Serum protein concentration and somatic index in relation to morphometric maturity for male snow crab (*Chionoecetes opilio*) in the Baie des Chaleurs.

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1993

Canadian Technical Report
of Fisheries and Aquatic Sciences
No. 1944

Fisheries and Oceans Pêches et Océans

Canada

Canadian Technical Report of Fisheries and Aquatic Sciences

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Cat. No. Fs97-6/1944E ISSN 0706-6457

Correct citation for this publication is:

Cormier, R.J., M. Comeau, and M. Lanteigne. 1993. Serum protein concentration and somatic index in relation to morphometric maturity for male snow crab (*Chionoecetes opilio*) in the Baie des Chaleurs. Can. Tech. Rep. Fish. Aquat. Sci. 1944: v + 17p.

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ABSTRACT

The serum protein concentration of juvenile and morphometrically mature male snow crab (*Chionoecetes opilio*) was measured using a refractometer. The somatic indices of juvenile and morphometrically mature male snow crab were calculated using the weight of the dry flesh of the chelae versus its wet weight. Results indicate that juvenile male snow crab have significantly higher serum protein concentrations and significantly lower somatic indices than their morphometrically mature counterparts. These observations are attributed to basic physiological differences that could be explained by a terminal molt when morphometrical maturity is achieved.

RÉSUMÉ

La concentration de protéines du sérum de crabes des neiges (*Chionoecetes opilio*) mâles juvéniles et morphométriquement matures a été déterminée à l'aide d'un réfractomètre. Des indices somatiques ont été calculés à partir du poids de la chaire sèche en relation à celui de la chaire humide de la pince des mâles juvéniles et morphométriquement matures de crabe des neiges. Les résultats démontrent que les crabes mâles juvéniles ont des concentations de protéines sériques significativement plus élevées et des indices somatiques significativement plus bas que ceux des mâles morphométriquement matures. Ces observations sont attribuées à des différences physiologiques fondamentales qui pourraient être expliquées par une mue terminale lorsque la maturité morphométrique est atteinte.

INTRODUCTION

Meat yield is an important factor in crab fishery. Snow crab (*Chionoecetes opilio*) in postmolt have a much lower meat yield than an animal which has attained intermolt (Taylor *et al.* 1989). At intermolt, most of the water absorbed during ecdysis has been replaced with muscular tissue in the chelae and pereiopods, thus providing the best meat yield and quality. Adequate meat yield has been a problem in the snow crab fishery in the southwestern part Gulf of St. Lawrence since the mid 1980's (Elner and Bailey 1986). In 1989, the southwestern Gulf of St. Lawrence snow crab fishery was closed only six weeks after its opening due to a high incidence of "white crabs" in the catch preventing fishers from catching their quota (Mallet *et al.* 1990). So called "white crabs" are animals in postmolt which have low meat yield and meat quality rendering them economically unprofitable for processing (Elner and Bailey 1986; Bailey and Elner 1989).

The physiology of molting in crustaceans has been described by Skinner (1985) and Chang and O'Connor (1988). Near the end of intermolt, the hemolymph concentration of ecdysteroids produced by the Y-organs increases sharply (Andrieux *et al.* 1976; Blanchet *et al.* 1979; Girard and Maissiat 1983). The epidermis withdraws from the cuticle marking the beginning of premolt (Drach 1939; Drach and Tchernigovtzeff 1967). A new cuticle forms underneath the old one during premolt and, depending on the species, calcium and magnesium is retrieved from the old cuticle and is either stored in the hemolymph, the midgut gland or the gastroliths (Skinner 1985; Chang and O'Connor 1988). Another increase of ecdysteroids production occurs at the end of premolt and, in most species, the concentration of hemolymph constituents increases. During ecdysis, the crab sheds its old carapace and takes in water to expand the new carapace. During postmolt the water is gradually replaced by new tissue, returning organs and muscles to their original density when intermolt is reached (Skinner 1985; Chang and O'Connor 1988). Thus, the internal physiology of the animal is continuously changing in a cycle designed to allow the animal to grow.

Reproduction is another physiological process having profound effects on the animal's life cycle. Numerous species of crustaceans undergo behavioral, physiological and morphological changes as they achieve sexual maturity (Charniaux-Coton and Payen 1988). Spermatogenesis and secondary male characteristics begin development once the androgenic gland is individualized and starts producing the androgenic hormone (Charniaux-Coton and Payen 1988). The androgenic hormone controls the development of external secondary sexual characteristics and plays an important role in decapod sexual behavior (Gleeson *et al.* 1987). In male snow crab, secondary sexual characteristics develop several molts after gonadal maturity (Comeau and Conan 1992). Conan and Comeau (1986) have shown that the chelae of male snow crab increases disproportionately in size in comparison to the carapace. This morphological change is obtained following one molt called the molt to maturity (Conan and Comeau 1986; Comeau and Conan 1992). The larger chelae of the mature males is seen as an advantage or a prerequisite to grasp the female during copulation (Conan and Comeau 1986).

The purpose of this study is to compare the serum protein concentrations and somatic indices of juvenile and mature male snow crab (*Chionoecetes opilio*) and explain these changes in relation with morphological maturity.

MATERIALS AND METHODS

Sampling

Male snow crab (*Chionoecetes opilio*) were collected using commercial rectangular and standard conical traps during two expeditions in the Baie des Chaleurs (48° 12'N: 64° 30'W), during May and June 1984. Traps were placed on commercial fishing grounds to investigate the biological and physiological characteristics of snow crab being commercially exploited. The appearance and condition of the carapace of captured crabs were noted qualitatively and only clean shell crabs were considered for the study.

Determination of molt stage

The molt stage of each crab was determined by histological section of the dactylopod of the fifth right pereiopod. These dactylopods were fixed in Bouin's solution for 24 hours and subsequently dehydrated and stored in a 70% ethanol/water solution. Each sample was set in paraffin and sectioned transversely to give 5 µm sections which were stained using the method of Mallory (Gabe 1968).

Molt stages were identified based on Drach and Tchernigovtzeff (1967) and Skinner (1985) descriptions.

Determination of serum protein concentration

Hemolymph samples were collected from live snow crab at the time of capture. Each sample was taken via a puncture of the joint between the coxopod and the ischiopod of one or more appendages using a 10 ml syringe. Special care was taken not to puncture the hepato-pancreas and contaminate the hemolymph sample. Any samples showing signs of contamination from hepato-pancreatic material (yellowish color) were discarded. All samples were frozen at -80°C until analyzed.

At the laboratory, each hemolymph sample was thawed and a subsample of 1 ml was transfered to a microcentrifuge tube (Eppendorf, 1.5 ml). The subsample was then centrifuged for 3 minutes at 1000 G in a microcentrifuge to separate the agglutinated material and the serum from the hemolymph. A few drops of serum were taken from the top of the subsample and were set on a refractometer. The refractometer was equipped with a reticle scaled primarily for the measurement of serum protein concentration. Serum protein concentrations were read directly from the refractometer in grams of protein/100 ml of serum.

Allometric measurements and statistical analysis

The morphometric maturity was determined using the allometric relationship between the overall carapace width and the dry weight of the chelae (Cormier *et al.* 1992). Flesh and the cuticle of the chelae were dried separately at a temperature of 60°C for a period of 48-h and weighed to the nearest mg. The combined weight was used as the dry weight of the chelae. The overall carapace width (CW) was measured to the nearest mm using a caliper. For consistency the terminology on maturity suggested by Comeau and Conan (1992) is used in this study.

The somatic index (SI) was defined as the ratio of the dried flesh weight of the chelae (DFW) and its corresponding wet flesh weight (WFW). This index was used to overcome the relationship between the animal size and the flesh weight, and allow comparison of flesh density between specimens.

Basic statistics (mean and standard deviation) were used to describe the data. The normality of the distribution and the homoscedasticity of the data were tested using the Kolmogorov-Smirnov test and the F_{max}-test respectively (Sokal and Rohlf 1981). A Mann-Whitney U-test was used to test the means of the serum protein concentrations and the somatic indices.

RESULTS

Molt stages and morphometric maturity

Only male snow crab considered to be in intermolt following histological observation of the cuticle were selected for this study. Animals in postmolt were not selected because it is impossible to identify morphometric maturity using the dry weight of the chela. A total of 42 juvenile males (lower swarms of points; Fig. 1) and 265 mature males (upper swarm of points; Fig. 1) were identified in the plot showing the natural logarithm (log) of the chelae dry weight versus log CW (Table 1, Fig. 1).

Somatic indices

The mean somatic indices are significantly lower (Mann-Whitney U-test; $p < 0.001$) for juvenile males compared to mature males (Table 1; Figure 2). The Mann-Whitney U-test was used to compare the somatic index of each category because of heterogeneity of variances (F_{max}-test; $p < 0.05$).

Serum protein concentrations

Serum protein concentrations are not correlated to carapace size for both juvenile ($r^2 = 0.0001$) and mature ($r^2 = 0.036$) males (Figure 3). The Mann-Whitney U-test was used to compare serum protein concentrations of both categories as the serum protein concentrations were not normally distributed (Kolmogorov-Smirnov test for the goodness of fit; $p < 0.05$) for mature males. The mean serum protein concentrations are significantly higher ($p < 0.05$) for juvenile males compared to mature males (Table 1; Figure 4).

DISCUSSION

In male snow crab, the chelae grows disproportionately in relation to the size of the carapace marking the beginning of morphometric maturity (Conan and Comeau 1986; Comeau and Conan 1992). Couples observed in nature during the reproductive period are composed of sexually mature males (differentiated chelae) and females. Generally, less than 2% of the sexually paired males can be identified as juveniles (Conan and Comeau 1986; Ennis *et al.* 1990; Comeau *et al.* 1991; Sainte-Marie and Hazel 1992). However, the differentiation of the chelae, *per se*, does not initiate the behavior and response of male snow crab to grasp and mate with mature females. Physiological and hormonal changes regulating the appearance of this secondary sexual character (Charniaux-Cotton and Payen 1988; Homola *et al.* 1991) are also factors involved in mating behavior. As mentioned by Comeau and Conan (1992), the differentiation of the chelae is only an external characteristic.

The concentration of hemolymph constituents vary in relation to the molt cycle (Teissier 1938; Drach and Teissier 1939; DeLeersnyder 1967; Stewart *et al.* 1967; Telford 1968; Barlow and Ridgeway 1969; Heppper 1978). Generally, the concentration rises rapidly during premolt in preparation for molting. During the molting process, large amounts of water are absorbed reducing the concentration of hemolymph constituents drastically. Afterwards, the concentration increases gradually during postmolt as the animal is in an active period of somatic growth to finally stabilize to a relatively static level during intermolt. In different species studied, the concentration of hemolymph constituents vary little in terms of size of the animals at intermolt (DeLeersnyder 1967). However, it is suggested that females have higher serum concentrations than males due to vitellogenesis processes (DeLeersnyder 1967). It can therefore be assumed that changes of serum protein concentration in the hemolymph are correlated with major physiological activities such as growth and reproduction.

In this study, juvenile males showed higher serum protein concentrations in the hemolymph than mature males. If serum protein concentration reflects physiological activity, it can be assumed that the energy budget of juvenile males is different than mature crabs. As juvenile males do not have a high gonadal production (Comeau and Conan 1992), it is conceivable that an important portion of the energy budget is directed toward short intermolt periods and hence somatic growth characterizing juvenile crabs. In mature males, lower serum protein concentrations indicate that the animal is in a somewhat low metabolic phase, resulting in a longer intermolt period, and/or great level of proteins are being used during gametogenesis. High concentration of serum constituents would not be required in an animal not actively growing.

Mature males have higher somatic indices (more flesh in their claws) than juvenile males. Throughout the postmolt phase of an actively growing individual, the water content is gradually replaced with tissue which is reflected by increasing somatic index values. The somatic index of an animal actively growing with short intermolt periods will not reach the same level of somatic index of an animal not actively growing or with long intermolt period. As noted for serum protein concentrations, mature males reach higher somatic indices than juvenile crabs indicating that these animals have longer intermolt periods.

Cormier *et al.* (1992) have shown that the concentration of ecdysteroids at intermolt (molting hormone) is significantly lower in the hemolymph of mature males compared to juvenile males *C. opilio*. They suggest that these observations are related to terminal molt. Bateller (1992) noted that the Y organs, responsible for the production of ecdysteroids, are much smaller in mature males compared to juvenile males. Although he does not directly link these observations to a terminal molt state, a reduction in size of a secreting organ is a strong indication of reduced activity. Similar observations are found in the isopod *Sphaeroma serratum* where the concentration of ecdysones and the size of the Y organs decreases after the onset of maturity (Charmantier *et al.* 1977; Charmantier and Trilles 1979). Terminal molt is a phenomenon observed and documented for various Majid crab species (Teissier 1935, 1960; Hartnoll 1963, 1982). Once sexual maturity is attained, a crab may never molt again and remain in a long intermolt.

Juvenile and mature males have different physiological states. As juvenile males are mainly growing in size preparing little for reproduction, mature males have acquired their mature sizes and are mainly involved in reproduction. Bateller (1992) and Cormier *et al.* (1992) provide physiological parameters in support of a terminal molt in mature male snow crab. Field observations of snow crab populations also suggest the existence of a terminal molt for the mature male snow crab as mature males are seldom observed in premolt (Conan and Comeau 1986; Conan *et al.* 1988; Conan *et al.* 1990; Yamasaki and Kuwahara 1991). There are however arguments, although contradictory, against the concept of terminal molt in male snow crab. Dawe *et al.* (1991) state that the duration of intermolt increases tremendously once male snow crab attain morphometric maturity and that mature male snow crab held in captivity have been induced to molt. However, special considerations and cautions are to taken when studying physiological processes in artificial conditions. Physiological parameters must be interpreted within the context of the natural life cycle of the animal studied. The molt to maturity can be considered terminal if the length of the intermolt after the molt to maturity is longer than the natural life span of the species.

ACKNOWLEDGMENTS

The authors wish to thank Dr T. W. Sephton, Mr. K. G. Davidson and F. Maynou for their critical comments, as well as Mr. A. Doiron for his laboratory assistance and Mr. P. Mallet for his expertise on the determination of molt stage by histology.

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Table 1. The mean size, mean somatic index, mean serum protein concentration and standard deviation of juvenile and mature males identified by the allometric relation of log of the dry chelae weight versus log CW.

Category	N	Mean CW (mm)	Mean somatic index	Mean Serum* Protein (g/100ml)
Juvenile	42	99.7 (5.8)	0.1327 (0.0094)	7.0342 (1.5163)
Mature	265	109.3 (9.6)	0.1367 (0.0153)	4.5745 (1.2954)

Standard deviation in parenthesis

* 38 juvenile and 251 mature males snow crab (*C. opilio*) were sampled for serum protein concentration

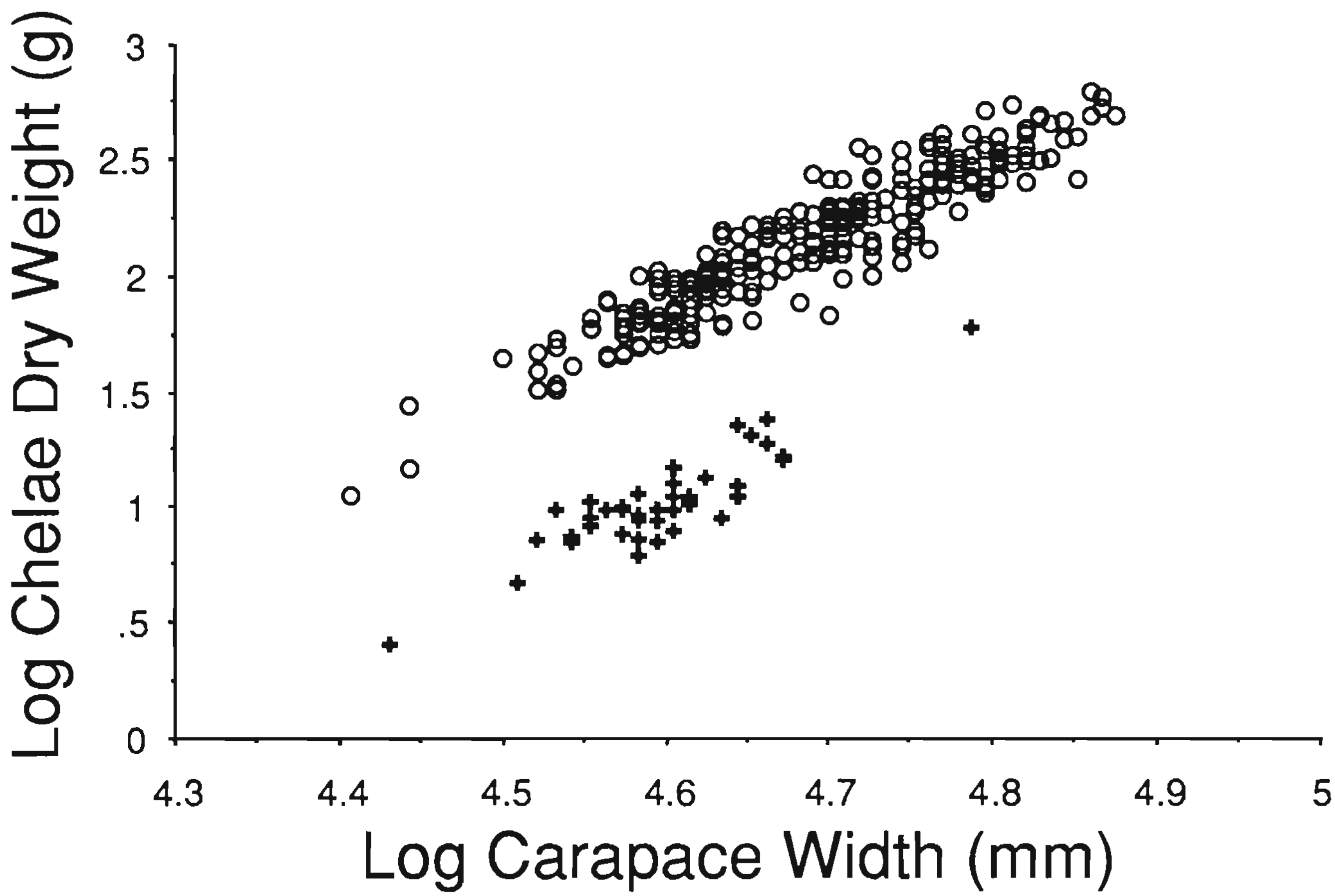

Figure 1. Relationship between log chelae dry weight and log carapace width of male snow crab, *Chionoecetes opilio* (+ = juvenile male, n = 42; o = morphometrically mature male, n = 265).

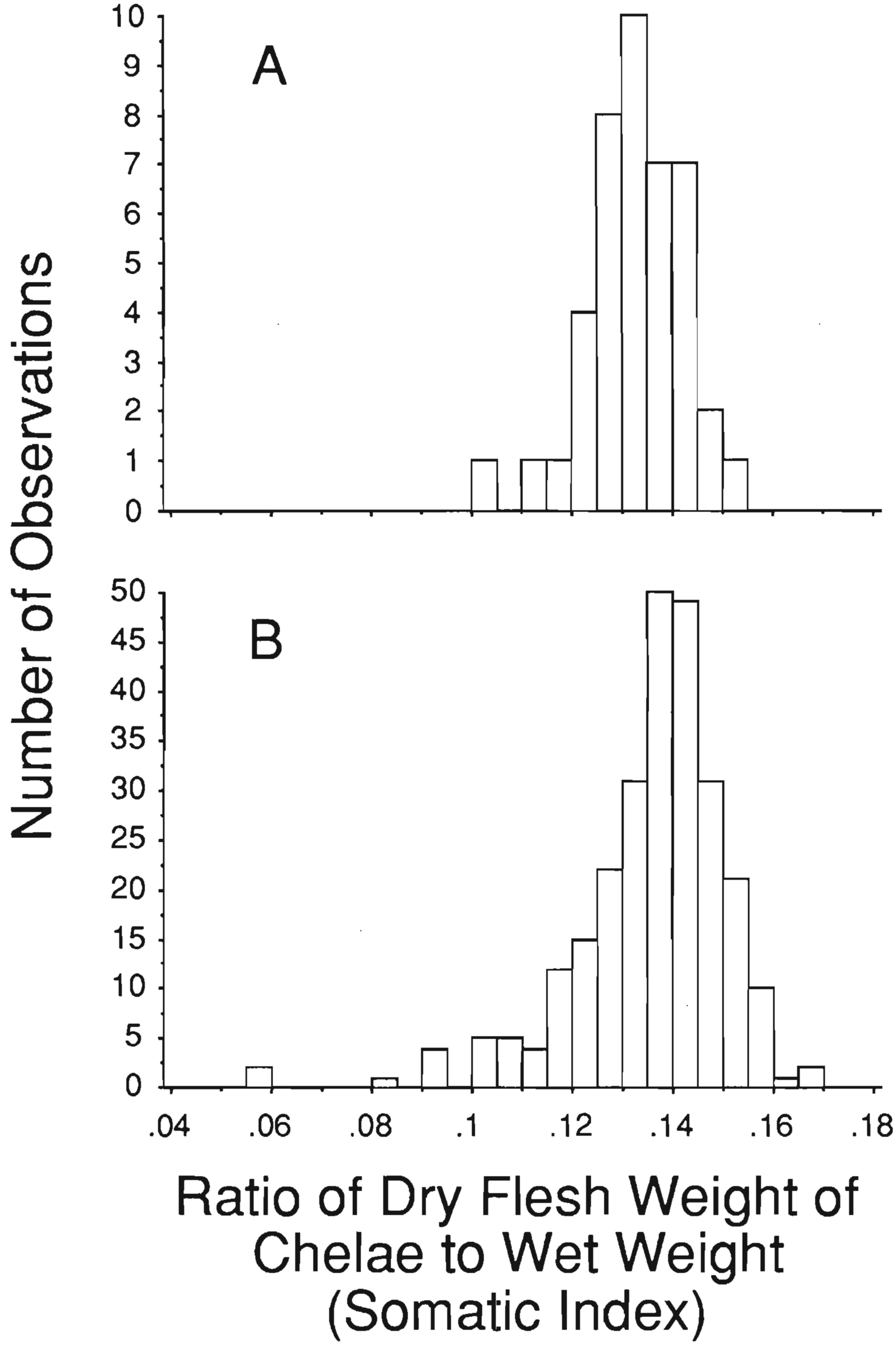

Figure 2. Histograms of the ratio of dry flesh weight of the chelae to its wet weight (somatic indices) of A) juvenile (n = 42) and B) morphometrically mature (n = 265) male snow crab (*Chionoecetes opilio*).

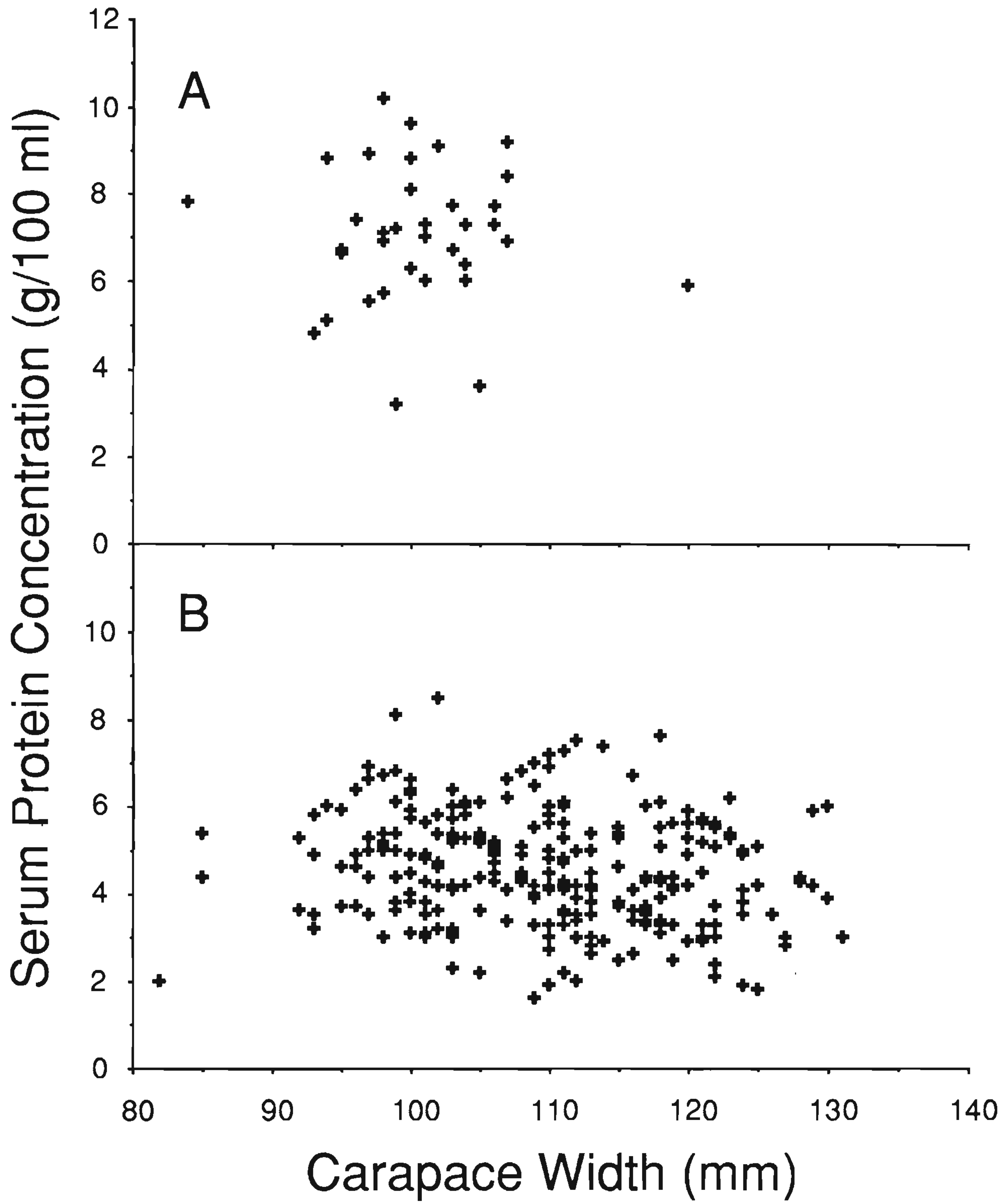

Figure 3. Relationship between the serum protein concentration and the carapace width of A) juvenile, $r^2 = 0.00014$, and B) morphometrically mature, $r^2 = 0.03605$, male snow crab (*Chionoecetes opilio*).

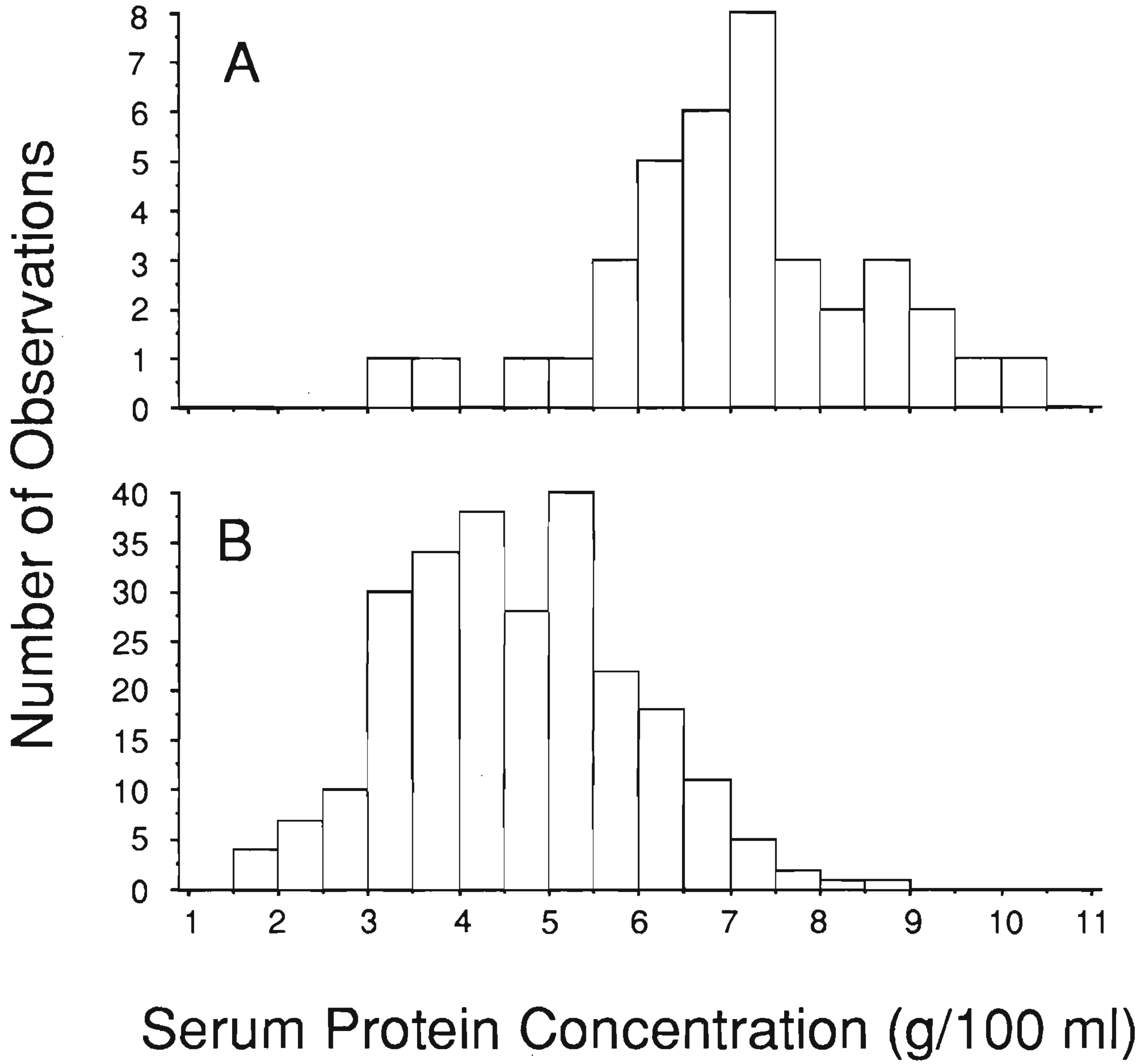

Figure 4. Histograms of the serum protein concentration of A) juvenile (n = 42) and B) morphometrically mature (n = 265) male snow crab (*Chionoecetes opilio*).