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RNA: DNA ratio as an indicator of growth, nutritional status and condition of fish: A review

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Abstract
The changes in the growth rate of fish with respect to nutrition, disease and environment are well documented. The growth rate in fish is estimated by simple length-weight calculations to complicated radiocarbon-dating methods. These growth estimation methods although commonly used but the factors like false indications, time consumption, labour and cost make most of the techniques less reliable. The RNA: DNA ratio is a simple estimator that provides a short-term measure of the condition of fish. The method is based on the notion that within individual cells, DNA concentrations remain constant and RNA varies, which in turn increases the protein synthesis. In general, the ratio is relatively high in a well-fed, metabolically active, growing fish compared to a starving, sluggish and metabolically inactive individual. The methodology to calculate the RNA: DNA ratio is simple, ranging from the extraction of nucleic acids to quantification. The post-processing of these methods is not needed, which make the technique simple, efficient and cost-effective. The present review highlights the importance, variations as well as the effects of nutritional status, stress and environment on the RNA: DNA ratio of culture and captured fishes.

Keywords: RNA; DNA; nucleic acids; fish; growth

1. Introduction
In the last two decades, the human population explosion has increased the need for food availability which some way has shown the drastic difference [1]. To fill the gap, there is a need for exploring the other ways for the production of the food. In this context, one simpler way is the use of aquaculture to meet the demands of human need. The aquaculture production has the potential to break the unavailability of the food line and can provide the nutritious diet to the growing population.

Protein is the most important nutrient for fish growth and plays a central role in the structure and functioning of all living organisms. Fish is an important component of total human food consumption and a principal source of animal protein for more than half of the world's population [2]. Fish growth, as defined by an increase in fish flesh, is mainly accomplished through the synthesis of protein [3]. Fish growth can also be defined as the investment of surplus energy into the production of somatic or reproductive tissues. However, growth is often defined in these simple terms of energy transfer as a life-history trait. Growth may be the single most important predictor of fish survival and fitness [4]. Measuring total length and weight of fish sometimes does not give a clear picture of the growth status of fish since they are not able to survive until it is measured at molecular levels.

The RNA: DNA ratio or simply the quantification of a nucleic acid ratio is a simple technique available that provides a short-term measure of the condition of fish. The technique is based on the simple notion that within individual cells, DNA concentrations remain relatively constant, and it is RNA that varies which in turn increase the protein synthesis [5, 6, 7]. Thus RNA: DNA ratio is an indicator of the protein- synthesising potential of a cell. In general, RNA: DNA is relatively high in a well-fed, metabolically active, growing fish compared to a starving, sluggish and metabolically inactive individual [8].

The RNA: DNA ratio has been applied widely to assess the individual condition of a variety of aquatic organisms ranging from small phytoplankton and zooplankton [9, 10] to fish larvae as well it's adult counterparts [11, 12, 13, 14]. This approach has provided significant results about the pure somatic growth status of the larval fish, which is defined as energy available purely for

the growth of tissues rather than used in molting or gonadal growth. Several studies have demonstrated that RNA: DNA ratios change with the change in the short-term growth of larval fish in a laboratory as well as different habitats and over time [11, 15, 16, 17, 18, 19].

It is assumed that in a fast-growing animal, the quantity of RNA is high. But, the synthesis of protein falls off with food limitation, and if this condition persists, attaining starvation, there will be degradation of ribosomes and loss of RNA [11]. Therefore, the RNA/DNA ratio is not only a sensitive index to starvation but can also be used to assess condition and growth rate [20, 21]. Furthermore, the fish demonstrate the continuous growth and the available approaches for the growth estimation involve a sampling which is highly susceptible to different errors [22]. DNA/RNA ratio, in turn, provide a point-in-time and potentially unbiased measure of individual condition and growth.

2. Need for this technique/advantages

There are lots of techniques available to measure the growth of fish, and commonly used method is the conventional length and weight measurement of an individual or group of individuals at a definite interval of time either in nature or in captivity. Here, the length quantifies an axial growth and weight quantifies growth in bulk; these two categories of growth are usually highly correlated with a few exceptions [23].

The techniques commonly available are generally time-consuming, and for the conclusion, the troublesome methodology needs to be employed.

The measurement of RNA: DNA ratios have several advantages than other methods to quantify the condition of the fish in which the most important is that it exactly provides the insights of the recent health status of a fish (i.e., past 1- 3 days). This prevents the cumbersome traditional measures of growth and condition in which the history of feeding is integrated with the energetic utilisation over the whole life-time of an organism [21]. By using this technique, it is possible to quantify samples of a large number of fish in a lesser period. 20-40 individual samples in a single microplate with the time from the preparation of samples to analysis as low as 8h can be analysed. The ability to create this vast data in less period of time makes this potentially cost-effective technique for the analysis of a large number of samples. RNA: DNA ratio can act as a diagnostic tool for assessing the nutrient deficiency or stress conditions of the ambient environment.

3. Major factors that influence RNA: DNA ratio

There are multiple sources of variations that reflect the RNA: DNA ratio and the primary sources include methodological, ontogenetic, and temperature effects [24].

3.1 Methodology

The methodologies for RNA: DNA evaluations should have the potential to influence resulting values and confound interpretation strongly. The RNA: DNA ratios have been demonstrated to vary with the body portions of fish larvae like the head portion having a lower ratio compared to the whole body [25]. The RNA: DNA ratio is also affected by the time of collection. The fish may show cyclical differences in the ratio throughout the day due to different feeding regime and endocrine activity rather than changes in temperature [26, 27]. It is very important that both the nucleic acids should have correct estimation, as errors in either the numerator (RNA) or denominator (DNA) can strongly change RNA: DNA ratios [28, 7].

3.2 Ontogenetic stage

The feeding of larval fish varies with ontogeny with both endogenous as well as exogenous feeding. Little changes may be observed on yolk sac stage between individual RNA: DNA values [29, 15] because the ratios are less likely to reflect environmental conditions. RNA: DNA values of newly hatched larvae may show differences in nucleic acid production conferred by individual parents [30, 31].

3.3 Temperature effects

Temperature influences physiological processes and affects RNA/DNA ratios and the somatic growth rate in a variety of larval fish species [32, 33, 34, 35, 36, 37, 38]. Temperature is considered as a dominant growth factor when food supplies are present in an adequate amount for larvae, and the availability of food becomes a predominant factor for growth and condition when the range is narrow [32].

4. Current Status of RNA: DNA ratio in fisheries and Aquaculture in the world, including India

RNA: DNA ratio has been successfully employed to evaluate the growth and nutritional status of higher animals, including fish. In aquaculture species, the technique is being widely used to estimate the smaller changes in growth that are otherwise difficult to assess by the other means. Some of the available published reports on fishes that used the technique have been presented below:

The application of RNA: DNA ratios evaluated the condition and growth of larval and juvenile Red drum (*Sciaenops ocellatus*) [26]. It was observed that RNA: DNA ratios decreased continuously over the entire five day starvation period, with relative reductions in RNA: DNA ratios with the increasing age. Diet variations in RNA:DNA ratios were investigated in controlled (constant) and natural (cyclical) temperature environments over a 48h period. RNA: DNA ratios were highest during daytime periods (0800, 1200, 1600, 2000 hours) and markedly reduced at night (0000, 0400 hours). Since RNA: DNA ratios from controlled and natural temperature treatments did not differ significantly, cyclical variations in temperature did not appear responsible for diet variations in biochemical conditions. Findings from the study support the use of nucleic acids as reliable indices of growth and condition and suggest that RNA: DNA ratios are potentially suitable measures to assess the condition of wild-caught *S. ocellatus*.

The larval condition and vulnerability to predation by mixed-prey experiments with larval capelin (*Mallotus villosus*) and predator (juvenile Lumpfish, *Cyclopterus lumpus)* were carried out. There were no significant differences in the mean condition (RNA/DNA ratios) or dry weights of larvae recovered from controls and treatments. These results of the study suggested that RNA/DNA ratios are not useful indicators of vulnerability to predation for larval capelin [39].

The RNA/DNA ratio in the rainbow trout during fasting and after fed was investigated with four feeding regimes [40]. The study concluded that the RNA/DNA ratios got changed from 1.72 to 0.73 for the starved, 1.96 to 1.91 for the delayed feeding, and 1.8 to 3-3 after fed three times a day, and 1.8 to 3.4 for the fed six times a day after 13 days. The RNA/DNA ratio was considered as the physiological index in rainbow trout fry when fed with different regimes.

The two different methods were compared to obtain RNA/DNA ratio in gobiid larvae in Portugal. The gobiid larvae were collected, and RNA/DNA ratio was analysed by two fluorometric methods. The study concluded that similar log DNA values were found, but at the same time different log RNA was detected, and the differences could be possible due to protocols used [41].

The effects of dietary protein level and protein particle size on the growth and protein turnover in *Salmo salar* were carried out. In the 3-month experiment having three dietary protein levels 30%, 35%, and 45% with two different fish meal particle sizes micro, coarse and grounded were fed to the adult fish. The RNA: DNA was measured in the muscle, which revealed that the ratio was not correlated with the growth rate due incorporation of the amino acids. The conclusion of the study was that protein catabolism was more necessary for net protein deposition and growth than protein anabolism in the fishes [42].

There was an attempt to determine the variability of RNA/DNA ratios during *Dicentrarchus labrax* larval development and it was observed that a two-phase modulation of the RNA/DNA ratio throughout seabass larvae development [43]. From day 10, after hatching, there was an increase in the RNA/DNA ratio with a maximum value at day 30 of age. Then, the RNA/DNA ratio decreased, reaching minimal values at day 60 after hatching. These results were consistent with a high rate of protein synthesis during the first 30 days of seabass development compared with the rest of the developmental period studied. The results were compared with that of otolith growth carried out for the same species larvae.

The seasonal variations of RNA/DNA and larval growth of (*Sardina pilchardus*) was evaluated from the southern coast of Spain [44]. The variables in the larvae were analysed in relation to different environmental changes. It was found that the higher temperature during spring season decreased the RNA/DNA ratio compared to the winter season. In conclusion, the study demonstrated that temperature although having a positive effect on the growth but at the same time affect the RNA/DNA ratio that should be taken into consideration when joint studies on RNA/DNA ratio and growth are carried out.

The growth variability of juvenile soles *Solea solea* and *Solea senegalensis* was studied, using RNA: DNA ratios in the Tagus estuary, Portugal and it was found that juvenile *S. senegalensis* showed significant differences between RNA: DNA ratios obtained for the two nursery areas ($P < 0001$). The decrease of seasonal growth rates with fish age was like seasonal variation of mean RNA: DNA values. Thus, the RNA: DNA pattern of juvenile *S. Solea* and *S. senegalensis* reflected growth and estuarine colonisation patterns [45].

The RNA/DNA ratio as an indicator of growth of three species, *Catla catla, Labeo rohita* and *Oreochromis mossambicus* was measured, when raised in ponds of a sewage-fed fish farm in the university of Kalyani, West Bengal, India. The RNA/DNA ratio was measured in the DNA and RNA in gill, liver and muscle tissue, respectively. In conclusion, the strong correlation was found between the RNA/DNA ratio and the growth of fish [46]. In general, the ratio was considered as the sophisticated technique for determination of growth in fish when reared in different environmental conditions.

The variations of RNA/DNA ratio in different tissues (muscle, head, eye, gut and the whole larvae) of different fishes like *Sardina pilchardus, Engraulis encrasicolus, Atherina presbyter and Paralichthys orbignyanus* was studied, collected from northeastern Atlantic and northwestern Mediterranean Sea. The results revealed that RNA/DNA ratio was low in the head region of larvae compared to gut and muscle which concluded that during RNA/DNA studies the conversion factors needs to be applied to data when different body sections are analysed [25].

The development, validation and field application of an RNA-based growth index in juvenile plaice *Pleuronectes platessa* was studied and found that RNA concentration began to respond to changes in feeding conditions within 8 days, suggesting that the index reflects growth rate in the short-term [47]. Furthermore, the index distinguished between rapid growth and negative growth of juvenile *P. Platessa* measured directly in laboratory and field enclosures, respectively. An application of the RNA-based growth index at two beaches on the west coast of Scotland suggested that the growth of juvenile *P. Platessa* varies considerably in space and time and is sub-maximum in late summer.

The RNA/DNA ratio was used for analysing the growth performance of Rohu, *Labeo rohita* after fed with a varied proportion of protein diet during intensive aquaculture in ponds of Nepal [48]. The four different diets with protein concentrations 20%, 30%, 40% and 50% were tested, and it was found that the RNA/DNA ratio was higher in later two treatments compared to the remaining protein concentration fed groups. The DNA and RNA were isolated by the simple procedures and the ratio was considered as a simple tool to evaluate the growth status of fish.

The ontogenetic changes in nucleic acids, protein contents and growth of larval and juvenile Japanese Flounder was studied and results revealed that the average RNA/DNA ratio in the daytime was higher than that in the dark and the ratio in fed larvae was higher compared to starved ones [49].

Yandi and Altinok (2018) studied the effect of irreversible starvation in lab-grown and field-caught larval anchovy, *Engraulis encrasicolus* caught from the Black Sea in Turkey on RNA/DNA ratio. The results exhibited that RNA/DNA of larvae was highly affected by the starvation and concluded that the ration could be effectively used for monitoring and comparing of nutritional status and recruitment probability of Black Sea anchovy.

The RNA/DNA ratio in the gills of *Channa striata* was measured after exposure to cypermethrin. The experiment was carried out in the fish collected from, the lake and markets of Amravati, Madhya Pradesh, India. The results revealed that the RNA/DNA ratio changed (2.23, 2.48, 2.27& 1.97), respectively at different exposure period compared to the control group. The conclusion of the study was that the RNA/DNA ratio serves as a biomarker for the fish growth after exposure to the different contaminants [51].

The RNA/DNA ratio was used to determine the requirement of leucine for growth *Catla catla* fingerlings in Aligarh Muslim University, Aligarh, India. It was found at 1.74 % dietary leucine, the RNA/DNA (4.62) ratio was higher indicating the better performance of fish when fed with the required concentration of leucine in the diet. Based on the results, the study concluded that inclusion of leucine ranging from 1.57 to 1.58 % in feed is necessary for the proper growth of *C. catla* in intensive culture in the commercial feed [52].

5. Conclusion

In the present review, we discussed that the nucleic acid

derived indices from fishes can be used to measure growth quickly, without laborious work of counting, measuring and identification. RNA/ DNA ratio has proven to be a reliable estimator of recent fish growth rate and food availability in their natural habitats. The ratio could be an important estimator of growth and stress over the changing temperatures. In general, RNA: DNA ratio might be a robust tool for the assessment of growth potential over a range of environments.

6. Conflict of interest

The authors declare no conflict of interest.

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