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EFFECT OF MORPHOMETRIC MATURITY AND SIZE ON ENZYME ACTIVITIES AND NUCLEIC ACID RATIOS IN THE SNOW CRAB *CHIONOECETES OPILIO*

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ABSTRACT

Indicators of metabolic capacity and the potential for protein synthesis in male snow crabs, *Chionoecetes opilio,* were measured to estimate how these factors are affected by morphometric maturity and body size. The metabolic capacity of muscle, as depicted by cytochrome C oxidase (CCO), citrate synthase (CS), and lactate dehydrogenase (LDH) activity, was greater in adults than in adolescents, while that of the digestive gland was not affected by maturity. These differences may reflect the different locomotory habits of adults and adolescents. Indicators of the potential for protein synthesis, the RNA:DNA and protein:RNA ratios, did not differ between adult and adolescent crabs, presumably because the animals were in late intermolt stage and flesh growth was already completed. The total mass of muscle, as estimated by the flesh content of the merus, and the total capacity of muscle CCO scaled isometrically with body mass, while that of CS scaled with an exponent <1. The mass of the digestive gland, as well as the total capacity of both mitochondrial enzymes in this organ, increased more slowly than body size. These results support the hypothesis that the negative allometry generally observed for aerobic metabolism may be caused by the decreasing size of the metabolically most active tissues with an increase of body mass. In contrast with the positive allometry of the anaerobic capacity of fast-swimming fish with body mass, we noted an isometric relationship between these two variables in the rather slow-moving *C. opilio.* This supports the hypothesis that an enhanced anaerobic capacity is necessary for the largest individuals of a fast-moving species in order to maintain their burst swimming speed despite the increased friction generated by increased speed and body size.

The effects of body size on the aerobic capacity of vertebrates have been thoroughly investigated during the last two decades (reviewed by Robinson *et al.,* 1983; Schmidt-Nielsen, 1984; Goolish, 1991). Far fewer allometric studies have been concerned with invertebrates, on the one hand, or with glycolytic capacity, on the other hand. Given the differences in locomotory strategies and dependency upon aerobic and anaerobic metabolism, the relationships between body size and metabolic capacities may differ between mammals, fish, and invertebrates.

Growth not only leads to increases in body size, but may also be associated with changes in maturity, morphology, and behavior. For example, immature males of many crustacean species, including crabs and shrimps, undergo molt cycles until they reach the adult stage. Whether the molt leading to morphometrical maturity is terminal or not in majid male crabs is still subject to controversy (Hartnoll, 1963; Conan and Comeau, 1986; Dawe *et al.,* 1991; Sainte-Marie and Hazel, 1992), although the general opinion favors the terminal molt hypothesis. Adult males have larger gonads as well as a higher chela height to carapace width ratio than adolescent males, and they display mating behaviors more frequently (Donaldson and Adams, 1989; Claxton, 1992; Cassier *et al.,* 1997). Adult males of various crab species also migrate over longer distances than immature males (Sainte-Marie and Hazel, 1992; Hines *et al.,* 1995). Maturity is thus likely to affect muscle metabolic capacities and should be considered in allometric studies. Whether an adjustment in enzyme activities with maturity or body size is strictly due to locomotory habits can be inferred by comparison with enzymatic responses in nonlocomotory tissues such as the digestive gland. To our knowledge, the effect of maturity on the relationship between metabolic capacity and body mass in invertebrates has not been investigated.

The equation describing the relationship between metabolic rate (Y) and body mass (M) is $Y = aM^b$, where a is a constant and b the scaling factor. Numerous studies have demonstrated that the oxygen consumption of a given endothermic species is proportional to the body mass to the power 0.67. Interspecifically, an exponent of 0.75 is often reported for ectothermic animals, although

much variation occurs among different species (Weymouth *et al.,* 1944; Von Bertalanffy and Krywienczyc, 1953; Zeuthen, 1953; Winberg, 1956; Hemmingsen, 1960; Schmidt-Nielsen, 1984). The activity of mitochondrial enzymes, cytochrome C oxidase (CCO), and citrate synthase (CS), in various tissues generally shows an allometry similar to aerobic capacity (e.g., Somero and Childress, 1980, 1990; Goolish and Adelman, 1988; Goolish, 1991; Pelletier *et al.,* 1993a). The relative decrease of oxidative metabolism with body size has been proposed to be caused by (1) O_2 uptake and transport constraints related to the low surface/volume ratio in large individuals (reviewed by Schmidt-Nielsen, 1984); (2) faster metabolic systems in smaller individuals (Lane and Lawrence, 1979); or (3) a decrease, with increasing body size, in the proportion of tissues that have a high metabolic rate (Calder, 1984). Faster metabolic systems can be approximately equated to a faster growth rate (Klein Breteler, 1975). An increase in mass-specific metabolic rate with decreasing body size should then be accompanied by an increase in the rate of protein synthesis. Nucleic acid ratios, such as RNA:DNA and protein:RNA, give indications of the potential of cell growth. The RNA:DNA ratio represents the quantity of RNA per cell which is available for protein synthesis, since 85–94% of the cellular RNA is ribosomal (McMillan and Houlihan, 1988). This ratio is a good indicator of growth in various species (e.g., Bulow, 1970; Buckley, 1984; Barron and Adelman, 1984; Mathers *et al.,* 1992), although this relationship does not always hold (Dagg and Littlepage, 1972; Jürss *et al.,* 1987; Mathers *et al.,* 1994). The ratio of RNA and protein concentration, an index of the synthetic activity of the RNA molecules, is better correlated with growth in some cases (Houlihan *et al.,* 1990).

In contrast with aerobic capacity, the activity of the glycolytic enzyme lactate dehydrogenase (LDH) in white muscle of pelagic fish increases faster than body size (Somero and Childress, 1980, 1990; Goolish, 1991). It has been suggested that this positive allometry of glycolytic capacity provides the power requirements for high-speed swimming of larger individuals, since these animals must overcome increased drag due to their greater body surface. Indeed, the glycolytic capacity is lower in skeletal muscle of benthic fish species than in that of pelagic fishes and shows a weaker positive allometry in the former group than in the latter (Somero and Childress, 1990). The respiration rate of the snow crab *Chionoecetes opilio* (O. Fabricius) indicates that this species has a low metabolic capacity, suitable for a sedentary life-style (Maynard, 1991). The mean walking speed in the wild varies between 130 and 400 m/day, depending on the depth and water temperature (Brêthes and Coulombe, 1989; Maynard, 1991). If the preceding hypotheses are correct, glycolytic enzyme activities in muscle should not scale positively with body size in this sluggish species which shows negligible bursts of locomotion, while oxidative metabolism should still scale negatively with body size.

The aims of this study are (1) to estimate the effect of morphometric maturity on biochemical properties of muscle and digestive gland in the snow crab *Chionoecetes opilio,* and (2) to investigate how mitochondrial and glycolytic enzyme capacities as well as nucleic acid ratios scale with body mass in the digestive gland and muscle of this slow-moving species.

Materials and Methods

Experimental Conditions.—In October 1992, male *Chionoecetes opilio* were collected by beam trawl and trap in the maritime estuary of the St. Lawrence River, near Mont-Joli, Québec, Canada. To avoid cannibalism and strong competition for food, the crabs were kept at low densities and were separated by size among six 1.8-m^3 circular tanks until they molted. A semi-open circulation system allowed sea water to remain at 4°C and 30 ppt salinity. The flow rate averaged 5–10 $l \cdot min^{-1}$. Crabs were fed with frozen capelin and shrimp ad libitum twice a week, ensuring that all the animals were in a similar physiological condition when molting began. Molting crabs were isolated in floating boxes until they reached hard-shell condition. They were then transferred to other tanks of similar size and rearing conditions until sampling. The smallest crabs (±40-mm carapace width before molt) molted from 18 January to 2 February 1993 and the largest (±70 mm) from 9 February to 12 March 1993. Molt-cycle stages were determined from the setal morphology of the basal endite of the maxilla (Moriyasu and Mallet, 1986) and from the morphology of the exopodites of the maxilla (O'Halloran and O'Dor, 1988). Only crabs in intermolt were selected, in order to avoid an effect of molt stages on the metabolism of the animals. Thirty-three adolescent and 17 adult crabs with body mass ranging from 54–360 g were sampled from 4 April to 16 August 1993. Maturity stages were determined by measuring the ratio of carapace width to chela height and using the equation obtained by Sainte-Marie and Hazel (1992) for a snow crab population of the nearby Baie Sainte-Marguerite. Following the terminology proposed by Sainte-Marie *et al.* (1995), adult males were defined as crabs with spermatophores and differentiated chelae, and ado-

lescents as crabs with spermatophores and undifferentiated chelae.

Dissection.—Carapace width, chela height, and body mass were measured and the number of missing legs noted. Each crab was drained before being weighed. Variations in the water content of tissues among the animals were not likely to affect significantly body mass, since snow-crab flesh has a density similar to that of sea water. When legs were missing, the mass they would have represented was estimated by weighing the corresponding legs on the opposite side of the animal and this value was added to body mass. The walking legs were cut open and muscles were dissected out. The total mass of wet muscle in the merus of the first walking leg was noted and used as an index of total muscle content of the crab. Muscles were then chopped and mixed together before subsamples of approximately 1 g each were taken for determination of enzyme activities, measurement of nucleic acid concentrations, total protein, and water content. The subsamples for biochemical analyses were immediately frozen in liquid nitrogen and later transferred to –80°C. One subsample was weighed before being dried at 90°C for 24 h and weighed again to determine the water content of the tissue. The digestive gland was dissected out, drained, and weighed before being subdivided in the same way as the muscle.

Enzyme Activity Measurements.—Approximately 30 mg of frozen digestive gland or muscle were homogenized in an ice-cold buffer solution of 50 mM imidazole, 2 mM $MgCl_2$, 5 mM Na_2EDTA, 0.1% Triton X-100, and 1 mM reduced glutathione in 40% glycerol, at pH 7.5, with a dilution 1:10 (W/W). The homogenates were then centrifuged at 1,000 *g* for 10 min, at <4°C. All enzymatic activities were measured in duplicate at 10°C, using a Beckman DU-600 spectrophotometer coupled with a circulating refrigerated water bath. The assays could not be conducted at 4°C, the experimental temperature at which the animals were held, because of condensation on the cuvettes. Enzyme-specific activity is expressed in international units (µmol of substrate converted to product per min) per g of wet tissue, and per g of protein and mg of DNA, which are both independent of variations in tissue hydration. Enzymes were measured in the following order, using the assay conditions previously described in Pelletier *et al.* (1993a, b).

Cytochrome C oxidase (CCO, E.C.1.9.3.1): 100 mM potassium phosphate, pH 7.0, and 50 µM reduced cytochrome C from horse heart. Reactions were run against a control of 50 µM cytochrome C oxidized with 0.0033% (W/V) potassium ferricyanide. The oxidation of reduced cytochrome C was followed at 550 nm and an extinction coefficient of 19.1 $mM^{-1} \cdot cm^{-1}$ was used for activity calculations.

Citrate synthase (CS, E.C.4.1.3.7): 100 mM Tris-HCl, 0.1 mM 5,5′ dithiobis 2-nitrobenzoic acid (DTNB), 0.2 mM acetyl CoA, 0.3 mM oxaloacetate, pH 8.6. The increase in absorbance due to the transfer of sulphydryl groups to DTNB was monitored at 412 nm. The extinction coefficient of DTNB is 13.6 $mM^{-1} \cdot cm^{-1}$.

Lactate dehydrogenase (LDH, E.C.1.1.1.27): 100 mM potassium phosphate, 0.16 mM NADH, 0.8 mM pyruvate, pH 7.0. LDH was assayed only in muscle, because its activity was negligible in the digestive gland. The use of the protease inhibitor PMSF (phenylmethanesulphonylfluoride) 1 mM did not enhance the LDH activity in this tissue. When purified enzyme was added to digestive gland homogenates, high LDH activity was detected, confirming that the very low activity in the samples was not due to technical problems. Lallier and Walsh (1991) also found no LDH activity in the digestive gland of *Callinectes sapidus*. The conversion of NADH to NAD was followed at 340 nm and an extinction coefficient of 6.22 $mM^{-1} \cdot cm^{-1}$ was used.

Nucleic Acids and Proteins.—Nucleic acid concentrations were determined by fluorimetry. The fluorochrome thiazole orange (TO, courtesy of Molecular Probes, Eugene, Oregon, U.S.A.) is more sensitive to small differences in DNA and RNA concentrations than ethidium bromide (Berdalet and Dortch, 1991). The specific DNA marker Hoechst 258, which Berdalet and Dortch (1991) used for phytoplankton cells, did not work well with animal tissues. For an unknown reason, the standard curve with Hoechst 258 became sigmoidal when a constant quantity of homogenate was added to the standards. Therefore, TO was used to measure both DNA and RNA in a subsample and DNA only in another subsample treated with RNAse (Sigma-Aldrich Ltd., Oakville, Ontario, Canada), according to the method of Karsten and Wollenberger (1972). Nucleic acids could not be quantified accurately in the digestive gland, possibly because of the high activity of RNAse in this tissue and of interference with carotenoid pigments. The percentages of nucleotide recovery in muscle tissue were evaluated by adding known quantities of purified DNA and RNA to one part of a homogenate, while the second part contained only endogenous nucleic acids. The percentages of recovery were 97 ± 5 (N = 3) and 98 ± 3 (N = 3) for DNA and RNA, respectively.

All glassware and pipette tips were autoclaved. All solutions were at pH 7.5 and were kept at <4°C, except TO which was conserved at room temperature in the dark. Buffer Tris-Ca was made up with 0.1 M NaCl, 0.1 M Tris-base, and 0.9 mM $CaCl_2$-2 H_2O in autoclaved 0.2 µm filtered distilled water. Solutions of 21µM TO, 16.5 units per ml heparin, and 4.8 units per ml RNAse were made in Tris-Ca buffer. Stock solutions of Bakers Yeast RNA type XI and Calf Thymus DNA type I were made up with 1 mg of nucleic acid per ml of Tris-Ca buffer and kept at –80°C. Working solutions of 200 µg/ml were used for standard curves.

A sample of approximately 250 mg of wet muscle was homogenized twice for 15 s in 5 ml of ice-cold Tris-Ca buffer and centrifuged at 3,000 *g* for 10 min. For each sample, 6 tubes were prepared: 2 for the determination of total nucleic acids (homogenate, heparin, and TO), 2 for the quantification of DNA (homogenate, heparin, RNAse, and TO), and 2 to determine the homogenate fluorescence (homogenate). Tris-Ca was added to attain a final volume of 3 ml in each tube. Two or 3 background tubes were prepared each day, with Tris-Ca, heparin, and TO. The "DNA" tubes of each sample were incubated at 37°C for 20 min, while the "total," "blank," and "background" tubes were kept at <4°C. After incubation, TO was added to all the tubes, except to the blanks. All tubes were mixed with a vortex and kept at 25°C before being read on the spectrofluorimeter (Perkin Elmer LS50, Perkin-Elmer Ltd., Rexdale, Ontario, Canada). The samples and standard curves were read at the same temperature, because the TO emission changes with temperature. Readings were made at EX 511 nm with a bandwidth of 3 mm and EM 533 nm with a bandwidth of 5 mm.

The total protein content was assessed by the method of Bradford (1976), using bovine albumin (fraction V, Sigma) as a standard. The 300 µl samples were first treated with 30 µl Triton 1%, 1.15 ml 10 M urea, and 40 µl glacial acetic acid to break down cell membranes and to solubilize proteins (Somero and Childress, 1990). Those products were used in the same proportions in blanks and standard curves. Glycogen content was quantified using the enzymatic method of Carr and Neff (1984) and lipid content with the gravimetric method of Bligh and Dyer (1959). Total lipids were solubilized and extracted with a Tecator Soxtec System H. T. (Tecator, Hoganas, Sweden). Lipids were measured in the digestive gland only, since they are consistently lower than 1% in muscle of *C. opilio* (Hardy, personal communication).

Statistics.—The log-transformed variables were normally distributed and homoscedastic. The effect of maturity and body mass was analyzed using ANCOVA with a significance level of 0.05, maturity being the treatment (adult versus adolescent) and body mass the covariable. Prior to analysis, we verified that there was no significant interaction between the covariate and the treatment (parallelism of slopes). Least-square linear regressions were fitted to the data for adults and adolescents separately when maturity had a significant effect on the relationship, or to pooled data when no significant effect of maturity was found. The resulting slopes were tested against theoretical slopes of 0 (the concentration not changing with body mass) and 1 (the total content increasing as fast as body mass).

RESULTS

Effect of Maturity

Maturity affected the enzymatic activities in muscle (Table 1), but did not affect any of the variables measured in the digestive gland ($P > 0.05$). Since adult crabs have relatively larger walking legs than adolescents, total wet muscle mass in the merus was highest in the first group. Nevertheless, the percentage of water in muscle was similar for the two groups. Nucleic acid contents and the RNA:DNA ratio in muscle were not affected by maturity. Neither glycogen, lipid, nor protein concentrations per g wet muscle or per mg DNA nor the total content of these molecules in the merus or digestive gland changed with morphometric maturity ($P > 0.05$). Total activities of CCO, CS, and LDH in the merus muscle were higher in adults. In the case of LDH, this was strictly due to the fact that adults have a larger merus than adolescents, since the specific activity of this enzyme was comparable in both groups. In contrast, the greater total activities of CCO and CS in adults were caused not only by the allometry of merus volume, but also by the higher specific activities of these enzymes.

Table 1. Effect of maturity on properties of merus muscle of *Chionoecetes opilio*. ANCOVA, with log body mass in g as the covariable and maturity stage as treatment. The log of the variables is used in the statistical tests and the ranges of untransformed values are given. The mass of wet tissues is given. Only variables which are significantly affected by maturity are shown. For all significant variables, adults have higher intercepts than adolescents. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. CCO = cytochrome C oxidase, CS = citrate synthase, LDH = lactate dehydrogenase.

Variable	*N*	Range of values	*F*
Total mass (g)	50	0.69–6.38	18.99***
CCO/DNA (units/mg)	43	8.02–22.75	4.48*
CCO/protein (units/g)	45	8.38–44.67	7.75**
CCO/merus (units)	45	1.45–10.23	8.05**
CS/g (units/g tissue)	47	1.49–3.25	26.84***
CS/DNA (units/mg)	48	12.22–29.44	10.89***
CS/protein (units/g)	47	12.16–46.45	10.16***
CS/merus (g)	46	2.52–16.33	39.37***
LDH/merus (units)	49	10.81–145.88	8.71**

Effect of Size on Muscle Properties

The wet mass of muscle in the merus scaled isometrically with body mass, for both adult and adolescent crabs (Fig. 1). Nevertheless, the b value for DNA content per merus was significantly lower than 1, and that for DNA concentration per g of wet muscle was significantly lower than 0 (Table 2). This suggests that the muscle of large crabs was composed of relatively fewer cells than that of small crabs, since the number of muscular fibers per merus increased more slowly than body mass. The decreasing concentrations of DNA, RNA, and protein per g of wet muscle with increasing body mass were not likely to be caused by a reduction in cell size, since the protein:DNA and RNA:DNA ratios varied isometrically with body mass. On the other hand, our results do not clearly indicate that water content in the intra- or extracellular compartments increased with body size, since the percentage of water in the merus muscle had an exponent value only slightly higher than 0 ($P = 0.08$, Table 2).

For the range of body mass under study, the size of the animal had only a slight effect on the potential of cell growth, as indicated by the following results. A marginally significant decrease in the synthetic activity of the RNA molecules (protein : RNA ratio) was noted as body mass increased (Table 2), but the relative quantity of RNA available for protein synthesis (RNA:DNA ratio) was independent of body size. Energetic reserves, as estimated

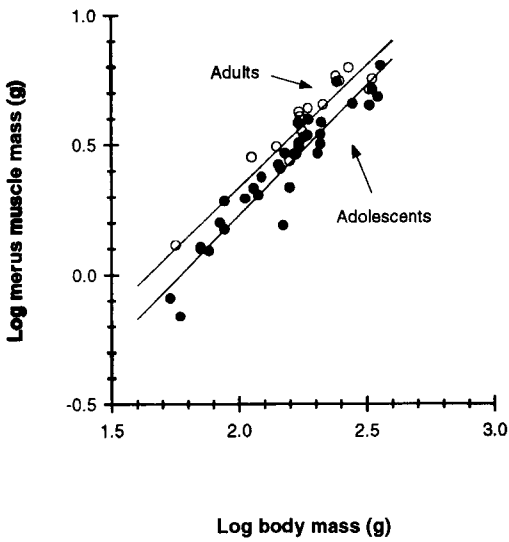

Fig. 1. *Chionoecetes opilio.* Total mass of merus muscle for adult (open circles) and adolescent (closed circles) males, versus body mass. Isometric relationships were: log muscle mass·merus ^{1} = –1.763 + 0.998 log M, for adolescents; log muscle mass·merus^{-1} = –1.541 + 0.940 log M, for adults. M = body mass in g.

by glycogen concentration per g of wet muscle and per mg of DNA, were also constant.

The components of the enzymatic pathways of muscle responded to size differently (Table 2). The total CCO capacity in merus muscle scaled isometrically with body mass of both adult and adolescent crabs (Fig. 2a). In adolescents, this was achieved through an increase of the CCO activity per cell and per protein, which kept the CCO activity per g constant, despite the fact that the number of cells per g, as estimated by DNA/g, was lowest in large animals. The same tendencies were observed in adults, but the relationships were not significant. The total CS activity in merus muscle increased more slowly than body size, with b values of 0.76 and 0.69 for adult and adolescent crabs (Fig. 2b). In adolescents, CS activity per g of wet tissue decreased with body mass. This allometric relationship was caused by the stability of CS activity per cell and per protein, which did not compensate for the lower number of cells per g in largest animals. The total glycolytic capacity, as estimated by the LDH activity in merus muscle, scaled isometrically with body mass of adult and adolescent animals (Fig. 2c), mainly because of the higher LDH concentration per protein in large animals.

Effect of Size on the Digestive Gland Properties

The digestive gland wet mass (Fig. 3) scaled with body mass with slope of 0.722 for both maturity stages, while the percentage of water increased faster than body mass (Table 3). Lipid and protein concentration being inde-

Table 2. Regression analyses for merus muscle of *Chionoecetes opilio,* fitting the model log Y = log a + b log M. M is the body mass in g. Units for the variables and symbols for the significance level are given in Table 1. † = 0.05 < P < 0.1. Regressions are done on adult (U) and adolescent (O) animals separately or on both groups (O + U), according to the results of the ANCOVA analyses. Parameters are given only for allometric relationships, i.e., b significantly different from 1 for total contents or from 0 for concentrations.

Variable	Maturity	Model parameters			H_0 test	
		Log a	b	r^2	b = 1	b = 0
Percentage water	O + U	1.888	0.011	0.06	***	†
DNA/g	O + U	2.380	–0.141	0.20	***	***
DNA/merus	O + U	0.710	0.831	0.70	*	***
RNA/g	O + U	3.499	–0.161	0.19	***	***
Protein/g	O + U	2.565	–0.261	0.17	***	***
Protein/RNA	O + U	2.171	–0.158	0.06	***	†
Protein/merus	O + U	1.093	0.622	0.38	**	***
CCO/DNA	O	0.548	0.264	0.20	***	*
CCO/Protein	O	0.425	0.349	0.25	***	***
CS/g	**O**	0.800	–0.225	0.36	***	***
CS/protein	**O**	0.809	0.221	0.11	***	†
CS/merus	**O**	–0.774	0.690	0.60	**	***
	U	–0.733	0.759	0.77	**	***
LDH/DNA	O + U	1.940	0.143	0.06	***	†
LDH/protein	O + U	1.769	0.263	0.12	***	*

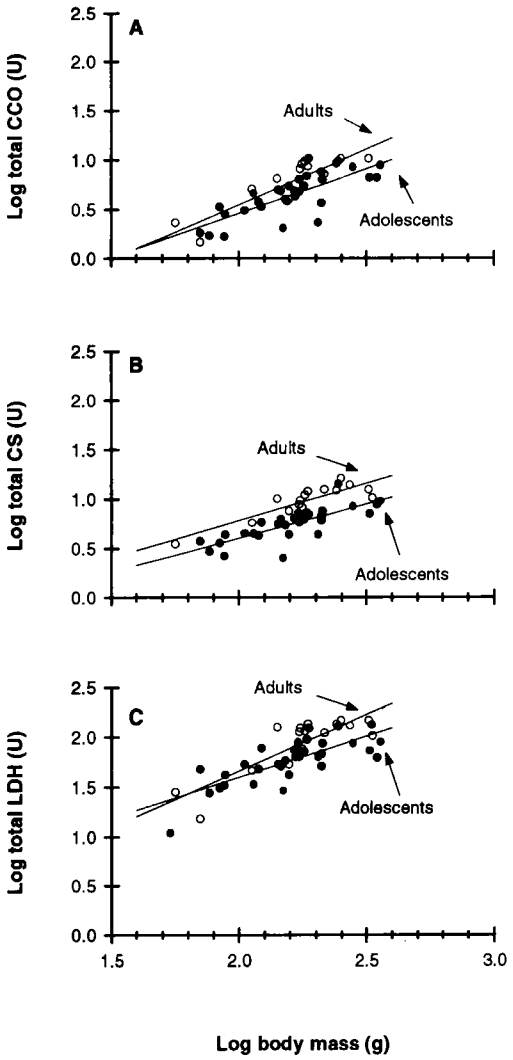

Fig. 2. *Chionoecetes opilio.* Change in enzymatic activities in whole merus muscle of adult (open circles) and adolescent (closed circles) males with increased body mass. (A) CCO activity. Isometric relationships were: log CCO·merus^{-1} = −1.340 + 0.901 log M, for adolescents; log CCO·merus^{-1} = −1.689 + 1.118 log M, for adults. (B) CS activity. Equations are given in Table 2. (C) LDH activity. Isometric relationships were: log LDH·merus^{-1} = −0.044 + 0.820 log M, for adolescents; log LDH·merus^{-1} = −0.608 + 1.134 log M, for adults. M = body mass in g.

pendent of body mass, the total content of these molecules scaled with size with exponents significantly lower than 1. On the other hand, glycogen concentration decreased significantly with increasing body mass, causing the slope of total glycogen reserves to be weaker than that of the total lipid and protein contents.

The specific activity of CCO and CS did not counterbalance the diminishing relative mass of the digestive gland with increasing body size. Total CCO and CS activity in this organ, therefore, had b values lower than 1, similar to the slope observed for the mass of the digestive gland.

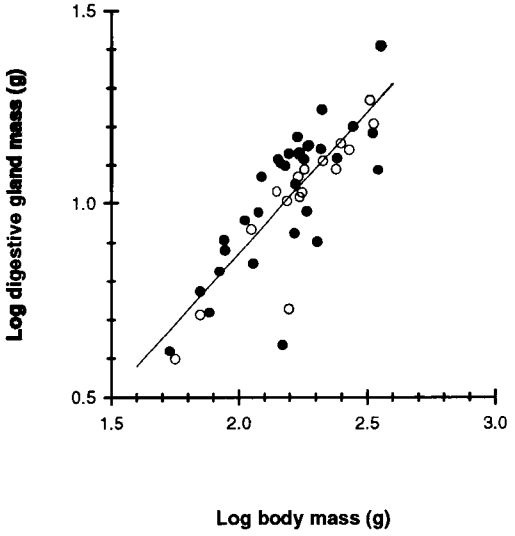

Fig. 3. *Chionoecetes opilio.* Total mass of digestive gland for adult (open circles) and adolescent (closed circles) males, versus body mass. Equation is given in Table 3.

Discussion

Effect of Morphometric Maturity

The higher aerobic capacity of muscle in adult male snow crabs compared with that of adolescent males was not only due to the more voluminous merus relative to the carapace width in adults, but also to the higher specific activity of CCO and CS. The levels of mitochondrial enzymes are known to be related to the capacity for sustained muscular effort (Darnell *et al.,* 1993). These observations, and the fact that CCO and CS activities in the digestive gland were not affected by maturity, suggest that different locomotory habits of adult and adolescent snow crabs could have caused these changes of enzymatic potentials in muscle. Adult male *Chionoecetes opilio* participate in 97–98% of the mating pairs observed in the wild (Sainte-Marie and Lovrich, 1994). Claxton (1992) noted that morphometrically mature male *Chionoecetes bairdi* Rathbun display more frequent and longer precopulatory behaviors

Table 3. Regression analyses for the digestive gland of *Chionoecetes opilio,* fitting the model log Y = log a + b log M. Units for the variables are given in Table 1. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. Regressions are done on both adult and adolescent animals (U + O) according to the results of the ANCOVA analyses. Parameters are given only for allometric relationships, i.e., b significantly different from 1, for total contents, or from 0, for concentrations.

		Model parameters			H_0 test	
Variable	Maturity	Log a	b	r^2	b = 1	b = 0
Total mass	O + U	−0.572	0.722	0.66	***	***
Percentage water	O + U	1.712	0.046	0.09	***	*
Total lipid	O + U	−1.104	0.651	0.51	***	***
Total protein	O + U	0.795	0.805	0.69	*	***
Glycogen/g	O + U	1.867	−0.366	0.09	***	*
Total glycogen	O + U	1.267	0.371	0.06	**	
Total CCO	O + U	−0.015	0.702	0.63	***	***
Total CS	O + U	−0.322	0.677	0.37	*	***

(e.g., high-on-legs behavior, grasping, bouncing, and body-lifting of females), and generally out-compete immature males in agonistic encounters. The term "morphometrically mature" utilized in Claxton's study corresponds to our use of the term "adult," and "immature" corresponds to "adolescent." According to Hartnoll (1969), brachyuran males carry a premolt female throughout a prolonged period. Paul and Paul (1996) indicated that small male *C. bairdi* have greater difficulty maneuvering multiparous females, compared to large males. Precopulatory relationships have been reported to last up to two months (Ennis *et al.,* 1985), but durations of 1–12 days seem to be more common (Watson, 1972; Donaldson and Adams, 1989; Claxton, 1992). Qualitative observations in the wild suggest that adult male *C. opilio* are more mobile than immatures and adolescents (Conan, personal communication). Sainte-Marie and Hazel (1992) showed that migration among depth strata is much more common in morphometrically mature than in immature males. Hines *et al.* (1995) used telemetry techniques to compare the movement patterns in immature and adult *Callinectes sapidus* Rathbun and *Maja squinado* (Herbst). They observed that, in both species, adults move over significantly longer distances than immatures. We propose that the higher CCO and CS capacities in adults may be necessary to support the sustained muscular effort associated with precopulatory behavior and more active locomotion. Adult and adolescent males may also differ by their activity levels during migration. Unfortunately, no quantitative data concerning frequency and duration of locomotion by adult and adolescent snow crabs during migration are available.

Anaerobic metabolism is related to brief and intense muscular effort (Somero and Childress, 1980; Goolish, 1991). The higher anaerobic capacity in adults suggests that an adult male may have a greater capacity to carry out bursts of locomotion than an adolescent of similar body mass. Once again, there are no available data concerning the speeds that can be attained by male *C. opilio* differing in maturity, although Hines *et al.* (1995) noted that in *C. sapidus* and *M. squinado* the mean maximum speed and mean speed of adults are at least twice those of immature crabs. Information on the changes in the various aspects of locomotion of snow crabs at the different stages of maturity is needed to interpret our results more thoroughly.

Shifts in the energetic reserves and protein content of muscle from adults and adolescents did not accompany the dissimilarities noted for the mitochondrial and glycolytic enzyme activities. However, we cannot conclude that maturity has no effect on these molecules, since we measured the final concentration of glycogen, lipid, and proteins resulting from anabolism and catabolism. Moreover, glycogen concentration is likely to have varied considerably among individuals, because some of them struggled considerably to avoid capture, prior to dissection.

Male snow crabs, having completed a terminal molt, rapidly increase their flesh content until they fill their new larger carapace. This process takes place mostly between day 25 and day 60 following molting, as has been shown in the course of another experiment.

During this period, the percentage of merus volume filled with wet flesh has been estimated to increase from 67.9% ± 6.2 to 86.0% ± 6.0 (Mayrand *et al.*, unpublished data). We can safely assume that flesh production is as fast in newly molted adolescent crabs as it is in adults. Once the process of rapid flesh growth is completed, further growth in adolescent crabs is possible only through another molt cycle. This may explain why, in the present study, the indicators of potential rate of protein synthesis (RNA:DNA and protein:RNA) did not differ among adult and adolescent males, all of which were in a late intermolt stage (108.6 ± 43.0 days postmolt). Since flesh growth was essentially completed at this period of the molt cycle, the potential for protein synthesis was probably used only for the needs for protein turnover. However, it is possible that rates of protein synthesis may vary in earlier molting stages between the two groups, such as postmolt stages A and B or early intermolt.

Effects of Size

Allometric effects do not appear to affect all sections of aerobic metabolism in a uniform fashion, since the total activity of CCO in merus muscle scaled isometrically with body mass, while that of CS scaled with b values inferior to 1. For a given ectothermic species, CS activity generally scales allometrically with body mass, with b values falling between 0.7 and 0.9, in equations using total enzyme activity, or with b values ranging from –0.30 to –0.10, in equations using weight-specific activity. Such allometric relationships were reported for total CS activity in *Artemia franciscana* (Kellogg) (see Berges *et al.*, 1990, 1993) and other aquatic crustaceans (Berges and Ballantyne, 1991), although the scaling factors did not significantly differ from 1 in the last study, and for specific CS activity in fish muscle (Somero and Childress, 1980, 1990; Pelletier *et al.*, 1993a) and in fish heart (Ewart *et al.*, 1988). Fewer studies have been concerned with CCO capacity. Pelletier *et al.* (1993a) noted a significant inverse relationship between CCO specific activity in muscle and body length in *Gadus morhua* L., but Goolish and Adelman (1988) noted that CCO specific activity in muscle is independent of body mass in cyprinids. Our results indicate that the relative levels of CS to CCO in muscle decrease as the size of the animals increases. In their study on the effect of thermal acclimation on muscle metabolic capacities in rainbow trout, Guderley and Gawlicka (1992) also described changes in the relative levels of CCO, CS, and β-hydroxyacyl CoA dehydrogenase (HOAD) in muscle with cold acclimation. A reorganization of mitochondrial capacities, rather than a change in mitochondrial abundance, occurs during cold acclimation of trout. Such adjustments seem to occur with a change in size in *C. opilio*. Since the total capacity of muscle CCO scaled isometrically with body mass, our results do not support the hypothesis that oxygen uptake and transport constraints, due to a diminished surface/volume ratio in large individuals, are the source of the negative allometry generally observed for the aerobic metabolism (reviewed by Schmidt-Nielsen, 1984). Weymouth *et al.* (1944) pointed out that the factors controlling the oxygen consumption of an animal are not likely to be a function of body surface. These authors computed the equations relating oxygen consumption and body mass for the kelp crab *Pugettia producta* (Randall) and, interspecifically, for various crustaceans. In both cases, the exponents were significantly different from 0.67, which is the expected value if the factors are affected by the surface/volume ratio.

An alternative hypothesis proposes that small individuals could have a higher metabolic rate than large ones, since feeding and excretion rates are highest in the smallest animals (e.g., Lane and Lawrence, 1979). This would probably lead tissue growth rates to be higher in smaller animals. Mathers *et al.* (1992) observed a diminution of the RNA concentration per g with increasing size in an open-sea fish species. The present study does not contradict this hypothesis, since the activity of protein synthesis, estimated by the protein:RNA ratio, tends to decrease with increasing body mass, although not significantly ($P = 0.09$).

A third mechanism has been proposed to explain the negative allometry of the aerobic capacity with body size (Schmidt-Nielsen, 1984; Calder, 1984). According to this hypothesis, a decrease in the relative mass of the metabolically most active organs, such as kidneys, heart, brain, and splanchnic organs, could be involved. However, in crustaceans, the relative mass of the metabolically inac-

tive carapace decreases as body size increases (Paul and Fuji, 1989). These authors reported that Tanner crabs 9.7 mm and 82 mm in width allocate 30% and 8%, respectively, of their assimilated energy to exuviae. Hence, as a crustacean grows, its proportion of metabolically active tissues is enhanced. Despite this fact, Sanchez *et al.* (1991) noted that, in 4 out of 6 crustacean species, the oxygen consumption scaled with the exoskeleton-free body mass with exponents <1. Therefore, the oxygen consumption of crustaceans is affected more strongly by the relative decrease of the most aerobically active organs than by the relative increase of total flesh content, mainly muscle, resulting from increase in body size. In the present study, the mass of the most aerobically active digestive gland scaled with body mass to the power 0.722 in both adults and adolescent crabs, while the estimated total muscular mass scaled isometrically. Taking into account that no augmentation of the specific enzyme activities in the digestive gland compensated for the diminished relative size of this organ, the observed negative allometry of the total aerobic capacity of the digestive gland may lead to a decrease in the oxygen consumption of the whole animal.

Sanchez *et al.* (1991) suggested that allometric studies dealing with crustaceans should relate physiological variables, such as respiration rates, to the mass of metabolically active tissues, by subtracting the carapace mass from the total body mass. This can be done by dissecting out all the flesh content, which is a very difficult task with crabs, or by measuring the dry mass and the ash-free dry mass of the whole animal, which does not allow enzymatic assays. If only the metabolically active tissues had been taken into account in the present study, the slopes of the relationships would have been steeper.

In *C. opilio,* the estimated total glycolytic capacity of muscle scaled isometrically with body mass, in contrast with the data reported for fish species. Ewart *et al.* (1988) and Sullivan and Somero (1983) reported a positive allometric relationship between specific activity of LDH in muscle and body mass or body length, in *Hemitripterus americanus* (Gmelin) and *Anoplopoma fimbria* (Pallas), respectively. On the other hand, Pelletier *et al.* (1993b) found no relationship between specific glycolytic enzyme activities in white muscle and body size of *Gadus morhua,* but the cod they studied were sampled at the end of the extended over-wintering period. At other periods of the year, following periods of greater food availability, positive relationships are found for glycolytic enzyme activities and body mass in cod (Martinez and Guderley, personal communication). Somero and Childress (1980) noticed a strong positive scaling of total LDH capacity with body length in white muscle of various fish species. For some of these species, the activity of LDH expressed per g of tissue did not change with body length, but the total capacity still scaled positively because muscle mass increased faster than body length. These authors suggested that larger fast-swimming fish need an enhanced anaerobic capacity to maintain their burst swimming speed despite the increase of friction drag with increased body surface and higher absolute speeds. Such an adjustment in LDH capacity may not be as crucial for snow crabs, which move rather slowly compared with pelagic fishes.

Since *C. opilio* feeds mainly on mollusks, small crustaceans, polychaetes, and vegetal detritus (Brêthes *et al.,* 1984, 1994), it does not need to move quickly to forage. Sainte-Marie and Hazel (1992) and Lovrich *et al.* (1995) suggested that snow crabs migrate to shallow waters during molting, their period of high vulnerability, possibly to avoid the principal predators. Hence, snow crabs do not have to rely on burst locomotion to escape cod and thorny skate. Independently of their molt stage, small immature crabs tend to stay in shallow waters while hard-shelled adult crabs return to bottoms deeper than 80 m (Sainte-Marie and Hazel, 1992). We do not know whether this spatial segregation reflects a strategy of the more vulnerable immature crabs to avoid predation and cannibalism or results rather from different environmental needs for mature and immature animals. This question is presently under study.

In conclusion, this study clearly indicated that morphometric stages affect the mitochondrial and glycolytic capacities of muscle in *C. opilio,* as was hypothesized from the different locomotory behaviors reported for morphometrically mature and immature crabs. The hypothesis that, in a slowly moving species, such as *C. opilio,* the glycolytic capacity of muscle should scale isometrically with body mass, while the oxidative capac-

ity should increase more slowly than body mass, was also supported by our study.

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