Changes in muscle of postmoult snow crabs *Chionoecetes opilio* (O. Fabricius) fed different rations

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Received 2 February 1999; received in revised form 28 June 1999; accepted 27 July 1999

Abstract

Somatic growth, muscle cell condition and metabolism, and gonadal production have been studied in male snow crabs *Chionoecetes opilio* fed 0, 0.4 and 2 g·animal⁻¹·day⁻¹ and sampled 5, 25, and 60 days after the terminal moult. Twenty-five days after moulting, muscle growth rate was low in crabs fed a high ration and negative in the other groups, and no gonadal production took place. This suggests that the energy was utilised in priority to sustain a high maintenance metabolism. Between 25 and 60 days post-moult, maintenance costs were apparently lower and more energy was directed, in decreasing order of priority, towards gonadal production, which was independent of food ration, muscle growth and reserve build-up in the digestive gland, the two latter being directly related to food ration. Starvation caused the death of merus muscle cells, as indicated by the significant decrease in DNA content, but after 60 days the size of the remaining cells (protein:DNA ratio) had increased as much as that of crabs fed a high ration. It may be a better strategy to maintain fewer cells, by using material and energy obtained from the sacrificed ones, than to keep all cells in a deficient state. Crabs fed a high ration filled the new larger exoskeleton through hypertrophy and hyperplasia. After 25 days of undernutrition, the reduced number of muscle cells relatively to the merus volume was counterbalanced by an enhanced activity of phosphofructokinase (PFK), citrate synthase (CS) and cytochrome C oxidase (CCO) per g of muscle dry weight. An inverse relationship between food ration and enzyme activity per g dry weight further attenuated the effect of food ration on the enzyme total activity in the whole merus muscle. After 60 days of starvation, the still high enzyme activity per g dry weight could no longer compensate for the continuing loss of cells and, at that time, the enzyme total activity was directly related to food ration. Our results suggest that the feeding status of wild crabs may affect their locomotor ability through effects on muscle capabilities. © 2000 Elsevier Science B.V. All rights reserved.
1. Introduction

Crustacean growth has been mainly quantified by increases in carapace size (Colby and Fonseca, 1984; Robichaud et al., 1989; Paul and Paul, 1995, 1996; Sainte-Marie et al., 1995). However, carapace enlargement is only a step in the growth trajectory. Numerous biochemical processes take place during the postmoult period, leading to changes in somatic and gonadal mass. Immediately after ecdisis, the animals have a very soft carapace and a reduced muscular mass. This is a critical time when crabs have a limited capacity to walk and eat and are vulnerable to predation (O’Halloran and O’Dor, 1988; Chang, 1995; Freire, 1996; West, 1997). How the muscle mass is increased and how the metabolism of muscle is adjusted during this period is thus of interest.

Few studies have examined physiological events in muscle of postecdysial crustaceans. Following ecdisis, muscles must grow to produce the larger forces required by the larger exoskeleton. Thus, the size and the number of muscle cells, as well as their capacity to synthesise proteins and ATP, are likely to increase during the course of the postecdysial period. Indeed, Skinner (1966) reported a marked increase in the rate of amino acid incorporation into proteins in the chela muscle of Gecarcinus lateralis, between 6 and 25 days postmoult. The addition of new sarcomeres, lengthening of existing ones, and transverse and longitudinal splitting of myofibrils have been identified as mechanisms underlying muscle growth in crustaceans (El Haj et al., 1984; El Haj and Whiteley, 1997; Whiteley and El Haj, 1997; West, 1997). O’Connor (1968) reported that lipid is stored in the abdominal muscle of premoult Orconectes virilis, probably to facilitate membrane synthesis during muscle growth following ecdisis.

In nature, marine crabs may go through periods of food shortage and may strongly compete for food (Scheibling, 1984; Wolff and Cerda, 1992; Wolff and Soto, 1992; Lovrich and Sainte-Marie, 1997). Our knowledge of the effects of undernutrition on crustacean muscle growth and of the possible role of energy reserves in sustaining muscle growth is limited. Houlihan et al. (1990) reported that k, the fractional rate of protein synthesis, in leg muscle of Carcinus maenas starved for 7 days was not significantly different from that of continuously fed animals, but these authors did not take the net rate of protein synthesis into account. Wang and Stickle (1986) noted a significant decrease in RNA and DNA concentrations of whole juvenile Callinectes sapidus, in response to starvation. They argued that the decrease in DNA concentration may have resulted from a differential conservation of cells, with muscle and nerve cells being conserved and cells having a rapid turnover not being replaced during starvation. In Chasmagnathus granulata, muscle glycogen and lipids are decreased by starvation (Vinagre and DaSilva, 1992). The digestive gland is an important storage site for lipids, polysaccharides, proteins and minerals (Renaud, 1949; Passano, 1960; Kameswaramma et al., 1990). Its decrease in mass during postmoult has been related to the transfer of materials for carapace thickening, but the importance of the digestive gland reserves for
muscle growth has not been assessed. In male *C. opilio*, germinal growth and reproductive activities seem to be costly enough to divert some energy from somatic growth, as the puberty moult leads to a smaller carapace size increment than that observed in immature males (Sainte-Marie et al., 1995).

Many questions concerning the muscle growth after moulting remain unanswered. Is muscle growth done in priority or do other events such as gonadal development take place first? By which process does muscle grow: through an increase in the number of cells, in the cell size, or both? How does food availability affect muscle growth? Is there a metabolic strategy that allows crabs to carry out all the transformations required in the postmoult period even in conditions of prolonged undernutrition? Such information would help understanding the physiology of the moult cycle as well as the effect of food availability on the fitness of wild crabs.

In an attempt to improve our knowledge of these matters, we investigated the effect of time and food ration on somatic growth, the condition and metabolic capacity of muscle cells and gonadal production in *Chionoecetes opilio* (O. Fabricius), following moulting. We measured the DNA content, RNA:DNA ratio, protein:DNA ratio and activity of glycolytic and mitochondrial enzymes, respectively as indicators of muscle cell number, capacity for protein synthesis (McMillan and Houlihan, 1988), cell size (Barron and Adelman, 1984) and capacity for the glycolytic and oxidative production of ATP. Tissue growth was followed by assessing the changes in muscle content of the merus (dry weight/volume) over the experimental period.

2. Materials and methods

2.1. Animals

Eighty seven male snow crabs were captured in the St. Lawrence Estuary, in September 1993. Animals with similar carapace width (mean width 54.1 ± 3.2 mm) were selected to avoid a size effect on the biochemical variables. Indeed, body mass and maturity have been shown to affect the metabolic capacity of muscle in *Chionoecetes opilio* (Mayrand et al., 1998). The crabs were held in 1.2 m × 1.8 m tanks, with a water depth of 0.35 m at the drain level. The tanks were supplied with natural seawater filtered by a flow-through system removing particles down to 20 μm. The temperature and salinity conditions were kept constant at 2°C and 30 ppt. The animals were fed *ad lib.* with frozen shrimp and capelin, twice a week, until they moulted. Moulting began on December 10, 1993 and ended on February 21, 1994, with a survival rate of 85%. Fifty-four of the 74 crabs having successfully moulted were adult, as determined by the chela height to carapace width ratio (Conan and Comeau, 1986), while 20 were still adolescents. Following the terminology proposed by Sainte-Marie et al. (1995), adult males are defined as crabs with spermatophores and differentiated chelae, and adolescents as crabs with spermatophores and undifferentiated chelae. The mean carapace width for adults was 66.2 ± 3.1 mm. The adult crabs were individually tagged and utilised in the following experiments.
2.2. Experimental conditions

As soon as they moulted, newly adult crabs were transferred to the experimental tanks. Tanks and experimental conditions were similar to those described in the previous section, except for the temperature which was 3°C. Recently moulted individuals were held in baskets floating in the experimental tanks for the first few days, to avoid cannibalism by crabs whose shell had already hardened. Crabs in tank A were not fed. Those in tank B were fed with 0.4 g \( \cdot \) animal\(^{-1} \cdot \) d\(^{-1} \) of frozen shrimp and capelin, which represents about 20% of the quantity of food ingested by crabs fed \( ad \ lib \). This is about 0.35% of the crab’s live weight and is similar to the amount of food consumed daily by wild \( C. opilio \) (Bréthes et al., 1984). Crabs in tank C were fed with 2 g \( \cdot \) animal\(^{-1} \cdot \) d\(^{-1} \), which approximates the quantity of food ingested by crabs fed \( ad \ lib \). In the following text, these food rations will be referred to as 0%, 20% and 100%. The food was distributed to the animals twice a week and, before each feeding, the shrimp and capelin remaining from the preceding feeding were retrieved and weighed. This procedure allowed us to evaluate the mean quantity of food that was ingested by the animals during the course of the experiment.

2.3. Dissection

Ten unfed animals were sampled 5 days after they had moulted, to assess the initial status of the animals. Twenty-one crabs which had had access to food rations of 0%, 20%, or 100% were sampled 25 days after moultling. Twenty-three more crabs were sampled 60 days after moultting. The number of crabs in the experimental groups ranged from 6 to 10.

Carapace width, chela height and body mass were measured. To estimate merus volume, we cut out the merus of the first left walking leg and slowly slid it into a 50 ml graduated cylinder filled with 30 ml of sea water, taking care that no air bubbles escaped from the merus before measuring the final volume. All the walking legs were cut open and muscles were removed. The total mass of wet muscle in the merus of the first left walking leg was noted. Muscles were then chopped and mixed together before sub-samples of approximately 1 g each were taken for determination of enzyme activities, nucleic acid concentrations, total protein, and water content. The sub-samples for biochemical analyses were immediately frozen in liquid nitrogen and later transferred to \( -80°C \). The digestive gland and gonads were dissected, drained and weighed. Intratissular water content in muscle and the digestive gland was measured as weight lost by drying at 60°C during 48 h. Since the gonads of many animals were too small to determine their dry weight, the wet weight was used.

2.4. Biochemical analyses

Nucleic acid concentrations were determined by fluorimetry, using thiazole orange (courtesy of Molecular Probes, Eugene, Oregon, U.S.A.) and RNAses, and the total protein content was assessed by the method of Bradford (1976), as described by Mayrand et al. (1998).
Approximately 30 mg of frozen muscle were homogenised in an ice cold buffer solution of imidazole 50 mM, MgCl₂ 2 mM, Na₂EDTA 5 mM, Triton 0.1% and reduced glutathione 1 mM in glycerol 40% (V/V), at pH 7.5, with a dilution 1:10 (W/V). The homogenates were then centrifuged at 1000 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. Citrate synthase (CS, E.C.4.1.3.7), cytochrome C oxidase (CCO, E.C.1.9.3.1), phosphofructokinase (PFK, E.C.2.7.1.11) and lactate dehydrogenase (LDH, E.C.1.1.1.27) were measured.

The DNA content and enzyme activity (international units: μmole of substrate converted to product per minute) were expressed per ml of merus. This form of expression was chosen because it corrects for the slight differences in merus volume among the animals and gives an indication of the DNA content or the enzyme activity in the whole merus muscle. This is interesting for interpreting the data from a mechanical point of view, as the number of cells per merus, the enzyme activity per cell and the extra- and intra-tissular water content are taken into account. Enzyme activity was also expressed per g of dry muscle as an indicator of the tissular metabolic capacity, independent of the variations in intra-tissular water content.

2.5. Growth rate

Muscle content in merus (MC) was estimated as the dry weight (mg) of total muscle tissue in the merus of the first left walking leg divided by the volume of this merus (ml). The growth rate (G) was determined for each crab by one of the following equations, depending on the time they were sacrificed after moulting:

\[
G_{\text{day 25-5}} = \frac{\text{Individual MC}_{\text{day 25 at ration } X} - \text{mean MC}_{\text{day 5 at ration 0%}}}{\text{Number of days}}
\]

\[
G_{\text{day 60-25}} = \frac{\text{Individual MC}_{\text{day 60 at ration } X} - \text{mean MC}_{\text{day 25 at ration } X}}{\text{Number of days}}
\]

Forty-four growth rates were obtained.

2.6. Statistical analysis

Normality and homoscedasticity were tested using Lilliefors’ and Bartlett’s tests, respectively. To meet these requirements, all variates, except G, had to be log transformed or square root transformed (DNA·ml merus⁻¹). Time (25 and 60 days) and food ration (0, 20, and 100%) effects, as well as the interaction of these factors, were tested by 2-way ANOVAs followed by a posteriori Tukey tests. T-tests were run to compare experimental values to those obtained on day 5. The significance level was 0.05.
3. Results

3.1. Food consumption

The crabs began feeding soon after moulting (Fig. 1). Ten days after moulting, the animals fed with a 20% ration were eating the entire ration, i.e. 0.4 g·animal$^{-1}$·day$^{-1}$. By day 15, the crabs fed with a 100% ration occasionally ate all the available food, i.e. 2 g·animal$^{-1}$·day$^{-1}$. By day 30, the maximal amount of food was consistently consumed.

3.2. Growth rate

Somatic growth rate ranged from $-0.732$ to $0.911$ mg dry flesh·ml merus$^{-1}$·d$^{-1}$. Food ration and time significantly affected growth rate (Table 1 and Fig. 2). The growth rate of starved animals was negative in both periods, with a greater daily loss of muscle occurring between day 5 and 25. In contrast, animals fed with a 100% ration had positive growth rates in both periods with faster growth rates between day 25 and 60. Snow crab fed with a 20% ration exhibited a slight loss in muscle content from day 5 to 25 and a slight increase from day 25 to 60.
Table 1  
*F* values from ANOVAs examining the effect of food ration and time on growth rate and physiological measurements in snow crab.  

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Factors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Food</td>
<td>Time</td>
</tr>
<tr>
<td>Growth rate</td>
<td>43.4***</td>
<td>18.9***</td>
</tr>
<tr>
<td>DNA·ml merus⁻¹</td>
<td>20.7***</td>
<td>0.0</td>
</tr>
<tr>
<td>Protein:DNA</td>
<td>0.7</td>
<td>24.3***</td>
</tr>
<tr>
<td>RNA:DNA</td>
<td>4.0*</td>
<td>14.5***</td>
</tr>
<tr>
<td>Digestive gland dry weight</td>
<td>81.0***</td>
<td>15.6***</td>
</tr>
<tr>
<td>Gonads wet weight</td>
<td>1.7</td>
<td>48.3***</td>
</tr>
<tr>
<td>PFK·ml merus⁻¹</td>
<td>10.9***</td>
<td>4.0*</td>
</tr>
<tr>
<td>PFK·g dry weight⁻¹</td>
<td>3.11†</td>
<td>0.17</td>
</tr>
<tr>
<td>LDH·ml merus⁻¹</td>
<td>6.6**</td>
<td>0.8</td>
</tr>
<tr>
<td>LDH·g dry weight⁻¹</td>
<td>3.06†</td>
<td>4.62*</td>
</tr>
<tr>
<td>CS·ml merus⁻¹</td>
<td>7.8***</td>
<td>5.1*</td>
</tr>
<tr>
<td>CS·g dry weight⁻¹</td>
<td>4.30*</td>
<td>0.73</td>
</tr>
<tr>
<td>CCO·ml merus⁻¹</td>
<td>3.9*</td>
<td>0.3</td>
</tr>
<tr>
<td>CCO·g dry weight⁻¹</td>
<td>6.61***</td>
<td>4.16*</td>
</tr>
</tbody>
</table>

*F* values are given. *** *p*<0.001, ** *p*<0.01, * *p*<0.05, † *p*<0.06. Variates were log transformed except for growth rate (not transformed) and DNA·ml merus⁻¹ (square root transformed). The results of Tukey’s *a posteriori* comparisons are given in the corresponding figures, except for enzyme activities which are given in the Results section.

### 3.3. Digestive gland and gonads

The total digestive gland dry weight was significantly affected by food ration and time, as well as by the interaction of these factors (Table 1, Fig. 3). At day 25, the digestive gland dry weight gradually increased with food ration, while at day 60 there was no significant difference between food ration 0 and 20%. In crabs starved or fed a low ration, the digestive gland weight decreased from day 25 to day 60, while it rose in crabs fed 100%. The digestive gland was heavier in the reference group than in crabs starved or fed a low ration for 25 and 60 days. Only the animals fed a 100% ration for 60 days had a heavier digestive gland than the reference group (*t*-test).

The gonad wet weight did not change with feeding status (Table 1, Fig. 4). Our results clearly indicate that, under these experimental conditions, this variable increased from day 25 to 60, whether the animals were fed or not. All the experimental groups sampled at day 60 had a significantly higher gonad wet weight than the reference group.

### 3.4. Muscle cells

Muscle cell abundance and cell size were affected by food ration and time in different ways. Cell abundance, estimated by the DNA content per ml of merus (Table 1 and Fig. 5), was significantly higher at day 25 than at day 60 in starved animals, but the reverse was true in crabs fed with a 100% ration. A significant effect of food ration was noted only at day 60, at which time the DNA content increased from food ration 0% to food
ration 100%. The DNA content in the reference group was lower than that in the group fed 100% for 60 days and higher than that in the group starved for 60 days. The size of cells, expressed as the protein:DNA ratio, varied only with time (Table 1, Fig. 6). Starved as well as fed crabs had higher protein:DNA ratios at day 60 than at day 25. While the protein:DNA ratio remained quite constant from day 5 to day 25, it had risen sharply by day 60, although only the value for crabs fed 100% was significantly higher than that in reference crabs.

The RNA:DNA ratio, an index of the cellular machinery available for protein synthesis, was greater at day 60 than at day 25 (Table 1, Fig. 7). Food ration also had a significant effect: the RNA:DNA ratio was higher in unfed crabs than in those fed 20%. By day 60, all the experimental groups had a higher RNA:DNA ratio than the reference group.

3.5. Enzymatic capacity

The metabolic capacity of the whole merus muscle, as represented by enzyme activity per ml of merus, increased with food ration either at day 25 and 60 (LDH and CS) or only at day 60 (PKF and CCO), as shown in Table 1 and Fig. 8a, c, e, g. In contrast, the activity of the four enzymes, expressed per g of dry weight, was inversely related to food ration (Table 1 and Fig. 8b, d, f, h), although the effect of food ration on PKF and LDH was only marginally significant ($p=0.059$ and 0.056 respectively).
Fig. 3. Effects of food ration and time on the digestive gland dry weight of adult male snow crab. At ration 0%: day 25 > day 60; at ration 20%: day 25 > day 60. At day 25: ration 0% < 20% < 100%; at day 60: (ration 0% = 20%) < 100% (Tukey’s test). Experimental group differs from the day 5 group: * p < 0.05, ** p < 0.01, *** p < 0.001 (t-test).

Time did not have such so strong an effect as food ration since only the CS activity per ml of merus, for food rations 20% and 100%, and that of PFK, for the 100% ration, were lower at day 25 than at day 60. On the other hand, CCO and LDH activities per g of dry weight were higher at day 25 than at day 60 (Table 1 and Fig. 8).

Significant differences were also noted between the reference and some of the experimental groups. After 60 days of starvation, the glycolytic (PFK and LDH) and mitochondrial (CCO) capacities per ml of merus were weaker than they were 5 days after molting. On the contrary, 60 days after molting, crabs fed 100% showed higher PFK and CS activities per ml of merus than the reference group. The LDH and CCO activity per g of dry weight were lower in the groups fed a 20% and a 100% ration for 60 days than in the reference group. The LDH activity in crabs fed 100% for 25 days and the CCO activity in crabs fed 20% for 25 days were also lower.

4. Discussion

4.1. Allocation of energy

Our results suggest that the allocation of energy to different physiological components
Fig. 4. Effects of food ration and time on gonad wet weight of adult male snow crab. Time: day 25<day 60 (Tukey’s test). Experimental group differs from the day 5 group: * $p<0.05$, *** $p<0.001$ ($t$-test).

varies with the time elapsed since moulting. During the first weeks following ecdysis (day 5 to 25), the energy expenditure for maintenance metabolism seemed to be high, as somatic and gonadal production were at their lowest (Figs. 2 and 4). Unfed crabs and crabs fed a low ration intensively mobilised the reserves from the digestive gland, as shown by the pronounced diminution in its dry weight (Fig. 3). The digestive gland of crustaceans is the primary organ for reserve storage. These reserves can be drawn upon whenever the energetic demands exceed the input of energy (Passano, 1960; Heath and Barnes, 1970). The role of this organ is particularly important during post-moult. For example, the chitin which is used to build the new endocuticle is mainly derived from the glycogen stock of the digestive gland (Vonk, 1960). Variations in the size of the digestive gland, as observed in our study, would thus reflect the equilibrium between energy expenditure and input. Unfed crabs and crabs fed a low ration also drew energy from the muscle, as the negative growth rate of muscle in the merus suggests. In crustacean muscle, glycogen and lipids are generally generally mobilised first when energy is needed, but proteins can also be catabolised (Vinagre and DaSilva, 1992). O’Connor (1968) reported that lipid is stored in the abdominal muscle of premoultng Orontectes virilis and is probably utilised during postmoult. At 3°C, crabs fed a high ration obtained enough energy from food to sustain the metabolic demands, since they did not mobilise reserves from the digestive gland and had a positive muscle growth rate.
In contrast, during the period from day 25 to 60, the energy demands for the maintenance metabolism seemed to be lower than during the weeks that immediately followed molting. Indeed, growth rate was higher (Fig. 2), reserves were stored in the digestive gland of crabs fed a high ration (Fig. 3) and gonadal production took place in all groups including the unfed crabs (Fig. 4). Why the animals fed with a 20% ration relied more heavily on the digestive gland reserves during the second experimental period while they could have used this source of energy more extensively during the first experimental period and thus have spared muscle tissue is not clear.

The differences in tissue production among the two experimental periods may have resulted from unequal food intake, but other factors may be involved. While crabs fed a high ration showed a lower rate of food consumption from day 5 to 25 than from day 25 to 60, the feeding rate of crabs fed a low ration was quite constant throughout the experiment (Fig. 1). During the first three weeks following molting, energy may have been allocated in priority to molecular and tissular reorganisation. It is well known that ionic regulation is intensified in postmoult crabs (Drilhon, 1935; Passano, 1960; Skinner, 1962, 1985; Mangum, 1992). O’Halloran and O’Dor’s (1988) examination of the maxillipeds internal structure revealed that tissues are disorganised in the first days following molting and subsequently undergo a gradual reorganisation. The post-moult period is considered as a period of progressive formation of tegument (Shafer et al.,
1995), during which endocuticle thickness can increase by 7 μm a day (Skinner, 1962, 1985). All these processes are energy consuming. A temporal sequence of events thus seems to exist after ecdysis. During the day 5 to 25 period, the energy obtained from reserves and/or food would be allocated in priority to carapace thickening and tissular reorganisation, leaving less energy for muscle growth. During the day 25 to 60 period, the decreasing order of priority would be: (1) gonadal production, (2) muscle growth if a minimal amount of food is available, and (3) accumulation of reserves in the digestive gland if the animals have access to a high ration.

The fact that gonadal development was independent of food ration (Fig. 4) suggests that the onset of this event is chronologically determined. The costs of seminal plasma production and spermatogenesis are not negligible in crustaceans and probably lead to competition between somatic and gonadal production, as it has been pointed out by Adiyodi (1985), Wolff and Soto (1992) and Sainte-Marie et al. (1995). Gonadal development in starved crabs would thus divert more energy away from the already impaired somatic growth. Our results suggest that, in the wild, the reproductive capacity of male snow crabs should not be strongly affected by the nutritional status of the animals, at least at 3°C. We used the equation obtained by Sainte-Marie et al. (1995) for wild *C. opilio* males in the Gulf of St. Lawrence to compute the expected wet weight of the gonads of “adult clean-soft males” of 66.2 mm of carapace width, which was the mean size of the animals in our study. The clean-soft quality applies to crabs having moulted 0 to 5 months ago. The expected value was higher (0.90 g) than that measured in crabs fed a 100% ration for 60 days (0.49 g±0.08). Considering that many of the wild adult clean-soft crabs had probably moulted more than 60 days ago, we can infer that
wild crabs and experimental ones have comparable gonad weight, even if wild crabs have presumably access to less food.

4.2. Muscle cell condition

Our results clearly demonstrate that muscle cells are lost under conditions of prolonged starvation, as DNA·ml merus$^{-1}$ decreased in crabs starved for 60 days. Despite this loss of cells, by day 60 the volume of the remaining cells had augmented as much as that of fed crabs, as shown by the rise in protein:DNA ratio (Fig. 6). According to Koumans et al. (1993), changes in protein:DNA ratio reflect modifications in the ratio of sarcoplasmic to nuclear volume. This increased net protein production may be ascribed to an enhanced cellular machinery for protein synthesis, since the RNA:DNA ratio was highest at day 60 for all food rations (Fig. 7). Actin mRNA reaches elevated levels in claw and leg muscles of crabs and crayfish in the postmoult stage, but does not vary significantly over the moult cycle of Homarus americanus (reviewed by West, 1997 and Whiteley and El Haj, 1997). Whether the increase in protein synthesis rate during postmoult occurred at the level of translation or at the level of transcription thus seems to depend on the species (Whiteley and El Haj, 1997). The preceding observations suggest that, in starved crabs, the molecular constituents and energy from the sacrificed

![Graph showing RNA:DNA ratio over time and food ration](image_url)
Fig. 8. Effects of food ration and time on enzyme activity in merus muscle of adult male snow crab. Filled bars: unfed crabs, hatched bars: crabs fed a 20% ration, empty bars: crabs fed a 100% ration. (a) PFK activity per ml of merus, (b) PFK activity per g of dry weight, (c) LDH activity per ml of merus, (d) LDH activity per g of dry weight, (e) CS activity per ml of merus, (f) CS activity per g of dry weight, (g) CCO activity per ml of merus, (h) CCO activity per g of dry weight. Results of Tukey’s tests are given in the Results section. Experimental group differs from the day 5 group: * \( p < 0.05 \), ** \( p < 0.01 \) (t-test).
cells may have been transferred to the remaining ones. It may have been physiologically more important for the animals facing a severe food shortage to maintain fewer normal muscle cells rather than a large number of deficient ones. This hypothesis contrasts with the general idea that crustacean muscle cells should be conserved during starvation to allow rapid recovery when food becomes available (Wang and Stickle, 1986).

The changes in DNA/ml merus \(^{-1}\) that were observed in the present study (Fig. 5) may reflect changes in muscle fibers and/or in myosatellite cell number. In vertebrates, striated muscular tissue is composed of multinucleated fibers and of myosatellite cells, which are small spindle-shaped cells lacking sarcomeres (Campion, 1984). Myosatellite cells are thought to be responsible for the addition of nuclei to existing muscle fibers and for the formation of new fibers (Akster, 1983; Campion, 1984; Koumans et al., 1994; Akster et al., 1995; Matschak and Stickland, 1995). The few studies on crustaceans indicate that myosatellite cells are also present in this group (Midsukami, 1981; Skinner, 1985; Novotova and Uhrlik, 1992; Pearce et al., 1997). Thus, we can hypothesise that myosatellite cells play a key role in supporting the considerable growth of muscle after molting.

The increased protein:DNA ratio and DNA content per ml of merus observed in crabs fed 100% during 60 days suggest that both hypertrophy and hyperplasia help fill the larger exoskeleton following molting. For snow crabs of similar size to those we studied, molting leads to an increase of 25.0\(\pm\)6.9 in carapace width (unpublished data), which is a considerable augmentation in volume (width increases by a factor of 1.25\(^3\) while volume increases by a factor of 1.25\(^4\)). Muscle fibers have to lengthen to fill the greater distance between the apodeme and the cuticle and also have to fill a wider space. Crustacean muscle fibres can grow in width by the addition of new myofibrils and by longitudinal splitting and enlargement of existing myofibrils. They can elongate by serial addition of sarcomeres and by transverse splitting of sarcomeres (Govind, 1982; El Haj et al., 1984; El Haj and Whiteley, 1997; West, 1997). The hypothesis of growth by the addition or enlargement of myofibrils is supported by the rise in protein:DNA ratio, which was observed at day 60 independently of food ration. However, the maximal volume of individual muscle fibres is probably limited since, at day 60, the protein:DNA ratio reached what seems its maximal value (Fig. 6). The production of new fibres through fusion of myosatellite daughter cells was thus probably necessary to fill the remaining space, as suggested by the rise in DNA content in crabs fed with a 100% ration during 60 days (Fig. 5).

Our results suggest that there is a general tendency to compensate for a reduced number of muscle cells, relative to the size of the merus, by having high glycolytic and mitochondrial tissular capacities. Such a relative reduced number of cells occurred either because muscle growth had not begun to fill the new larger carapace, as in the reference group, or because some of the cells had died, as in starved crabs. These groups generally had higher enzyme activities per g of dry muscle (Fig. 8b, d, f, h) than crabs in pre-moulst stages \(D_2\) to \(D_4\) fed \textit{ad lib.} and kept in similar conditions of temperature and salinity. In pre-moulst animals, the activity per g of dry weight was 23.78 units\(\pm\)6.38 for PFK, 110.99\(\pm\)27.38 for LDH, 8.81\(\pm\)1.35 for CS, and 6.28\(\pm\)1.63 for CCO (unpublished data). The enhanced activity per g of dry weight in post-ecdysial crabs, joined to the inverse relationship between enzyme activities and food ration (Table 1 and Fig. 8b, d, f,
h), counterbalanced the relatively low number of muscle cells in the merus of starved crabs and crabs fed a 20% ration. This lead to an attenuation of the effect of food ration on the enzyme total capacities in the merus muscle during the 25 days immediately following ecdysis, as shown by the enzyme activity per ml of merus (Fig. 8a, c, e, g).

Sixty days after moulting, the compensatory effect described above was no longer sufficient to counterbalance the continuing loss of muscle cells in starved crabs, and the effect of food ration on the enzyme activities per ml of merus became more pronounced (Fig. 8a, c, e, g). The enzyme total activity in muscle of fasting crabs no longer compared with that of fed animals, the loss of cells being too important to be compensated for by the high enzyme activity per g of dry muscle. Consequently, the total PFK, LDH and CCO capacities in merus muscle of starved crabs, as represented by the activity per ml of merus, fell below the values observed at day 5. In contrast, animals fed a 100% ration had an enhanced total metabolic capacity in the merus muscle, PFK and CS activities per ml of merus reaching higher values than those in the reference group (Fig. 8a, e). This was achieved by the addition of muscle cells rather than an increased specific tissular activity, since the enzyme activity per g of dry weight tended to return to lower values (Fig. 8b, d, f, h). This decrease in the tissue metabolic capacity with an increasing food ration may be related to a dilution effect by the storage of energy reserves in muscle of fed crabs and/or to the fact that these animals no more needed to maintain a high specific metabolic capacity since the muscle cells were now abundant. Our observations contrast with those reported for fish. A positive relationship between LDH and PFK activity per g of wet muscle and food consumption has been noted in sablefish (Sullivan and Somero, 1983), barred sand bass (Lowery et al., 1987), and cod (Dutil et al., 1998). For comparative purposes, we expressed the enzyme activities per g of wet weight, but we still found that enzyme activities were either unrelated (PFK, LDH, CS), or inversely related (CCO) to food ration, despite the fact that water % was lower in crabs fed a high ration than in starved ones (82.01±0.74 and 84.41±0.50, respectively). Enzyme adjustments which counterbalance a loss of cells thus seem to be a particularity of crustaceans.

The feeding status of wild crabs is likely to affect their locomotor ability via modifications of muscle capacities. Since well fed crabs had a higher metabolic capacity in the whole merus muscle and probably had a larger cross sectional area of muscle due to the more numerous cells, they may have a better locomotor ability than those starved for 60 days. Thus, the nutritional state of wild crabs would modify their fitness. Well fed crabs would forage more efficiently than undernourished ones, and would thus obtain more food. Such a positive feedback has been proposed for fish (Sullivan and Somero, 1983; Pelletier et al., 1993). The fact that snow crab kept at 3°C began feeding as early as four or five days after moulting (Fig. 1) is in contrast with the observations of O’Halloran and O’Dor (1988) who report that C. opilio held at 8°C did not eat during the first two weeks following ecdysis. This indicates that, in nature, recently moulted C. opilio may have the physical ability to eat earlier than what is generally believed, although we cannot infer that they have the opportunity to do so. In fact, wild crabs have to search for their food and must be able to escape predators. Those activities require locomotion and, again, the positive feedback suggested above would play a determinant role.
In conclusion, some of the events that follow moulting in *C. opilio*, such as gonadal development and net protein synthesis in muscle cells, seem to be chronologically determined rather than to be influenced by the feeding status of the animals. Others, such as the production of new muscle cells, changes in glycolytic and mitochondrial capacities in the merus muscle, and the reserve build-up in the digestive gland appear to be affected by both time and food ration. Cellular and metabolic adjustments may counterbalance the effect of the feeding status on the whole merus muscle metabolic capacity.

Acknowledgements

We are grateful to Mr. Mario Peloquin and Mrs. Claudie Vigneault for their technical help. This work was supported by funds from the Université Laval, from the Ministère des Pêches et des Oceans, Canada, and from the Natural Sciences and Engineering Research council, Canada.

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