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Influence of salinity and food deprivation on growth and *RNA-DNA* ratio in red drum *Sciaenops ocellatus* (Pisces: Sciaenidae)

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Abstract: Juvenile Red drum *Sciaenops ocellatus* held in 30 ppt natural seawater or fresh water were starved or fed *ad libitum* for six weeks, and sampled each week for growth and for determination of *RNA, DNA,* and protein contents of white and red muscles and liver. Specific growth rate and condition factor were used to compare changes in nucleic acid and protein concentrations in fish tissues. Salinity had little effect on growth, while starvation caused a significant reduction in all growth parameters and in the *RNA-DNA* ratio after three weeks. The *RNA-DNA* ratios of red and white muscles of the fed fish were greater than those of the starved fish and also were related to specific growth rate and fish condition factor. Nucleic acid concentrations in liver were highly variable. Since white muscle predominates and is more easily sampled in red drum than red muscle, and because it has less variable *RNA-DNA* ratios than liver, it is the tissue recommended for nucleic acid analysis of physiological condition and growth.

Key words: *RNA-DNA* ratio, growth, physiological condition, red drum, nucleic acid, starvation.

Growth is an increase in body mass accomplished by addition of new cells or by an increase in cell size, both accompanied by an increase in protein synthesis. If this physiological activity could be measured and standardized, it would be a useful tool for determining fish condition and growth rates.

The concentration of deoxyribonucleic acid *(DNA)* per unit weight of tissue is an index of cell number, as quantitative values do not change in response to acute environmental alterations or short term variation in feeding conditions (Mustafa and Mittal 1982, Bulow 1987). In contrast, ribonucleic acid *(RNA)* is a measure of metabolic activity related to the rate of protein synthesis (Buckley 1984, Bulow 1987) and can vary depending upon nutritional conditions and environmental factors. The RNA-DNA ratio has been used for determination of fish condition and growth (Robinson and Ware 1988, Clemmesen 1989, Miglavs and Jobling 1989, Mugiya and Oka 1991). The *RNA-DNA* ratio has also been used to determine physiological and nutritional conditions in molluscs (Wright and Hetzel 1985, Nusetti and Morales 1988), crustaceans (Ota and Landry 1984, Wang and Stickle 1986), hydrothermal-vent vestimentiferans (DeBevoise and Taghon 1988), and planktonic marine organisms (Berdalet and Dortch 1991, Mordy and Carlson 1991). A relationship between the *RNA-DNA* ratio and condition factor in the catfish *Heteropneustes fossilis* was demonstrated by Mustafa and Zofair (1983). The rate of protein synthesis is reduced by starvation and a decrease in *RNA* as an indicator of recent growth (Spiegelman 1965). The two most

important factors demonstrated to influence *RNA-DNA* ratios are temperature and feeding conditions (Bulow 1987, Jurss *et al.* 1987).

Red drum are successfully spawned in our laboratory using the photoperiod and temperature manipulation technique (Arnold 1978) and juveniles are reared in both seawater and fresh water. Salinity and temperature requirements for larval development and growth were reported by Holt *et al.* (1981) and Lee *et al.* (1984);growth rate was positively related to temperature within a range of 20-30°C. Salinity tolerance increased with larval development and although juveniles (50 mm standard length) were successfully adapted to fresh water, they grew less than those in seawater (Crocker *et al.* 1981).

Nucleic acid and protein contents in the tissue of juvenile red drum subjected to different growth conditions have not been investigated. The objective of this study was to determine the influence of salinity and food deprivation on growth, protein, *RNA* and *DNA* concentrations in white muscle, red muscle, and liver of cultured red drum juveniles, and to compare the *RNA-DNA* ratio to condition factor and specific growth rate.

MATERIAL AND METHODS

Laboratory spawned red drum were raised in fresh water and seawater in continuously circulating flow-through systems at the Fisheries and Mariculture Laboratory of the University of Texas Marine Science Institute. Six month old juveniles, 100-150 mm standard length and 15-40 g wet weight adapted to 12 ppt salinity, were randomly assigned and acclimated to either fresh water or 30 ppt seawater over three days. The fish were marked with plastic tags by tying plastic-rubber thread through the epaxial muscle under the first dorsal fin before all experiments began, and individual standard length and wet weight were measured.

Four treatments (two fresh water and two seawater, with one fed *ad libitum* frozen shrimp, and one starved in each salinity) were used to measure growth and changes in nucleic acid and protein concentrations in fish tissues over a six-week experimental period in March and April, 1986. Each circular tank (1.5 m diameter, 0.7 m depth, 1800 l) was stocked with 50 fish. Tanks were outdoors on continuous flow-through circulation with a flow of approximately 100 l/hour.

Water temperature and salinity were monitored on a daily basis, and pH, ammonia, and nitrite were analyzed twice a week. Every week two fish from each tank were quickly frozen in 95% ethyl alcohol and dry ice after measuring standard length and wet weight. Whole fish were stored at -80oC until nucleic acid and protein contents were determined. The Comassie blue method (Bradford 1976, Spector 1978) was used to measure protein concentrations, and a modification of the enzymatic assay of Bentle et al. (1981) was used to determine RNA and DNA. The liver was excised, and white and red muscle tissues were taken from dorsal epaxial and lateral line areas, respectively. Tissues were homogenized in 1 M NaOH, subsampled for protein assays and then centrifuged; the supernatant was taken for nucleic acid analyses following procedures outlined in Westerman and Holt (1988).

Protein and nucleic acid concentrations in tissue of fed versus starved fish during the experimental period were tested. Comparisions were made among white muscle, red muscle, and liver. Condition factor *K* (Busacker *et al.* 1990) and specific growth rate *SGR* (Ricker 1979) were calculated by the following formulae:

$$K = (W \times 10{,}000) / l^3$$

$$SGR = (\ln W2 - \ln W1) \times 100 / (t2 - t1)$$

where *L* is standard length in cm, *W* is wet weight in g (*W1* before the experiment; *W2* after treatment), and *t* is time in days (*t1* at initiation, *t2* at termination).

Student's t-test and regression analysis were used to compare the relationships of *RNA*, *DNA*, protein and *RNA-DNA* ratio to growth, over both treatments and among tissue types. Unless noted otherwise, a 95% confidence level was employed for statistical decision making.

RESULTS

Fig. 1 shows the variation in several environmental parameters. Water temperature decreased during the first week from 25 to 15°C (in the morning) and 20°C (afternoon), but after two weeks increased to 20 (AM) and 25°C(PM) and similar values were maintained throughout the experimental period. The pH was almost constant (near 8) in all tanks, although tanks receiving feed showed a slightly higher pH value. Ammonia values were very

low up to the third week (less than 0.3 ppm). During the third and fourth weeks, values in tanks receiving feed increased to 0.7 ppm during a shutdown of water circulation, but then returned to the low values when flow through was restored. Nitrite values were always lower in seawater tanks (less than 0.05 ppm) than in fresh water (about 0.05 to 0.1 ppm), and they steadily increased in fresh water tanks (up to 0.35 ppm) during the last two weeks (Fig. 1).

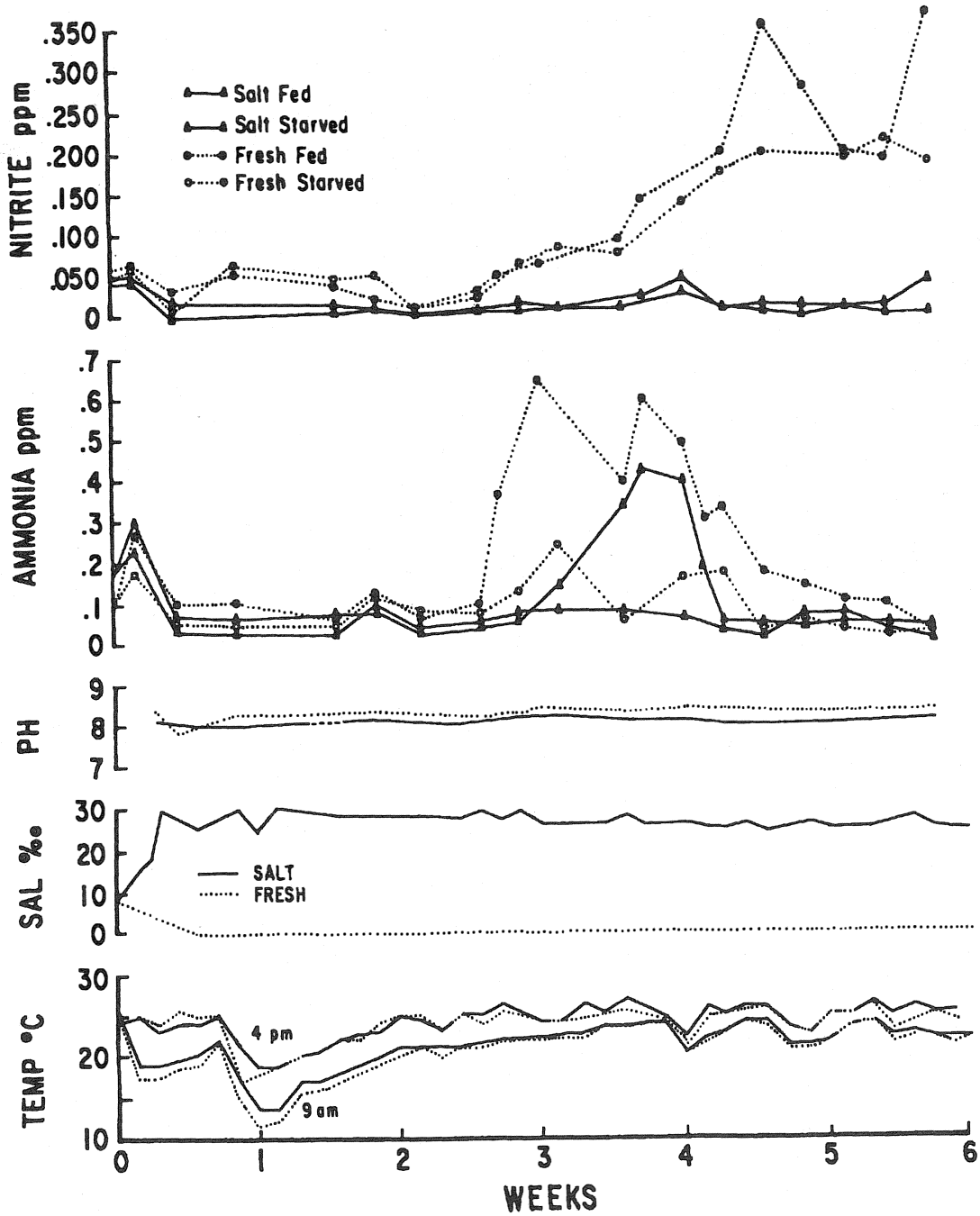

Fig. 1. Temperature, salinity, pH, amonia, and nitrite values measured in fish tanks during the 6-week experimental period.

During the first two weeks, wet weight of both fed and starved fish decreased, but wet weight of the fish fed *ad libitum* continuously increased from the third week in both fresh water and seawater. Conversely, weight of starved fish in fresh and seawater decreased throughout the six-week period (Fig. 2). After six weeks there was an average increase of 23.2 g in the fed fish and a decrease of 14.1 g in starved fish. There were no consistent differences in growth between fresh and seawater treatments in either the fed or starved fish (Fig. 2).

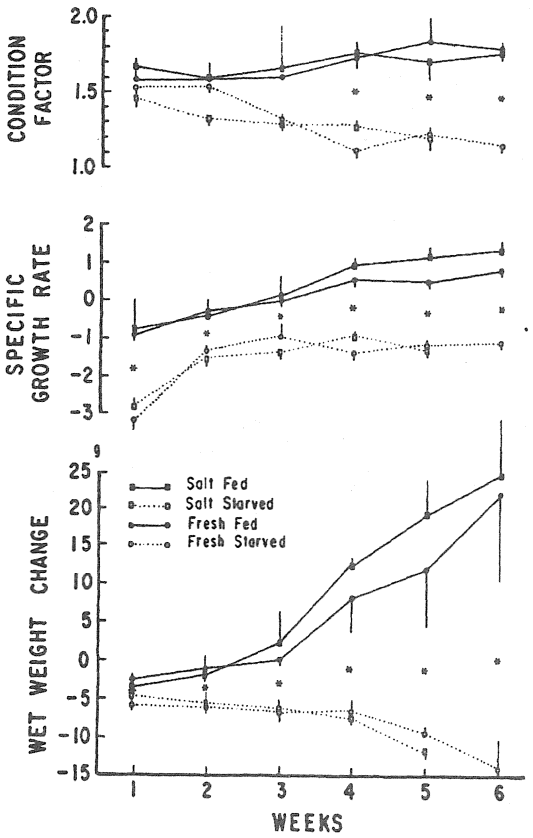

Fig. 2. Wet weight change *(g)*, specific growth rate *(SGR)*, and condition factor *(K)* of fed and starved red drum in fresh water and seawater during 6 weeks. Mean and standard error are given for each tank and condition. Significant differences between fed and starved fish are indicated by * (5% level).

Values for specific growth rate *(SGR)* of starved fish were negative during the experimental period due to the wet weight losses. *SGR* values for fed fish also decreased during the first and second weeks but increased thereafter. Condition factors for satiated fish in both fresh and saline conditions were similar during the six weeks, showing a slight increase, while values for starved fish decreased considerably (Fig. 2).

White muscle *DNA* concentrations of fed fish in fresh water and seawater were similar throughout the experimental period, while µg *DNA*/mg tissue of the starved fish increased slightly during the six weeks (Fig. 3). *RNA* concentrations in the white muscle of starved fish decreased (less than 2 µg/mg tissue) fairly constantly throughout the six weeks, while those of the fed fish increased (more than 3 µg/mg) after the first two weeks. *RNA* values of fed fish were higher than those of the starved after 2 weeks and accordingly the *RNA-DNA* ratios were higher (Fig. 3).

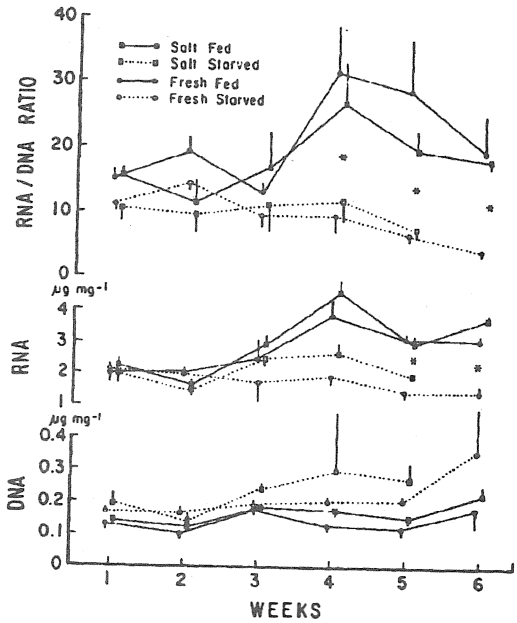

Fig. 3. Changes in *DNA*, *RNA* and *RNA-DAN* ratio in white muscle of red drum under all conditions. Mean and standard error are given for each tank and condition. Significant differences between fed and starved fish are indicated by * (5% level).

The relationship between specific growth rate and *DNA* concentrations in white muscle was not significant. However, *RNA* concentrations were positively correlated with specific growth rates as were *RNA-DNA* ratios (Table 1). Similar trends in fish condition factor with *RNA* and *RNA-DNA* ratio were noted.

Red muscle nucleic acid concentrations exhibited similar patterns to the white muscle; *DNA* values of the starved fish were

TABLE 1

Linear regression of nucleic acid concentration in the three tissues examined with specific growth rate (SGR), condition factor (K), and significance test. H_0: r = 0, and overall test conditions (N = 92)

Tissue	Nucleic acid (μg mg-1)	Specific growth rate a	b	r	F	Condition factor a	b	r	F
White muscle	RNA	y = 2.73 + 0.49 x		0.68	8.79**	y = -0.77 + 2.12x		0.60	7.11**
	DNA	y = 0.18 – 0.01x		0.15	1.43Ns	y = 0.44 – 0.17x		0.50	5.47*
	RNA-DNA	y = 17.20 + 3.40x		0.56	6.41*	y = 17.95 + 21.77x		0.69	9.04**
Red muscle	RNA	y' = 2.95 + 0.70x		0.72	9.97**	y = -2.92 + 3.60x		0.76	11.09**
	DNA	y = 0.56 – 0.36x		0.29	2.87Ns	y = 0.99 – 0.27x		0.45	4.78*
	RNA-DNA	y = 5.77 + 1.57x		0.63	7.69**	y = 7.36 + 8.05x		0.65	8.11**
Liver	RNA	y = 0.86 - 0.43x		0.23	2.24Ns	y = 9.20 - 0.21x		0.35	3.54Ns
	DNA	y = 0.71 - 0.15x		0.36	3.66Ns	y = 2.63 - 1.22x		0.59	6.93**
	RNA-DNA	y = 15.46 + 1.59x		0.25	2.45Ns	y = 1.97 + 10.89x		0.34	3.42Ns

*: 5% of significance
**: 1% of significance
Ns: no significance*

consistently higher than those of the fed fish throughout the experiment. In contrast, *RNA* concentrations of fed fish were always greater than those of the non-fed; hence *RNA-DNA* ratios of the fed fish were greater than in starved fish (Fig. 4).

Data obtained from liver tissue exhibited similar trends to white and red muscles with the exception that there was increased variation in the measured nucleic acid concentrations in liver (Fig. 5). *DNA* values of fed fish were almost constant after two weeks, while *DNA* concentration in the starved fish liver increased. There was little difference between fed and starved fish in liver *RNA* concentrations due to the wide variations in values, but *RNA-DNA* ratios were higher in the satiated fish after three weeks (Fig. 5). Nucleic acid values in liver tissue were not significantly correlated to specific growth rate or condition factor (Table 1).

Generally, protein levels in white and red muscles and liver of fed fish were higher than values in starved fish but they were not statistically significant except during the sixth week (Fig. 6). Significant positive relationships were seen between *RNA* and protein in white (y = 64.20 + 11.43x; r = 0.45) and red muscle (y = 33.49 + 10.22x; r = 0.47), but protein in the liver was not correlated with *RNA* concentration.

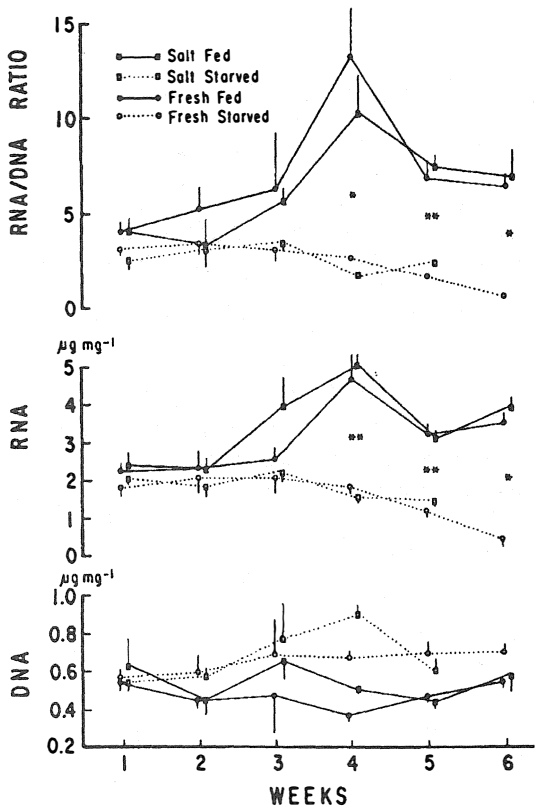

Fig. 4. Changes in *DNA*, *RNA* and *RNA-DNA* ratio in red muscle of red drum under all conditions. Mean and standard error are given for each tank and condition. Significant differences between fed and starved fish are indicated by * (5% level) and ** (1% level).

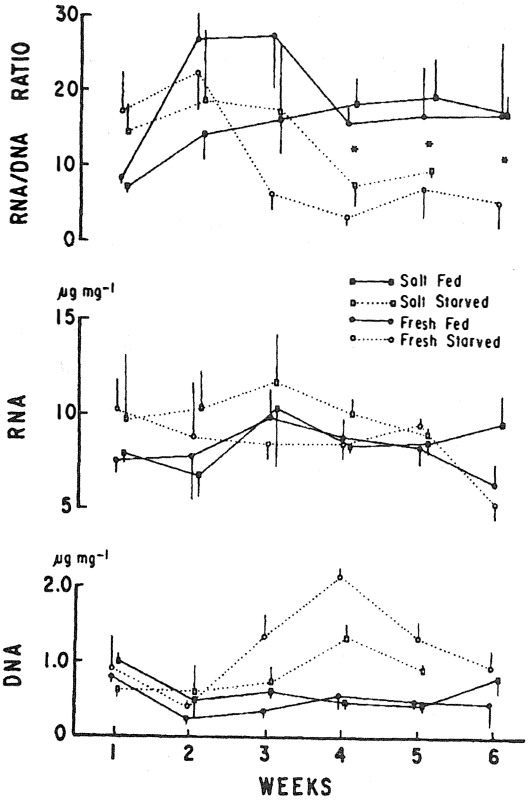

Fig. 5. Changes in *DNA, RNA* and *RNA-DNA* ratio in liver of red drum under all conditions. Mean and standard error are given for each tank and condition. Significant differences between fed and starved fish are indicated by * (5% level).

DISCUSSION

Salinity effects on the *RNA-DNA* ratio of fish were not apparent. Generally, nucleic acid values and growth parameters were higher in fish grown in seawater but the lack of notable differences suggest red drum juveniles (15-40 g) are well adapted to euryhaline conditions and can easily be cultured to fresh water with little change in overall growth. Crocker *et al.* (1981) reported high survival but decreased growth in red drum juveniles raised for one month in fresh water and attributed the slow growth to appetite depression. They used smaller juveniles (average initial weight was 2.5 g compared to our 25 g fish) which may not adapt to fresh water as readily as older red drum.

Red and white muscle *RNA-DNA* ratios in starved fish differed from values in well fed fish due to an increase in the amount of *DNA* per unit weight of tissue in starved fish, and an

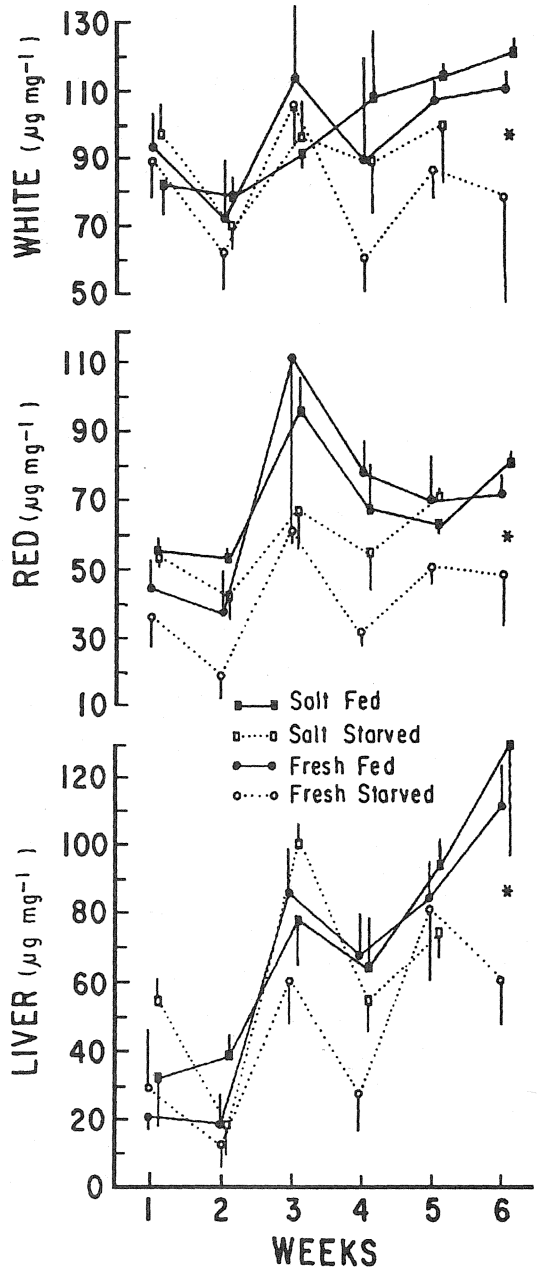

Fig. 6. Protein contents of liver, white muscle, and red muscle of red drum under all conditions. Mean and standard error are given for each tank and condition. Significant differences between fed and starved fish are indicated by * (5% level).

increase in p73 μg *RNA*/mg tissue in fed fish. High *DNA* concentrations reflect small cell size resulting in a large number of cells per unit weight of tissue (Hinegarder and Rosen 1972). Previous studies have shown similar results.

RNA concentrations of rainbow trout *Salmo gairdneri* decreased significantly and *DNA* values per g liver weight increased significantly as a result of starving and thus, the *RNA-DNA* ratio decreased considerably as a result of these changes (Jurss *et al.* 1987). A significant reduction in protein and *RNA* in liver and muscle of starved rainbow trout was reported by Smith (1981). Food deprivation resulted in reduced *RNA-DNA* ratio in the catfish *Clarias batrachus* which was reversed by subsequent feeding (Mustafa and Mittal 1982). They explained that high *DNA* values in starved fish muscle occurred because intracellular substances were catabolized during starvation, decreasing cell volume and thus increasing the number of cells per unit tissue weight. Generally, constant *DNA* values in muscles of fed fish indicate that intracellular materials such as water, protein, fat, and carbohydrate and the individual cell volumes maintain a steady state as the fish grows. Nucleic acid concentrations in liver were highly variable and do not correlate well with growth rate or condition factor. This may reflect a different function of protein synthesis in liver, and the use of this tissue for evaluating growth in red drum is not recommended. White muscle is more easily sampled in red drum than the red muscle, and has less variable *RNA-DNA* ratios than liver, and is the tissue we suggest for nucleic acidanalysis.

Protein concentrations in liver were not sensitive to changes in *RNA*, and in all tissues there was an inconsistent pattern between fed and starved fish. In another study, protein synthesis in both red and white muscles were measured using a constant infusion technique in fed and starved rainbow trout (Loughna and Goldspink 1984); protein synthesis rates, in both muscles, fell during starvation and the reduced rates of protein synthesis were related to reduced levels of *RNA* in the tissue.

The *RNA-DNA* ratio of muscle is a sensitive measure of nutritional condition in juveniles red drum showing significantly higher values in fed fish than in starved fish and is significantly correlated with specific growth rate *(SGR)* and condition factor *(K)*, and therefore, correlation coefficient values are reasonably high (Table 1). *RNA*, particularly in red muscle, was also highly correlated with these growth parameters and explained more than 72% of the correlation in *SGR* and *K*. Wilder and Stanley (1983) concluded the growth rates of brook trout and Atlantic salmon were significantly correlated with *RNA-DNA* ratio. Kayes (1979) reported a closer relationship betweeen growth rate and *RNA* concentration per unit weight of muscle tissue and criticized the use of the *RNA-DNA* ratio, arguing that adding *DNA* only increased the error term. Our results show this to be true; with the addition of *DNA* to the correlation with *SGR* or *K*, the correlation coefficient is reduced. In studies of fish of the same species and age of similar size, *RNA* concentration may be a more sensitive measure of growth than the ratio of *RNA-DNA*. In comparisons between species or among fish of unknown age, use of the ratio is necessary to reduce potential interspecific or interage variation in cell size and number.

The *RNA-DNA* technique described here is relatively simple, sensitive and useful to evaluate growth and therefore particularly valuable for use with fish of unknown age where calculation of growth rate is impossible. The fact that *RNA-DNA* is significantly correlated with *K* and *SGR*, calls for further investigations concerning the nature of this relationship and whether the *RNA-DNA* ratio is indeed more indicative of physiological condition of juvenile fishes at the time of sampling.

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RESUMEN

Se cultivaron los juveniles del pez rojo, *Sciaenops ocellatus*, durante seis semanas en aguas de mar natural al 30 ppm y en agua dulce, alimentando unos *ad libitum* y manteniendo

otros en ayunas, para analizar *ARN*, *ADN*, y proteínas del músculo blanco, rojo e hígado cada semana. La tasa de crecimiento específico y el factor de condición fueron los parámetros usados para comparar los cambios de los ácidos nucleicos y proteínas en tejidos del pez. La salinidad presentó poco efecto sobre el crecimiento, mientras que la inanición causó una disminución significativa en todos los parámetros de crecimiento y en la relación de *ARN-ADN* después de tres semanas. La tasa *ARN-ADN* en el músculo blanco y rojo de los peces alimentados fue mayor y también estuvo en relación con la tasa de crecimiento específico y el factor de condición. Las concentraciones de ácidos nucleicos de hígado fueron altamente variables. La musculatura blanca es dominante, más fácil de obtener que la roja, y menos variable en la tasa *ARN-ADN* que el hígado; por lo tanto, este músculo es recomendable para el análisis de ácidos nucleicos para evaluar la condición fisiológica y el crecimiento del pez.

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