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RNA-DNA Ratios as an Indicator of Fish Growth in Golden Mahseer (*Tor putitora*)

G. K. SIVARAMAN*, RAJEEV KAPILA and P. C. MAHANTA
Nainital, National Research Centre on Coldwater Fisheries,
Bhimtal- 263136, Uttarakhand, India.

Abstract

In this investigation, Golden Mahseer (*Tor putitora*) having an average body weight ranging between 3.5 and 650 gms were collected from Experimental Mahseer Hatchery and lake, Bhimtal in order to assess the food availability in their natural habitat as well as for the prediction of growth rate. Commonly used molecular indices for fish growth and feeding conditions include RNA concentration (µg/mg tissue) and its ratio to DNA (RNA/DNA). Most of the isolated DNA had no sign of degradation and the spectrophotometer analysis of absorbance at 260 and 280nm provided ratios ranging between 1.70 and 1.90 indicating that quality of DNA was good. The DNA and RNA concentration ranged between 4.06 and 8.86 and 0.92-to 23.70 µg/ mg respectively. The RNA/ DNA ratios ranged from 0.10 to 4.83 with the mean average of 2.52. The means of the RNA/ DNA ratio of the wild fish were relatively higher than that of the reared fry indicating that they were generally in better condition. A positive correlation was also observed between body mass/size of the fish with RNA/ DNA ratio. The RNA concentration and ratio of tissue RNA/ DNA have proven to be a reliable estimator of recent fish growth rate and food availability in their natural habitat.

Introduction

Most of the studies had confirmed that food availability is a limiting factor for survival of larvae (Setzler-Hamilton *et al*., 1987, Leggett and Deblois, 1994 and Cushing, 1995). In the beginning, the researchers used a variety of morphometric, histological and biochemical indices to measure, growth and nutritional condition of fish species. Measuring of total length and weight of fish sometimes does not give clear picture of growth status of fish since they are not able to survive until it is measured at molecular levels by estimating rates of protein synthesis. Currently the most commonly used indices for fish growth and nutritional status are RNA concentration (µg/mg tissue) and its ratio to DNA (R/D) (Mathers *et al.* 1994; Clemmesen *et al.* 1997; Buckley *et al*., 1999; Gwak and Tanaka, 2001; Smith and Buckley, 2003; Peck *et al*., 2003 and Caldarone *et al*., 2001). RNA is required for protein synthesis and final outcome as biomass growth

*Corresponding Author: Tel No: 05942-247279, 247280 (O) Ext 121
Email address: gkshivraman@gmail.com

fluctuates in response to food availability and physiological condition of fish (Bulow 1987, and Clemmesen, 1993). DNA content is an index of cell number or biomass (Dortch *et al*., 1983). An increase or decrease in the RNA/ DNA or RNA: mg tissue ratios would indicate a concomitant change in protein synthesis, growth rate and nutritional condition of fishes (Buckley, 1979 and 1984; Martin *et al*., 1985; Fukuda *et al*., 1986; Buckley and Lough, 1987; Clemmesen, 1987; Raae *et al*., 1988; Robinson and Ware, 1988; Westerman and Holt, 1988). Major advantage of a nucleic acid based growth estimate is rapid response to time and R: D ratio in post yolk-sac larvae can reflect changes in growth rates and nutritional condition over a period of time. Growth rate estimation of field- caught fish is a powerful tool for evaluating the survival potential of an individual and for identifying environmental variables which may affect recruitment success. In this study an attempt is made to investigate the natural food availability and growth condition of the coldwater fish *Tor putitora* in relation to their body weight as compared with the laboratory maintained fish in terms of RNA/DNA ratio and their concentrations.

Materials and Methods

Golden Mahseer (*Tor putitora*) of different body weight were collected randomly from the catch in natural lake of Bhimtal (Nainital) and Mahseer Experimental Hatchery, Bhimtal, Nainital, Uttarakhand. R/ D ratios were estimated from the extracts of muscle tissues of the fingerlings and adults fish (n= 10). Muscle tissue (150mg) was collected just below the dorsal fins. Total DNA/ RNA was extracted using lysis buffer (50mM Tris-HCl buffer pH 8.0 having 50mM EDTA, 100mM NaCl, 1.5% sarcosyl and proteinase K) (Sambrook *et al.* 1989). Phenol- chloroform- isoamylalcohol method (25:24:1) was used for purification of DNA. The DNA and RNA concentrations were measured at 260nm in an UV Spectrophotometer followed by removal of RNA by RNase treatment (30 µg) for 150 mg of tissues. R/D ratio was estimated as the difference between pre and post RNase treatment.

DNA concentration (µg/ µl) was calculated by OD260 x 50 x Dilution Factor/1000.

Statistical Analysis

Correlations were calculated among the different body weights with the amount of RNA concentrations and with RNA/ DNA ratios. Comparisons between different sizes were using one-way ANOVA (Snedecor and Cochran, 1989).

Results

Most of the isolated DNA was in good quality as seen in 0.8% agarose gel electrophoresis (Fig 1a - 1d). Spectrophotometer comparison of absorbance at 260 and

280nm provided a DNA/RNA and protein contamination and relationship of 1.55 to 2.02 indicating good quality of isolated DNA. DNA and RNA concentration ranged from 4.06 to 8.86 (5.370 ± 0.012) and 0.90 to 23.73 (77.93 ± 0.23) µg/g of muscle tissue (Tab.1). The mean RNA and R/ D of juveniles (body weight of 3.0-5.0 to 6.0 - 10.0g) and adult of (body weight of 500.0- 550.0 to 560.0- 650.0g) were differing significantly ($P < 0.01$). The RNA concentrations in the juveniles were lower (18.63 ± 0.04, 12.74 ± 0.27, 1.36 ± 0.14 and 0.92 ± 0.09) as compared to adult Mahseer (19.65 ± 0.77 and 23.73 ± 0.64). Similarly the RNA/ DNA ratios were found lower in the juvenile (2.47 ± 0.14, 2.69 ± 0.22, 0.20 ± 0.03 and 0.10 ± 0.01) as compared to the adult (and 4.84 ± 0.64 and 4.83 ± 0.78). The growth in body weights was significantly ($P < 0.01$) correlated with both RNA concentration and RNA/DNA ratio ($r^2 = 0.54$) and with more genetic variability whereas it was not statistically significant in the pond-reared fish having the body weights ranging from 11.0- 14.0 to 15.0-20.0gms.

No significant correlation between DNA content and size of the fish was found. Where as positive correlations were observed between body mass/size of the fish with RNA concentration and RNA/ DNA ratios (Table1). The RNA: DNA ratio was proportionately increased as the increase of body weight except in 11-20g body weight sizes (Fig.2), which was fed with normal feed with CP of 42 - 45%. Whereas the fry was fed with goats liver *ad libidum* and wild caught fishes had higher body weight reflecting the food availability in the natural habitats.

Table1. The DNA concentration, RNA concentration and R/D ratio of Mahseer fish population from wild and experimental hatchery sources

Life stage	Sampling Location (n = 10)	Body weight (g) Concentration (ìg/ mg)	DNA Concentration (ìg/ mg)	RNA Concentration	R/D Ratio
Juvenile	Hatchery	3.0- 5.0	7.54 ± 0.034	18.63 ± 0.04**	2.47 ± 0.14**
Juvenile	Hatchery	6.0- 10.0	4.74 ± 0.036	12.74 ± 0.27**	2.69 ± 0.22**
Juvenile	Hatchery	11.0- 14.0	6.73 ± 0.051	1.36 ± 0.14	0.20 ± 0.03
Juvenile	Hatchery	15.0- 20.0	8.86 ± 0.040	0.92 ± 0.09	0.10 ± 0.01
Adult	Lake	500.0- 550.0	4.06 ± 0.043	19.65 ± 0.77**	4.84 ± 0.64**
Adult	Lake	560.0- 650.0	4.91 ± 0.068	23.73 ± 0.64**	4.83 ± 0.78**
Overall			4.06 to 8.86 (5.370 ± 0.012)	0.90 to 23.73 (77.93 ± 0.23)	0.10 to 4.84 (15.13 ±0.86)

Discussion

To assess the nutritional status/ physiological condition of the field caught mahseer fish population it is necessary to determine the amount DNA, RNA and RNA/ DNA

** Significant at P <0.01

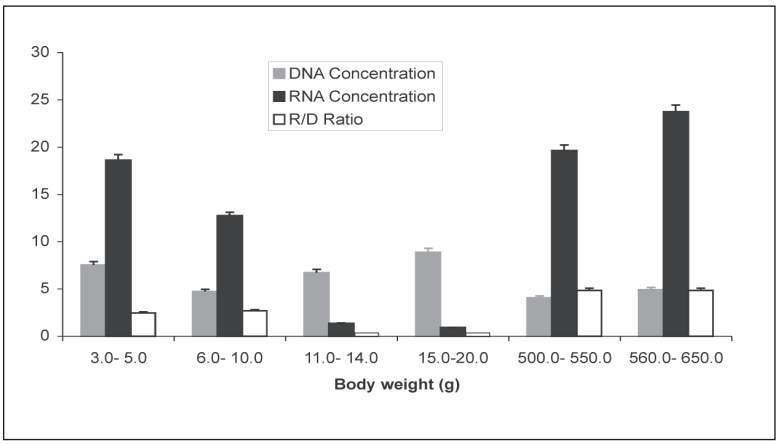

Figure.1. Relationships among DNA concentration, RNA concentration, and RNA/ DNA ratios in different size of Mahseer population

ratio and with their body weights. In the present investigation mean value of RNA/ DNA ratios ranged from 0.10± 0.01 to 4.84 ± 0.64 and the similar type of values (2.22 to 2.56) was observed by Smith and Buckley (2003) in juvenile Atlantic cod *Gadus morhua* and demonstrated that the R/D of scale tissue also reflects the feeding condition and growth of the fish. The whole-body R/D of fish larvae has been shown to be a reliable measure of nutritional status and growth (Buckley, 1984 and Clemmesen, 1987). Whereas Malloy and Targett (1994) and Rooker *et al*., (1997) studied the juvenile and adult fish using whole body, liver and muscle homogenates and suggested that R/D can provide a useful index of feeding condition and growth. The means of the RNA/DNA ratio were relatively high, so the Mahseer fish collected from the lake were generally in good condition. Larvae in good condition tend to have higher RNA/DNA ratio than those in poorer condition (Robinson and Ware, 1988 and Clemmesen, 1994). It is further suggested that this ratio can respond quickly to changes in environmental condition (Martin and Wright, 1987), and reflects the instantaneous growth in the field caught fish (Buckley, 1984).

This study shows the high correlation between the body weights of the fishes with RNA concentration and RNA/DNA ratio, except in fishes having the body weights of 11.0- 14.0 to 15.0-20.0gms, which was reared under pond condition. Similar to this study Malloy and Targett (1994) and Rooker *et al*., (1997) obtained higher correlations between growth in weight and R/D (r^2= 0.66) in the white muscle tissue of wild caught juvenile summer flounder *Paralichthys dentatus* and was significant ($P < 0.05$) in fed juvenile drums than starved fish, respectively. In contrast, Smith and Buckley (2003) found mean R/D of scale extracts was more highly correlated with growth in length (r^2=

0.59) and Rooker and Holt, (1996) also observed (r^2 = 0.65) in juvenile *Sciaenops ocellatus.* As clearly indicated the Mahseer fishes are well adapted to the natural water resources (lakes/ rivers/ streams) and are highly active fish of marginal bottom feeding habits. It may need the natural condition for active growth since it guts contains the macrophytes, filamentous algae, molluscus, insects, debris and sand and mud (Annual report, NRCCWF, Bhimtal, 2007).

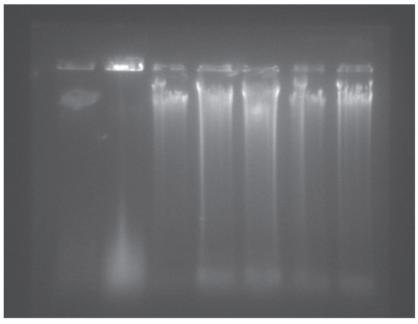

Fig.2a.Pre- RNase Treatment

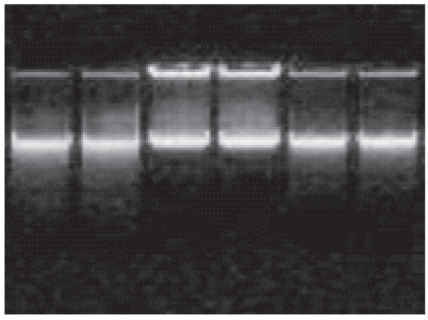

Fig.2c Post- RNase Treatment

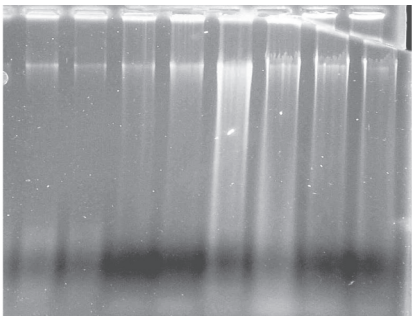

Fig.2b Pre- RNase Treatment

Figure 2. Quality of isolated DNA and RNA from muscle tissue of the collected samples

Highly positive correlation was measured between RNA/DNA ratio with fish size and weight indicating growth status of fish. But Chicharo *et al*., (1998) found non-significant correlation between the RNA/ DNA ratios in field caught *Sardina pilchardus* larvae of the north Spain. Moreover, strong correlation between RNA/DNA ratio and growth have been observed in a variety of species such as, *Clupea harengus*, *Ammodyles spp*, *Theragra chalcogramma*, *Paralichthys dentatus*, *Pseudopleuronectes americanus*, *Gadus morhua*, *Scomber scombrus* and *Morone saxatilis* (Buckley, 1984). The results of our study indicate that it is indeed possible to conduct such surveys on large scale in natural habitats of fish so as to estimate growth and feeding status of wild specimens during various seasons and were carried out for the first time in coldwater fish species of this region. The study was carried out during the breeding season so that fry and fingerlings were not collected from the wild but as the work progressed, it was found

that significant differences existed between the wild caught fry and fingerlings which were hatchery- reared (data not shown). The RNA: DNA ratio could therefore become the method of choice for determining the growth and nutritional status of coldwater fishes.

Conclusion

The RNA concentration and ratio of tissue RNA/ DNA have proven to be a reliable estimator of recent fish growth rate and food availability in their natural habitats.

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