.42
12-15	N	232	62	294	4	5	9	236	67	303
	Mean	14.6	13.9	14.5	12.5	13.2	12.9	14.6	13.9	14.4
	S.D.	2.71	3.43	2.88	2.38	2.39	2.26	2.71	3.35	2.87
24-27	N	148	42	190	0	2	2	148	44	192
	Mean	14.2	14.9	14.4	—	13.0	13.0	14.2	14.8	14.3
	S.D.	3.29	2.03	3.06	—	4.24	4.24	3.29	2.13	3.07
36-38	N	25	8	33	0	0	0	25	8	33
	Mean	15.4	15.8	15.5	—	—	—	15.4	15.8	15.5
	S.D.	3.13	1.98	2.87	—	—	—	3.13	1.98	2.87
12-38	N	405	112	517	4	7	11	409	119	528
	Mean	14.5	14.4	14.5	12.5	13.1	12.9	14.5	14.4	14.5
	S.D.	2.96	2.93	2.95	2.38	2.61	2.43	2.96	2.92	2.95

Only values of 6-23 mm were used to estimate growth from a single molt.

Table 2. Mean and standard deviation (S.D.) of estimated growth in carapace length (mm) from two molts for male golden king crabs tagged and released in the Unaska Island area, Alaska, July-August 1997 and recovered during the commercial fishery 12-15, 24-27, and 36-38 months after release.

Months after release	Legal status at release			
	Statistic	Sublegal	Legal	All
12-15	N	2	0	2
	Mean	25.0	—	25.0
	S.D.	1.41	—	1.41
24-27	N	34	0	34
	Mean	30.1	—	30.1
	S.D.	2.73	—	2.73
36-38	N	48	1	49
	Mean	31.3	36	31.4
	S.D.	3.39	—	3.42
12-38	N	84	1	85
	Mean	30.6	36	30.7
	S.D.	3.26	—	3.29

Only values greater than 23 mm were used to estimate growth from two molts. Estimated two-molt growth data were obtained from crabs released in new-shell condition.

The probability that new-shell males molted within 12-15 months after release was negatively related to size at release (Fig. 2). Ninety-six percent (45 of 47) of those ≤119 mm CL at release molted, whereas only 8% (4 of 48) of those ≥150 mm CL at release molted. A logistic regression was used to estimate the dependency of molting probability on CL:

$$P(molt) = \exp(17.930–0.129CL)/[1+ \exp(17.930–0.129CL)].$$

Based on the estimated logistic regression parameters, we estimated that at 139 mm CL, 50% of new-shell males could be expected to molt within 12-15 months (S.E. = 0.81).

The probability that a sublegal new-shell male (≥90 mm) molted to legal size within 12-15 months after release increased with carapace length at release (Fig. 3). None of the 34 sublegal-sized new-shell males released at ≤118 mm CL and recovered 12-15 months after release had molted to

Table 3. Percentage by shell condition and legal status at release of male golden king crabs tagged and released in the Yunaska Island area, Alaska, July-August 1997 and recovered during the commercial fishery 0-4, 12-15, 24-27, and 36-38 months after release. Crabs were classified as not molted (% Not), molted once (% One), or molted twice (% Two) before recovery.

		Shell condition at release								
Months after release	Statistic	New shell			Old shell			All shell conditions		
		Sublegal	Legal	All	Sublegal	Legal	All	Sublegal	Legal	All
0-4	N	221	520	741	3	34	37	224	554	778
	% Not	98.6	98.5	98.5	100.0	97.1	97.3	98.7	98.4	98.5
	% One	1.4	1.5	1.5	0.0	2.9	2.7	1.3	1.6	1.5
	% Two	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12-15	N	283	184	467	4	6	10	287	190	477
	% Not	17.3	66.3	36.5	0.0	16.7	10.0	17.0	64.7	36.0
	% One	82.0	33.7	63.0	100.0	83.3	90.0	82.3	35.3	63.6
	% Two	0.7	0.0	0.4	0.0	0.0	0.0	0.7	0.0	0.4
24-27	N	187	49	236	0	2	2	187	51	238
	% Not	2.7	14.3	5.1	—	0.0	0.0	2.7	13.7	5.0
	% One	79.1	85.7	80.5	—	100.0	100.0	79.1	86.3	80.7
	% Two	18.2	0.0	14.4	—	0.0	0.0	18.2	0.0	14.3
36-38	N	74	9	83	0	0	0	74	9	83
	% Not	1.3	0.0	1.2	—	—	—	1.3	0.0	1.2
	% One	33.8	88.9	39.8	—	—	—	33.8	88.9	39.8
	% Two	64.9	11.1	59.0	—	—	—	64.9	11.1	59.0

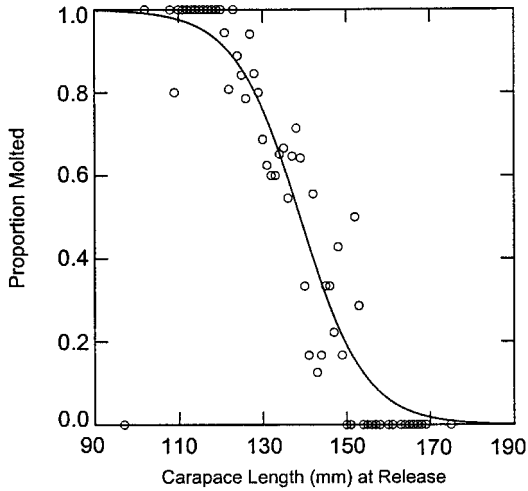

Figure 2. Proportion molting by carapace length at release of 467 new-shell male golden king crabs tagged and released in the Yunaska Island area, Alaska, July-August 1997 and recovered within 12-15 months during the commercial fishery. The curve is a logistic regression fit to the data.

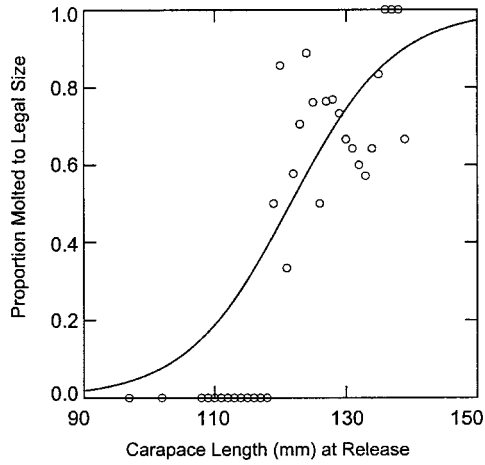

Figure 3. Proportion by carapace length at release of 281 sublegal, new-shell male golden king crabs tagged and released in the Yunaska Island area, Alaska, July-August 1997, that molted to legal size before recovery 12-15 months later during the commercial fishery. The curve is a logistic regression fit to the data.

legal size. However, 91% (10 of 11) of sublegal-sized new-shell males released at ≥136 mm CL and recovered 12-15 months after release had molted to legal size. The logistic regression estimate for the relationship between probability of molting to legal size and carapace at release was

$$P(\text{molt to legal size}) = 1 - \exp(15.541 - 0.127CL)/[1 + \exp(15.541 - 0.127CL)].$$

Based on the estimated logistic regression parameters, we estimated that at 123 mm CL, 50% of sublegal-sized new-shell males could be expected to molt to legal size within 12-15 months (S.E. = 1.54).

Growth, Molting Probability, and Reproductive Cycle of Females

The percentages of females estimated to have molted before recovery is summarized by recovery period and maturity status at release in Table 4. Estimated growth of females by recovery period is plotted against size at release in Fig. 4. Twenty females that were immature when tagged and released were recovered with data for analysis of growth (Table 4). Size at release of those 20 females ranged from 93 to 137 mm CL. The two females >130 mm CL in this sample may have been mature females whose egg case remnants were no longer visible. Forty-nine of the females recovered were released as mature animals and ranged in size at release from 106 to 142 mm CL.

Females Released as Immature

Of 13 immature females recovered 0-4 months after release, 12 remained in immature condition and had estimated growths of –3 mm to 2 mm. We considered those 12 females to have not molted and attributed any nonzero estimates of growth to measurement error. One female released in immature condition was recovered 0-4 months later with a clutch of embryos, indicating that it molted, mated, and grew 10 mm (from 99 mm CL) between release on August 2, 1997 and recovery on October 13, 1997.

Of the five females that were immature at release and recovered 12-15 months later, three molted prior to recovery. They ranged in size from 103 to 104 mm CL and had a mean estimated growth of 9.3 mm. One grew 11 mm (from 103 mm CL) and carried a clutch of embryos at recovery, indicating that it had molted to maturity. Two females had molted but showed no embryos and no empty egg cases at recovery, indicating that they remained immature females. Those two females were released at 103 mm and 104 mm CL and were recovered with estimated growth increments of 9 mm and 8 mm, respectively. The two remaining females were released at 110 mm CL, grew 1 mm, and were recovered without embryos or without empty egg cases, indicating that they did not molt between release and recovery.

Both immature females recovered after 24-27 months had molted to maturity and grew a mean of 7 mm prior to recovery. One female grew 6 mm (from 99 mm CL) and was recovered with empty egg cases, indicating

Table 4. Percentage by maturity at release of female golden king crabs tagged and released in the Unalaska Island area, Alaska, July-August 1997 and recovered during the commercial fishery 0-4, 12-15, 24-27, and 36-38 months after release that were estimated to have not molted or to have molted at least once before recovery.

Months after release	Statistic	Maturity status at release		
		Immature	Mature	All
0-4	N	13	22	35
	% Not molted	92.3	100.0	2.9
	% Molted	7.7	0.0	97.1
12-15	N	5	10	15
	% Not molted	40.0	70.0	60.0
	% Molted	60.0	30.0	40.0
24-27	N	2	9	11
	% Not molted	0.0	0.0	0.0
	% Molted	100.0	100.0	100.0
36-38	N	0	7	7
	% Not molted	—	0.0	0.0
	% Molted	—	100.0	100.0

that it had molted and carried a clutch of embryos through hatching at least once. The other female grew 8 mm (from 106 mm CL) and was carrying a clutch of embryos at its recovery.

Females Released as Mature

The 22 mature females that were released and recovered 0-4 months later ranged in size at release from 116 to 136 mm CL. Growth increments of ≤2 mm and comparison of their clutch conditions at release and recovery indicated that none of them had molted prior to recovery. Four crabs released without embryos remained barren at recovery. The time between release and recovery for those four females ranged from 35 to 62 days. Of the 18 that were released with clutches of embryos, 5 were recovered without embryos (3 with and 2 without empty egg cases), indicating that their clutches had hatched before recovery. Those five females were at large for as little as 41 days (July 30, 1997 to September 9, 1997) and as much as 101 days (10 August 1997 to 19 November 1997).

Of the 11 mature females released with clutches of embryos, 7 were recovered 12-15 months later and were barren when recovered. Six of

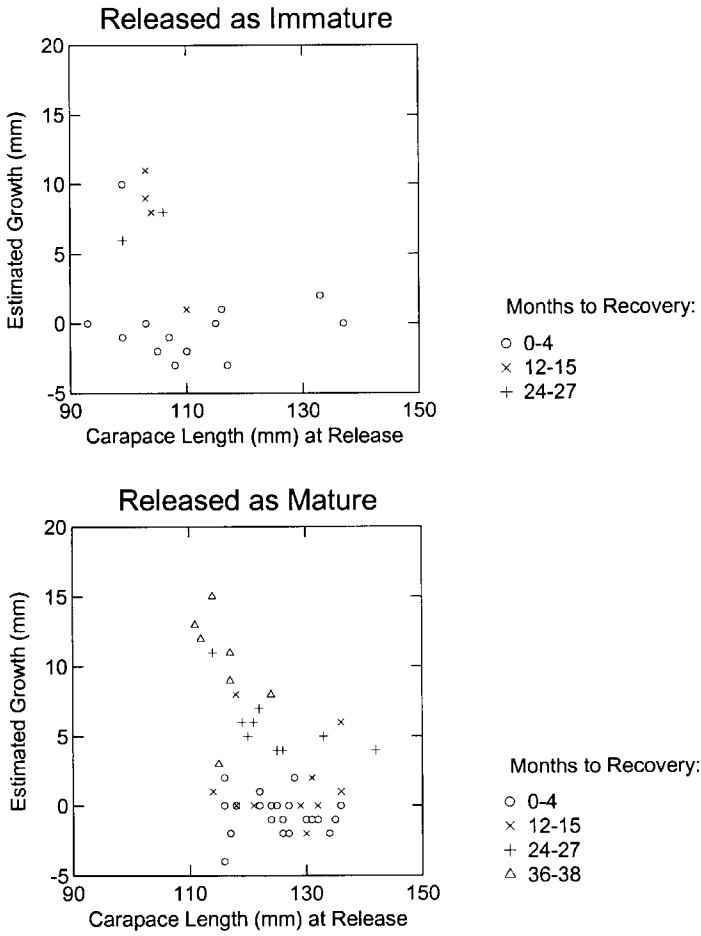

Figure 4. Estimated growth in carapace length by time since release as related to carapace length at release from 20 female golden king crabs tagged and released as immature (top panel) and 49 female golden king crabs tagged and released as mature (bottom panel). All crabs were tagged and released in the Yunaska Island area, Alaska, during July-August 1997.

those, with size at release ranging from 114 to 136 mm CL, had growth increments of 0-1 mm that we attributed to measurement error of nonmolting animals. One female that was released carrying uneyed embryos was recorded as barren with empty egg cases and matted setae at recovery, but was estimated to have grown 10 mm (from 106 mm CL). We suspect the data recorded for this female are in error and did not include them in our analysis. For that female to have grown between release and recovery and to be recovered with empty egg cases and matted setae, it would first have to hatch the clutch it was carrying when released, molted and grown, and then extrude and hatch another clutch. We consider it unlikely that that female hatched two clutches and molted once during the 430 days (August 2, 1997 to October 6, 1998) between its release and recovery. Four barren mature females were recovered 12-15 months after release. Three carried clutches of embryos at recovery, with growth increments of 8 mm (from 116 mm CL), 6 mm (from 136 mm CL), and 2 mm (from 131 mm CL), for a mean growth of 5.3 mm CL. One mature female released as barren was in barren condition when recovered 12-15 months later with a growth increment of –2 mm (from 130 mm CL). We judged that female to have not molted during the 420 days between release and recovery (August 3, 1997 to September 27, 1998).

All nine females released as mature and recovered 24-27 months later had molted at least once, with estimated growth increments ranging from 4 to 11 mm. Growth for those females was negatively related to size at release (Fig. 4). The largest growth was observed in the animal that was smallest at release (114 mm CL). The mean estimated growth was 7 mm for the five animals released at sizes <125 mm CL, and only one of those had an estimated growth of ≤5 mm. The mean estimated growth for animals with size at release ≥125 mm CL was 4.3 mm and all had estimated growth ≤5 mm. The two females with the largest growth increments (11 mm CL from 114 mm CL at release and 7 mm CL from 122 mm CL at release) were released with clutches of eyed embryos and were recovered with empty egg cases 744 and 804 days later. The five females that were carrying uneyed embryos at release were recovered with new clutches of eyed embryos. The two barren females that were largest (133 mm and 142 mm CL) at release were recovered with empty egg cases, indicating that they had carried and hatched a clutch between release and recovery.

All of the seven mature females recovered 36-38 months later had molted. Two molts are suggested from the data for the six females where growth was ≥8 mm. Release size of these crabs ranged from 111 to 124 mm CL, with a growth range of 8-15 mm. Growth was negatively related to size at release in those six females (Fig. 4). The three females with release sizes of 111-114 mm CL had a mean growth of 13.3 mm, the two females with a release size of 117 mm CL had a mean growth of 10 mm, and the female with a release size of 124 mm CL grew 8 mm. Of those crabs, five had clutches of embryos at release and at recovery (the sixth crab had a clutch at recovery but not at release). Data from the remaining one female

was indicative of a single molt. That female, released at 115 mm CL with a clutch of eyed embryos, was recovered barren and grew only 3 mm.

Discussion

Prior to initiation of 100% observer coverage in the Aleutian Islands king crab fisheries, recoveries of tagged golden king crab were so infrequent that estimation of growth and growth per molt was not possible. The ADFG tagged and released 3,612 male and 1,188 female golden king crabs in the Yunaska Island area during 1991, but only 190 of the males and 3 of the females were recovered during the two subsequent fishery seasons (Blau and Pengilly 1994). Of the 190 males that were recovered, carapace length was recorded for only 105, and of those, only 21 molted and grew. Estimated growth for those 21 males captured within 2 years after release ranged from 10 to 34 mm, but the data were too sparse to attribute growth to either one or two molts. No assessment of female growth or molting cycle was possible for the three females recovered from the 1991 release. The high number of tag recoveries from releases in the Yunaska Island area during 1997 made assessment of growth, molting probabilities, and the female reproductive cycle possible.

The mean estimated growth increments in one molt (14.5 mm) and two molts (30.7 mm) for males in this study were consistent with each other, indicating that males within the size range examined (91-183 mm CL) grew about 15 mm per molt. Our estimates are slightly less than those provided from a tagging study of golden king crabs in southeastern Alaska, in which males 126-152 mm CL were estimated to grow an average 16.3 mm in one molt and 31.3 mm in two molts (Koeneman and Buchanan 1985). Average growth estimated from a single molt of new-shell males varied from 14.4 to 15.5 mm and was similar for legal and sublegal crabs that were recovered 12-15 months, 24-27 months, or 36-38 months after release. However, crabs released as new-shell males that were recovered 0-4 months after release had a 10 mm disparity in mean growth from one molt between crabs released as sublegals and those released as legals. The disparate results for recoveries that occurred 0-4 months after release may reflect both the small sample sizes and the size-selectivity of the fishery. In fisheries prosecuted under size limits in which fishers concentrated more effort on large male crabs, sublegal-sized crabs that molted to larger sizes shortly after release are more likely to be represented in recoveries than those that did not. Males released in new-shell condition tended to have a higher mean growth per molt than those released in old-shell condition, as has been reported for red king crabs *P. camtschaticus* (Weber and Miyahara 1962, McCaughran and Powell 1977, Schmidt and Pengilly 1990, Zheng et al. 1995). No strong relationship between carapace length at release and growth per molt of males was evident in the size range that we examined, however, indicating that growth per molt is relatively constant in relationship to premolt size for male golden king crabs ≥90 mm CL.

Unlike red king crabs, golden king crabs may have an asynchronous molting cycle (McBride et al. 1982, Otto and Cummiskey 1985, Sloan 1985, Blau and Pengilly 1994). Although asynchronous molting can make assessment of molting probabilities difficult, our recoveries of tagged crabs at roughly 1-year intervals provide some insights into annual molting probabilities and the factors that affect them. As in red king crabs (Weber and Miyahara 1962, McCaughran and Powell 1977, Schmidt and Pengilly 1990, Zheng et al. 1995), annual probability of molting in male golden king crabs was dependent on the shell condition at release. Regardless of size at release, old-shell males were more likely to molt within 12-15 months after release than new-shell crabs, indicating that most males in the size range examined typically have an intermolt period longer than 1 year. Two males recovered 12-15 months after release grew ≥24 mm, evidence that males molt twice in 1 year or that these crabs exhibited unusually large growth from a single molt. One male golden king crab released in 1991 was recovered 41 days later with a 26 mm increase in size (Blau and Pengilly 1994). Some new-shell males, particularly those released at legal size, had intermolt periods greater than 2 years. Only 1% of the males recovered 36-38 months after release may have had an intermolt period in excess of 3 years.

Annual molting probability of males was dependent on size. The high proportion of new-shell males ≤119 mm CL that molted within 12-15 months is consistent with a 1-year intermolt period for small new-shell males. On the other hand, growth per molt for males ≥150 mm CL released in new-shell condition was consistent with an intermolt period >1 year. We estimated that 50% or less of the new-shell males ≥139 mm CL will molt within 1 year. This size of 50% annual molting probability for new-shell males corresponds with the 135-137 mm CL range at which 50% of male golden king crabs are legal-sized in the Aleutian Islands (Blau and Pengilly 1994, Blau et al. 1998). That is somewhat consistent with the composite shell condition of sublegal and legal males encountered during the 1997 tagging survey; more than 99% of the sublegal crabs were in new-shell condition whereas 92% of the legal males were new-shells (Blau et al. 1998).

Tag loss during molting could affect estimates of molting probability. Although we have no estimates for such tag loss in this study, it has been shown that tag loss for molting red king crabs is negligible (Gray 1964). We recovered a high proportion of molted males released at sizes <120 mm CL and recovered 12-15 months after release, indicating that tag loss may be negligible for molting golden king crabs, as well.

The size class at which male crabs can be considered 1 year away from recruiting to legal size (the "prerecruit-one" size class) is often of interest to fishery managers and population assessment biologists. Blau and Pengilly (1994) and Blau et al. (1998) considered sublegal males ≥121 mm CL to be prerecruit-ones, based on growth per molt estimates of male golden king crabs from southeastern Alaska (Koeneman and Buchanan 1985) and the Aleutian Islands (R.S. Otto, National Marine Fisheries Service, Kodiak, pers. comm.). The analyses presented here provide two ways for specifying a

lower bound for the prerecruit-one class of golden king crabs in the Aleutian Islands, both of which are about 121 mm CL. The first is to subtract the estimated growth per molt from the carapace length at which 50% of male crabs are of legal size (135-137 mm CL; Blau and Pengilly 1994, Blau et al. 1998). That provides an estimated lower bound of 120-122 mm CL. However, all sublegal males ≥120 mm CL cannot be expected to molt within 1 year. The other way to compute a lower bound for the prerecruit-one size class is to estimate the size at which sublegal males have a 50% probability of being recovered as legal-sized 1 year later. We estimated that size to be 123 mm CL based on recovery data from males released as new-shell sublegals. Although based only on recoveries of males released as new-shells, that estimate may be considered applicable for determining the lower size for prerecruit-ones, given the high frequency of new-shell crabs in the sublegal male class.

Our tag recoveries of female golden king crabs provide baseline information on their growth and reproductive cycle. It is the only body of data we are aware of on golden king crab growth and reproductive cycle that is based on a tag-recovery study. Growth per molt is known to decrease with attainment of maturity in red king crabs (Gray 1963), and the size distribution of mature female golden king crabs has been interpreted as reflecting diminished growth with maturity (Otto and Cummiskey 1985). Our tag-recovery data for females also indicated a negative relationship between growth increment and premolt size. That, coupled with low sample sizes, the present poor understanding of the reproductive cycle of female golden king crabs, and values of growth per molt that can be obscured by measurement error, hindered a full assessment of female golden king crab growth. Nonetheless, some general conclusions may be drawn and scenarios for later testing developed.

Only 60% of the females released as immature and 30% of the females released as mature were judged to have molted within 12-15 months after release. Female crabs recovered 24-27 months and 36-38 months after release all showed evidence of growth. Those data are consistent with a molting period >1 year and ≤2 years. Although we cannot be certain of the number of times females molted within 36-38 months after release, we interpreted data from those females as representing two molts.

Female lithodids molt before copulation and egg extrusion (Nyblade 1987). Otto and Cummiskey's (1985) observations on embryo development in golden king crabs indicated that time between successive oviposition was roughly twice that of embryo development, and suggested that spawning and molting of mature females occurs every 2 years. Sloan (1985) also suggested a reproductive cycle >1 year with a protracted barren phase for female golden king crabs. In the context of a molt period ≤2 years, evidence in our tag recoveries that the females carry embryos for less than 2 years is given by the prolonged period in which some mature females remained in barren condition. All five of the mature females released in barren condition and recovered within 0-4 months after release

remained barren for 35-62 days. Those values give the lower bound for which mature females may be expected to remain barren after hatching embryos. Evidence that embryos are carried for less than 2 years is also seen in the two mature females released in barren condition that molted and grew, and were recovered in barren condition 24-27 months later. A single female released in barren condition that was recovered 420 days later in barren condition with no evidence of growth may be indicative of an unusually long intermolt period for some larger (130 mm CL) mature females or, perhaps, to data recording errors.

Numerous observations on clutch and embryo condition of mature female golden king crabs captured during surveys have been consistent with asynchronous reproduction (Otto and Cummiskey 1985, Hiramoto 1985, Sloan 1985, Somerton and Otto 1986, Blau and Pengilly 1994, Blau et al. 1998). The appearance of molted and unmolted females in the recoveries 0-4 months and 12-15 months after release in this study was also consistent with asynchrony in the molting and reproductive cycle. Based on data from Japan (Hiramoto and Sato 1970), McBride et al. (1982) suggested that spawning of golden king crab in the Bering Sea and Aleutian Islands occurs predominately during the summer and fall. We could not assess whether a peak spawning period exists because our recoveries of tagged animals occurred only in the fall of four successive years. We do note, however, that one female released as immature on August 2, 1997, was recovered with a clutch of embryos on October 13, 1997, indicating that molting and mating could occur in the August-October period. However, 5 of the 18 recoveries of mature females that were released with clutches of embryos indicated hatching of embryos in the period August-November 1997. Asynchronous reproductive activity was also indicated by the recoveries made a year or more after release. For example, within 12-15 months after release, three mature females that were barren at release were recovered with clutches of embryos, whereas seven mature females that were released carrying clutches were recovered in barren condition.

Growth increments of females were markedly lower than male growth increments. In fact, some growth increments were so low that in some cases it was difficult to distinguish true growth from measurement error without referring to changes in clutch condition. Notable here is the estimated growth of only 2 mm for a released barren mature female that was recovered with a clutch of embryos approximately 1 year later. Aside from that female, growth ranged from 3 to 15 mm. Greatest values of growth tended to be associated with females that were released as immature or with females that were released as mature and recovered 36-38 months after release. Although average growth was higher for released immature females than for mature females, that comparison is confounded by a negative relationship between estimated growth and size at release. Growth of immature females was estimated from crabs <107 mm CL at release. Mature females for which estimates of growth were available had a broader range of sizes at release, and most were released at sizes >110 mm CL.

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Larval Culture of the King Crabs *Paralithodes camtschaticus* and *P. brevipes*

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Abstract

Paralithodes camtschaticus and *P. brevipes* hatch out as zoeae, metamorphose into glaucothoe, and molt into juveniles. Zoeae were cultured and fed with *Artemia* nauplii and diatoms of the genus *Thalassiosira* (dominant species: *T. nordenskioldii*) in combination, while glaucothoe were nonfeeding. *Artemia* and *Thalassiosira* had sufficient content of eicosapentaenoic acid (EPA) but low content of docosahexaenoic acid (DHA). Freeze-dried *Thalassiosira* were enriched with tuna oil which contained DHA at 23.3%. Hatched *Artemia* nauplii were fed with enriched *Thalassiosira* for about 16 hours beginning 8 hours after hatching. The enriched *Artemia* were fed to zoeae of *P. brevipes*. The survival rate was 75.9% (A), which was much better than 43.1% (B) fed control *Artemia* without enrichment. The food value of *Artemia* fed with enriched *Spirulina* (C) and enriched beer yeast (D) was also examined. The survival rate was 59.3 and 55.6% for C and D, respectively. Although enriched *Artemia* feeding improved the survival rate of zoeal stages, survival rate during the glaucothoe stage was poor at 16.2, 8.4, 6.1, and 5.0% for A, B, C, and D, respectively. Heavy mortality of the glaucothoe was apparently due to the white-turbid midgut gland disease. The disease may be controlled by antibiotic treatment; however, water quality control is more important for improving survival rate associated with nutritional enrichment of food.

Red king crab, *Paralithodes camtschaticus,* and Hanasaki king crab, *P. brevipes*, are important marine resources in the North Pacific Ocean. *Paralithodes camtschaticus* is distributed widely in the Okhotsk Sea and Bering Sea while *P. brevipes* inhabits waters around the Nemuro Peninsula, the Northern Four Islands, and some limited areas around Sakhalin (Sato 1958). The resources have been extensively fished; however, the results of an annual trawl survey (e.g., Stevens et al. 2001) indicate the population of *P. camtschaticus* in the eastern Bering Sea has been well managed. Another approach to resource management may be stock enhancement by restocking juvenile king crabs. About 200,000 first instar juveniles of *P. brevipes* have been released annually in Nemuro waters. A serious problem with this technique is heavy mortality after release. King crabs hatch out as zoea larvae. After three molts in *P. brevipes* and four in *P. camtschaticus*, they metamorphose into the glaucothoe, and then molt into the first instar juvenile (Kurata 1956, Sato 1958, Kurata 1959). Valuable information has been published on larval feeding of *P. camtschaticus* (Kurata 1959, 1960; Paul and Paul 1980; Nakanishi and Naryu 1981; Nakanishi 1987, 1988; Paul et al. 1989). They undergo a drastic developmental change during metamorphosis: the mouthparts, appendages, and foregut atrophy and the glaucothoe do not eat (Kittaka, unpubl., presented to the Fifth International Working Group on Crustacean Nutrition Symposium, April 22-24, 1995, Kagoshima, Japan; Abrunhosa and Kittaka 1997a,b). Therefore, the physiological condition of the glaucothoe and early juveniles is primarily affected by nutrition during the zoeal stages.

We carried out larval and postlarval culture experiments in 1996. Food given to zoeae was generally *Artemia salina* nauplii and cultured diatoms, *Thalassiosira nordenskioldii*, in combination (Iwamoto et al. 1982; Nagakura et al. 1983; Kittaka 2000; Abrunhosa 1998). However, culture results fluctuated widely between tanks. Both foods do not necessarily contain the necessary amounts of essential fatty acids for marine decapod crustaceans (Kanazawa 2000). Another cause of fluctuation in survival between years may be disease such as midgut gland necrosis found commonly in cultured crustacean larvae (Liao 1988). Heavy mortality was reported in *P. camtschaticus* and *P. brevipes* larval culture due to the midgut gland disease (Watanabe et al. 1998). In order to establish larval and postlarval culture methods for king crabs, we examined the effect of nutritional enrichment of foods to survival rate in 2000.

Materials and Methods

Larval Culture with Ordinal Foods in 1996

Preparation of Foods (Thalassiosira and Artemia)

Ambient seawater of about 1 m^3 was filtered using a plankton net of 23 μm opening. Collected diatoms were introduced into a 100 L transparent container placed near a window, filled with seawater filtered through a 10 μm

Table 1. Composition of the culture medium for *Thalassiosira nordenskioldii*

Ingredients	Contents	Ingredients	Contents
Solution A		**Solution B**	
Distilled water	100 ml	Distilled water	100 ml
$NaNO_3$	10 g	$MnCl_2$	4 g
Sodium glycerophosphate	1.7 g	$CaCl_2$	0.1 g
Thiamine HCl	10 mg	$ZnCl_2$	0.4 g
Vitamin B_{12}	0.1 mg	$FeCl_2$	1 g
$Fe(NH_4)_2(SO_4)_2 \bullet 6H_2O$	0.7 g		
H_3BO_3	0.2 g	**Solution C**	
EDTA-2Na	0.3 g	Distilled water	100 ml
Solution B	1 ml	$Na_2SiO_3 \bullet 9H_2O$	4.5 g

PES culture medium: (Solution A) 1 ml and (Solution C) 3 ml into 1,000 ml filtered and sterilized seawater.
PES = Erdschreiber medium as modified by Provasoli.

cartridge filter and sterilized with an ultraviolet sterilizer. Nutrients as shown in Table 1 were added in the container. The diatoms were cultured at 8-10ºC under about 8,000 lux with vigorous aeration. In about 7 days, *Thalassiosira* spp. were dominated by *T. nordenskioldii* at about 50×10^3 cells per ml.

One to ten g of *Artemia* eggs (Great Salt Lake *Artemia* cysts provided by Sanders Brine Shrimp Company, Inc.) were put into a 30 L *Artemia* hatching container filled with filtered and sterilized seawater and kept at 28ºC with aeration for 24 h. Hatched *Artemia* were collected and fed to zoeae at about 20-60 nauplii per day per zoea from early to late stages.

Rearing of Egg-Carrying Females in 1995/1996

Ovigerous females of both *P. camtschaticus* and *P. brevipes* were transported from Russia in November and December 1995 and reared in a tank (capacity: 2 m^3) with aeration at water temperatures of 5-6ºC until hatching. Daily, 10-30% of the tank water was changed with ambient seawater. Crabs were fed mussels, *Mytilus edulis,* every 2-3 days. Hatching usually began in mid-January and ended in mid-April with approximately 100,000-200,000 and 10,000-50,000 zoeae per female of *P. camtschaticus* and *P. brevipes*, respectively.

Culture of Larvae and Postlarvae in 1996

Hatched zoeae were scooped with a 1.5 mm mesh net and placed into a 10 L plastic container. The total number was either counted (if below 2,000) or estimated by sampling two or three times with a 200 ml beaker, and computing the average. About 700-9,800, 10,000, and 35,000 hatched zoeae

were introduced into a 100 L transparent plastic container with a conical bottom, a 200 L plastic rectangular container, and a 1.5 m^3 FRP (fiber resin plastics) rectangular tank, respectively, filled with filtered seawater with aeration. Water temperature was maintained at 8-10ºC. *Artemia* (eggs: 1-5 g) nauplii hatched at 28ºC for 24 hours, and *Thalassiosira* were fed in combination or separately. The culture water was changed every 2 days for 100 L and 200 L containers and about 20% every 5 days for the 1.5 m^3 tank. Mortality and molting were observed daily. After metamorphosis, the glaucothoe grasped the mesh net hung in the containers. The attached glaucothoe were transferred daily into separate tanks of water at 8-10ºC and were cultured without feeding. Survival rate of glaucothoe was monitored using a 10 L plastic container, into which glaucothoe were exactly counted (around 100) on the day of metamorphosis.

Larval Culture Experiment with Enriched Artemia Nauplii in 2000

Twelve ovigerous females of *P. brevipes* caught off Ochiishi, Nemuro Peninsula in June and August 1999 were reared in similar conditions as mentioned previously. Hatching began in mid-February and ended in early April 2000. Hatched first zoeae with positive phototaxis were introduced into eight 10 L plastic containers. Numbers of zoeae in each container ranged between 103 and 174. Water temperature was maintained at 8-10ºC. *Artemi*a nauplii were fed once per day at about 20-60 individuals per zoea. The culture water was changed daily. Dead zoeae and molts were removed and recorded daily. After metamorphosis, the glaucothoe were transferred daily into a separate 10 L container and cultured at 8-10ºC without feeding, until molting to the first instar crab. The following four kinds of *Artemia* nauplii were provided for feeding experiment of zoeae.

1. *Artemia Enriched with Thalassiosira Enriched with Tuna Oil*
   The 100 L culture of *Thalassiosira* was inoculated into a transparent 1 m^3 tank and cultured in similar conditions as the 100 L container. In about 10 days, *Thalassiosira* increased to maximum concentration and were collected after precipitating with aluminum potassium sulfate, anhydrous at about 250 ppm.

   Freeze-dried *Thalassiosira* (20 g) was put into a vinyl bag. Tuna oil (trade name: Sun Omega DHA33 produced by Nippon Yusi Co. Ltd., containing eicosapentaenoic acid (EPA) at 6.9% and docosahexaenoic acid (DHA) at 23.3%) of 1.5 g was dissolved with 4.5 g diethyl-ether and sprayed onto the freeze-dried *Thalassiosira* in the bag, and then both were mixed well (hereinafter referred as enriched *Thalassiosira*).

   Artemia eggs were put into an *Artemia* hatching container (capacity: 30 L) filled with filtered and sterilized seawater and kept at 28ºC with aeration for 24 hours. Hatched *Artemia* nauplii were kept in a separate container (capacity: 30 L) for about 8 hours and then were fed

with the enriched *Thalassiosira* at 200 ppm for about 16 hours at 20ºC (hereinafter referred to as *Artemia* T.).

2. *Artemia Enriched with Enriched Spirulina*
   Artemia nauplii were fed with *Spirulina* sp. (Class: Cyanophycea) enriched with oil containing n-3 highly unsaturated fatty acid (HUFA). The trade name is "Super Artemia," produced by Higashimaru Co. Ltd., Japan. Similar procedures used to produce *Artemia* T. were applied (hereinafter referred to as *Artemia* S.).

3. *Artemia Enriched with Enriched Beer Yeast*
   Artemia nauplii were fed with beer yeast, in which squid oil (contained n-3 HUFA) was encapsulated. The trade name is "Yugen," produced by Kirin Beer Co. Ltd., Japan. Similar procedures used to produce *Artemia* T. were applied (hereinafter referred to as *Artemia* B.Y.).

4. *Control Artemia*
   The controls were *Artemia* nauplii without enrichment (hereinafter referred to as *Artemia* C.).

Statistical Procedures

Culture in 1996

For both species, results of culture in low density tanks (≤ 25 zoeae per L) were compared to culture in high density tanks (>25 zoeae per L) by *t*-test. Proportional survival of zoeae and glaucothoe was transformed to the arcsine (square root [*p*]) before analysis. Although glaucothoe were cultivated at low densities of about 10 per L and not fed, similar comparisons were made because nutritional status and survival may have been affected by culture density during zoeal stages.

Culture in 2000

The proportions of zoeae surviving to the glaucothoe stage, and the first crab stage, were compared by analysis of variance, after arcsine transformation (as above), using two replicates for each food combination. Post-hoc comparisons were made using Tukey's Honestly Significant Difference (HSD) technique (Zar 1984).

Fat Analysis of the Cultured and Enriched Thalassiosira and Hatched Artemia

Thalassiosira and *Artemia* were analyzed for moisture (loss in drying at 105ºC for 12 hours), crude protein (Kjeldahl nitrogen × 6.25), crude lipid (Bligh and Dyer 1959), and crude ash contents (combustion at 500ºC for 12 hours). The fatty acid profile of food materials was determined using gas chromatography (GC), Shimadzu GC 17A fitted with a flame ionization detector (column temperature 220ºC, detector temperature 260ºC, helium

as carrier gas at 30 ml per min), and capillary column (OmegawaxTM320, 30 m length × 0.32 mm ID, 0.25 mm film thickness) (Supelco Inc., Japan). Peaks were recorded using a Shimadzu Chromatopac C-R4A data processor. They were identified by comparison to a known standard such as pollock liver oil and quantified by means of response factor to the internal standard, tricosanoic acid (Querijero et al. 1997).

Lipids used for GC analysis were extracted from samples using the method of Bligh and Dyer (1959). They were separated into neutral (NL) and polar lipid (PL) fractions using column chromatography with Sep-Pak silica cartridge (Waters, USA) eluted using chloroform-methanol (98:2 v/v) and methanol solvent systems for NL and PL, respectively (Juaneda and Rocquelin 1985). The fatty acid components of the NL and PL fractions were prepared into fatty acid methyl esters (FAME) using boron trifluoride in methanol (BF3-MeOH; Wako Pure Chemicals, Japan) as a catalyst (Morrison and Smith 1964). FAME samples were purified using SEP-PAK silica cartridges eluted with diethyl ether:petroleum ether (5:95 v/v). Distilled hexane was added to the purified methylesters at 20 mg per ml just before injection into the GC port (Querijero et al. 1997).

Results

Larval and Postlarval Culture in 1996

Culture results of *P. camtschaticus* and *P. brevipes* fed *Artemia* and *Thalassiosira* in combination in 1996 are shown in Tables 2A and 3A, respectively. The survival rate of zoeae cultured in 100 L containers ranged between 0 and 87.2% for *P. camtschaticus* and between 1.0 and 78.9% for *P. brevipes*, and that of glaucothoe was 40.0-80.0% for the former and 33.0-72.5% for the latter. Survival rate of zoeae fluctuated widely during the zoea stage compared to the nonfeeding glaucothoe stage.

Two tanks (#2 and 3, Table 2) of *P. camtschaticus* with zero survival were concluded to be the result of spillage or other error and were excluded from analysis. For *P. camtschaticus*, there was no difference in weight, survival, or duration of zoeae or glaucothoe between low density and high density treatments (Table 2B). For *P. brevipes* (Table 3B), survival of zoeae was greater at low density than at high density, but other parameters did not differ.

Thalassiosira Culture in 1999

Thalassiosira nordenskioldii were cultured during the period from May to September 1999. The culture results are shown in Table 4. Beginning at 2,000-5,000 cells per ml, they increased to 31,000-56,000 cells per ml in 1 or 2 weeks. Production of cultured *Thalassiosira* ranged between 29.4 and 137.8 g with an average 71.1 ±34.2 g in dry weight. The culture period was divided into two series: early (No. 1-11, starting date of April 30-June 29) and late (No. 12-22 , July 1-September 7). The production per culture was

93.0 ±30.8 and 43.7 ±9.1 g for the early and late series, respectively. *Thalassiosira* production decreased after July.

Lipid Content and Fatty Acid Composition of the Cultured Thalassiosira

The lipid content and fatty acid content and composition of the cultured *Thalassiosira* of 1999 and those of *Artemia* are shown in Table 5. Those of the cultured *Thalassiosira* before and after enrichment with tuna oil are shown in Table 6 with its proximate composition in Table 7. The lipid content of the cultured *Thalassiosira* ranged between 75 and 180 μg per mg with an average 131.5 ±45.8 μg per mg dry weight.

Total fatty acid content averaged 58.3 ±43.7 μg per mg dry weight with 47.2 ±24.6% total lipids. Major components of the cultured *Thalassiosira* were 16:1n-9 (POA [palmitoleic acid]) and 20:5n-3 (EPA). EPA was contained at 3.39-34.68 μg per mg dry weight with an average of 18.3 (range:13.3-29.9)% in fatty acid composition. Lipid contents and fatty acid composition varied presumably by culture condition.

Cultured *Thalassiosira* in 1999 was enriched with tuna oil (Table 6). After enrichment, the lipid content increased from 95 to 195 μg per mg dry weight and fatty acid content from 68 to 116 μg per mg dry weight. In fatty acids, 18:1n-9 increased drastically from 0.09 to 14 μg per mg dry weight and 22:6n-3 from 0.3 to 11.5 μg per mg dry weight. Enrichment of tuna oil was effective for increasing DHA content of *Thalassiosira*. *Artemia* contained EPA at the same level as *Thalassiosira*, but contained smaller amounts of DHA than *Thalassiosira*.

Survival Rate in 2000

Survival during zoea and glaucothoe stages of *P. brevipes* in 2000 is shown in Table 8, Fig. 1, and Fig. 2. Survival during zoeal stages was highest for those fed *Artemia* T. (75.9%), followed by *Artemia* S. (59.3%), *Artemia* B.Y. (55.6%), and *Artemia* C. (43.1%). Survival rate during the glaucothoe stage was generally poor, although survival of those fed *Artemia* T. (16.2%) was higher than those fed *Artemia* S. (8.4%), *Artemia* B.Y. (6.1%) and *Artemia* C. (5.0%). These differences in survival were not significant for either zoea [$F_{(0.05,\ 3,4)} = 4.594$, $P = 0.087$] or glaucothoe stages [$F_{(0.05,\ 3,4)} = 4.754$, $P = 0.083$].

However, total survival rate from first zoea to the first instar crab of *P. brevipes* was significantly greater for those fed *Artemia* T. (12.2%) than for those fed *Artemia* S. (5.0%), *Artemia* B.Y. (3.6%), or *Artemia* C. (1.9%) [$F_{(0.05,\ 3,4}$] = 24.643, $P = 0.005$). The HSD test showed that survival of the latter 3 groups was homogeneous.

Period of Zoea and Glaucothoe Stages

Zoeae metamorphosed into glaucothoe during the period from 23 to 27 days after hatching with an average of 24.5, 24.6, 24.5 and 25.1 days for

Table 2A. Survival rate and duration of zoea stage (fed *Artemia* and *Thalassiosira* in combination) and those of glaucothoe stage (nonfeeding) of *Paralithodes camtschaticus* in 1996.

Number of exp.	Number of zoeae	Number of glaucothoe	Zoea survival rate (%)	Zoea Duration (days)	WT[a] (ºC)	Glaucothoe survival rate (%)	Glaucothoe duration (days)	Capacity of zoea culture container (L)
1	700	72	10.3%	35.0	8.8			100
2	1,200	0	0.0%					100
3	1,000	0	0.0%					100
4	1,200	68	5.7%	33.0	8.9	80.0%	17.0	100
5	2,000	608	30.4%	28.0	9.3	74.5%		100
6	2,000	162	8.1%	27.9	9.3			100
7	2,500	1,121	44.8%	31.9	8.5	73.0%		100
8	2,500	2,181	87.2%	26.0	8.8	40.0%	17.0	100
9	10,000	1,093	10.9%	28.0	9.4	40.0%	17.0	200
10	35,000	8,674	24.8%	28.0	9.6	62.0%	22.5	1,500
All (exp. 1-10)			24.4%	29.7	9.1	61.6%	18.4	
	<2,501		30.7%	30.3	8.9	66.9%	17.0	
	>2,500		17.9%	28.0	9.5	51.0%	19.8	

[a]Water temperature

Table 2B. Results of *t*-test comparisons between low density (<2,501 zoeae) and high density culture conditions.

Comparison	*t*-value	d.f.	*P*-value	Significance
Zoea duration	0.880	6	0.413	n.s.
Zoea weight	2.373	6	0.055	n.s.
Zoea survival	0.403	6	0.701	n.s.
Glaucothoe duration	1.000	2	0.423	n.s.
Glaucothoe survival	1.070	4	0.345	n.s.

Table 3A. Survival rate and duration of zoea stage (fed *Artemia* and *Thalassiosira* in combination) and those of glaucothoe stage (nonfeeding) of *Paralithodes brevipes* in 1996.

Number of exp.	Number of zoeae	Number of glaucothoe	Zoea survival rate (%)	Zoea Duration (days)	WT[a] (ºC)	Glaucothoe survival rate (%)	Glaucothoe duration (days)	Capacity of zoea culture container (L)
1	1,200	799	66.6%	23.9	8.9	48.5%	17.0	100
2	2,400	1,735	72.3%	25.6	8.8	49.5%	23.7	100
3	4,960	3,097	62.4%	24.4	8.7	56.6%	17.6	100
4	4,800	47	1.0%	29.3	8.2	46.2%	17.0	100
5	9,840	1,127	11.5%	29.7	8.0	33.0%	23.1	100
6	5,880	854	14.5%	28.4	8.3	71.8%	26.1	100
7	1,060	829	78.2%	27.6	8.1	68.4%	19.0	100
8	1,000	469	46.9%	21.3	11.0	72.5%	22.2	100
All (exp.) (1-8)			44.2%	26.3	8.8	55.8%	20.7	
	<2,501		66.0%	24.6	9.2	59.7%	20.5	
	>2,500		29.5%	28.0	8.3	53.8%	21.0	

[a]Water temperature

Table 3B. Results of *t*-test comparisons between low density (<2,501 zoeae) and high density culture conditions.

Comparison	*t*-value	d.f.	*P*-value	Significance
Zoea duration	1.857	6	0.113	n.s.
Zoea weight	1.400	6	0.211	n.s.
Zoea survival	2.793	6	0.031	s.
Glaucothoe duration	0.178	6	0.865	n.s.
Glaucothoe survival	0.758	6	0.477	n.s.

s. = significant

Table 4. Culture of *Thalassiosira nordenskioldii*.

Number of cultures	Date of beginning in 1999	Initial concentration (cells per ml)	Water temperature (ºC)	Date of harvest in 1999	Final concentration (cells per ml)	Dry weight (g)
1	Apr 30	4,800	10.0	May 12	38,000	104.6
2	May 11	3,600	10.0	May 24	34,000	123.5
3	May 13	4,200	9.4	May 27	35,000	67.9
4	May 25	4,600	10.0	Jun 4	34,000	78.6
5	May 28	3,800	9.4	Jun 9	55,000	137.8
6	Jun 4	1,200	9.8	Jun 11	56,000	64.8
7	Jun 9	2,200	9.7	Jun 21	[a]	
8	Jun 11	3,100	10.0	Jun 21	40,000	131.5
9	Jun 21	2,100	9.4	Jun 29	43,000	56.9
10	Jun 21	3,000	9.4	Jul 1	34,000	102.7
11	Jun 29	4,400	9.4	Jul 8	41,000	61.8
12	Jul 1	4,000	9.4	Jul 13	31,000	45.3
13	Jul 8	3,800	9.8	Jul 16	40,000	49.7
14	Jul 14	3,200	10.0	Jul 21		48.0
15	Jul 16	2,500	10.4	Jul 23	35,000	52.2
16	Jul 21	3,000	10.4	Jul 30	[a]	
17	Jul 30	3,600	10.8	Aug 11	[a]	
18	Aug 1	3,400	10.8	Aug 11		44.0
19	Aug 11	3,800	11.0		[a]	
20	Aug 11	4,200	11.2	Aug 23		30.0
21	Aug 27	3,600	10.8	Sep 6	8,900	29.4
22	Sep 7	4,800	10.4	Sep 14	37,000	51.0

Note: Capacity of culture tank: 1 m^3.

[a] Discontinued.

Table 5. Fatty acid content (ng per mg dry weight) of *Artemia* nauplius and cultured *Thalassiosira*.

Fatty acid	*Artemia*	*Thalassiosira* Jan 24, 1998[a]	*Thalassiosira* Apr 8, 1998[a]	*Thalassiosira* May 26, 1998[a]
14:0	1,250	4,190	3,580	6,900
14:1n-5	1,400	tr.	tr.	tr.
15:0	tr.	246	230	298
16:0	15,600	4,360	4,100	10,140
16:1n-9	6,600	8,100	6,970	51,110
16:2n-6+16:2n-4	tr.	920	120	tr.
17:0	tr.	tr.	650	1,870
17:1	1,440	661	523	2,250
16:4n-3	tr.	tr.	840	4,800
18:0	5,500	147	250	109
18:1n-11+18:1n-9	29,400	205	380	190
18:1n-7	6,400	157	220	238
18:2n-6	7,300	130	164	390
18:3n-3	34,000	28	166	73
18:4n-3	4,600	1,630	1,390	5,150
20:1n-11	610	tr.	tr.	tr.
20:4n-6	1,210	tr.	14	49
20:3n-3	580	8	tr.	tr.
20:4n-3	650	60	59	190
20:5n-3	4,700	4,000	3,390	34,680
22:5n-3	tr.	tr.	tr.	tr.
22:6n-3	76	300	360	900
Total	102,000	25,200	23,800	116,100
Total lipid (μg per mg dry weight)	220.9	121.0	74.8	180.3

[a]Date of harvest after about 10 days culture.

Table 6. Total lipids and fatty acid contents (ng per mg dry sample) and fatty acid composition of diatom *Thalassiosira nordenskioldii*.

	Before enrichment				After enrichment			
	ng per mg diatom[a]		% of total FA[b]		ng per mg diatom[a]		% of total FA[b]	
Fatty acid	Average	S.D.[c]	Average	S.D.[c]	Average	S.D.[c]	Average	S.D.[c]
14:0	5,840	596	8.6	0.2	7,070	91	6.1	0.2
14:1n-5	104	15	0.2	0.0	tr.	tr.	tr.	tr.
15:0	543	71	0.8	0.0	791	37	0.7	0.0
16:0	6,760	488	9.9	0.1	20,900	1,750	18.0	0.6
16:1n-9	36,100	2,610	53.1	0.4	27,000	834	23.3	0.5
16:2n-6+16:2n-4	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
17:0	832	0	1.2	0.0	515	62	0.4	0.0
17:1	1,930	203	2.8	0.1	2,750	64	2.4	0.1
16:4n-3	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
18:0	551	2	0.8	0.1	3,390	442	2.9	0.2
18:1n-11+18:1n-9	92	23	0.1	0.0	14,000	2,600	12.0	1.6
18:1n-7	408	43	0.6	0.0	17,80	368	1.5	0.2
18:2n-6	231	12	0.3	0.0	1,080	167	0.9	0.1
18:3n-3	91	14	0.1	0.0	442	27	0.4	0.0
18:4n-3	1,070	174	1.6	0.1	1,570	74	1.4	0.0

[a] *Thalassiosira nordenskioldii*: cultured May 1999 and enriched with tuna oil (EPA 6.9% and DHA 23.3%). Two samples were analyzed.

[b] Fatty acid.

[c] Standard deviation.

Table 6. (Continued.) Total lipids and fatty acid contents (ng per mg dry sample) and fatty acid composition of diatom *Thalassiosira nordenskioldii*.

	Before enrichment				After enrichment			
	ng per mg diatom[a]		% of total FA[b]		ng per mg diatom[a]		% of total FA[b]	
Fatty acid	Average	S.D.[c]	Average	S.D.[c]	Average	S.D.[c]	Average	S.D.[c]
20:1n-11	tr.	tr.	tr.	tr.	1,220	95	1.1	0.0
20:4n-6	tr.	tr.	tr.	tr.	827	115	0.7	0.1
20:3n-3	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
20:4n-3	tr.	tr.	tr.	tr.	325	16	0.3	0.0
20:5n-3	9,130	687	13.4	0.1	11,700	110	10.1	0.6
22:5n-3	tr.	tr.	tr.	tr.	531	74	0.5	0.0
22:6n-3	308	27	0.5	0.0	11,500	986	10.0	0.3
Others	3,420	371	5.1	0.9	8,450	1,620	7.3	1.8
Total	68,000	5,440	100.0	0.0	116,000	6,050	100.0	0.0
Total lipids (μg per mg dry weight)	95.0				195.0			

[a]*Thalassiosira nordenskioldii*: cultured May 1999 and enriched with tuna oil (EPA 6.9% and DHA 23.3%). Two samples were analyzed.
[b]Fatty acid.
[c]Standard deviation.

Table 7. Proximate composition (%) of diatom *Thalassiosira nordenskioldii*.

	Before enrichment	After enrichment
Crude protein	10.6	15.1
Crude lipid	9.5	19.5
Crude ash	42.8	36.4
Others	37.1	29.0

Three samples were analyzed.

Table 8. Survival of larvae and postlarvae of *Paralithodes brevipes* fed *Artemia* nauplii.

		Number of larvae and postlarvae			Survival rate (%)		
Food	Experiment	Zoea 1	Glaucothoe	Crab 1	Zoea	Glaucothoe	Total
Artemia T.	No. 1	124	96	15	77.4	15.6	12.1
	No. 2	113	84	14	74.3	16.7	12.4
	Average				75.9	16.2	12.3
Artemia S.	No. 1	174	91	8	52.3	8.8	4.6
	No. 2	113	75	6	66.4	8.0	5.3
	Average				59.3	8.4	5.0
Artemia B.Y.	No. 1	130	69	5	53.1	7.3	3.9
	No. 2	119	81	4	58.1	4.9	3.4
	Average				55.6	6.1	3.6
Artemia C.	No. 1	137	49	4	35.8	8.2	2.9
	No. 2	103	52	1	50.5	1.9	1.0
	Average				43.1	5.0	1.9

Note: *Artemia* T.: *Artemia* enriched with *Thalassiosira* enriched with tuna oil. *Artemia* S.: *Artemia* enriched with enriched *Spirulina*. *Artemia* B.Y.: *Artemia* enriched with enriched beer yeast. *Artemia* C.: *Artemia* without enrichment (Control). Zoeae metamorphosed into glaucothoe 23-27 days after hatching with peaks at 25 days. Capacity of culture container: 10 L.

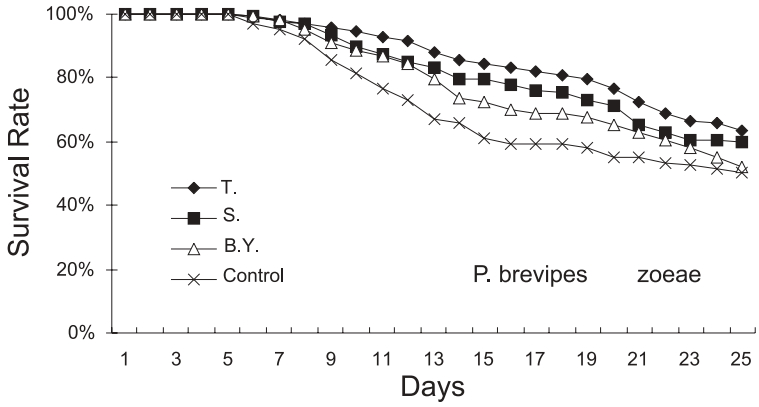

Figure 1. Survival rate of Hanasaki king crab, Paralithodes brevipes, during zoeal stage fed Artemia nauplii enriched with various kinds of food. T.: Artemia enriched with Thalassiosira enriched with tuna oil (Artemia T.); S.: Artemia enriched with enriched Spirulina (Artemia S.); B.Y.: Artemia enriched with enriched beer yeast (Artemia B.Y.); Control: Artemia without enrichment (Artemia C.). The survival rate was averaged with duplicated test as shown in Table 8.

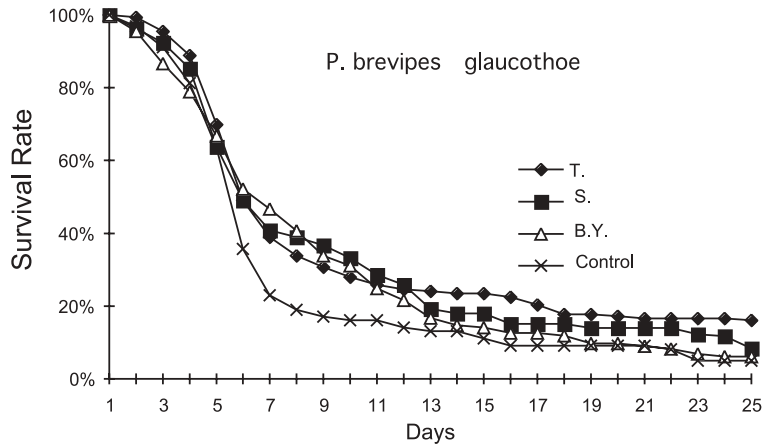

Figure 2. Survival rate of Hanasaki king crab, Paralithodes brevipes, during glaucothoe stage fed Artemia nauplii enriched with various kinds of foods during zoea stage. T.: Artemia enriched with Thalassiosira enriched with tuna oil (Artemia T.); S.: Artemia enriched with enriched Spirulina (Artemia S.); B.Y.: Artemia enriched with enriched beer yeast (Artemia B.Y.); Control: Artemia without enrichment (Artemia C.). The survival rate was averaged with duplicated test as shown in Table 8.

those fed *Artemia* T., *Artemia* S., *Artemia* B.Y. and *Artemia* C., respectively (Table 8). The majority of the glaucothoe molted into the first instar crab for 23-25 days after metamorphosis at water temperatures of 8-10ºC.

Discussion

Larval development of some decapod crustaceans is entirely dependent on the yolk stored in the eggs. Larvae of the golden king crab *Lithodes aequispinus* successfully undergo the entire larval and postlarval stages without feeding (Shirley and Zhou 1997, Paul and Paul 1999). Anger (1996) also concluded that the northern stone crab *Lithodes maja* is nonfeeding during larval and postlarval stages. In *P. camtschaticus*, larvae are not lecithotrophic although hatched larvae contain reserve egg yolk (Kurata 1959, Paul and Paul 1980). However, *P. camtschaticus* and *P. brevipes* are unique in their development because they undergo a nonfeeding postlarval stage (Kittaka, unpubl.; Abrunhosa and Kittaka 1997a,b; Abrunhosa 1998). Substantial morphological alterations occur in the mouthparts and foregut during the metamorphosis from the zoea to the glaucothoe and then to the first instar juvenile. Functional mouthparts and foregut in zoea stages transform to nonfeeding structures in the glaucothoe stage. The mandibles of the glaucothoe, especially the lateral lobes, undergo remarkable reduction in size. The gnathal processes are uncalcified, lack denticles and teeth, and the mandibular palps are nonsetose. The endites and endopods of the maxillae are also rudimentary, having only minute setae in the glaucothoe. Numerous setae are present in the cardiac and pyloric stomachs of the zoea, which aid in the movement of food through the foregut. A complex filter press is present in the pyloric stomach, through which processed food is filtered into the midgut gland for digestion (Abrunhosa and Kittaka 1997a). The foregut of the glaucothoe is drastically changed compared to the zoea stage. The setae in the stomachs are reduced and the filter press is atrophied, which indicate food processing does not occur in the glaucothoe. In the first instar juvenile, however, the mouth parts transform to a fully functional calcified organ and the foregut becomes highly complex with a well developed gastric mill as in the adult form (Abrunhosa and Kittaka 1997a).

The survival rate of larvae varies in relation to environmental and nutritional conditions in culture. Particularly, food is an important factor for the zoea stage directly and for the nonfeeding glaucothoe stage in connection with stored nutrients during the zoea stage. Compared to the glaucothoe, the survival rate of zoeae fluctuated over a wider range, suggesting that larvae of *Paralithodes* spp. may be vulnerable and have strict environmental and nutritional requirements.

For the larvae of the swimming crab *Portunus trituberculatus*, the suitable level of n-3 HUFA content was estimated at 0.9-1.7 g per 100 g dry weight of food (Takeuchi et al. 1999a). Takeuchi et al. suggested that EPA

was effective for improving survival rate while DHA helped to shorten the intermolt period and to increase body size (Takeuchi et al. 1999b).

Thalassiosira originally contained sufficient amounts of EPA at 0.34-3.46 g per 100 g dry weight and small amounts of DHA at 0.03-0.09 g per 100 g dry weight (Table 5 and 6). In the present experiment, 1.5 g tuna oil (DHA content: 23.3%) was absorbed in 20 g freeze-dried *Thalassiosira*. The DHA content of *Thalassiosira* before enrichment was 0.308 mg per g dry weight (Table 6). After adding tuna oil, DHA increased to {(1500 mg × 0.233) + (0.308 mg × 20)}/ (20 g + 1.5 g) = 16.5 mg per g dry weight of enriched *Thalassiosira*, assuming that 100% DHA was absorbed by *Thalassiosira*. However, analysis of the fatty acid contents showed that enriched *Thalassiosira* contained DHA 11.5 mg per g dry weight, suggesting that about 73-74% of added DHA was absorbed by freeze-dried *Thalassiosira*. After enrichment with tuna oil, DHA increased to 1.15 g per 100 g and EPA to 1.17 g per 100 g of dry weight *Thalassiosira* (Table 6). In the present experiment, control *Artemia* contained EPA and DHA at 0.47 and 0.007 g per 100 g dry weight, respectively (Table 5). It is apparent that enriched *Artemia* used in the present experiment contained higher levels of both EPA and DHA. For *Paralithodes brevipes*, an increase of these n-3 HUFA, particularly DHA, had a positive effect on the survival rate of zoeae when compared to control *Artemia*, although this was not evident for zoeae of *Portunus trituberculatus* (Takeuchi et al. 1999a). Structure of the mouthparts and foregut in the megalopae (metamorphosed form) of *P. trituberculatus*, which takes food actively, is quite different from those of glaucothoe of *Paralithodes* spp. The requirement for DHA in larval stages may be different between king crabs and true crabs.

Both *Artemia* and *Thalassiosira* contained appropriate amounts of EPA, but DHA content was less (Table 5). Lipid contents in beer yeast and *Spirulina* which were enriched with HUFA were about 40.4 and 48.5%, respectively. Assuming that the total fatty acid contents are equal or less than the total lipid contents, enriched beer yeast contained at maximum EPA 50.1 and DHA 57.4 mg per g dry weight (Ishiwaki M. Kirin Beer Co. Ltd., Tokyo, Japan, Jan. 2001, pers. comm.) and enriched *Spirulina* contained at maximum roughly EPA 78.1 and DHA 130.0 mg per g (Deshimaru O., Higashimaru Co. Ltd., Kagoshima, Japan, Jan. 2001, pers. comm.). The survival rate of zoeae was better for those fed *Artemia* T., followed by those fed *Artemia* S. and *Artemia* B.Y., while it was least for those fed *Artemia* without enrichment. These results indicate that enrichment of *Artemia* with HUFA is effective for improving the survival rate of zoeae.

In the present experiment, control *Artemia* contained EPA and DHA at 0.47 and 0.007 g per 100 g dry weight, respectively (Table 5), but these values increased after enrichment with either enriched *Thalassiosira*, *Spirulina,* or beer yeast. *Artemia* S. was estimated to contain EPA and DHA at a maximum of 2.1 and 1.0 g per 100 g dry weight, respectively (Deshimaru O., Higashimaru Co. Ltd., Kagoshima, Japan, Jan. 2001, pers. comm.). Cultured

Thalassiosira originally contained a sufficient amount of EPA at 0.9 g per 100 g dry weight and small amount of DHA at 0.03 g per 100 g dry weight (Table 6). After enrichment, EPA and DHA increased to 1.2 and 1.2 g per 100 g dry weight, respectively (Table 6). In *Paralithodes brevipes*, increased DHA increases the survival rate of zoeae, which was not evident for zoeae of *Portunus trituberculatus* (Takeuchi et al. 1999b). The suitable level of EPA and DHA in the food must be determined by further experiments.

Lipids are thought to be the primary energy sources used during the nonfeeding glaucothoe stage of king crabs (Abrunhosa and Kittaka 1997a,b). By morphological observations, the first zoea hatches out with a large amount of lipid reserve (yolk) in the epithelial cells of both the midgut gland and the anterior midgut ceca. Accumulation of lipids is observed in the medial portion of the midgut gland as well as in the distal portion of the anterior midgut ceca. The large lipid droplets observed in the early glaucothoe are not present in the late phase glaucothoe. Small lipid droplets are abundant in the outer margin of almost all epithelial cells of the midgut gland tubules in the late phase glaucothoe. By chemical analysis, accumulation of lipid during the zoeal stage and consumption during the glaucothoe stage were clearly shown in *P. camtschaticus* (Ishikawa et al., unpubl.).

Better survival rate for zoeae of *Paralithodes brevipes* was evident in diets with various kinds of enriched *Artemia* nauplii (Fig. 1). However, survival rate of glaucothoe was extremely poor (Fig. 2). Within a 10 day period after metamorphosis, heavy mortality occurred in the glaucothoe regardless of the kinds of food. The cause of mortality is apparently due to the white-turbid midgut gland necrosis which occurred commonly in cultured larvae of marine crustaceans. Numerous *Vibrio* spp. were isolated from the larval midgut gland and midgut of cultured *P. brevipes* (Watanabe et al. 1998). High mortality of zoeae in 100 L culture containers (Tables 2 and 3) may have been the result of pathogenic bacteria, although density was also a factor for *P. brevipes*. Treatment with oxytetracycline hydrochloride (OTC) at 5 mg per L was effective in preventing the disease. The bacteria number in the zoea culture water of *P. brevipes* was 1.4×10^4 CFU per ml (CFU: colony forming units on ZoBell's 2216E agar medium inoculated aerobically at 20ºC for 2 days) at the beginning (3 days after the second instar), and increased to $1.2\text{-}1.7 \times 10^6$ CFU per ml after 6-7 days feeding with *Artemia* nauplii (Watanabe et al. 1999), whereas it varied from 3.6×10^3 to 1.8×10^5 CFU per ml in the zoea culture water of *P. camtschaticus* (Igarashi and Kittaka 2000). In the larval culture water, it is apparent that cultured *Thalassiosira* control water quality, including microflora. However, accumulation of *Thalassiosira* on the tank bottom may accelerate bacterial growth, including pathogenic *Vibrio* spp. Use of nutritionally enriched *Artemia* with freeze-dried cultured *Thalassiosira* will be critical for controlling disease in mass-cultured larvae of *Paralithodes* spp.

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Injuries and Aerial Exposure to Crabs during Handling in Bering Sea Fisheries

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Extended Abstract

Large numbers of bycatch crabs in some eastern Bering Sea commercial fisheries can be subject to handling-related injuries and prolonged aerial exposure prior to release. Studies that examined handling mortality on several crab species identified lethal and sublethal effects attributable to cold air temperatures and injuries and simulation models have shown that consequences from crab bycatch mortality may negatively affect fishery yields and add significant mortality to stocks in decline. To document aerial exposure and identify the prevalence and nature of handling injuries in the Bering Sea fisheries, Alaska Department of Fish and Game observers sampled female and undersized male bycatch crabs and recorded pot catch sorting times between 1997 and 1999. Vessel crew sorting practices were also documented. The relationship between aerial exposure and total catch was examined and data results were tested for significant differences between fisheries and individual vessels.

Results from the study indicated that injury type, rates, and aerial exposure of bycatch crabs can be highly variable between fisheries and individual fishing vessels. The data suggest that factors influencing the susceptibility of nonretained crabs to injury and excessive time onboard vessels prior to being returned to the sea may at least include directed catch and bycatch rates and timing of fishing seasons. Other prevailing factors, such as competition between harvesters, may also be important.

Directed catch and bycatch rates of crabs were expected to correlate strongly to maximum aerial exposure times based on the logical assumption that as crabs per pot increase, additional time will be required for

catch sorting by vessel crews. However, in data results from observed vessels, combined catch rates of retained and nonretained crabs appear to influence aerial exposure in some fisheries but not others. Exposure times were most poorly correlated to catch rates in the Pribilof Islands hair crab fishery, which also had the lowest overall and least variable individual catch rates of retained and nonretained crabs. Significant correlation between catch rates and on-deck exposure was most apparent in open access fisheries for Bering Sea snow crabs and Bristol Bay red king crabs.

A number of generalized statements can be made based on the results of injury assessment sampling regarding the nature and extent of injuries inflicted on crabs during handling. In each case the overwhelming percentage of injuries observed were those defined in the study as minor. Minor injuries included punctures, cracks, or shell damage without exposure of integument, muscle, or vital organs. Internal tissues and viscera were visible through the damaged shells of crabs with major injuries. Major injuries were rare and, when observed, most often consisted of severely damaged walking legs. Bycatch crabs seldom incurred more than a single injury during handling, as those with multiple injuries composed less than 5% of sample sizes in all fisheries except the open access fishery for Bering Sea snow crab where 6.9% had more than one injury. Broken rostrums were the most prevalent minor injury inflicted on king crabs, while autotomized legs were the most common minor injury observed for snow crabs. New-shelled crabs were typically subject to injuries at much lower rates than those for old- and very old-shelled crabs.

Trends in catch sorting practices were not readily apparent, with methods and equipment employed varying among individual vessels and, occasionally, between fisheries. Some vessel crews utilized a semi-mechanized means for handling crabs, while others sorted catches entirely by hand. Numbers of crew assisting in catch sorting also varied, most often between fisheries. Differences in crab aerial exposure times due to variable sorting practices were difficult to detect, since interactive factors such as changes in target catch and bycatch rates and gear configurations may also have a significant effect.

Size at Maturity of Kodiak Area Female Red King Crab

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Abstract

Size at maturity for female red king crabs in the Alaska Department of Fish and Game Kodiak Management Area, Alaska, was estimated using data collected by pot surveys during 1972-1986. Based on a sample of 186,468 females, the size at which 50% are mature ("size at 50% maturity," SM50) for the Kodiak Management Area was estimated as 101.9 mm carapace length. The estimated female SM50 for the Kodiak Management Area is among the largest of those estimated for red king crab stocks in Alaska. Estimates of SM50 were also computed for samples representing individual management districts for each year, for all management districts for each year, and for each management district over all years. We relate annual variation in SM50 to changes in population size and recruitment over the period studied and discuss factors that can bias estimation of SM50 in our samples.

Introduction

Red king crabs (*Paralithodes camtschaticus*) supported an important commercial fishery in the Alaska Department of Fish and Game Kodiak Management Area, Alaska from 1950 to 1982 in which 78 million male crabs, worth an ex-vessel value of approximately $286 million, were harvested (Nippes 1983; Blau 1986, 1988a). The size at maturity for male and female Kodiak red king crabs has not been documented, although several authors have provided informal approximations (Gray 1963, Powell and Nickerson 1965, Gray and Powell 1966, Kingsbury and James 1971, Powell et al. 1973, Paul et al. 1991). Size at maturity provides an index for comparing growth and productivity among stocks and for estimating maturity status of animals on the basis of size. The size at which 50% are mature ("size at 50% maturity," SM50) has been estimated for female red king crabs in Alaska from the Norton Sound (Powell et al. 1983, Otto et al. 1989), Pribilof Islands (Otto et al. 1989), Bristol Bay (Weber 1967), and Adak (Blau 1989) areas.

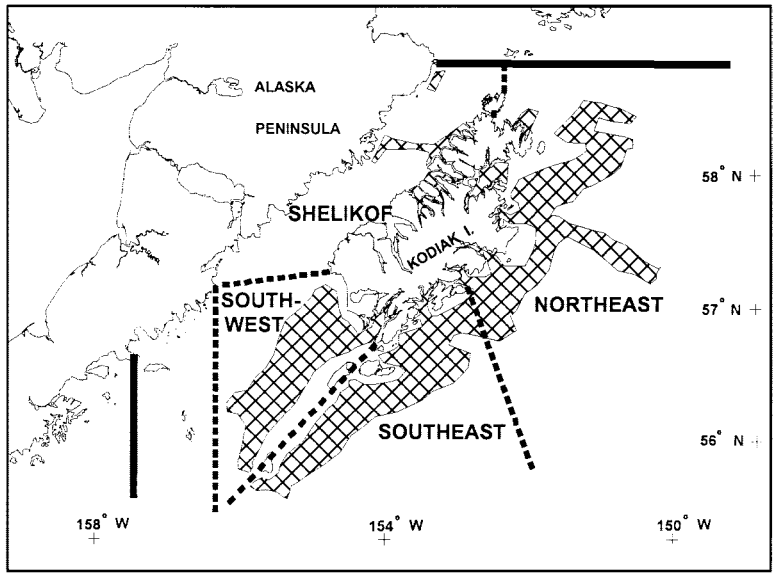

Figure 1. ADFG Kodiak Management Area, between heavy lines, and four king crab management districts, separated by dotted lines. The shaded areas are a composite of where the annual king and Tanner crab pot surveys occurred during 1972-1986.

This paper provides an estimate of the SM50 for female red king crabs from the Kodiak Management Area and describes the size distribution of mature female red king crabs from that area. We also describe the annual variation of SM50 in the Kodiak Management Area over the 15-year period, 1972-1986, and the variation in SM50 among four individual management districts in that area.

Methods

Data for this analysis were collected from female red king crabs captured in king crab pots during the 1972-1986 annual king and Tanner crab surveys conducted by the Alaska Department of Fish and Game (ADFG) in the Kodiak Management Area (Blau 1986, 1988b; Peterson et al. 1986). Surveys were conducted during late June through early September. That survey timing followed the season of hatching, molting, and oviposition by female red king crabs in the Kodiak Management Area, which occurs during January through May (Powell et al. 2002). Data were recorded from all crabs in a survey pot or from random subsamples of crabs by sex in a pot depending

on the catch or time constraints. The carapace length (CL) of each sampled crab was measured to the nearest millimeter from the posterior margin of the right eye socket to the midpoint of the rear margin of the carapace (Wallace et al. 1949). Presence or absence of embryos or empty egg cases was recorded for females.

Only new-shell (exuviant) females with a CL measurement and noted as having either presence or absence of embryos or of empty egg cases were used in our analysis. A new shell indicates a recent molt, which is a prerequisite for ovulation and mating (Marukawa 1933, Wallace et al. 1949). Additionally, it is not always possible to discern if old-shell females have previously ovulated, because attached egg cases and funiculi degrade after the larvae are released. New-shell crabs with embryos or empty egg cases attached to the pleopodal setae were recorded as "mature" for our analysis; otherwise, they were recorded as "immature." Because the surveys followed the period of hatching, molting, and mating, old-shell females and females with empty egg cases were only rarely captured during the survey. Incidence of females with empty egg cases did increase during the surveys in the 1980s, but that has been attributed to the effects of infestation by brood symbionts rather than to incidence of unmated mature females (Kuris et al. 1991). Females with unfertilized external eggs were unlikely to occur during the Kodiak pot surveys because unmated mature females would have either reabsorbed or released and lost their unfertilized external eggs prior to the survey season.

Data were grouped by year and Kodiak king crab management district (Northeast, Southeast, Southwest, or Shelikof; Fig. 1). Data from the Southeast District for the 1973 survey and from the Shelikof District for the 1985 survey were not available for analysis. No surveys were performed in the Northeast and Shelikof districts during 1978. In all, data from 186,468 females (31,391 immature and 155,077 mature) were available for analysis. Sample sizes of females for each district and year are provided in Table 1. Only data from measured crabs were used in the analysis; we did not attempt to adjust size frequency distributions from measured crabs to reflect subsampling of the catch for measuring that occurred during any survey.

The SM50 (mm CL) was estimated by first using a logistic model (Cox and Snell 1989) to fit the proportion of females that were mature at given carapace lengths:

$$p_m(CL) = \frac{e^{\beta 0 + \beta 1 CL}}{1 + e^{\beta 0 + \beta 1 CL}},$$

where $p_m(CL)$ is the predicted proportion of females with carapace length CL that are mature. The maximum likelihood estimates (MLEs) of the logistic regression parameters, β_0 and β_1, were obtained using the logit regression routine of SYSTAT 9 (SPSS Inc. 1999). The size at which 50% of females are

Table 1. Estimated size at 50% maturity (SM50) in mm carapace length for female red king crabs by management district and year in the Kodiak Management Area, Alaska, collected during 1972-1986.

Year		Northeast	Southeast	Southwest	Shelikof	All
1972	*N*	1,946	3,228	3,648	551	9,373
	SM50	108.1 (0.34)	98.9 (0.29)	101.3 (0.24)	106.6 (0.69)	101.9 (0.30)
1973	*N*	4,917	0	5,925	660	11,502
	SM50	102.2 (0.32)	—	98.8 (0.27)	99.0 (1.12)	99.9 (0.22)
1974	*N*	6,257	4,552	9,793	456	21,058
	SM50	102.7 (0.51)	98.2 (0.31)	97.2 (0.30)	101.6 (1.97)	97.5 (0.22)
1975	*N*	2,554	2,481	3,869	127	9,031
	SM50	102.8 (1.34)	99.2 (0.70)	100.2 (0.48)	N/E	100.2 (0.35)
1976	*N*	5,559	3,710	3,329	318	12,916
	SM50	107.3 (0.37)	97.0 (0.08)	101.8 (0.15)	108.9 (0.99)	101.7 (0.17)
1977	*N*	11,592	11,407	8,584	1,243	32,826
	SM50	106.8 (0.14)	104.0 (0.12)	102.1 (0.15)	107.3 (0.27)	104.5 (0.07)
1978	*N*	0	7,808	4,400	0	12,208
	SM50	—	101.1 (0.16)	97.1 (0.53)	—	100.5 (0.15)
1979	*N*	3,976	3,829	5,422	533	3,760
	SM50	99.1 (0.53)	98.1 (0.45)	101.6 (0.33)	103.5 (0.72)	99.9 (0.23)

Estimated size at 5% maturity (SM05) and 95% maturity (SM95) are provided for all years combined. Values in parentheses are estimated standard errors. N/E indicates the parameter was not estimated.

Table 1. (Continued.)

Year		Northeast	Southeast	Southwest	Shelikof	All
1980	*N*	5,432	6,216	11,109	603	23,360
	SM50	97.4 (0.78)	100.4 (0.36)	103.0 (0.23)	98.9 (2.19)	101.4 (0.18)
1981	*N*	3,740	5,286	7,810	477	17,313
	SM50	99.8 (1.32)	105.2 (0.20)	102.7 (0.35)	99.9 (2.02)	103.9 (0.16)
1982	*N*	2,618	1,906	1,927	160	6,611
	SM50	104.3 (2.04)	N/E	101.3 (0.65)	N/E	99.4 (0.71)
1983	*N*	1,985	1,474	2,370	235	6,064
	SM50	110.0 (0.82)	N/E	N/E	N/E	102.0 (0.73)
1984	*N*	1,762	1,017	3,402	478	6,659
	SM50	109.3 (0.77)	N/E	N/E	N/E	105.8 (0.63)
1985	*N*	529	243	2,074	0	2,846
	SM50	N/E	N/E	N/E	—	N/E
1986	*N*	229	271	419	22	941
	SM50	N/E	N/E	N/E	N/E	N/E
All	*N*	53,096	53,428	74,081	5,863	186,468
	SM05	94.9 (0.13)	89.1 (0.11)	89.7 (0.11)	94.7 (0.35)	91.0 (0.07)
	SM50	104.7 (0.08)	101.3 (0.06)	100.2 (0.08)	104.8 (0.19)	101.9 (0.05)
	SM95	114.4 (0.15)	113.5 (0.16)	110.6 (0.17)	115.0 (0.39)	112.9 (0.09)

Estimated size at 5% maturity (SM05) and 95% maturity (SM95) are provided for all years combined. Values in parentheses are estimated standard errors. N/E indicates the parameter was not estimated.

mature (SM50) was estimated from the estimated logistic regression parameters by

$$\hat{SM50} = -\frac{\hat{\beta}_0}{\hat{\beta}_1}$$

where $\hat{\beta}_0$ and $\hat{\beta}_1$ are the MLEs of β_0 and β_1, respectively. We also estimated the size at which 5% of females are mature (SM05) and the size at which 95% are mature (SM95) from the estimated logistic regression parameters by

$$\hat{SM05} = \frac{\ln(0.05/0.95) - \hat{\beta}_0}{\hat{\beta}_1}$$

and

$$\hat{SM95} = \frac{\ln(0.95/0.05) - \hat{\beta}_0}{\hat{\beta}_1} \quad .$$

We estimated standard errors for the estimates of SM05, SM50, and SM95 by applying the variance and covariance estimates for the MLEs of β_0 and β_1 to the Taylor series approximation of the variance of a ratio of two random variables (e.g., Mood et al. 1974).

Estimates of SM50 were computed for each district in each year that data was available. We also estimated SM50 for each year over all districts, and SM05, SM50, and SM95, for each district over all years and for all districts over all years. We did not estimate SM50 from data sets for which the logistic regression was not significant ($P > 0.05$) or provided estimated logistic regression parameters of the wrong sign or from data sets that did not conform adequately to the logistic regression model.

Results

Immature females ranged in size from 43 to 164 mm CL (Fig. 2) and only 1.0% of all immature females were larger than 115 mm CL. However, larger immature females were more commonly collected during the 1980s, and 31% (216 of 691) of all the immature females measured during 1982-1986 were larger than 115 mm CL. Mature females in the entire Kodiak Management Area data set ranged in size from 85 to 196 mm CL (Fig. 2). Mean and median size of the mature females was 125.4 and 124.0 mm CL, respectively.

Estimates of SM50 were not computed from 16 of the 56 district-year data sets because the size distribution of immature and mature females did not overlap (i.e., the 1975 Shelikof District and 1986 Southwest District

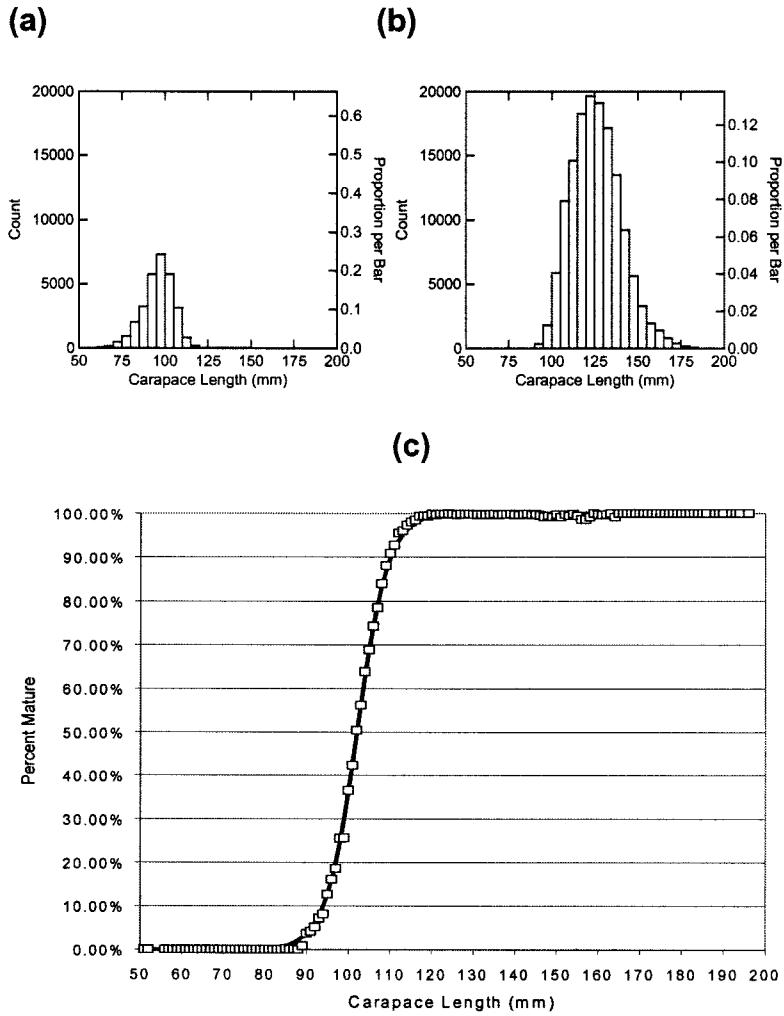

Figure 2. Carapace length frequency distributions for 31,391 immature (a) and 155,077 mature (b) red king crab females from the Kodiak Management Area, Alaska, collected during 1972-1986 and size-at-maturity curve estimated from those data (c). Open squares in (c) denote the percent mature at a given carapace length in the sample of 186,468 females.

samples) or because of the paucity of immature females in the post-1981 data. Estimation of SM50 by our method from the post-1981 district-year data sets, the pooled 1985 data, and the pooled 1986 data was also precluded by the incidence of abnormally large (130-164 mm CL) females among the few that were classified as immature.

Over all years and districts, the female SM50 for the Kodiak Management Area was estimated as 101.9 mm CL (Table 1), although only 2.7% of the entire sample of 155,077 mature females were smaller than 102 mm CL. The female size-at-maturity curve for the Kodiak Management Area estimated by logistic regression fits the data well, with sample values of percent mature generally within 1% of the value predicted by the fitted curve (Fig. 2). The curve, however, tends to slightly overestimate percent mature at size for females <95 mm CL and 125-165 mm CL and tends to slightly underestimate percent mature for females 107-125 mm CL.

Estimates of SM50 varied among districts and years from 97.0 mm CL for the Southeast District in 1976 to 110.0 mm CL for the Northeast District in 1983 (Table 1). Within years, no consistent trends in the estimated SM50s were apparent, but over all years combined estimated SM50 was largest in the Northeast and Shelikof districts (104.7 mm CL and 104.8 mm CL, respectively) and smallest in the Southwest District (100.2 mm CL). For all districts combined, estimated SM50 increased from 97.5 mm CL in 1974 to 104.5 mm CL in 1977 before returning to values <102 mm CL during the period 1978-1980. The Northeast District had the longest series of SM50 estimates during the 1980s. In that district, estimated SM50 increased from 97.4 mm CL in 1980 to 110.0 and 109.3 mm CL in 1983 and 1984.

The estimated SM05s and SM95s in Table 1 indicate the range of size overlap of immature and mature females. For the entire Kodiak Management Area over all years, SM05 and SM95 were estimated as 91.0 mm CL and 112.9 mm CL, with estimated values varying among districts. Sizes of immature and mature females in district-year data sets generally overlapped in the range of 85-120 mm CL during the 1970s and early 1980s. The smallest mature females in most district-year data sets collected after 1981 were typically greater than 100 mm CL and size-at-maturity parameters could not be estimated. However, the range of overlap in carapace length for immature and mature females increased in the 1982-1986 data due to the presence of 169 large (130-164 mm CL) females that were scored as immature.

Discussion

The SM50 of 101.9 mm CL for Kodiak Management Area female red king crabs estimated here is similar to that assumed by other investigators (Gray 1963, Powell and Nickerson 1965) and to that estimated from data collected prior to 1972 (Kingsbury and James 1971). Powell and Nickerson (1965) considered 100 mm CL as “a practical average” for the minimum

length of Kodiak red king crab female maturity. Kingsbury and James (1971) estimated SM50 for female red king crabs from Alitak Bay in the Kodiak Southwest Management District to be 100 mm CL, essentially equal to our estimate of 100.2 mm CL from Southwest District samples pooled over all years. Gray (1963) noted Kodiak female red king crabs ranged from 95 to 113 mm CL at sexual maturity, close to the range between our estimated SM05 (91.0 mm CL) and SM95 (112.9 mm CL).

Compared with most estimates of SM50 for female red king crabs from other areas in Alaska, the SM50 of 101.9 mm CL we estimated for the Kodiak Management Area is large. Female red king crab SM50 has been estimated as 68 mm CL (Powell et al. 1983) and 71.4 mm CL (Otto et al. 1989) for Norton Sound, 88.8 mm CL for Bristol Bay (Otto et al. 1989), 89 mm CL for the Adak (Aleutians) Area (Blau 1989), and 102.1 mm CL for the Pribilof Islands (Otto et al. 1989). Although the northernmost stock examined, Norton Sound at 64ºN, has the lowest estimate for SM50, there is no consistent north-to-south trend. Bristol Bay and the Pribilof Islands are at roughly the same latitude as Kodiak (56-58ºN), but the estimated SM50s for the Pribilof Islands and Kodiak both exceed that for Bristol Bay by 12 mm. The SM50 estimated for the southernmost area examined, the Adak Area at 52ºN, is comparable to that for Bristol Bay. In the Kodiak Management Area, estimates of SM50 from the two northern management districts were larger than for the two southern management districts.

Estimates of SM50 in the Kodiak Management Area varied by management district and year by as much as 13 mm (97-110 mm CL). The estimated SM50s for Bristol Bay and Norton Sound female red king crabs also varied interannually by as much as 7 mm during the period 1975-1989 (Otto et al. 1989). Data from 8,439 female red king crabs collected from Middle Bay in the Northwest Kodiak Management District during December-March of 1971 (Powell et al. 1973) also indicate that a sample from a more restricted area from one time period could produce a larger estimate of SM50 than any of the data sets we examined. Mature females exceeded 50% of a size class only at 111 mm CL or greater in the 1971 Middle Bay sample.

Annual variation in female red king crab SM50 can be expected due to changes in the size frequencies and relative abundance of immature and mature females that occur with changes in age composition and cohort strength. During periods of poor recruitment, when the adult population is aging and its size frequency distribution is shifting to larger carapace lengths, the presence of a few large, immature females can increase SM50. Our estimates of SM50 for the Northeast District increased from 97.4 mm to over 109 mm CL during 1979-1984, a period of poor recruitment and an aging adult population (Blau 1985, 1986). Recruitment events that result in dominance of immature females at the size range where immature and mature females overlap can also increase SM50. Our estimates of SM50 for the pooled-district sample increased from 97.5 to 104.5 mm CL, during 1974-1977, a period when abundance of immature females in the Kodiak

red king crab population increased relative to mature females (Blau 1985, 1986). Such trends indicate that size and maturity data from only one or a few years may not be sufficient to estimate SM50 under "average" conditions for populations with highly variable recruitment.

Disproportionate sampling of immature or mature females can artificially influence estimates of SM50, as noted earlier by Weber (1967) and Blau (1989). In our sample of 186,468 females, only 17% (31,391) were immature. Hence, it is evident that disproportionately more mature females were sampled than immature females during the pot surveys from which our data were collected. The higher catchability of mature females during the pot survey may be due to the gear used for the survey and to the areas surveyed. We are uncertain, however, as to what extent the sampling of immature females ≥ 85 mm CL (the size of the smallest mature female in our sample) was disproportionately low during the pot survey and to the influence that it may have had on our estimates. We also do not know if subsampling of the catch for measurements during the surveys affected our estimates.

Estimation of size at maturity can also be artificially influenced by the presence of large females that were classified for this study as immature. The red king crab females considered immature in our analysis with sizes well above our estimated SM95, such as those in the range of 130-164 mm CL, were probably mature. Such "immature" females were relatively more common in the samples collected during the 1980s. They may represent mating failures of mature animals due to senescence or disease in an aging population or to lack of available mates due to fishery removals of males (Orensanz et al. 1998). Those large, questionably immature females only constituted a small portion of our overall sample, so they have little influence on our overall estimate of SM50 for the Kodiak Management Area. However, their presence prohibited our estimation of SM50 from most of the samples collected after 1981.

Multiyear ADFG pot survey data also exist for red king crabs in the Dutch Harbor (Aleutians), Alaska Peninsula, Cook Inlet and Southeastern areas. Determining the female red king crab size at maturity for each of those areas would provide a statewide perspective on size at maturity across Alaska's varied oceanographic conditions.

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