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Role of lipid, protein, carbohydrate and nucleic acid as indicators

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INTRODUCTION Growth in crustaceans is a discontinuous process consisting of consecutive stages, distinguished to anatomic level (Drach & Tchernigovtveff, 1967), which constitute the moult cycle. This study attempts to describe the moult process in this species, according to the main biochemical changes in the different tissues (muscle and hepatopancreas), to complement the existing information at anatomical level.

METHODOLOGY **Samples**.- Adult females of *Nephrops norvegicus* in intermoult stage were obtained from a commercial fishing ground in the Mediterranean Sea, located 6 miles offshore Barcelona harbour and 300-600 meters deep. Only individuals in stage C (intermoult) were analyzed after being collected. They were maintained in tanks, under controlled temperature (13±1 ºC) and salinity (36±1 psu) conditions, until they reached a selected moult stage. A total of 50 females were analyzed, 10 by each moult stage (Fig. 1).

Clasification.- Moult stages were selected by anatomical criteria (according to Sardà, 1983) as shown in Fig. 1. For biochemical analysis five stages were used: stages D_0 and D_3 (preecdysis), stages A and B (postecdysis) and stage C (intermoult) (Fig. 1).

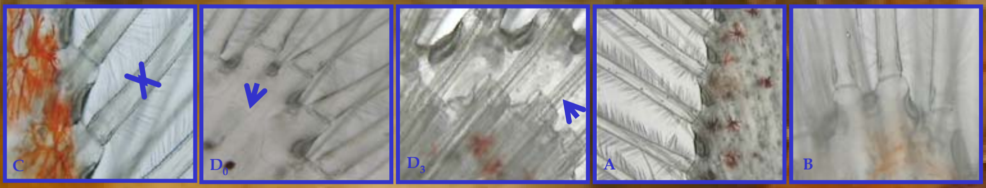

Figure 1. The characteristic pleopod setogenesis of *Nephrops norvegicus* for each moult stage.
Stage C : central nerve is clearly observed.
Stage D_0 : epidermal retraction is observed.
Stage D_3 : the fully formed setae are observed.
Stage A : matrix occupies the whole seta.
Stage B : matrix constriction starts.

Biochemical analysis.- Freeze-dried hepatopancreas and muscle samples were used to determinate elemental composition (CNH), lipids (Bligh & Dyer, 1959) and nucleic acids (Clemmesen, 1988 and 1993). The conversion factor 6.25 (FAO/WHO, 1973) was used for protein quantification from nitrogen obtained in elemental analysis. Carbohydrates were estimated as the difference of weight between the total sum of the analyzed fractions and the total sample.

RESULTS Lipid content in hepatopancreas increased along the premoult period (stages D_0 and D_3; Fig. 2) to cover the increase in energetic requirements in later stages. This increase in requirements results from starvation from stage D_3 until the end of postmoult period, altogether with the formation of the new exoskeleton (Mayrand *et al.,* 2000). Both factors caused a decrease of lipid proportion along the postmoult (% dry weight; ANOVA, p<0,05) and an increase of protein proportion (% dry weight) due to the synthesis of new tissues, primarily muscle, during growth.

In stage D_0 hepatopancreas presented high values of protein:DNA ratio, which shows the increase in the number of cells in the tissue (Fig. 3), coinciding with the period of highest protein synthesis. This result is corroborated with RNA/DNA ratio, a metabolic cellular activity index (Buckley, 1984), which shows high values in stages C and D_0 (Fig. 4; ANOVA, p<0,05), during which the animal is feeding.

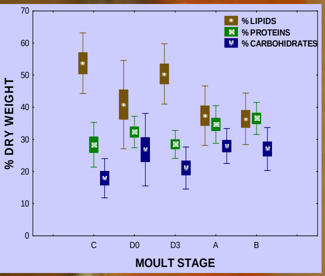

Figure 2. Mean biochemical composition (lipids, carbohydrates and proteins) in *N. norvegicus* hepatopancreas (% dry weight) in each moult stage. Box: Mean ± SE; Whisker: Mean ± 0,95*SD

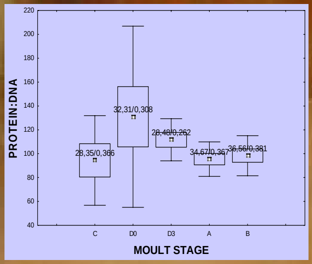

Figure 3. Mean Protein:DNA ratio values in *N. norvegicus* hepatopancreas in each moult stage.
Box: Mean± SE; Whisker: Mean ± 0,95*SD

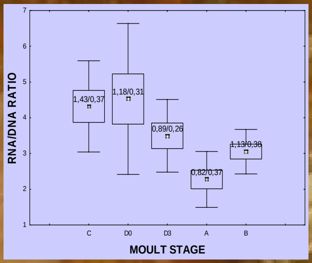

Figure 4. Mean RNA:DNA ratio values in *N. norvegicus* hepatopancreas in each moult stage.
Box: Mean ± SE; Whisker: Mean ± 0,95*SD

Growth process in muscle was observed to take place fundamentally in premoult stage, which is confirmed by an increase in protein:DNA and RNA:DNA ratios (Fig. 5 and 6). This growth is produced by an increase in both the number and volume of cells.

At the studied biochemical level, hepatopancreas is more sensitive than muscle to changes produced during the moult cycle.

RNA:DNA and protein:DNA ratios and lipid content are the parameters that describe best the biochemical changes along the moult cycle, both for hepatopancreas and muscle.

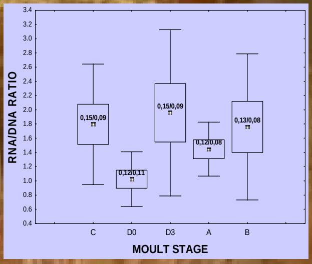

Figure 5. Mean RNA:DNA ratio values in *N. norvegicus* muscle in each moult stage.
Box: Mean ± SE; Whisker: Mean ± 0,95*SD

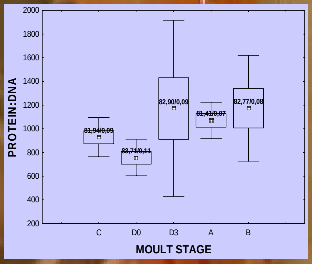

Figure 6. Mean Protein:DNA ratio values in *N. norvegicus* muscle in each moult stage.
Box: Mean ± SE; Whisker: Mean ± 0,95*SD

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