Biochemical characterization of eggs of the Norway lobster, *Nephrops norvegicus* (Decapoda:Nephropidae), during the embryonic development

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INTRODUCTION Egg-bearing females of the Norway lobster, *Nephrops norvegicus,* incubate their eggs for several months. Depending on the geographic area this period may vary. In the NW Mediterranean it lasts for 6-7 months. In order to gain information on egg development we studied the major changes in the biochemical composition of the eggs during the entire incubation period.

METHODOLOGY **Sampling**.- A total of 70 egg-carrying females of *N. norvegicus* were caught in August before spawning from a commercial fishing ground in the Mediterranean Sea, located 6 miles offshore Barcelona harbour and 300-600 meters deep (Fig. 1). The Norway lobsters were maintained in tanks, under controlled temperature (13±1 ºC) and salinity (36±1 psu) conditions and fed with frozen mussels three times per week. Eggs belonging to 10 individuals/month were analyzed.

Figure 1. Location of sampling area in the Spanish Mediterranean coast.

Classification.- Embryonic development was classified according to anatomical criteria (modified from Fariña, 1996); Stage I (months 1 and 2), stage II (months 3 and 4), stage III (month 5), stage IV (month 6) and stage V (month 7; Fig.2).

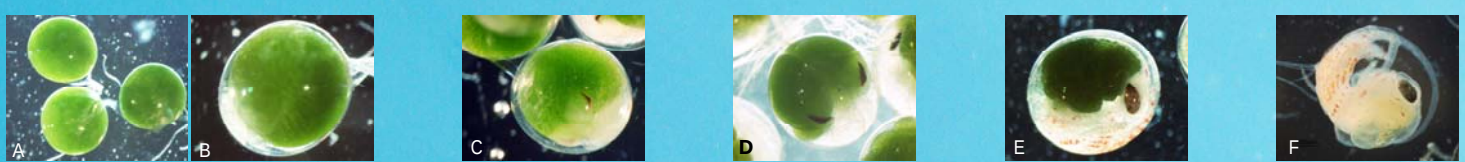

Figure 2. Stages of embryonic development of *N. norvegicus* (x7).
Stage I: uniform yolk and no embryonic development visible in early stage (A) and at the end of the stage the first organized structures appear (B). *Stage II*: lateral eye pigment spot is seen as a small dark crescent lining the posterior part of the lobes (C). *Stage III*: abdomen appendages and eyes clearly visible (D). *Stage IV*: embryo fully developed and red pigment (chromatophores) appear (E). *Stage V*: characteristics of first larval stage (prezoea) fully developed (F).

Biochemical analysis.- Inorganic matter (ash) was obtained after heating in an incinerator (Hobersol HK-11) at 450ºC during 4 hours. Freeze-dried eggs were analyzed for elemental composition (CNH), proteins (Lowry, 1951), lipids (Bligh & Dyer, 1959), carbohydrates (Dubois, 1956) and nucleic acids (Clemmesen, 1988 and 1993). Additionally, protein content was calculated from elemental nitrogen by applying a conversion factor of 5.78 (Gnaiger & Bitterlich, 1984) and subtracting the nucleic acid amount. A freeze-dryer (Telstar Cryodos 50) was used for the extraction of water.

RESULTS Two different phases were distinguished in the embryonic development of *N. norvegicus* with respect to the biochemical composition. The first one covered the early 4 months in which the biochemical changes in any parameter were small (stages I and II) showing no significant difference. The second phase covered the last 2-3 months (stages III, IV and V) in which significant biochemical changes appeared. Especially the lipid content decreased significantly during the last three months (Tukey test, $p<0.05$; Fig. 3). The nucleic acid content increased significantly during the last months and, thus, showed an inverse course compared to the lipids (Tukey test, $p<0.05$; Fig. 3).

A decrease in lipids and an increase of nucleic acids are due to principal process in the larval morphogenesis during the last months (Hulley & Beltz, 1991; Silbert *at al.,* 2004). This was also reflected by an increase of ash (Fig. 3).

Protein content was constant during the egg development until stage IV. In the stage V (month 7) a decrease was present when protein content was measured after Lowry (1951) method. This decrease did not appear when protein was calculated from CHN-values (Fig. 4), which is coincident with previous studies (Rosa *et al.*, 2003; Silbert *et al.,* 2004).

Furthermore, we observed a decrease in egg dry weight (ANOVA; $p>0.05$; Fig. 5) and an increase in egg volume or egg diameter (Fig. 6). This is associated with an increase in water content throughout the entire development period with rapid increase prior to hatching. This observation has previously also been made by Petersen and Anger (1997) and Silbert e*t al.* (2004).

Our results show that the energy reserves were not consumed at a constant rate during the embryonic development. The development of the embryos was accelerated prior to spawning, i.e. cell proliferation and thus increase of nucleic acids and simultaneously the consumption of storage products due to increased metabolic demands.

From a methodological point of view Lowry method was less suitable for determining proteins than CHN calculated values in the latest embryonic stages (Fig. 3).

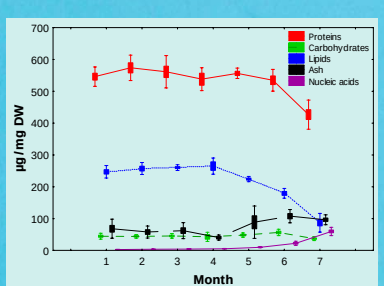

Figure 3. Biochemical composition of the development stages of *N. norvegicus* eggs (lipids, carbohydrates, proteins and nucleic acids) and inorganic matter (ash).
Box: Mean ± SE; Whisker: Mean ± 0,95*SD

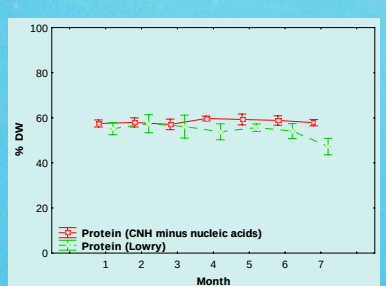

Figure 4. Protein content during the embryonic development determined by the Lowry method and by elemental CHN analysis (conversion factor 5.78, N from nucleic acids was subtracted).
Whisker: Mean ± 0,95*SD.

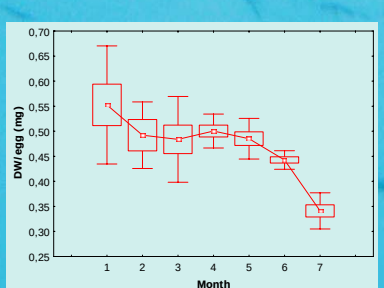

Figure 5. Egg dry weight in *N. norvegicus* during embryonic development.
Box: Mean ± SE; Whisker: Mean ± 0,95*SD

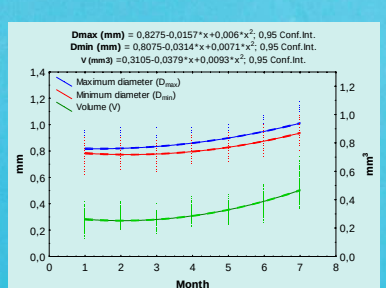

Figure 6. Egg volume and diameter in *N. norvegicus* eggs during embryonic development.
Box: Mean ± SE; Whisker: Mean ± 0,95*SD

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