Aquat. Living Resour., 1995, **8**, 171-179

Osmoregulation and salinity tolerance in zoeae and juveniles of the snow crab *Chionoecetes opilio*

Guy Charmantier and Mireille Charmantier-Daures

Laboratoire d'Ecophysiologie des Invertébrés, Université des Sciences-Montpellier 2, place Eugène Bataillon, 34095 Montpellier Cedex 05, France.

Received October 10, 1994; accepted December 12, 1994.

Charmantier G., M. Charmantier-Daures. *Aquat. Living Resour.*, 1995, **8**, 171-179

Abstract

Osmoregulation and salinity tolerance were studied in zoeae 1 and instar IX juveniles of *Chionoecetes opilio*. In zoeae 1, the lower and upper lethal salinities for 50% of the animals (LS 50) at 14°C were about 10 and 42 $^0/_{00}$ at 24 h, 18 and 41 $^0/_{00}$ at 48 h, 25 and 38 $^0/_{00}$ at 96 h. In juveniles, the approximate 48 h LS 50 s at 6°C were 13.5 and 46 $^0/_{00}$. Both developmental stages are able to withstand relatively ample but brief variations of salinity. Their short-term euryhalinity is discussed in relation to the salinity of their habitat. Zoeae were hyper-osmoconformers. Juveniles were osmoconformers and isoionic to the external medium except for Mg^{++} which was hypo-regulated. Isosmotic and isoionic regulation in post-metamorphic stages are presumably an attribute of the family Majidae. The pattern of osmoregulation in zoeae 1 and juveniles relate *C. opilio* to the first of three groups of species previously characterized by their pattern of ontogeny of osmoregulation.

Keywords: Osmoregulation, salinity tolerance, zoea, juvenile, snow crab, *Chionoecetes opilio*, Majidae.

Osmorégulation et tolérance à la salinité chez les stades zoés et les juvéniles du crabe des neiges Chionoecetes opilio.

Résumé

L'osmorégulation et la tolérance à la salinité sont étudiées chez les zoés 1 et les juvéniles en stade IX de *Chionoecetes opilio*. Chez les zoés 1, les valeurs des salinités inférieures et supérieures léthales pour 50% des animaux ((LS 50) à 14°C sont d'environ 10 et 42 $^0/_{00}$ à 24 h, 18 et 41 $^0/_{00}$ h à 48 h, 25 et 38 $^0/_{00}$ à 96 h. Chez les juvéniles, LS 50 48 h à 6°C est évaluée à 13,5 et 46 $^0/_{00}$. Les deux stades de développement sont capables de supporter des variations de salinité importantes mais brèves. Leur euryhalinité à court terme est discutée en fonction de la salinité de leur milieu. Les résultats montrent que les zoés sont des hyper-osmoconformeurs. Les juvéniles sont osmoconformes et isoioniques au milieu extérieur, sauf pour le Mg^{++} qui est hypo-régulé. La régulation isosmotique et isoionique chez les stades post-métamorphiques est sans doute une caractéristique de la famille des Majidae. Les types d'osmorégulation des zoés 1 et des juvéniles rattachent *C. opilio* au premier des trois groupes d'espèces préalablement définis par leur type d'ontogenèse de l'osmorégulation.

Mots-clés : Osmorégulation, tolérance à la salinité, zoé, juvénile, crabe des neiges, *Chionoecetes opilio*, Majidae.

INTRODUCTION

The snow crab, *Chionoecetes opilio* (O. Fabricius, 1788), is a spider crab (Decapoda, Reptantia, Brachyura, Majidae) which inhabits deep, cold (below 5°C: Williams, 1984) waters in the Sea of Japan, the north Pacific Ocean, including the Okhotsk Sea and the Bering Sea, and the northwest Atlantic Ocean. Off the east coast of North America, snowcrab distribution extends from West Greenland to Labrador, Newfoundland, the Gulf of St. Lawrence, the Canadian Atlantic Provinces and Maine (Squires, 1966, 1990), and it corresponds closely with the distribution of sub-arctic waters (Dunbar, 1968). Snow crabs are usually found from 20 to 700 m depth (Adams, 1979) and occasionally occur in shallower waters (Sainte-Marie and Hazel, 1992). This species sustains important fisheries, particularly in Japan, Alaska, and Atlantic Canada, which prompted extensive ecological studies (reviews in Adams, 1979; Davidson, 1983; Jamieson and McKone, 1988). Other related species include the tanner crabs *C. bairdi* Rathbun, also known as Alaskan snow crab and harvested in the Gulf of Alaska (Donaldson *et al.*, 1981; Somerton, 1981) and *C. tanneri* Rathbun living in deeper waters (Tester and Carey, 1986).

The general biology and life cycle of *C. opilio* have been studied by several authors (reviews in Adams, 1979; Elner, 1988). Developing eggs remain attached to the pleopods of the female for one year or more (Ito, 1967). Hatching occurs from February to April in the Sea of Japan (Ito, 1967), in August and September off the Pacific coast of Japan (Kurata, 1963), from May to July in the Okhotsk Sea (Kurata, 1963), from April to June in the Gulf of St. Lawrence (Powles, 1966; Watson, 1969; Davidson, 1983; review in Adams, 1979). The early post-embryonic development comprises one prezoea, two zoeae, one megalopa, before the end of metamorphosis to young crab stages (Aikawa, 1937; Kurata, 1963; Kon, 1967, 1970; Kuwatani *et al.*, 1971; Haynes, 1973; Motoh, 1973; Davidson, 1983; Lanteigne, 1985; reviews in Adams, 1979; Elner, 1988). The duration of development from prezoea to the end of megalopa ranges from 2 to 8 months and is influenced by temperature (Fukataki, 1969; Kon, 1970; Davidson, 1983; reviews in Adams, 1979; Elner, 1988), feeding (Kon, 1979; Incze and Paul, 1983) and salinity (Kon, 1973). Young and juvenile crabs pass through juvenile instars (Watson, 1969; Kon, 1970) I to X (Robichaud *et al.*, 1989); the last instars last about one year each (Ito, 1970; Robichaud *et al.*, 1989). Female snow crab undergo a terminal molt to maturity between 47 and 95 mm cephalothoracic width (ctw) (Ito, 1963, 1967; Watson, 1969, 1970; Somerton, 1981). Males apparently also attain a terminal molt to morphometric maturity, within the range of 52-137 mm ctw (Conan and Comeau, 1986), although the existence of terminal molt in males is still controversial (Dawe *et al.*, 1991; Cormier *et al.*, 1992; Comeau and Conan, 1992).

Although snow crabs usually live in environments which are not subjected to ample salinity fluctuations (Kon, 1980; Incze *et al.*, 1987), it was recently reported that some of their populations can be found in shallow waters where salinity is potentially more variable (Sainte Marie and Hazel, 1992) for bottom-dwelling juveniles and adults and for planktonic larvae. In addition, in order to provide live crabs to the market off the short fishing season, current investigations are directed to the maintenance of crabs in land-based facilities (Hardy *et al.*, 1994) supplied with coastal sea water possibly subjected to salinity fluctuations. The determination of salinity tolerance and of osmoregulatory patterns in different developmental stages of *C. opilio* is thus necessary for both ecological and practical approaches. However, little is known about the physiology of *Chionoecetes* sp., particularly with reference to their hydromineral metabolism. The only related reports include the effect of salinity on larval and postlarval development in *C. opilio* (Kon, 1973), the salinity tolerance and osmoregulation in commercial size male *C. opilio* (Hardy *et al.*, 1994), and the measurement of hemolymph osmotic pressure and ionic composition in *C. tanneri* (Mackay and Prosser, 1970).

The general objective of this study was to determine the salinity tolerance and the ability to osmoregulate in zoeae and late juveniles of the snow crab *C. opilio*. This would expand our knowledge of the physiology of this commercially important species and of majid crabs in general. Our data could also contribute to an explanation of the distribution of snow crabs. Finally this study is part of a general survey of the ontogeny of osmoregulation in crustaceans (Charmantier *et al.*, 1988; Charmantier and Charmantier-Daures, 1991).

MATERIALS AND METHODS

Animals

Two berried females and several male and female juvenile *C. opilio* caught in the Gulf of St. Lawrence and kept in running sea water at ambient temperature at the Shippagan Marine Center, New brunswick, were transferred in early April to the culture facility at the Biological Station, St. Andrews, New Brunswick, Canada. They were kept before experiments in separate tanks supplied with running sea water (S∼28-32 $^0/_{00}$; T=3.5-6.5°C) under natural photoperiod, and were fed cod, squid and shrimp (*Pandalus borealis*). According to their cephalothoracic width, respectively 48.3±2.5 mm (extremes: 43-54 mm) and 50.3±1.8 mm (46-54 mm), male and female juveniles were in instar IX (Robichaud *et al.*, 1989) and under the commercial size (ctw>95 mm). These crabs were in molt stage C throughout the experiments (Drach, 1939).

Larvae were obtained in early April from the two females. After hatching, larvae from one of the females

were transferred to two 40-l planktonkreisels (Hughes *et al.*, 1974) modified for the culture of crab larvae. Sea water was filtered to 50 μm; flow-rate was set at 2.5-3 l.min^{-1}; a 280 μm mesh screen was used around the overflow system (Charmantier-Daures and Charmantier, 1991). Two culture temperatures were selected within the tolerable range of snow crabs (Kon, 1970; Davidson, 1983; Elner, 1988), 9.5±0.5°C in one planktonkreisel, 14.0±0.5°C in the other. Crab larvae were fed three times a day with live *Artemia* nauplii (California brand). Dead larvae were removed and the filters were cleaned daily. Only actively swimming larvae were selected for survival bioassays and osmoregulation experiments.

Preparation of media

Experiments were conducted in compartmented 250-*l* tanks with charcoal-filtrated recirculated medium kept at 6±0.5°C (minimum technically obtainable temperature) for juveniles, and in 0.5-l plastic containers kept at 14±0.5°C for larvae. Dilute media were prepared by adding dechlorinated tap water to sea water, and high salinity media were prepared by adding "Instant Ocean Synthetic Sea Salts" (Aquarium Systems, Inc.) to sea water. Sea water salinity was checked before use since it varied with the Bay of Fundy tides. Salinities were expressed according to the osmotic pressure in mosm.kg^{-1}, and to the salt content in the medium in $^0/_{00}$. A value of 3.4 $^0/_{00}$ is equivalent to 100 mosm.kg^{-1}. Osmotic pressure was measured with an Advanced Instruments 31 LA or Wescor 5000 osmometer, and salinity on a YSI 33 salinometer.

Salinity tolerance

Due to the small number of available animals, salinity tolerance in juveniles was evaluated only from the number of surviving and dead animals in media of different salinities. Juvenile crabs were progressively adapted from sea water to diluted or concentrated media by adding freshwater or Instant Ocean salts to the original medium; each change of 100 mosm.kg^{-1} in the salinity required about 24 h. Between two changes of salinity, they were kept for two days at constant salinity in each test medium, which differed from one another by increments of 100 mosm.kg^{-1} (~3.4 $^0/_{00}$), or 50 mosm.kg^{-1} in the lowest salinity media.

Acute static 96 h bioassays were conducted with zoeae 1 at days 1 and 10 of development (D1, D10), that were held in test media ranging from 100 mosm.kg^{-1} to sea water (~900-1 000 mosm.kg^{-1}) and to 1 500 mosm.kg^{-1}, and differing by increments of 100 mosm.kg^{-1}. Each bioassay was run on a group of 10 individuals and replicated. Animals were counted and dead animals were removed at 0.5, 1, 3, 6, 12, 24, 36, 48, 72, 96 h according to the prescriptions of Sprague (1969) in toxicity studies. The criteria for death were total lack of movement, immobility of appendages and heart, and lack of response after repeated touches with a probe. Median lethal salinities (lower and upper LS50s) and 95% confidence intervals were calculated by techniques of probit analysis (Lichtfield and Wilcoxon, 1949; Finney, 1952) computerized on the Letcur program (Zitko, 1982; Lieberman, 1983) adapted by R. Mounet-Guillaume (unpubl.). LS 50s were calculated at 24, 48 and 96 h.

Osmotic and ionic regulation

The hemolymph was collected from juvenile crabs via a hypodermic needle inserted through the articulation membrane at the basis of the fifth pereiopods. At least seven days elapsed between hemolymph collections from the same animal.

Zoeae at D1 and D10 were quickly dried on filter paper and immersed in mineral oil to avoid evaporation and desiccation. The hemolymph was then sampled with a glass micropipette inserted in the heart.

Osmotic pressure of hemolymph was measured on individual samples, on an Advanced Instruments 31 LA or Wescor 5000 osmometer respectively requiring 200 μl and 10 μl samples (adults), or on a Kalber-Clifton micro-osmometer, with reference to the osmotic pressure of the medium, requiring 30-50 nl samples (zoeae). At each salinity, the osmoregulatory capacity OC was calculated as the difference between the osmotic pressures of the hemolymph and of the medium.

On hemolymph samples taken from juveniles, Cl^- ion concentration was determined using a Cotlove Chloride Titrator, and Na^+, K^+, Ca^{++} and Mg^{++} ions were determined using a Perkin Elmer 5000 atomic absorption photometer. Student t tests were used for statistical comparisons.

RESULTS

Larval development

Time of development and percent of survival of zoeae are given in table 1. At higher temperature (14°C compared to 9.5°C), the shortest duration of zoea 1 stage decreased from 24 to 15 days, *i.e.* by ~38%. Mortality rate was high (20-30%) in both conditions of temperature during the first three days

Table 1. – Times of development and percent of survival of zoeae of *Chionoecetes opilio* at two different temperatures.

Temperature (°C)	9.5	14
Number of zoeae 1 initially stocked	6 000	6 000
Duration of stage zoea 1 (days)	24-38	15
Survival at first molt to zoea 2 (%)	34	20
Survival at last molt to zoea 2 (%)	23	0
Duration until 100% mortality (days)	42	23

Table 2. – Salinity tolerance in zoeae 1 of *Chionoecetes opilio* at days 1 and 10 of development (Z1D1, Z1D10) at 14°C. Variations in lower (low) and upper (up) LS 50s in mosm.kg^{-1} and $^0/_{00}$, with 95% confidence interval values in brackets.

Stage		Lethal salinity LS 50s					
		24 h		48 h		96 h	
		low	up	low	up	low	up
Z1D1	mosm.kg^{-1}	280 (269-302)	1 250 (1 208-1 294)	530 (456-638)	1 194 (1 135-1 285)	655 (379-744)	1 140 (1 098-1 182)
	$^0/_{00}$	9.5 (9.1-10.3)	42.5 (41.1-44.0)	18.0 (15.5-21.7)	40.6 (38.6-43.7)	22.3 (12.9-25.3)	38.8 (37.3-40.1)
Z1D10	mosm.kg^{-1}	288 (214-381)	1 223 (1 175-1 280)	528 (445-641)	1 205 (1 120-1 440)	736 (663-894)	1 125 (996-1 369)
	$^0/_{00}$	9.8 (7.3-13.0)	41.6 (40.0-43.5)	18.0 (15.1-21.8)	41.0 (38.1-49.0)	25.0 (22.5-30.4)	38.3 (33.8-46.5)

following hatching, then it tended to stabilize, and survival of zoeae 1 was subsequently higher at low temperature. Zoeae 1 were active and were observed to feed on *Artemia* nauplii which were captured by sudden folding and flipping of the abdomen. Mortality increased again immediately before the molt from zoea 1 to zoea 2 (at 14°C) or in late stage zoea 2 (at 9.5°C). Late zoeae 2 died before reaching the molt to megalopa. Dying and dead larvae were partly or totally covered by whitish patches apparently of bacterial origin.

Salinity tolerance

Juveniles of *C. opilio* survived for at least two days without mortality in media ranging from 500 to 1 300 mosm.kg^{-1} (~ 17 to 44 $^0/_{00}$) at 6°C. The lower and upper LS 50s were respectively about 400 and 1 350 mosm.kg^{-1} (~ 13.5 and 46 $^0/_{00}$) (*fig.* 1). No difference in salinity tolerance was detected between males and females.

In larvae, LS 50s were measured only in active zoeae 1, 1 and 10 days (D1 and D10) after hatching, at 14°C. The different values of LS 50s were not significantly different between zoeae 1 at D1 and D10 (*table* 2). In low salinity media, the lower LS 50 increased from about 280 mosm.kg^{-1} (~ 10 $^0/_{00}$) at 24 h to 530 mosm.kg^{-1} (~ 18 $^0/_{00}$) at 48 h and to 650-740 mosm.kg^{-1} (~ 22-25 $^0/_{00}$) at 96 h. In high salinity media, upper LS 50s at 24, 48 and 96 h decreased, and were about 1 250, 1 200 and 1 140 mosm.kg^{-1} respectively (~ 42, 41 and 38 $^0/_{00}$).

Osmotic and ionic regulation

Adaptation time

The time of osmotic adaptation after a change in environmental salinity was evaluated in juveniles and zoeae 1 at D1. After a rapid transfer from seawater at 835 mosm.kg^{-1} (~ 28.4 $^0/_{00}$) to a dilute medium of 697 mosm.kg^{-1} (~ 23.7 $^0/_{00}$), the hemolymph osmotic pressure stabilized within 24 h in juveniles. In zoeae 1 at D1 transferred from seawater (920 mosm.kg^{-1}, ~ 31.3 $^0/_{00}$) to a dilute medium of 500 mosm.kg^{-1} (~ 17 $^0/_{00}$), the corresponding time was 1 h (*fig.* 2). The time of adaptation to concentrated media was not tested; in other species, it was shorter than the time of adaptation to dilute media (Charmantier *et al.*, 1988). In subsequent experiments, we kept the zoeae 6 h and the juveniles 2-3 days in each medium before sampling.

Osmotic and ionic regulation in juveniles

Juveniles of *C. opilio* were osmoconformers over the entire range of experimental salinities, from 500 to 1 300 mosm.kg^{-1} (~ 17-44 $^0/_{00}$). The differences between the osmotic pressures of hemolymph and medium, i.e. the osmoregulatory capacity OC, were consistently below 10 mosm.kg^{-1} (*table* 3, *fig.* 3). No difference of regulation was detected between males and females.

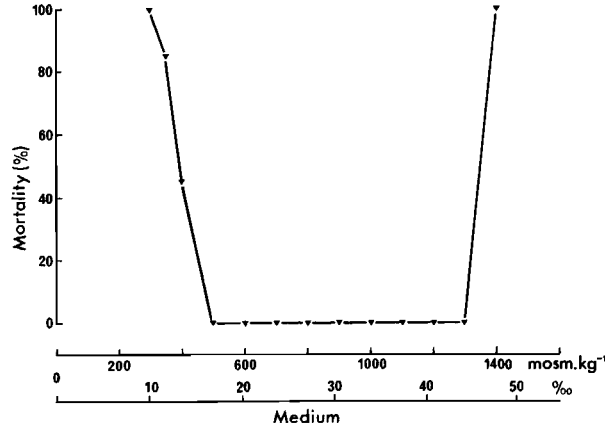

Figure 1. – Salinity tolerance in juveniles of *Chionoecetes opilio* at 6°C over 48 h. Percent mortality of animals according to the salinity of the medium. Number of animals at the start of the experiments in sea water (900 mosm.kg^{-1}): 20 (to low salinity media) and 7 (to high salinity media).

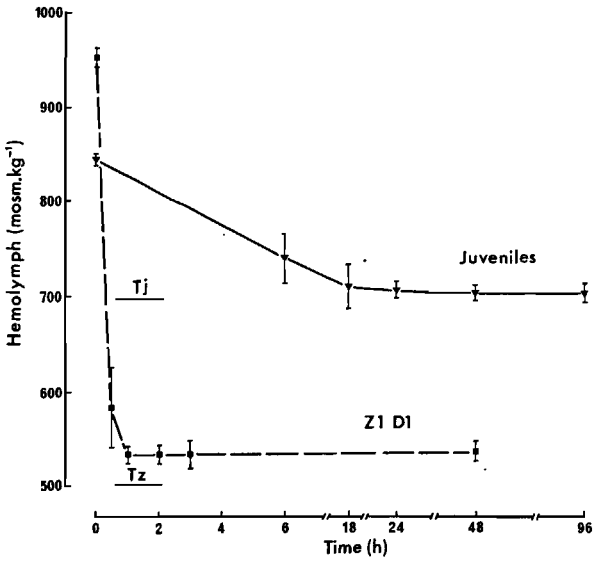

Figure 2. – Change in hemolymph osmotic pressure in juveniles of *Chionoecetes opilio* after rapid transfer from sea water (835 mosm.kg^{-1}, ~28.4 $^0/_{00}$) to 697 mosm.kg^{-1}, ~23.7 $^0/_{00}$ (Tj) at 6°C, and in zoeae 1 at D1 after rapid transfer from sea water (920 mosm.kg^{-1}, ~31.3 $^0/_{00}$) to 500 mosm.kg^{-1}, ~17 $^0/_{00}$ (Tz) at 14°C. Each point represents the mean value of determinations form 3-8 juveniles or 5-10 zoeae, with 95% confidence interval.

The hemolymph was isoionic with the medium for most of the measured ions. The osmotic effect of Na^+ and Cl^- was calculated using 0.9 as dissociation coefficient. In all media, these ions accounted for 86-90% (*table* 3) of the total hemolymph osmotic pressure. At some salinities, hemolymph Cl^- and K^+ concentrations were slightly lower than those of the media, and Na^+ and Ca^{++} concentrations in the hemolymph were slightly higher than in the media. Regulation of hemolymph Mg^{++} concentrations was hypo-ionic at all salinities, by 15-25 mEq.l^{-1}: hemolymph Mg^{++} were about 88-55% that in the medium according to the salinity (e.g. 70-80% in sea water).

Osmoregulation in zoeae 1

Zoeae 1 at D1 and D10 hyper-osmoconformed, *i.e.*, their hemolymph osmotic pressure varied as a function of external osmotic pressure but remained above by about 30 mosm.kg^{-1}. At the lowest and highest tested salinities (300 and 1 300 mosm.kg^{-1}, ~ 10 and 44 $^0/_{00}$), zoeae tended to isosmoticity (*fig.* 3).

DISCUSSION

Larval development

The time of hatching, early April in this study, is in agreement with previous observations on the eastern coast of Canada (Powles, 1966; Watson, 1969; Davidson, 1983). Mass culture of *C. opilio* in modified Hughes *et al.* (1974) planktonkreisels was only possible up to stage zoea 2 and then failed apparently due to bacterial infection. The same equipment had been previously used to culture *Cancer irroratus* from zoeae to young crabs (Charmantier-Daures and Charmantier, 1991). Successful culture of *C. opilio* up to the megalopa and crab 1-stages was previously conducted either on a small number of individuals kept in static condition with daily change of water (Kon, 1970; Motoh, 1973) or by individual rearing in screen bottomed cups immersed in running sea water (Davidson, 1983). These authors observed heavy mortality in zoeae 2 and megalopae (Motoh,

Table 3. – Hemolymph (HL) osmotic pressure and ion concentrations relative to those of the medium (MD) in juveniles of *Chionoecetes opilio* at 6°C. Corresponding salinities are given in $^0/_{00}$. Each point represents the mean value of determinations from n individuals (n indicated in brackets) with 95% confidence interval.

	Salinity ($^0/_{00}$)	Osm. pressure (mosm.kg^{-1})	Cl^- (mEq.l^{-1})	Na^+ (mEq.l^{-1})	K^+ (mEq.l^{-1})	Ca^{++} (mEq.l^{-1})	Mg^{++} (mEq.l^{-1})
MD	44.5	1 309	651	560	12.2	22.0	128
HL		1 314±2 (7)	636±14 (7)	568±17 (5)	11.0±2.4 (5)	22.4±1.6 (5)	113±6 (5)
MD	33.9	996	521	417	10.0	16.4	101
HL		1 000±4 (7)	485±5 (6)	424±23 (5)	9.4±1.7 (5)	16.9±1.1 (5)	82±5 (5)
MD	28.4	835	427	348	8.0	14.2	84
HL		844±6 (7)	393±6 (7)	364±15 (5)	7.1±1.2 (5)	15.8±0.9 (5)	60±5 (5)
MD	23.7	697	350	299	6.8	11.3	70
HL		703±3 (8)	319±5 (6)	313±7 (5)	6.3±1.1 (5)	12.2±0.9 (5)	49±5 (5)
MD	16.6	487	243	211	4.9	8.3	49
HL		492±3 (9)	231±9 (7)	216±15 (5)	4.4±1.4 (5)	9.1±2.4 (5)	24±4 (5)

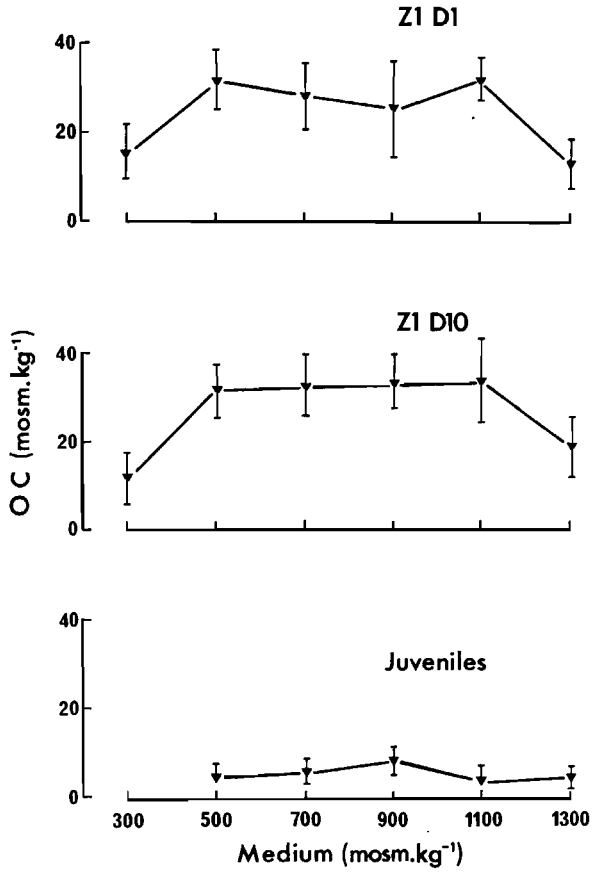

Figure 3. – *Chionoecetes opilio*: variations in the difference between osmotic pressures of hemolymph and medium (osmoregulatory capacity OC) in relation to the osmotic pressure of the medium in juveniles at 6°C and in zoeae 1 at D1 and D10 at 14°C. Each point represents the mean value of determinations from 7-9 juveniles or 10 zoeae, with 95% confidence interval.

1973; Davidson, 1983), due in one case to an epizootic of ciliates (Davidson, 1983).

Although these experiments were not oriented toward experimental larval culture under different conditions, they revealed that lower temperature (9.5°C vs 14°C) increased the survival rate in zoeae 1 and at the beginning of stage zoea 2. These results are in agreement with earlier reports: the optimum temperature for molting to zoea 2 ranged from 7.9 to 12.9°C (average: 9.6°C) according to Kon (1970) and was 8.2°C according to Davidson (1983). Higher temperatures, 10-15°C, were necessary to reach the megalopa and first crab stages (Kon, 1970).

Salinity tolerance

In *C. opilio*, the range of salinities tolerated depends on the time of exposure and the stage of development. In zoeae 1 at days 1 and 10 of their development, the lower and upper LS 50s at 14°C are about 10 $^0/_{00}$ and 42 $^0/_{00}$ at 24 h, 18 $^0/_{00}$ and 41 $^0/_{00}$ at 48 h, 25 $^0/_{00}$ and 38 $^0/_{00}$ at 96 h. Zoeae 1 are thus able to tolerate wide variations of salinity, but for only short durations. Our results are in agreement with earlier data on *C. opilio* larvae from the Sea of Japan (Kon, 1973). This author reported a minimum intermolt period for zoeae 1 and 2 in water with a specific gravity range of 1.020-1.022 (corresponding salinities at 11°C: 24-27 $^0/_{00}$); survival of zoeae 1 was over 70% in sea water of sp. gr. 1.022-1.027 (27-34 $^0/_{00}$), a range of salinity which is strictly encompassed in our values of 96 h LS 50s. Most zoeae 1 failed to molt or molted and died when maintained at a sp. gr. of 1.012 (14 $^0/_{00}$), and this salinity is intermediate between the 24 h and 48 h lower LS 50s reported in this study.

In the field, zoeae are usually found in the upper layers of the water column, in the 0-50 m depth range in the Sea of Japan (Kon, 1980), in the upper 20 m in the Bering sea with salinity of 31-32 $^0/_{00}$ (Incze *et al.*, 1987). They were observed at deeper depths, between 20 and 80 m (Lanteigne, 1985), in the Gulf of St. Lawrence. In this area, *C. opilio* larvae are found in waters ranging from 30.2 to 32 $^0/_{00}$ salinity (Roff, in Davidson, 1983), which is in the range of their long-term tolerable salinities. Their deeper habitat in the Gulf is perhaps related to the avoidance of upper layers where the salinity could be lowered by continental freshwater, particularly in spring from the St. Lawrence river.

In juveniles, the approximate 48 h lower and upper LS 50s at 6°C were 13.5 and 46 $^0/_{00}$ and no mortality was observed for 48 h between 17 and 44 $^0/_{00}$. This relatively wide euryhalinity demonstrated by juveniles could be partly related to the short time of exposure to different media and to the progressive adaptation of experimental crabs to changing salinities. Long term exposure to extreme salinities could yield more restrictive results: over 96 h, mortalities occurred at salinities below 21 $^0/_{00}$ at 4°C in larger, commercial size *C. opilio* (Hardy *et al.*, 1994). Juvenile snow crabs usually live in a benthic habitat, down to depths of 250 m in the Gulf of St. Lawrence (Watson, 1969, review in Adams, 1979), where variations of salinity are unlikely. However, snow crabs are also found in shallow waters, particularly near shores of the Gulf of St. Lawrence (Sainte Marie and Hazel, 1992) where salinity is potentially more variable. As demonstrated by the present results, they can sustain brief and relatively ample salinity changes in such habitats.

Adaptation time

In *C. opilio*, the time of osmotic equilibration in a dilute medium is about 1 h in zoeae 1 and 24 h in juveniles. These times are similar to those of the corresponding stages of other species (Charmantier *et al.*, 1988; Charmantier and Charmantier-Daures, 1991). Short (a few hours) variations of salinity have thus more physiological impact in larvae than in juveniles and adults. The potential adverse effects of

exposure to variable salinity in larvae could be lowered by behavioral avoidance of low salinity, as in other species (Scarrat and Raine, 1967). This hypothesis could be assessed through field and experimental studies of the larval movements in the water column.

Osmotic and ionic regulation

Juveniles of *C. opilio* are osmoconformers and isoionic to the external medium (except for Mg^{++}), particularly for Na^{+} and Cl^{-} ions which are the main hemolymph osmotic effectors, for external salinities ranging from 17 to 44 $^{0}/_{00}$. Commercial size *C. opilio* were also osmoconformers for salinities in the range 20-30 $^{0}/_{00}$ (Hardy *et al.*, 1994). Hemolymph ionic composition of *C. opilio* is similar to that of the Alaska Tanner crab, *C. tanneri*, measured in sea water (Mackay and Prosser, 1970).

Isosmotic and isoionic regulation is presumably a physiological feature among the crabs of the family Majidae. This pattern of osmotic and/or ionic regulation has also been reported in other species of majid crabs, as *Maja verrucosa* (Fredericq, 1922), *M. squinado* (Drilhon, 1935; Robertson, 1960), *Hyas araneus* (Schlieper, 1929; Robertson, 1960), *Libinia emarginata* (Gilles, 1970). These species are generally presented as stenohaline (review in Mantel and Farmer, 1983) but some of them, as juvenile *C. opilio* in the present study, are relatively euryhaline. Specimens of *L. emarginata* were able to withstand diluted media down to 40% sea water (~ 12 $^{0}/_{00}$) (Gilles, 1970), which is close to the 48 h LS 50 in *C. opilio*. In *L. emarginata*, the mechanism of osmoregulation does not implicate an extracellular regulation (since the hemolymph remains isosmotic to the medium at all salinities) but an isosmotic intracellular regulatory mechanism in which the intracellular free amino acids play a prominent part (Gilles, 1970). A similar mechanism is probably present in *C. opilio*.

Mg^{++} concentration in the hemolymph of *C. opilio* is hyporegulated at all salinities; it represents 70-80% of the Mg^{++} concentration of sea water. Similar degrees of Mg^{++} hyporegulation have been observed in *H. araneus* (Robertson, 1960), *Pugettia producta* and *L. emarginata* (Cornell, 1979). It has been noted since Robertson (1960) that relatively "unresponsive" species, such as majid crabs, have high concentrations of Mg^{++}, that are about 80% that in sea water, while more "active" species have concentrations less than 50% of sea water, although there are a few exceptions (Mantel and Farmer, 1983; Morritt and Spicer, 1993). High hemolymph Mg^{++} concentrations are thought to have an anaesthetic effect on the neuromuscular junction (Robertson, 1960) and would affect the functional properties of hemocyanin (Mangum, 1983).

Zoeae 1 of *C. opilio* hyper-osmoconform in all but extreme tested salinities. The short-term euryhalinity reported at this stage is presumably related to an isosmotic intracellular regulatory mechanism similar to that postulated in juveniles. Most decapod larvae that have been studied hyper-osmoconform in salinities that they normally encounter in their environment. By favouring the turgescence of the body, particularly of the buoyancy-involved appendages and exopodites of the larvae through an osmotic influx of water, the positive difference in osmotic pressure could be considered an adaptation of small organisms to planktonic or pelagic life (Charmantier, 1988; Charmantier and Charmantier-Daures, 1991). In *L. emarginata*, zoeae 1 are also hyper-osmoconformers but a slight capacity to hyper-regulate in dilute media and to hyporegulate in sea water is gradually aquired in zoeae 2 and early megalopae; this ability is slowly lost in late megalopae that present the adult osmoconformer pattern of regulation before the completion of metamorphosis (Kalber, 1970). Further studies on zoeae 2 and megalopae would be required to known whether such progressive changes occur in *C. opilio*. The patterns of osmoregulation in zoeae 1 and juveniles of the snow crab relate it to the first of three groups of species, determined by their pattern of ontogeny of osmoregulation (Charmantier *et al.*, 1988; Charmantier and Charmantier-Daures, 1991), in which early larval stages hyper-osmoconform whereas the juveniles and adults are osmoconformers.

Acknowledgments

Part of this study was supported by a grant from NATO. We thank Dr. D. E. Aiken and S. L. Waddy for providing lab space and facilities, Dr. M. Moriyasu for supplying the animals, David Robichaud, Wilfred Young-Lai and M. A. Garcia for their help.

REFERENCES

Adams A. E. 1979. The life history of the snow crab, *Chionoecetes opilio*: a literature review. *Alaska Sea Grant Rep.* 8-13, 141 p.

Aikawa H. 1937. Further notes on Brachyuran larvae. *Rec. Oceanogr. Works Japan* **9,** 87-162.

Charmantier G., M. Charmantier-Daures 1991. Ontogeny of osmoregulation and salinity tolerance in *Cancer irroratus*; elements of comparison with *C. borealis* (Crustacea, Decapoda). *Biol. Bull.* **180,** 125-134.

Charmantier G., M. Charmantier-Daures, N. Bouaricha, P. Thuet, D. E. Aiken, J.-P. Trilles 1988. Ontogeny of osmoregulation and salinity tolerance in two decapod crustaceans: *Homarus americanus* and *Penaeus japonicus*. *Biol. Bull.* **175,** 102-110.

Charmantier-Daures M., G. Charmantier 1991. Mass-culture of *Cancer irroratus* larvae (Crustacea, Decapoda): adaptation of a flow-through sea-water system. *Aquaculture* **97,** 25-39.

Comeau M., G. Y. Conan 1992. Morphometry and gonad maturity of male snow crab, *Chionoecetes opilio*. *Can. J. Fish. Aquat. Sci.* **49,** 2460-2468.

Conan G. Y., M. Comeau 1986. Functional maturity and terminal molt of male snow crab. *Chionoecetes opilio*. *Can. J. Fish. Aquat. Sci.* **43,** 1710-1719.

Cormier R. J., A. R. Fraser, R. F. Bailey, N. Raymond 1992. Hemolymph ecdysone concentration as a function of sexual maturity in the male snow crab (*Chionoecetes opilio*). *Can. J. Fish. Aquat. Sci.* **49,** 1619-1623.

Cornell J. C. 1979. Salt and water balance in two marine spider crabs, *Libinia emarginata* and *Pugettia producta*. I. Urine production and magnesium regulation. *Biol. Bull.* **157,** 221-233.

Davidson K. 1983. Stock delineation and larval taxonomy of the snow crab, *Chionoecetes opilio* (O. Fabricius) (Decapoda, Brachyura, Majidae) in Atlantic Canada. M. Sci. Thesis, University of Guelph, Ont., 134 p.

Dawe E. G., D. M. Taylor, J. M. Hoenig, W. G. Warren, G. P. Ennis, R. G. Hooper, W. E. Donaldson, A. J. Paul, J. M. Paul 1991. A critical look at the idea of terminal molt in male snow crab (*Chionoecetes opilio*). *Can. J. Fish. Aquat. Sci.* **48,** 2266-2275.

Donaldson W. E., R. T. Cooney, J. R. Hilsinger 1981. Growth, age and size at maturity of tanner crab, *Chionoecetes bairdi* M. J. Rathburn, in the northern Gulf of Alaska (Decapoda, Brachyura). *Crustaceana* **40,** 286-302.

Drach P. 1939. Mue et cycle d'intermue chez les Crustacés Décapodes. *Ann. Inst. Océanogr. Monaco* **19,** 103-391.

Drilhon A. 1935. Etude biochimique de la mue chez les Crustacés Brachyoures (*Maja squinado*). *Ann. Physiol. Physicochim. Biol.* **11,** 301-326.

Dunbar M. J. 1968. Ecological development in polar regions. Prentice-Hall, Englewood Cliffs, N.J., 119 p.

Elner R. W. 1988. Review of snow crab biology. A stock definition and larval mixing in the snow crab (*Chionoecetes opilio*), 67-74. *In:* Proc. Int. Workshop on Snow Crab Biology, G. S. Jamieson, W. D. McKone eds., December 8-10, 1987, Montréal, Qué., *Can. Ms. Rep. Fish. Aquat. Sci.*, 2005, 145 p.

Finney D. J. 1952. Probit analysis: a statistical treatment of the sigmoid response curve. Second Edition, Cambridge Univ. Press, London, 318 p.

Fredericq L. 1922. Action du milieu marin sur les Invertébrés. *Arch. Int. Physiol.* **19,** 309-351.

Fukataki H. 1969. Occurrence and distribution of planktonic larvae of edible crabs belonging to the genus *Chionoecetes* (Majidae, Brachyura) in the Japan Sea. *Bull. Jpn. Sea Reg. Fish. Res. Lab.* **21,** 35-54.

Gilles R. 1970. Osmoregulation in the stenohaline crab "*Libinia emarginata*" Leach. *Arch. Int. Physiol. Biochim.* **78,** 91-99.

Hardy H., J. Munro, J.-D. Dutil 1994. Temperature and salinity tolerance of the soft-shell and hard-shell male snow crab. *Chionoecetes opilio*. *Aquaculture* **122,** 249-265.

Haynes E. 1973. Descriptions of prozoeae and stage I zoeae of *Chionoecetes bairdi* and *C. opilio* (Oxyrhyncha, Oregoniidae). *Fish. Bull.* **71,** 769-775.

Hughes J. T., R. A. Shleser, G. Tchobanoglous 1974. A rearing tank for lobster larvae and other aquatic species. *Progress Fish. Cult.* **36,** 129-133.

Incze L. S., D. A. Armstrong, S. L. Smith 1987. Abundance of larval tanner crabs (*Chionoecetes* spp.) in relation to adult females and regional oceanography of the southeastern Bering Sea. *Can. J. Fish. Aquat. Sci.* **44,** 1143-1156.

Incze L. S., A. J. Paul 1983. Grazing and predation as related to energy needs of stage I zoeae of the tanner crab *Chionoecetes bairdi* (Brachyura, Majidae). *Biol. Bull.* **165,** 197-208.

Ito K. 1963. Some observations on the degree of ripeness of the eggs of the snow crab (*Chionoecetes opilio*). *Bull. Jpn. Sea Reg. Fish. Res. Lab.* **11,** 1-12.

Ito K. 1967. Ecological studies on the edible crab. *Chionoecetes opilio* (O. Fabricius) in the Japan Sea. I. When do female crabs first spawn and how do they advance into the following reproductive stage. *Bull. Jpn. Sea Reg. Fish. Res. Lab.* **17,** 67-84.

Ito K. 1970. Ecological studies on the edible crab. *Chionoecetes opilio* (O. Fabricius) in the Japan Sea. II. Age and growth as estimated on the basis of the seasonal changes in the carapace width frequencies and the carapace hardness. *Bull. Jpn. Sea Reg. Fish. Res. Lab.* **22,** 81-116.

Jamieson G. S., W. D. McKone 1988. Proceedings of the International Workshop on Snow Crab Biology, December 8-10, 1987, Montréal, Québec. *Can. Ms. Rep. Fish. Aquat. Sci.*, 2005, 145 p.

Kalber F. A. 1970. Osmoregulation in decapod larvae as a consideration in culture techniques. *Helgol. Wiss. Meeresunters.* **20,** 697-706.

Kon T. 1967. Fisheries biology of the tanner crab. *Chionoecetes opilio*. I. On the prezoeal larva. *Bull. Jpn. Soc. Scient. Fish.* **33,** 726-730.

Kon T. 1970. Fisheries biological studies of the tanner crab. IV. Estimation of the duration of planktonic stages by rearing experiments of larvae. *Bull. Jpn. Soc. Scient. Fish.* **36,** 219-224.

Kon T. 1973. Fisheries biology of the Japanese tanner crab. V. Effect of salinity on survival and growth of the larvae reared in the laboratory. *Bull. Jpn. Soc. Scient. Fish.* **39,** 945-950.

Kon T. 1979. Ecological studies on crabs belonging to the genus *Chionoecetes*. I. The influence of starvation on the

survival and growth of the zuwai crab. *Bull. Scient. Fish.* **45,** 7-9.

Kon T. 1980. Studies on the life history of the zuwai crab, *Chionoecetes opilio* (O. Frabricius). *Spec. Publ. Sado. Mar. Biol. Stat. Niigata Univ.* Ser. 2, 64 p.

Kurata H. 1963. Larvae of Decapoda Crustacea of Hokkaido. 2. Majidae (Pisinae). *Bull. Hokkaido Reg. Fish. Res. Lab.* **27,** 25-31.

Kuwatani Y., T. Wakui, T. Nakanishi 1971. Studies on the larvae and post-larvae of a tanner crab. *Chionoecetes opilio elongatus* Rathbun. 1. On the protozoeal larvae. *Bull. Hokkaido Reg. Fish. Res. Lab.* **37,** 32-40.

Lanteigne M. 1985. Distribution spatio-temporelle des larves de crabe appartenant aux genres *Chionoecetes* et *Hyas*, dans la Baie des Chaleurs, Canada. Thèse de Maîtrise, Université de Moncton, N.B., 161 p.

Lichtfield J. T., F. Wilcoxon 1949. The reliability of graphic estimates of relative potency from dose-percent effect curves. *J. Pharmacol. Exp. Theor.* **108,** 18-25.

Lieberman H. R. 1983. Estimating LD 50 using the probit technique: a basic computer program. *Drug. Chem. Toxicol.* **6,** 111-116.

Mackay W. C., C. L. Prosser 1970. Ionic and osmotic regulation in the king crab and two other North Pacific crustaceans. *Comp. Biochem. Physiol.* **34,** 273-280.

Mangum C. P. 1983. Oxygen transport in the blood. *In:* Internal Anatomy and Physiological Regulation, D. E. Bliss ed. in chief, The Biology of Crustacea, Vol. 5, L. H. Mantel ed., Academic Press, New York, 373-429.

Mantel L. H., L. L. Farmer 1983. Osmotic and ionic regulation. *In:* D. E. Bliss ed. in chief, Internal Anatomy and Physiological Regulation, The Biology of Crustacea, Vol. 5, L. H. Mantel ed., Academic Press, New York, 53-161.

Morritt D., J. I. Spicer 1993. A brief re-examination of the function of extracellular magnesium and its relationship to activity in Crustacean Arthropods. *Comp. Biochem. Physiol.* **106**A, 19-23.

Motoh H. 1973. Laboratory-reared zoeae and megalopae of zuwai crab from the Sea of Japan. *Bull. Jpn. Soc. Scient. Fish.* **39,** 1223-1230.

Powles H. W. 1966. Observations on the biology of two species of spider crabs, *Chionoecetes opilio* and *Hyas araneus*, in the Gulf of St. Lawrence. *Fish. Res. Board Can. Ms. Rep. Ser. (Biol.)* **884,** 36 p.

Robertson J. D. 1960. Osmotic and ionic regulation. *In*: The Physiology of Crustacea, Vol. 1. Metabolism and Growth, T. H. Waterman ed., Academic Press, New York, 317-339.

Robichaud D. A., R. F. J. Bailey, R.W. Elner 1989. Growth and distribution of snow crab, *Chionecetes opilio*, in the southeastern Gulf of St. Lawrence. *J. Shellf. Res.* **8,** 13-23.

Sainte-Marie B., F. Hazel 1992. Moulting and mating of snow crabs, *Chionoecetes opilio* (O. Fabricius), in shallow waters of the northwestern Gulf of Saint Lawrence. *Can. J. Fish. Aquat. Sci.* **49,** 1282-1293.

Scarratt D. J., G. E. Raine 1967. Avoidance of low salinity by newly hatched lobster larvae. *J. Fish. Res. Board Can.* **21,** 661-680.

Schlieper C. 1929. Uber die Enwirkung niederer Salzkonzentrationen auf marine Organismen. *Z. vergleich Physiol.* **9,** 478-514.

Somerton D. A. 1981. Regional variation in the size of maturity of two species of tanner crab (*Chionoecetes bairdi* and *C. opilio*) in the eastern Bering Sea, and its use in defining management subareas. *Can. J. Fish. aquat. Sci.* **38,** 163-174.

Sprague J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. *Water res.* **3,** 793-821.

Squires H. J. 1966. Distribution of decapod crustacea in the northwest Atlantic. *Ser. Atlas Mar Environ. Am. geogr. Soc.*, Folio 12.

Squires H. J. 1990. Decapod Crustacea of the Atlantic Coast of Canada. *Can. Bull. Fish. Aquat. Sci.* **221,** 532 p.

Tester P. A., A. G. Carey Jr. 1986. Instar identification and life history aspects of juvenile deepwater spider crabs. *Chionoecetes tanneri* Rathbun. *Fish. Bull.* **84,** 973-980.

Watson J. 1969. Biological investigations on the spider crab *Chionoecetes opilio. Can. Fish. Rep.* **13,** 23-47.

Watson J. 1970. Maturity, mating, and egg laying in the spider crab *Chionoecetes opilio. J. Fish. Res. Board Can.* **27,** 1607-1616.

Williams A. B. 1984. Shrimps, lobsters and crabs of the Atlantic coast of the United States, Maine to Florida. Smithsonian Institution Press, Washington DC, 550 p.

Zitko V. 1982. Letcur, the lethality curve program. *Can. Tech. Rep. Fish. Aquat. Sci.*, 1134, 10 p.