INDIVIDUAL ESTIMATES OF RNA/DNA RATIOS OF ANCHOVY LARVAE (*ENGRAULIS ENCRASICOLUS*) OF THE NORTHWESTERN MEDITERRANEAN (CATALAN SEA AND GULF OF LIONS)

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The survival of young fish is conditioned to several factors; namely, predation, inanition and hydrographic entrainment to unfavourable areas, or through an interaction of several of these factors. Larvae under starving conditions are more vulnerable to predation (PURCELL *et al.*, 1987). Therefore, the nutritional state of fish larvae must play a key role in understanding recruitment variability. The use of RNA/DNA indices for studying larval condition in fish larvae is justified, taking in account that the total amount of desoxy-ribonucleic acid (DNA), per cell must be constant in individuals of the same species, and that it does not vary apparently with starvation or with environmental factors. However, the amount of ribonucleic acid (RNA) present in the cell is variable, because it is directly related to protein synthesis. Thus, RNA/DNA ratios is an index of the metabolic rate of the cell. Larvae under starving condition present lower RNA/DNA indices than well-fed larvae; decreasing linearly during periods of inanition (BUCKLEY, 1980; CLEMMESSEN, 1988).

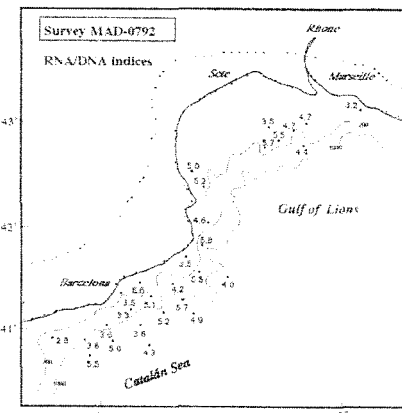
RNA/DNA ratios have been used for the first time, on individual larvae of anchovy (*Engraulis encrasicolus*), sampled in the northwestern Mediterranean Sea. RNA/DNA indices of 191 anchovy larvae from 28 stations, distributed between the Catalán Sea and Gulf of Lions were estimated (between the meridians 1°E and 6°E). The size length distribution of the analized larvae varied between 6 and 12 mm. The individual indices RNA/DNA varied between 1 and 7.8. The samples were collected during the anchovy egg and larval survey "MAD-0792", on board the R/V Garcia del Cid, during 27/6/92-26/7/92. Anchovy larvae were collected by Bongo 40 mouth opening plankton nets equipped with 200 µm. The RNA/DNA indices have been determined by measurements of fluorescence, using specific nucleic acid fluorescent dyes. Ethidium bromide was employed for the joint determination of the RNA and DNA, while the bisbenzimidazole was used exclusively for the determination of RNA following the method described by CLEMMESSEN (1988) with some modifications described in CORTÈS & RAMIREZ (1994). Larvae were sorted quickly from the plankton collectors and only the well conserved and good condition ones were destined to RNA/DNA analysis. These were measured on board through micronmetric eyepieces fitted on stereoscopic microscopes, set permanently at 10 X magnitude. Immediately after measurement these were stored in liquid nitrogen. The chemical reactants used in the extraction of nucleic acids and the analytical procedures are in the following table.

Tris buffer, pH=8.8	Chloroform/isoamylalcohol 24:1
Tris 0.5 M, pH=8	Ethidium bromide
Proteinase K	Bisbenzimadazol
SDS 20%	DNA standard (calf thymus)
Saturated phenol (pH=8)	RNA standard (yeast)

An ultrasonic generator Branson Sonifer 250 was used to homogenize the larvae. Fluorescence measurements were done with a Perkin Elmer LS-5 spectrofluorometer equipped with a data processing system, the Perkin Elmer 3600 Data station. For absorbance measurements in UV at 260 nm, the spectrophotometer Perkin Elmer mod. Coleman 55 was used. The maximum emission for DNA/RNA-EB is located at 589 nm for a wave length of excitation at 324 nm. The latter differs from that proposed by CLEMMESEN (1988). For DNA-Bis, the maximum emission is located at 447 nm for a wave length of excitation at 352 nm. The data for building the calibration curves were fit to a linear functions with the results as shown in the joint table.

DNA-Bis	y= 0.65354 + 33.733x	R = 0.99
DNA-EN	y = 1.033 + 31.044x	R = 0.99
RNA-EB	y = 0.029603 + 11.108x	R = 0.99

The total quantity of DNA is an index of the number of cells. This is assumed to be independent of environmental factors. The DNA content is aproximately constant for larvae of practically the same size. However, RNA values present variations, since these are dependent of the metabolic rate of cells. Nucleic acid content (DNA, RNA) varied exponentially with size. No differences have been observed in DNA content from larvae sampled in the Catalán Sea and Gulf of Lions. High RNA/DNA ratios (> 6) seem to related with the existing larval abundances (larvae/m^2), differentiating the two areas studied. In the Catalán Sea, high values of RNA/DNA ratios (> 6) are associated with areas where larval abundances are in the range of 0 to 40 larvae/m^2. In the Gulf of Lions these high values (> 6), correspond to the abundances range 0 to 140 larvae/m^2. The stations with maximum values of RNA/DNA indices seem to be associated to the Ligur-Provençal-Catalán front. This front has some permanent hydrographic features (FONT *et al.*, 1988). Related with this front, maximum zooplankton abundances have been observed by (SABATÈS *et al.*, 1989), even when compared to the coastal areas. During summer, PALOMERA (1992) also found anchovy spawning grounds associated to this front. RNA/DNA ratios between 4.5 and 5.5 in the Catalán Sea are found in areas close to the slope, while in the Gulf of Lions these are in the shelf border (Fig. 1).



Spatial distribution of mean RNA/DNA indices by station

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