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Ribonucleic acid content and RNA-DNA ratio of Black Sea anchovy and sprat larvae

Дослідження вмісту нуклеїнових кислот (РНК, ДНК), а також вмісту білка в гомогенатах тіла личинок чорноморської хамси і шпрота дозволяє досить точно визначити інтенсивність білкового синтезу та росту личинок у морі. При цьому виявляються відмінності в інтенсивності білкового синтезу і росту як у окремих видів різноманітних вікових стадій, так і у одного виду в різні роки.

The study of the RNA content and nucleic coefficient (RNA-DNA ratio) in fishes has become one of the widespread research areas over recent decades. With the help of these indicators, assessments of the intensity of protein synthesis in fish can be made and then can be applied to understanding the present growth rate. These investigations are especially applicable to early stages of ontogenesis (eggs, larvae, and fries) as the protein synthesis and growth mostly closely correlate just at these stages [1, 2]. In the last years, therefore, the usage of these indices became especially useful for the aquaculture industry. This method allowed one to exert more control over the development of juveniles, to test the effect of food rations, temperature, and other rearing conditions (3). They used also for estimation of the growth processes of juveniles in the field in connection with food supply which define "the degree of well-being" of animals [1, 4–6].

For the Black Sea, such investigations are very important as the unstable regime of this marine basin caused considerable fluctuations of the ecosystem parameters, which often have disastrous results on its bioresources. The estimation of the intensity of protein synthesis and growth of fish juveniles will allow us not only to define the condition of a large part of the population (their recruitment) but to consider other processes occurring in the sea as a whole. Fishes being one of the terminal linkages of the food chain are sensitive indicators of the general welfare of the marine environment.

As the species to be investigated, we chose larvae of anchovy *Engraulis encrasicholus ponticus Aleksandrov* and of sprat *Sprattus sprattus phalericus Risso* which are the main mass fishes of the Black Sea, play a substantial role in the trophodynamics of the pelagic ecosystem, and are the most important fishery objects for all the Black Sea countries. Our interest in the juvenile stages of these species stems also from their being planktophages with opposing characteristics in relation to temperature. Anchovy is a warm-requiring form, reproduces in summer, and sprat is a cold-requiring one reproducing in winter. The main time for the growth of anchovy juveniles is summer and autumn, and it is winter and spring for sprat juveniles. Thus, studying nucleic indices of juveniles of both species, it would be possible to estimate the conditions of growth and habitat of pelagic fishes in opposite seasons of the year. The most important ecological factors determining these conditions for pelagic fishes of the Black Sea are, undoubtedly, food supply and temperature [7].

The work was carried out on anchovy larvae of different sizes caught in July 1997 and September 1998 along the Turkish coast during cruises of the RV "Bilim" and on sprat larvae

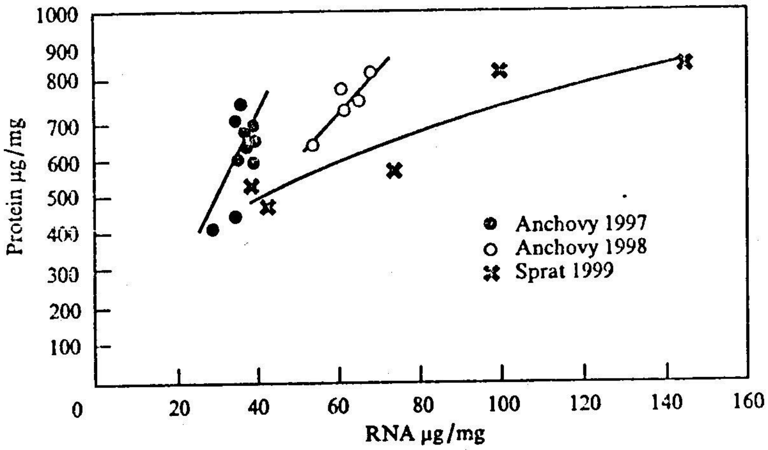
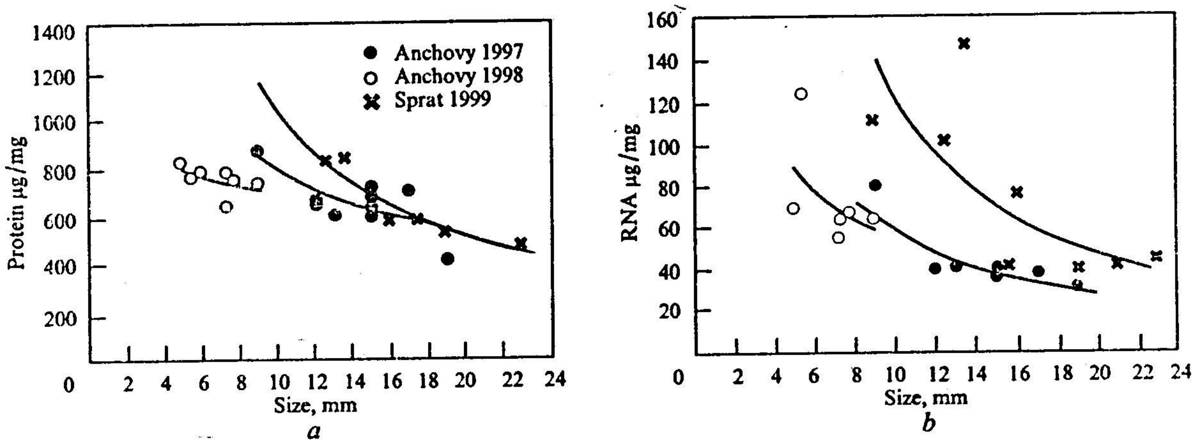


Fig. 1. Relation between the protein and RNA content in larvae of different sizes



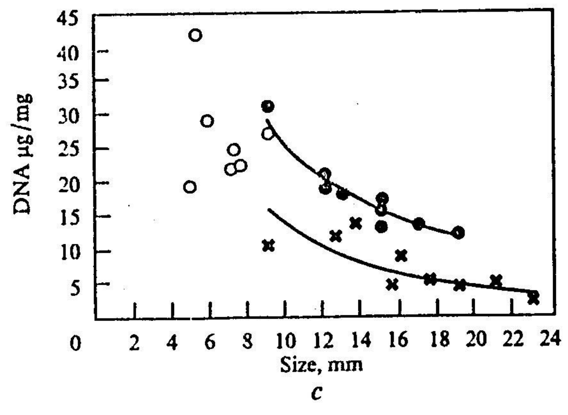


Fig. 2. Protein (a), RNA (b), and DNA (c) content (per mg dry weight) in larvae of different sizes

caught along the Crimean coast in March 1999 during the cruise of the RV "Professor Vodyanitsky". Larvae were caught using a Hensen plankton net (300 μm mesh size, 0.7 m diameter) with horizontal tow in the subsurface water. They were identified immediately with a binocular microscope, and the length of anchovy and sprat larvae was measured from the tip of the upper jaw to the end of the tail fin. The length of anchovy larvae varied from 4.9 to 9 mm in July 1997 and from 9 to 19 mm in September 1998. The length of sprat larvae ranged from 9 to 23 mm, Samples comprising 1–3 larvae of the same size each were placed in chlorophorm : methanol

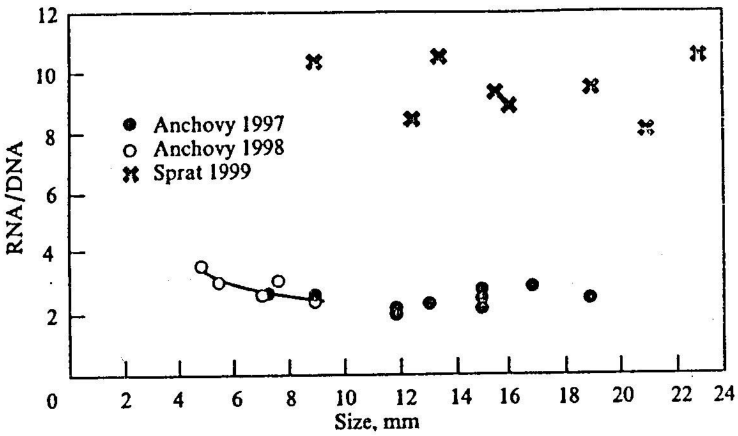


Fig. 3. RNA-DNA ratio in larvae of different sizes

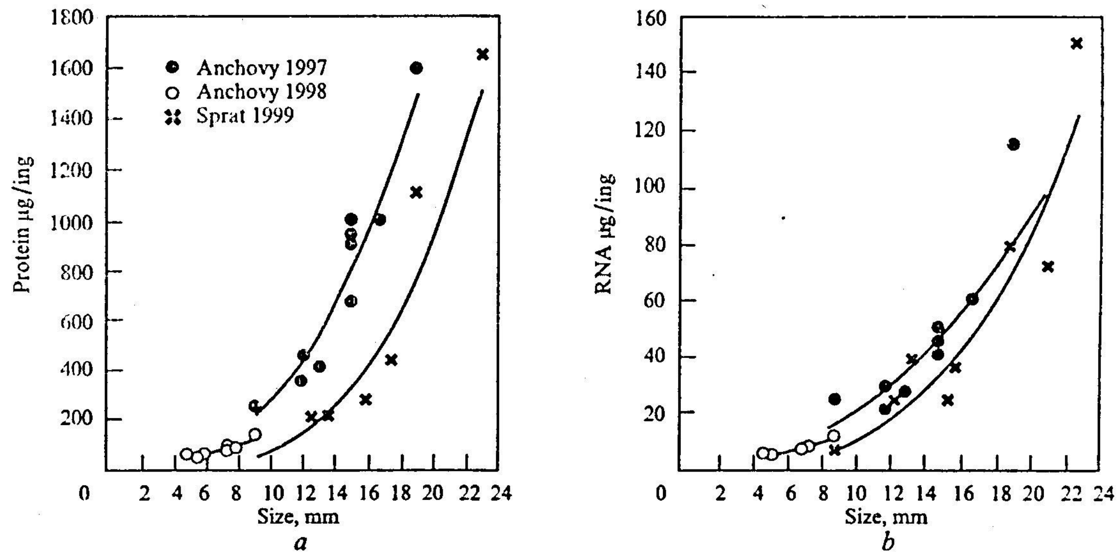
mixture (2 : 1 v/v) [8] for preliminary lipid extraction. Defatted samples (dry weight was at most 250 μg), were used for further determinations of nucleic acids and protein in the laboratory. The content of RNA and DNA was determined spectrophotometrically after the extraction of free nucleotides with 0.5 N $HClO_4$ by using the methods of Divavin and Kopytov [9] based on the original method of Schmidt – Tangausen. The protein content was determined with Lowry's method [10]. The content of nucleic acids and protein was given as μg per individual (μg ind^{-1}) and as μg per mg defatted dry weight (μg mg^{-1}).

The research results are represented in Figs. 1–4. A high correlation between the RNA and protein contents in larvae revealed a close connection between protein synthesis and protein growth (Fig. 1; $r = 0.92$–0.97; $P < 0.01$). The intensity of protein synthesis in sprat (according to the RNA content) was higher than in anchovy. The protein and RNA content (μg per mg) in larvae of both species decreased with an increase of their size (Fig. 2, *a*, *b*). So, the protein growth and synthesis of juveniles were the most intensive at the early stages of the larva development [1] and then became slower. The DNA content in juveniles of both species also decreased as their size increased (Fig. 2, *c*). It means that cell duplication moderated with growth. In contrast to RNA, the DNA content in sprat juveniles was lower than that in anchovy juveniles. The ratio of RNA-DNA on the contrary to both nucleic acids (excluding anchovy of 4–10 mm) did not change with an increase in the juvenile size (Fig. 3). Because of the higher RNA and lower DNA contents, this ratio in sprat was much higher than that in anchovy. A correlation between it and protein growth (appraised with protein content in μg per mg) was not found.

It is especially necessary to mention the calculations of RNA, DNA, and protein in the whole organism (Fig. 4, *a*, *b*, *c*). With increasing the size of juveniles, all observed indices increased ($r = 0.89$–0.96; $P < 0.01$). It is curious that the protein growth of anchovy juveniles was higher than that in sprat (Fig. 4, *a*); at the same time, the protein synthesis is similar (Fig. 4, *b*). It is also worth noting that the curves of all indices in anchovy juveniles in 1997 were somewhat lower than those in 1998.

In the discussion of the results obtained, we consider it important to pay attention to the following:

1. The data on the relative RNA content (μg mg^{-1}) in our research better defined the intensity of protein synthesis and growth than the RNA-DNA ratio. Evidently, the reason is that juvenile stages show the very expressed cell proliferation and also cell duplication connected with formation of the muscular and digestive systems as well as the systems of enzyme and hormonal



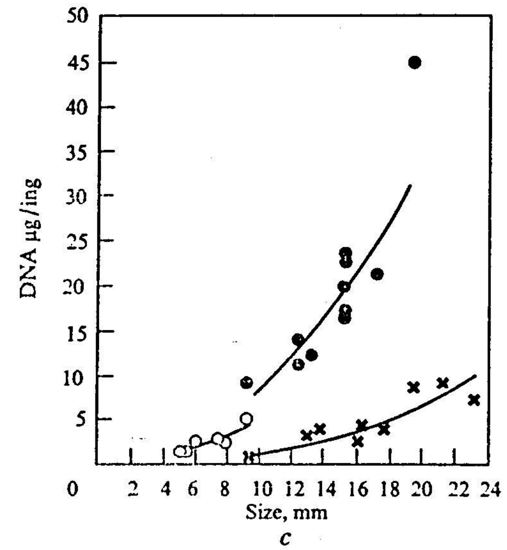


Fig. 4. Protein (a), RNA (b), and DNA (c) content (per individual) in larvae of different sizes

regulations of metabolism [1, 2]. Thus, both parts of the fraction (numerator and denominator) in nucleic coefficients changed radically. The RNA-DNA ratio defines biosynthesis and growth at the early and late stages of life history (i.e., during the development of eggs and larvae as well as adults) but may not be useful for the intermediate developmental stages in which the duplication of DNA is more or less stabilized, and the cardinal processes of protein synthesis are connected with growth [1, 5, 11].

2. The interesting fact that sprat juveniles have the higher indices of protein synthesis (RNA content) in comparison with anchovy juveniles, but the similar indices of protein growth for both species obviously show that sprat must have a higher level of protein catabolism. In the balance equation $\Delta P = A_p - K_p$, where ΔP is the protein growth or decrease, A_p is the protein anabolism (defined by RNA content), and K_p is the protein catabolism which was absent in the present study. K_p can be estimated, for example, from the excretion of the terminal product of protein catabolism (i.e., ammoniacal nitrogen) [12]. Unfortunately, in the fish growth estimations using RNA or the RNA-DNA ratio, the data on protein catabolism are generally very scarce. However,

in [3]. while studying the American catfish *Clanas gariepinus* in which the enzyme degradation of amino acids and oxidative catabolism were defined, the protein dissimilation was determined. In other studies carried out at the Institute of Biology of the Southern Seas on the mussel *Mytilus galloprovinciahs*, it was shown that the RNA content or the nucleic coefficient and the excretion of ammoniacal nitrogen (namely, protein anabolism and catabolism) are inversely related [13, 14]. It means that there is not always a positive relationship between protein synthesis and growth.

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