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Effects of variable ration levels on direct and indirect measures of growth in juvenile red drum (*Sciaenops ocellatus*)

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Abstract

Relationships between somatic growth (length and weight) and two indirect measures of growth (otolith growth, RNA/DNA ratio) were assessed for red drum (*Sciaenops ocellatus*) under different feeding rations [0%, 2.5%, 5%, 10%, 15%, and 20% body weight (BW)/day] for 30 days. Representative samples from each ration level were taken in 10-day intervals between Day 0 and Day 30 for evaluation of direct and indirect growth measures. Positive correlations were observed between ration levels, somatic growth, and otolith growth. Statistical differences in weight and length of red drum were observed among ration levels by Days 10 and 20, respectively. Statistical differences for measures of otolith growth among ration levels were evident by Days 20 and 30. In addition, RNA/DNA ratios showed clear separation between fish that were starved and fish that were fed but demonstrated minimal separation among ration levels. Overall, the combination of a measure of somatic growth (weight) and a measure of otolith growth (otolith weight) resulted in the most statistical separation among ration levels. Findings from this study suggest that somatic growth, otolith growth and RNA/DNA ratios are suitable measures of relative growth of red drum; however, due to differences in sensitivity, caution must be exercised when using indirect growth (otolith growth, RNA/DNA ratios) measures to estimate recent growth.

Keywords: Otolith growth; Ration levels; RNA/DNA ratio; *Sciaenops ocellatus*; Somatic growth

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1. Introduction

During the last quarter century, the ability of fisheries scientists to evaluate different measures of growth and condition has expanded greatly and become an integral part of fisheries management. Variability in growth and condition influences survival and recruitment success (Leggett and Deblois, 1994); therefore, these measures are often used to evaluate habitat quality and assess the impact of changing environmental conditions (Rooker and Holt, 1996; Thorrold et al., 1997). Growth and condition are commonly evaluated by direct measurements of somatic growth (length and weight) (Bradford and Geen, 1992). However, otolith growth (morphometric index) and RNA/DNA ratios (biochemical index) have been increasingly implicated as viable and sensitive indirect measures of relative growth (Bergeron, 1997). Use of these indirect measures enable researchers to make retrospective inferences about previous life history and environmental conditions. Nonetheless, empirical studies describing the relationship between direct and indirect measures of growth under variable environmental conditions are lacking and these data are needed to fully understand the reliability of indirect assessments.

In recent years, relationships between somatic growth and otolith growth have been examined and several studies have demonstrated that somatic growth is strongly coupled with otolith growth (Radtke, 1989; Sogard, 1991; Peters and Schmidt, 1997; Iglesias et al., 1997; Narimatsu and Munehara, 1997). However, other findings suggest that somatic and otolith growth uncouple (i.e. time lag effects) during times of stress, resulting in the breakdown of the somatic growth/otolith growth relationship (Maillet and Checkley, 1990). As a result, it cannot be assumed that otolith growth has a linear relationship to somatic growth. Therefore, caution should be exhibited when using otolith measurements as a proxy for recent somatic growth. In addition to otolith growth, a suite of biochemical indices have been used as indirect measures of condition and growth for marine fishes. In particular, RNA/DNA ratios are commonly used to estimate recent growth (Westerman and Holt, 1988; Clemmesen, 1994, 1996; Folkvord et al., 1996; Garcia et al., 1998; Kawakami et al., 1999). This technique is based upon the premise that DNA is constant while RNA fluctuates, depending upon the amount of protein synthesis (Clemmesen, 1996; Folkvord et al., 1996). Because growth occurs through protein synthesis, RNA levels appear to be reliable indicators of somatic growth. Due to the rapid response of RNA/DNA ratios to less-than-optimal conditions, this technique appears useful for assessing recent growth history and condition (Bergeron, 1997).

Red drum (*Sciaenops ocellatus*) is one of the most highly sought-after game fishes in the Gulf of Mexico, and recruitment success or failure of this fish has been linked to differential growth and survival during early life stages (Rooker and Holt, 1997; Marine Recreational Fisheries Statistics, 1999; Rooker et al., 1999). Several authors have reported relationships between direct and indirect measures of growth for red drum and have used indirect measures to evaluate the importance of abiotic and biotic factors on growth (Bass and Avault, 1975; Peters and McMichael, 1987; Rooker et al., 1997; Stunz, 1999). Nevertheless, a thorough assessment of the reliability of indirect measures under different environmental conditions (i.e. variable ration levels) has not been attempted and these data are required to validate the procedure. In response, the objective of this study was to determine the effects of variable ration levels on both direct (somatic growth) and indirect

(otolith growth, RNA/DNA ratio) measures of growth of juvenile red drum. More specifically, the goal of the proposed work was to assess the sensitivity and reliability of both direct and indirect measures of growth. This research will better clarify the relationships between otolith and somatic growth and aid in the accurate prediction of previous growth and condition of red drum.

2. Methods

Juvenile red drum approximately 30 days old were collected from the Texas Parks and Wildlife Department Sea Center Texas hatchery located in Lake Jackson, TX. These fish were transported to the Texas A&M Aquacultural Research and Teaching Facility near College Station, TX. In order to simulate estuarine conditions, red drum were acclimated from 40 ppt natural seawater at 27 °C to 18 ppt artificial sea salt (Fritz Aquaculture) at 27 °C over a period of 24 h. Thirty-three red drum were then randomly chosen for a length–weight regression. This length–weight relationship can be described as SL=44.675(WT)+13.467 (r^2=0.90) where units for standard length (SL) and weight (WT) are millimeters and grams, respectively. At 24 h post-acclimation, all individuals were immersed in a solution of alizarin complexone (ALC) at a rate of 100 mg l^{-1} for 2 h in order to mark otoliths (Thomas et al., 1995).

Groups of 10 randomly chosen red drum were weighed and stocked into eighteen 110-l aquaria (each containing 80 l of water). In addition, baseline lengths were calculated from our initial length–weight regression and 10 fish were randomly sampled to represent baseline otolith and RNA/DNA measurements. Each tank was connected with water recirculation through a common biofilter that maintained water quality within favorable ranges for red drum. Temperature was maintained at 27±1 °C, salinity was at 18±1 psu, and light/dark cycle was 12 h/12 h.

Each tank was randomly assigned one of six ration levels (0%, 2.5%, 5%, 10%, 15%, and 20% body weight (BW)/day), and each level was replicated in a total of three tanks (30 fish/level). In order to standardize feed amounts, ration levels were assigned as a percentage of the mean group BW. Red drum within each treatment were fed one half ration in the morning and one half in the evening. Feed consisted of a nutritionally complete artificial diet containing 52% crude protein and 15% lipid (Rangen). Periodically, each day, mortalities were removed, weighed, measured, and stored in 95% ethanol (ETOH). In order to account for mortality, feed was adjusted accordingly at subsequent feedings. Experimental design included representative samples of each treatment being taken in 10-day intervals between Day 0 and Day 30. Sample size (n) was dependent on mortality for that treatment and the sample day (Table 1).

At each sample period, randomly chosen red drum were first weighed to the nearest 0.01 g, standard length was then measured to the nearest 0.1 mm, and, finally, each fish was dissected into two pieces along a line connecting the anal fin and posterior end of the dorsal fin. The head region was placed in 95% ETOH and held for otolith analysis. The trunk section was frozen in liquid nitrogen and transferred to a freezer (−80 °C) for subsequent RNA/DNA analysis. For the 0% treatment level, the experiment was terminated after 10 days due to the poor condition of the fish.

Table 1
Sample sizes for all ration levels (expressed as percent body weight per day) at the corresponding sample days for *S. ocellatus*

	Ration level					
	1 (0%)	2 (2.5%)	3 (5%)	4 (10%)	5 (15%)	6 (20%)
Day 10	6	8	8	8	8	8
Day 20	n/a	5	7	5	7	7
Day 30	n/a	3	4	6	13	12

Otolith analysis was conducted at the Texas A&M Fisheries Ecology Laboratory in Galveston, TX. Sagittal and lapillar otoliths were removed according to the procedures outlined in Secor et al. (1989) and Malony and Choat (1990). Using a digital image analysis system (Optimus 4.0, Edmonds, WA), two measurements were taken in order to assess recent sagittal and lapillar growth (Fig. 1). Measurement A represents the amount of rostrum growth, while Measurement B represents the otolith growth along the dorsal edge of the otolith. In addition to these measurements, each sagittal otolith was cleaned, dried, and weighed.

RNA/DNA analysis was carried out at the University of Texas Fisheries and Mariculture Laboratory in Port Aransas, TX. DNA and RNA measurements were made using an ethidium bromide fluorometric technique described by Westerman and Holt

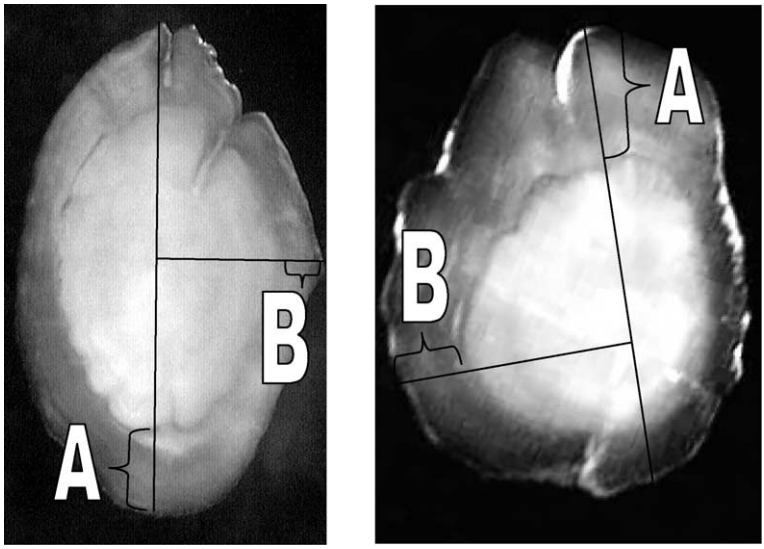

Fig. 1. Sagittal (left) and lapillar (right) otoliths of juvenile red drum after treatment with ALC. Sagitta is 2 days after treatment with ALC and lapillus is 30 days after treatment with ALC. A represents the measurements of rostrum growth along the rostrum/postrostrum axis through the primordia. B represents the measurement of either the farthest point along the dorsal edge of the otolith from the primordia (sagittal) or 90° to the rostrum–postrostrum axis (lapillus).

(1988). Individual trunk muscle samples were homogenized in 800 μl ice-cold 1 M NaCl. Homogenates were centrifuged at 3000×*g* for 45 min at 4 °C. Aliquots of homogenates (100 μl) were used to estimate DNA and RNA concentrations. Calculations were based on comparisons with DNA-EB and RNA-EB calibration curves from known standards. Calf thymus DNA and yeast (Type III) RNA were used as standards.

At each sample period (Days 0, 10, 20, and 30), mean weight, mean length, mean otolith size, and RNA/DNA ratios were calculated for each sample. Because stress has been linked to asymmetry between otoliths (Somarakis et al., 1997), all paired values for otolith measurements were averaged together. When only a single measurement was available, that measurement was used in place of the mean. Sagittal otolith weight was analyzed by looking at the ratio between otolith weight and body weight [(otolith weight/body weight)×100].

A one-way analysis of variance (ANOVA) was used to examine effects of variable ration levels on a several dependent variables (mean fish length and weight, otolith measurements, and RNA/DNA ratios). Significant (α=0.05) results were examined further with Tukey's Honestly Significantly Difference (HSD) multiple comparison test to determine which ration levels differed (Sokal and Rohlf, 1981; Day and Quinn, 1989). Statistical testing was completed using the statistical software package SYSTAT (Wilkinson, 1989).

3. Results

3.1. Baseline values

Average values for weight at Day 0 ranged from 0.19 to 0.22 g, while average length values ranged between 22.13 and 23.22 mm. Mean baseline values (±S.E.) for sagittal otolith Measurement A and Measurement B were 2.207±0.153 μm and 4.135±0.547, respectively. Mean sagittal otolith weight had an initial value of 0.78±0.066 mg. Mean baseline values (±S.E.) for lapillar otolith Measurement A and Measurement B were 2.776±0.217 μm and 1.490±0.197, respectively; and the mean RNA/DNA ratio baseline value was 8.449±0.517.

3.2. Weight

Throughout the 30-day period, mean weight (g) increased positively with increasing ration levels (Fig. 2). By Day 10, weight values showed a statistical separation of ration levels occurring between the 0% ration level and all other ration levels. Between Day 10 and Day 20, mean weights for the 0% ration level showed a decline from 0.20 to 0.18 g; however, mean increases of between 0.10 and 0.63 g were seen for each of the remaining ration levels. Statistically, the 2.5% and 5% ration levels became distinct from the 15% and 20% ration levels. Between Day 20 and Day 30, mean weight declined for the 10%, 15%, and 20% ration by 0.17, 0.20, and 0.03 g, respectively; however, the mean weight of the 2.5% and 5% ration levels continued to increase. Statistical differences were evident

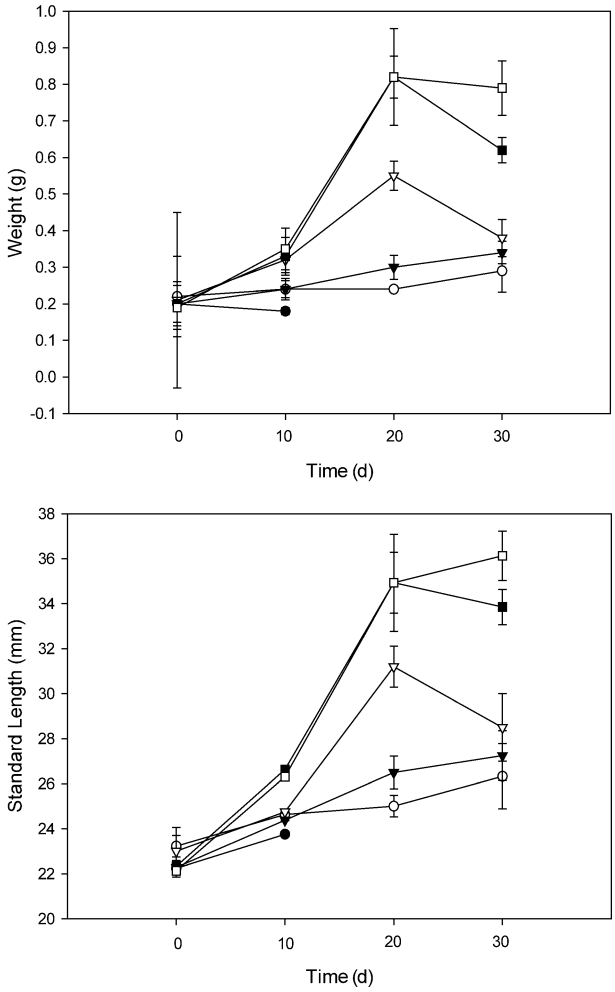

Fig. 2. Mean values±S.E. of weight and standard length for all ration levels of *S. ocellatus*. Experimental ration levels were 0% (●), 2.5% (○), 5% (▼), 10% (▽), 15% (■), and 20% (□) percent body weight per day at time of sampling. Due to high mortality rates, the 0% ration level was terminated after Day 10. Differences in mortality rates resulted in sample sizes that were based on the number of fish alive at the time of sampling and the duration of the experiment. Sample sizes (*n*) varied for each sample and can be found in Table 1.

between the 2.5% ration level and the 15% and 20% ration levels, as well as between the 5% and 10% ration levels and the 20% ration level (Table 2).

3.3. Length

Between Day 0 and Day 10, standard length failed to show statistical separation among ration levels; however, increases in mean length values remained positive (Fig. 2). By Day 20, red drum lengths in the 10%, 15%, and 20% ration levels increased significantly

Table 2

	Day	p (ANOVA)	Tukey's HSD (ration level)
Weight	10	0.034	1 and 6, 1 and 5
			1 and 4, 1 and 3
			1 and 2
	20	0.000	2 and 6, 3 and 6
			2 and 5, 3 and 5
	30	0.000	2 and 6, 3 and 6
			2 and 5, 4 and 6
Length	10	0.159	
	20	0.000	2 and 6, 3 and 6
			2 and 5
	30	0.000	2 and 6, 3 and 6
			4 and 6, 2 and 5
			3 and 5, 4 and 5

ANOVA comparisons mean weight (g) and mean standard length (mm) of *S. ocellatus* by ration level. Significant results ($p<0.05$) were further compared by Tukey's HSD post hoc tests. Paired numbers are comparisons that were significant ($p<0.05$). Results from Days 10, 20, and 30 were examined for relationships between ration levels 1 (0% BW/day), 2 (2.5% BW/day), 3 (5% BW/day), 4 (10% BW/day), 5 (15% BW/day), and 6 (20% BW/day).

compared to those in the 2.5% and 5% ration levels. Statistical separation among treatments occurred between the 2.5% and 20% ration levels, 5% and 20% ration levels, and 2.5% and 15% ration levels (Table 2). At Day 30, mean lengths separated into two distinct groups. The lowest ration levels (2.5%, 5%, and 10%) became statistically smaller compared to the higher ration levels (15% and 20%) (Table 2). Mean lengths declined for the 10% and 15% ration levels, while the 2.5%, 5%, and 20% ration levels continued to increase positively (Fig. 2).

3.4. Sagittal otolith

Sagittal otolith measurements responded in a positive manner to variable ration levels between Day 0 and Day 30. Statistically, there was no differences between ration levels for Measurement A at Day 10; however, mean length values (μm) at Day 10 did increase positively. Mean values for Measurement A ranged between 3.63 μm for the 0% ration level and 8.99 μm for the 20% ration level (Fig. 3). By Day 20, responses of Measurement A to variable ration levels did result in the 15% and 20% ration levels becoming statistically distinct from the 2.5%, 5%, and 10% ration levels (Table 3). Mean lengths of Measurement A ranged between 8.12 μm (2.5% ration level) and 35.26 μm (20% ration level). All length values increased according to ration level except for the 15% (24.29 μm) and 10% (24.67 μm) ration levels; however, these values did fall between the 20% (35.29 μm) and the 5% (14.51 μm) (Fig. 3). Day 30 results show statistical differences between the lowest (2.5%, 5%, 10%) and the highest (15%, 20%) ration levels (Table 3).

At Day 10, mean lengths of Measurement B failed to produce significant differences among ration levels (Table 3); however, there was a positive increase for all ration levels (Fig. 2). Mean lengths ranged between 9.57 and 12.52 μm for all ration levels. By Day 20,

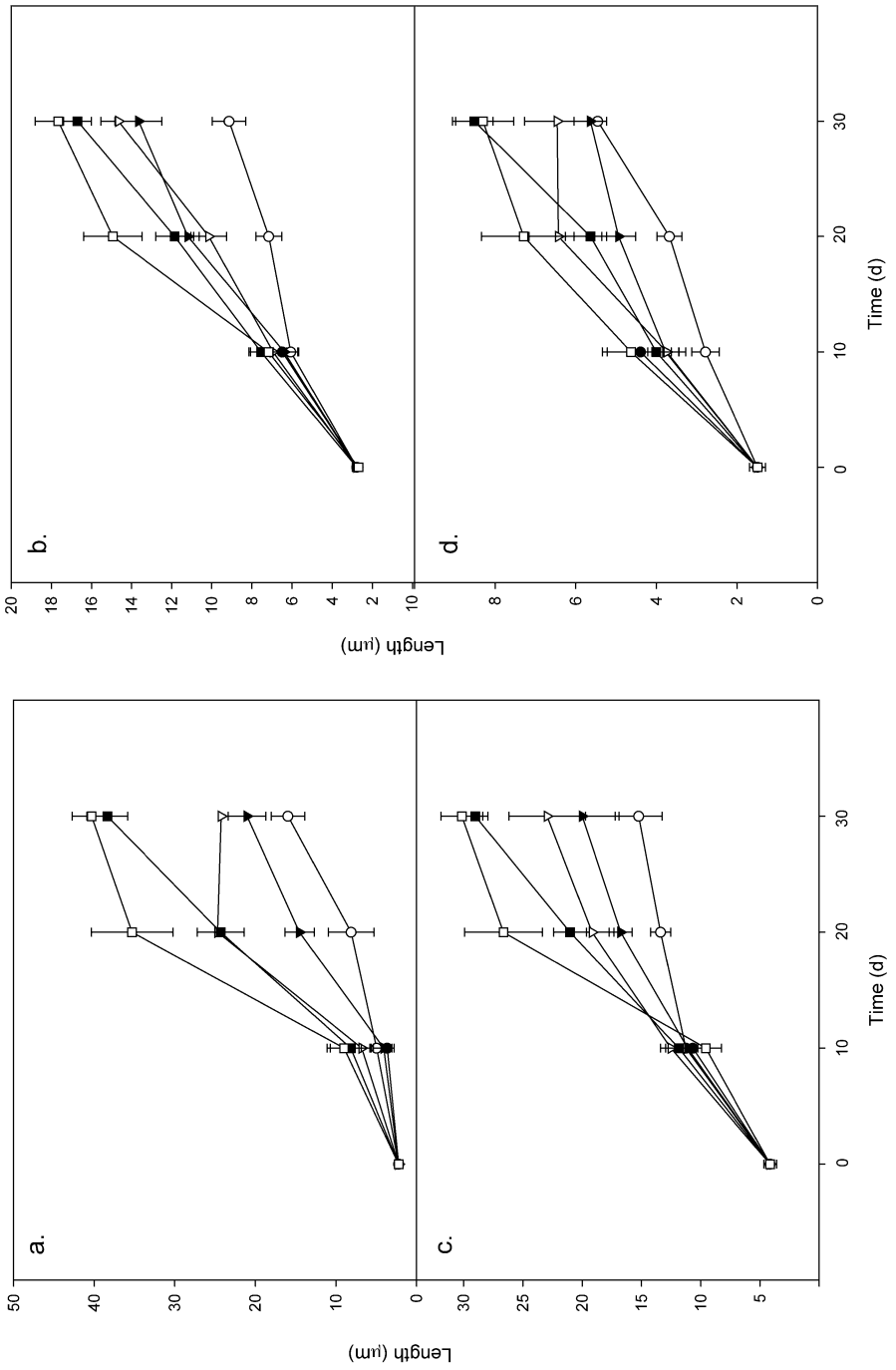
a.
b.
c.
d.
Length (μm)
Time (d)

Table 3

Otolith measurement	Day	p (ANOVA)	Tukey's HSD (ration level)
Sagittal A	10	0.150	
	20	0.000	2 and 6, 3 and 6
			2 and 5, 2 and 3
	30	0.000	2 and 6, 3 and 6
			4 and 6, 2 and 5
			3 and 5, 4 and 5
Sagittal B	10	0.339	
	20	0.001	2 and 6, 3 and 6
	30	0.000	2 and 6, 3 and 6
			2 and 5
Lapillar A	10	0.561	
	20	0.000	2 and 6, 2 and 5
			4 and 6
	30	0.001	2 and 6, 2 and 5
Lapillar B	10	0.265	
	20	0.011	2 and 6
	30	0.020	

ANOVA comparisons of mean lengths (μm) for sagittal and lapillar otolith measurements for *S. ocellatus* by ration level.

Significant results ($p<0.05$) were further compared by Tukey's HSD post hoc tests. Paired numbers are comparisons that were significant ($p<0.05$). Results from Days 10, 20, and 30 were examined for relationships between ration levels 1 (0% BW/day), 2 (2.5% BW/day), 3 (5% BW/day), 4 (10% BW/day), 5 (15% BW/day), and 6 (20% BW/day).

statistical separation occurred between the 2.5% and 20% ration levels and the 5% and 20% ration levels (Table 3). Mean length values for Measurement B continued to increase with increasing ration levels. Mean values ranged between 13.38 μm for the 2.5% ration level and 26.64 μm for the 20% ration level (Fig. 3). At Day 30, statistical separation among ration levels occurred between the 2.5% ration level and the 15% and 20% ration levels, as well as between the 5% ration level and the 20% ration level (Table 3). Mean lengths of Measurement B continued to increase positively according to ration level. Mean values ranged between 15.22 and 30.15 μm (Fig. 3).

When sagittal otolith weight was expressed as a ratio of body weight, there was clear statistical separation among ration levels as early as Day 10. At Day 10, values showed statistically that the 0% ration level was distinct from all other ration levels (Table 4). Mean values of sagittal weight at Day 10 were 0.62% BW for the 0% ration level and between 0.40% BW and 0.32% BW for all other ration levels (Fig. 4). At Day 20, the 2.5%

Fig. 3. Mean values±S.E. of sagittal otolith Measurement A (a), sagittal otolith Measurement B (b), lapillar otolith Measurement A (c), and lapillar otolith Measurement B (d) for all ration levels of *S. ocellatus*. Measurement A evaluated rostrum growth for both the sagittal and lapillar otoliths. Experimental ration levels were 0% (●), 2.5% (○), 5% (▼), 10% (▽), 15% (■), and 20% (□) percent body weight per day at time of sampling. Due to high mortality rates, the 0% ration level was terminated after Day 10. Differences in mortality rates resulted in sample sizes that were based on the number of fish alive at the time of sampling and the duration of the experiment. Sample sizes (n) varied for each sample and can be found in Table 1.

Table 4

	Day	p (ANOVA)	Tukey's HSD (Ration Level)
Mean Weight	10	0.000	1 and 6, 1 and 5
			1 and 4, 1 and 3
			1 and 2
	20	0.000	2 and 6, 3 and 6
			2 and 5, 3 and 5
			2 and 4, 3 and 4
	30	0.000	2 and 6, 3 and 6
			2 and 5
RNA/DNA	10	0.019	1 and 5, 1 and 4
	20	0.001	2 and 6, 3 and 6
			4 and 6, 5 and 6
	30	0.620	

ANOVA comparisons of mean weight (% BW) of sagittal otolith weight and RNA/DNA ratios for *S. ocellatus* by ration level.

Significant results ($p<0.05$) were further compared by Tukey's HSD post hoc tests. Paired numbers are comparisons that were significant ($p<0.05$). Results from Days 10, 20, and 30 were examined for relationships between ration levels 1 (0% BW/day), 2 (2.5% BW/day), 3 (5% BW/day), 4 (10% BW/day), 5 (15% BW/day), and 6 (20% BW/day).

and 5% ration levels separated statistically from the 15% and 20% ration levels; however, no differences were detected between intermediate ration levels (e.g. 10% vs. 15%) (Table 4). Mean sagittal otolith weight declined for the 10%, 15% and 20% ration levels, while increases were seen for the 2.5% and 5% ration levels (Fig. 4). At Day 30, statistical differences among ration levels occurred between the 2.5% ration level and the 20% ration and between the 5% ration level and the 20% ration level (Table 4). Sagittal otolith weight increased as a percentage of body weight for each ration level; however, mean weight values reflected the appropriate ration level. Mean weights ranged between 0.51 for the 0% ration level and 0.35 for the 20% ration level (Fig. 4).

3.5. Lapillar otolith

Day 10 values for lapillar otolith length (μm) for Measurement A showed no statistical separation among ration levels (Table 3). Measurements of mean lengths for Measurement A showed a positive relationship with increases in length ranging between 7.54 and 6.05 μm (Fig. 3). At Day 20, statistical separation among ration levels for Measurement A occurred between the 2.5% ration level and the 15% and 20% ration levels and between the 10% ration level and the 20% ration level (Table 3). Mean lengths also continued to increase positively according to ration level (Fig. 3). By Day 30, statistical differences occurred between the 2.5% and 5% ration levels and the 20% ration level for Measurement A (Table 3), while mean lengths increased positively (Fig. 3).

Measurement B showed no statistical differences among ration levels at Day 10 (Table 3); however, mean values of Measurement B showed a positive increase according to ration level. Mean values ranged between 4.6 and 2.78 μm (Fig. 3). By Day 20, Measurement B statistical differences were evident between the 2.5% ration level and

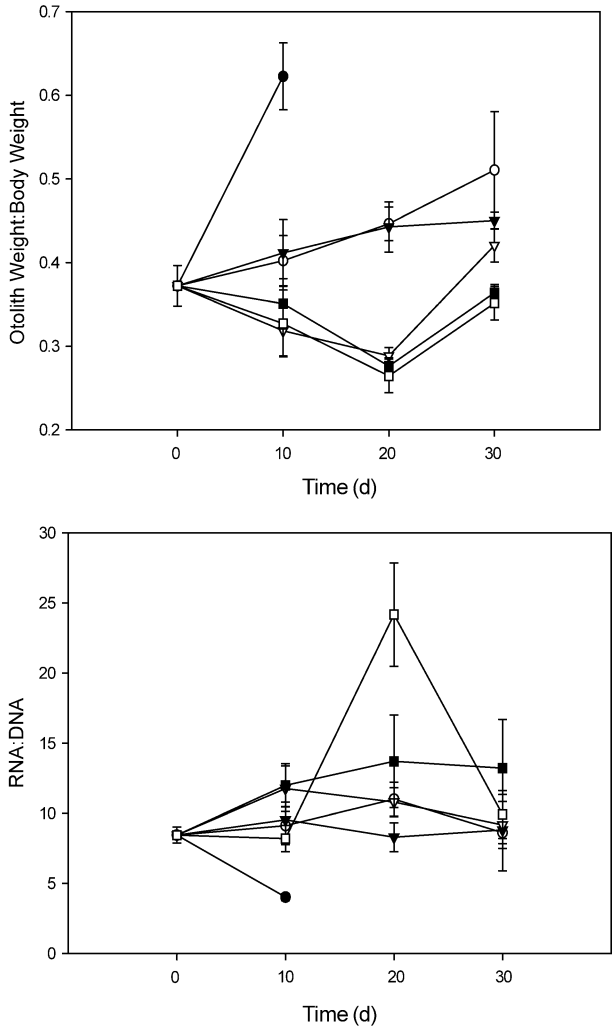

Fig. 4. Mean values±S.E. of sagittal otolith weight/body weight and RNA/DNA ratios for all ration levels of *S. ocellatus*. Sagittal otolith weight was derived from the mean of both the right and left otoliths. Experimental ration levels were 0% (●), 2.5% (○), 5% (▼), 10% (▽), 15% (■), and 20% (□) percent body weight per day at time of sampling. Due to high mortality rates, the 0% ration level was terminated after Day 10. Differences in mortality rates resulted in sample sizes that were based on the number of fish alive at the time of sampling and the duration of the experiment. Sample sizes (*n*) varied for each sample and can be found in Table 1.

the 20% ration level (Table 3); in addition, increases in mean length for all ration levels remained positive and with the exception of the 5% ration level (Fig. 3). At Day 30, there were no statistical differences among ration levels for Measurement B (Table 3); however, mean values of Measurement B remained positive for all ration levels except the 15% ration level (Fig. 3).

4. RNA/DNA

RNA/DNA values at Day 10 resulted in significant differences occurring between the 0% ration level and the 10% and 15% ration levels (Table 4). Mean RNA/DNA value for the 0% ration level was 4.02, while all other ration levels were between 8.20 and 12.00 (Fig. 4). By Day 20, the 20% ration level statistically separated from the 2.5%, 5%, 10%, and 15% ration levels (Table 4, Fig. 4). The RNA/DNA value for the 20% ration level increased from 8.20 to 24.18, while only moderate increases occurred in any the remaining ration levels. Day 30 values no longer resulted in any statistical differences among ration levels (Table 4). The 20% ration level declined from 24.18 to 9.92, while all values of RNA/DNA ranged between 8.62 and 13.23 (Fig. 4).

5. Discussion

Somatic growth of red drum responded in a positive manner to increased ration levels. This correlation between ration level and growth is not unique to fisheries research (Bradford and Geen, 1992; Narimatsu and Munehara, 1997; Dickey et al., 1997; Pedersen, 1997; Bestgen and Bundy, 1998); however, few studies have evaluated the effects of gradual increases or decreases in ration level. Paperno et al. (1997) addressed this issue for juvenile weakfish (*Cynoscion regalis*) at multiple ration levels based on body weight and found that growth was positively correlated to ration level. In this study, weight responded more quickly than length to reduced ration levels, but over time, the response of length became more pronounced. In this study, differences in values of weight demonstrated some separation among ration levels at Day 10, while length showed no separation at any ration level; however, Day 20 and Day 30 length values did show separation between the highest ration levels and the lowest ration levels. Consequently, weight may be a more sensitive indicator of condition than length for early stages of feeding stress, while both length and weight appear to be useful indicators of nutritional stress for durations in excess of 20 days. These results can be explained by the ability of fishes to mobilize energy reserves during times of reduced food intake. In studies examining white sturgeon (*Acipenser transmontanus*) and rainbow trout (*Oncorhynchus mykiss*), it was determined that carcass and visceral protein are better conserved than lipids during extended periods of starvation (Reinitz, 1983; Hung et al., 1997). Combined with the fact that metabolic rates can decline for several weeks before stabilizing after the onset of feeding stresses, losses in body weight should be evident before reductions in standard length (Jobling, 1980).

Otolith growth measurements did not respond as rapidly to reduced ration levels as somatic growth measurements. By Day 10, there was no clear separation among ration levels for any measure of otolith growth. Statistical separation among ration levels took between 20 and 30 days to manifest, suggesting a delay between the onset of stress and a reduction in otolith growth (i.e. uncoupling). This delay in otolith growth is not uncommon and has been documented for numerous species with uncoupling between the otolith growth and somatic growth relationship taking up to 21 days (Neilson and Geen, 1985; Molony and Choat, 1990; Bradford and Geen, 1992; Paperno et al., 1997;

Thorrold et al., 1997; Bestgen and Bundy, 1998). This change in the deposition of otolith material supports the daily increment packing (DIP) hypothesis proposed by Secor and Dean (1989). This hypothesis states that as long as suboptimal conditions are not prolonged, otolith growth will continue independent of somatic growth. The breakdown of this relationship continues until some point in time where otolith deposition ceases due to the lack of energy reserves. The amount of time it takes for these changes to manifest are variable and dependent on the species and energy reserves of each individual fish (Hoff and Fuiman, 1993). In addition, Folkvord et al. (2000) described otolith growth as being suitable for detecting recent improvements but stated that in addition to otolith growth, other indices may be needed to better assess deteriorating conditions.

Several studies have documented the influence of temperature over otolith growth (Neilson and Geen, 1985; Bradford and Geen, 1992); therefore, in order to reduce this influence on otolith deposition, we maintained a constant temperature throughout the duration of the experiment. This allowed for ration levels to produce salient differences in otolith growth without the influence of metabolic rates (Hoff and Fuiman, 1993). The lack of temperature fluctuation does not accurately reflect actual environmental conditions experienced by juvenile red drum in the wild, but rather, it demonstrates a slower reaction of the otolith to decreased ration levels as compared to somatic changes. This delay in the cessation of otolith deposition may help explain some of the variability in studies using otolith deposition as a determinate of recent growth (e.g. Suthers et al., 1989; Mugiya and Oka, 1991; Gallego et al., 1996; Rooker and Holt, 1997; Stunz, 1999; Folkvord et al., 2000). For example, a reduction in growth for juvenile red drum as a result of feeding stress would not be evident in the otolith record for approximately 20 days.

Of the four otolith measurements obtained, growth along the rostrum–postrostrum axis (Measurement A) of the sagittal otolith was the most sensitive to a reduction in ration levels; however, differences among ration levels for any otolith measurement were not evident until Day 20. This lack of sensitivity in the three remaining otolith measurements (sagittal B, lapillar A and B), highlights the ability of fishes to continue otolith growth independent of somatic growth (Hoff and Fuiman, 1993; Folkvord et al., 2000). Because of their relative size and ease of extraction, sagittal otoliths are most often used in experimental assays (David et al., 1994). This size, however, may explain the increased sensitivity of sagittal A. Sagittal otoliths are often much larger than the lapillar and asteriscus otoliths (Secor et al., 1989); as such, maintenance and growth (protein deposition) should be the most physiologically expensive. As a result, energetically, it may be easier to maintain deposition to the smaller otoliths during extended periods of feeding stress. Ecologically, it may also be advantageous to maintain energy to the smaller otoliths; however, little literature has been devoted to these otoliths and their role beyond balance and hearing. Lack of sensitivity by sagittal otolith Measurement B can be explained by the unique growth characteristics of sagittal otolith. Sagittal otoliths, and more specifically, red drum sagittal otoliths, grow along the sagittal plane to a greater extent than the transverse plane (Secor and Dean, 1992; Hoff and Fuiman, 1993). As a result, continued growth in the transverse plane (Measurement B) may be maintained during times of increased feeding stress as a result of lower physiological costs to the fish.

Assessments of condition can be made using techniques such as length–weight regressions, Fulton-type condition factors, relative condition factors, and relative weights.

One problem with these techniques is that somatic growth is the only measurement used and can be subject to variability within and among populations (Murphy et al., 1992). In an effort to better describe the condition of fishes, techniques combining somatic growth with others measures (gonads, liver, and intraperitoneal fat) have been developed and utilized [e.g. gonadosomatic index (GSI), hepatosomatic index (HSI), and intraperitoneal fat ratio (IPF)] (Craig et al., 2000). By combining measures of somatic growth and otolith growth, we determined that the most sensitive measure of condition as it relates to variable ration levels was the ratio of sagittal otolith weight to body weight. This expression enabled different ration levels to be separated out as early as Day 10, continued through Day 20, but then lost sensitivity by Day 30. Loss of sensitivity between Day 20 and Day 30 correlates with the "recoupling" of somatic and otolith growth. This analysis may be useful when other techniques (i.e. single measures of somatic or otolith growth) have failed to detect differences among treatments and/or sampling locations as a result of species specific uncoupling of somatic and otolith growth.

RNA/DNA ratios have been an effective tool for assessing conditions of fish under different environmental situations (Bergeron, 1997; Rooker et al., 1997); in addition, white muscle has been shown to be extremely sensitive tissue to feeding stress (Loughana and Goldspink, 1984; Black and Love, 1986). Results from this study suggest that RNA/DNA ratios can effectively separate starved red drum (0% ration level) from other ration levels as early as Day 10. These findings are similar to previous studies that have shown that RNA/DNA ratios are moderate predictors of protein and useful indicators of nutritional stress in red drum (Brightman et al., 1997; Rooker et al., 1997). According to Rooker and Holt (1996), RNA/DNA ratios decreased to significantly different levels two days after the onset of starvation. Similarly, for Atlantic cod (*Gadus morhua*), a decrease in RNA/DNA values appears to occur between 3 and 4 days of starvation (Clemmesen and Doan, 1996). In this study, differences were evident at Day 10 between red drum experiencing starvation (0% ration level) and the two highest ration levels (15% and 20% ration levels), confirming the trend that RNA/DNA ratios can be used to assess condition. Nonetheless, ration levels that were less than optimal (2.5%, 5%, and 10%) maintained constant RNA/DNA ratios throughout the duration of the experiment. Consistent with the findings of Clemmesen (1996), these results suggest that this indirect measure may not be sensitive to modest changes in condition of red drum.

In conclusion, findings from this study demonstrate that during periods of feeding stress, both somatic growth and otolith growth of red drum growth maintain a positive relationship to ration level. However, the onset of this relationship is often delayed for certain otolith measurements, demonstrating a breakdown in the otolith growth–somatic growth relationship. In addition, RNA/DNA was an inefficient tool for delineating among ration levels, but it was good at discriminating between fish that had been feeding and those that had not. Overall, a combination of measurements of somatic growth (weight) and otolith length (Measurement A) was the most sensitive to small differences among ration levels regardless of length of stress experienced by the fish. This combination is recommended for use in future studies for comparative purposes. Recent growth along the rostrum–postrostrum axis of the sagittal otolith, weight, and length measurements were also sensitive to feeding stress; however, length and otolith measurements should be used as a measure of condition 10–20 days after the onset of stress.

Future studies utilizing these relationships will enhance our understanding of otolith growth–somatic growth relationship for juvenile red drum. By understanding these relationships, information stored in the otolith may be accessible and decipherable, allowing for its use to analyze the red drum in its natural environment. Future analysis of reduced ration levels on fish growth should utilize the descriptive techniques of the daily otolith increment widths found on all three otoliths to better describe the otolith growth–somatic growth relationship.

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