Mar. Freshwater Res., 1996, **47**, 283–90

Application of RNA:DNA Ratios to Evaluate the Condition and Growth of Larval and Juvenile Red Drum (*Sciaenops ocellatus*)

Jay R. Rooker and G. Joan Holt

The University of Texas at Austin, Marine Science Institute, 750 Channelview Drive, Port Aransas, Texas 78373, USA.

Abstract. RNA:DNA ratios in individual red drum (*Sciaenops ocellatus*) were measured in laboratory-reared larvae and juveniles (6–20 mm standard length; age 16–40 days) to assess the effects of growth, starvation, and diel periodicity on biochemical condition. RNA:DNA ratios were correlated positively with both absolute (mm day^{-1}) and instantaneous (% day^{-1}) growth rates. The effect of starvation was evaluated daily over 5 day periods at three ontogenetic stages (20, 30, and 40 days). Significant differences in RNA:DNA ratios of fed and starved larvae were observed and changes in biochemical condition were detected statistically within 1 to 2 days of food deprivation. RNA:DNA ratios decreased continuously over the entire 5 day starvation period, with relative reductions in RNA:DNA ratios decreasing with increasing age. Diel variations in RNA:DNA ratios were investigated in controlled (constant) and natural (cyclical) temperature environments over a 48 h 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 diel variations in biochemical condition. Findings from this 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*.

Introduction

Effective management of marine fish populations is contingent on understanding the nature and causes of variability in recruitment and year-class strength. Variability in annual recruitment success is thought to be largely controlled by starvation and predation during early life-history stages (Houde 1987; Sinclair 1988; Jones 1990; Cushing and Horwood 1994). Starvation and predation are inherently interrelated and individual effects are difficult to separate in the field. Poor nutritional condition leads to reduced growth rates (Buckley 1984) and prolongs the duration of the larval stage. As a result, susceptibility to predation is increased, causing reductions in cohort survivorship (Houde 1987; Rice *et al.* 1993). Small changes in mortality due to variations in growth and larval stage duration can ultimately determine recruitment success (Houde 1987).

Uncertainties resulting from a poor understanding of condition and growth of natural populations during early life-history stages have limited the ability to adequately evaluate factors affecting larval survival and juvenile recruitment success. Consequently, unbiased estimates of nutritional condition and relationships to growth for natural populations are needed by fisheries workers to properly evaluate early life-history processes. In recent years, a variety of techniques has been developed to assess the nutritional condition of larval and juvenile fishes. These include morphological, histological, and biochemical indices (see review by Ferron and Leggett 1994). The need for sensitive indicators of nutritional condition and growth has led to extensive research on biochemical indicators, particularly nucleic acids (Buckley 1979, 1980, 1981, 1984).

The ratio of ribonucleic acid (RNA) concentration to deoxyribonucleic acid (DNA) concentration in body tissue is a useful indicator of nutritional condition. The quantity of DNA per cell is a species constant in somatic tissue, whereas the quantity of RNA (primarily associated with the ribosomes) varies with the rate of protein synthesis. Since growth in larvae is accomplished through protein synthesis, the ratio of RNA to DNA can potentially be used to assess nutritional condition and growth at any given time. The RNA:DNA ratio has proven to be a useful indicator of growth rate for a variety of species (Buckley 1979, 1980, 1981; Westerman and Holt 1994). Moreover, studies on the effect of food availability have demonstrated a strong relationship between nutritional and biochemical indices. RNA:DNA ratios of several species of larval fishes have been positively correlated with prey density (Buckley 1979; Buckley *et al.* 1984). Furthermore, during periods of starvation, RNA:DNA ratios have been shown to continually decrease (Wright and Martin 1985; Richard *et al.* 1991; Clemmesen 1994).

The present study was designed to evaluate the condition and growth of larval and early juvenile red drum (*Sciaenops*

1323-1650/96/020283$05.00

ocellatus) by biochemical techniques. RNA:DNA ratios were quantified for individual *S. ocellatus* to determine the effects of size, age, and growth rate on biochemical condition. Laboratory-reared larvae were used to examine the effect of starvation on the RNA:DNA ratio at different ages. The effect of diel periodicity under controlled and natural temperature conditions was also investigated. Information from this study will assist in determining the applicability of nucleic acids as reliable measures of nutritional condition and provide the foundation for future condition studies on wild-caught *S. ocellatus* larvae and juveniles.

Materials and Methods

Sciaenops ocellatus eggs were obtained from laboratory spawns induced by photoperiod and temperature cues (Arnold 1988) at the University of Texas Marine Science Institute, Port Aransas, Texas, USA. Eggs were treated with a 10 ppm formalin bath for 1 h and placed in 150 L cone-bottom, vertical tanks with internal biofilters (Holt 1993). Eggs hatched within approximately 24 h and larvae began feeding at 3 days after hatching following the depletion of the yolk sac. After 20 days larvae were transferred to a 1100 L, modified V-bottom raceway located in a greenhouse with removable partitions and an external biological filter (Holt 1992). Larvae were fed live rotifers (*Brachionus plicatilis*) at a rate of 3–5 mL^{-1} from 3 to 12 days and brine shrimp nauplii (*Artemia salina*) at 1–2 mL^{-1} from 12 to 25 days. Beyond 25 days, larvae and juveniles were fed a combination of yellowtail snapper eggs (*Ocyurus chrysurus*) and shrimp slurry. The laboratory was kept under controlled temperature and photoperiod conditions of 26°C and 12L–12D, respectively. During the day, approximately 675 μE [675 μmol] m^{-2} s^{-1} of light were available at the surface of the tanks. Salinity was maintained at PSS 29 and total ammonia nitrogen below 0.5 ppm. The effects of age, size, and growth rate on the RNA:DNA ratio were measured from collections taken at 2–5 day intervals over the rearing period (16–40 days). Absolute (mm day^{-1}) and instantaneous (specific) growth rates (% day^{-1}) were calculated as:

$$\text{Absolute growth rate} = (L2–L1)/(T2–T1)$$

$$\text{Instantaneous growth rate} = [(\ln L2 – \ln L1)/(T2 – T1)] \cdot 100,$$

where T1 and T2 are the beginning (0 days) and end (20 days) of a time interval, respectively, and L1 and L2 are the respective lengths at those times. Growth rates were based on mean population length (L1) against individual final lengths at 20 days (L2). Growth rates and RNA:DNA ratios were calculated individually for 48 *S. ocellatus* specimens. In addition, mean population growth rates and RNA:DNA ratios were estimated at 16, 18, 20, 25, 30, 35, and 40 days. Final lengths (L2) for each time period were based on individual lengths, and initial lengths (L1) were based on the mean population length at the first day of the interval.

Starvation experiments were conducted on variously aged larvae and juveniles: 20–25, 30–35, and 40–45 days. A sample of 8 individuals was taken 1 day before starvation (T_0) as a control and the remaining larvae were placed individually in 1-L beakers with similar water conditions. Samples of 8 larvae were collected daily from randomly selected beakers for 5 days: T_1, T_2, T_3, T_4, and T_5. Larvae were sampled at about 1200 hours for each sampling interval. A second control sample (fed larvae) was also collected at T_5 for trials beginning at 20 and 30 days. Survivorship during all starvation trials was greater than 90%.

The effects of diel periodicity on RNA:DNA ratios were investigated for 20-day-old larvae over a 2-day period. Samples were taken from 150-L cone tanks at 4-h intervals over a 48-h period beginning at 0800 hours. At each sampling interval, 6 larvae were collected from each of two replicate tanks located in a temperature-controlled (26±0·05°C) wet laboratory. To evaluate the potential effects of natural cyclical variations on RNA and DNA, replicate tanks (partitioned 1100 L V-bottom) were also set up in a greenhouse and stocked with individuals from the cone tanks described above. In a manner similar to the sampling protocol described above, 6 individuals were sampled from two replicate tanks every 4 h. Larvae in both experiments (constant and fluctuating temperature) were from the same spawn. Individuals in the greenhouse received natural lighting and experienced daily temperature fluctuations of approximately 6°C (about 23–29°C). Continuous temperature monitoring (5 min intervals) was conducted in greenhouse tanks with a Hydrolab Data Sonde III.

Larvae and juveniles from all trials were anaesthetized with tricaine methanesulfonate (MS-222) and measured (standard length) to the nearest 0·1 mm. After length was obtained, individuals were placed immediately on dry ice and stored at –80°C. Nucleic acid and protein determinations were made on sections of epaxial and trunk white muscle tissue from larval and juvenile *S. ocellatus*. Individual white muscle samples were homogenized in 800 μL ice-cold 1 M NaCl. Homogenates were immediately centrifuged at 3000 *g* for 45 min at 4°C. Aliquots (100 μL) were drawn for fluorometric and spectrophotometric analyses.

DNA and RNA measurements were made by the ethidium bromide (EB) fluorometric technique described by Westerman and Holt (1988). Aliquots of homogenates (100 μL) were used to estimate DNA and RNA values. Calculations were based on comparisons with DNA–EB and RNA–EB calibration curves from known standards. Calf thymus DNA and yeast RNA (Type III) were used as standards. Aliquots were also assayed for total soluble protein concentration as described by Bradford (1976). Proteins were estimated from standard curves derived from known quantities of bovine serum albumin. Proteins were measured as primary amines after acid hydrolysis by staining with Coomassie brilliant blue and spectrophotometric detection.

A one-way analysis of variance (ANOVA) was used to examine the effect of starvation on RNA:DNA ratios. A three-way factorial ANOVA with day, time of day, and temperature as main effects was used to examine diel changes in RNA:DNA ratios in constant and fluctuating temperature treatments. Significant (α = 0·05) results were examined further with Tukey's HSD multiple-comparison test to determine which levels of the main effect(s) differed from other levels. Regression analysis was used to investigate the effects of growth rate and starvation on RNA:DNA ratios by the least-squares linear method (Sokal and Rohlf 1981). Differences in regression lines were examined by analysis of covariance (ANCOVA). The software package SYSTAT was used for statistical testing (Wilkinson 1989).

Results

RNA:DNA ratios of white muscle tissue from individual larval and juvenile *S. ocellatus* were affected by age and size. Ontogenetic changes in RNA:DNA ratios were most pronounced during early development. RNA:DNA ratios increased continuously with size for *S. ocellatus* between 6 and 10 mm (Fig. 1). Values observed for 6 and 10 mm individuals ranged from 11·6 to 20·0, respectively. For late larval and early juvenile *S. ocellatus* (10–20 mm), RNA:DNA ratios remained relatively constant, with slight reductions occurring for larger individuals (>14 mm). Similar trends were present when ratios were compared with age (days). RNA:DNA ratios increased during early development (16–25 days) and reached maximum values at 25 days. Beyond 25 days, RNA:DNA ratios ranged from 15 to 20. Curvilinear relationships (second-order polynomial) comparing ratios with size and age are expressed as

$y = -13{\cdot}33 + 5{\cdot}04x - 0{\cdot}19x^2$ ($r^2 = 0{\cdot}82$) and $y = -10{\cdot}32 + 1{\cdot}88x - 0{\cdot}03x^2$ ($r^2 = 0{\cdot}69$), respectively.

Ontogenetic changes in mean RNA and DNA concentrations were also observed for *S. ocellatus*. RNA and DNA concentrations per protein decreased with size (Fig. 2). RNA and DNA concentrations were extremely high for small larvae (6 mm) and declined drastically over a narrow size range (6–8 mm). Similar trends in reduction of RNA and DNA concentrations were also observed with changes in age. To facilitate size and age comparison with RNA:DNA ratios, the age–length equation ($r^2 = 0{\cdot}93$) for laboratory-reared *S. ocellatus* is $L = 2{\cdot}10\ \exp(0{\cdot}0645t)$, where L and t represent standard length (mm) and age (days), respectively.

The RNA:DNA ratio was a significant predictor of growth rate for individual *S. ocellatus*. Estimations of RNA:DNA ratios for 20-day-old individuals ($n = 48$) were correlated with absolute and instantaneous growth rates measured in mm day^{-1} and % day^{-1}, respectively (Figs 3*a* and 3*b*). Linear regression of absolute growth rate on the RNA:DNA ratio was highly significant ($P < 0{\cdot}001$) and described by the equation $y = 1{\cdot}98 + 44{\cdot}92x$ ($r^2 = 0{\cdot}65$), where RNA:DNA ratio is the dependent variable. In like manner, the relationship between RNA:DNA ratio and instantaneous growth rate was highly significant ($P < 0{\cdot}001$) and described as $y = -7{\cdot}88 + 3{\cdot}80x$ ($r^2 = 0.61$). Thus, over 60% of the variation in the RNA:DNA ratio of individual late larval and juvenile *S. ocellatus* appears related to changes in length-based growth rate.

In addition to individual estimates, mean RNA:DNA ratios as a function of mean growth rates were determined at

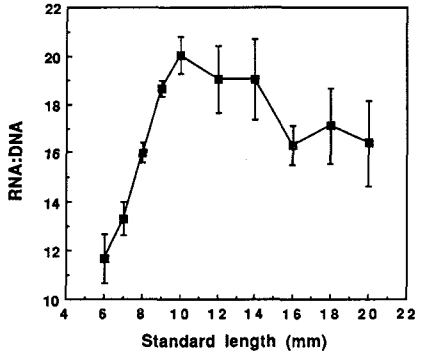

Fig. 1. Relationship between RNA:DNA ratios and standard length (mm) for larval and juvenile *Sciaenops ocellatus* ($n = 144$). Values are means from 1–2 mm size class categories ± standard error. Size class intervals above 10 mm represent 2 mm groupings (e.g. 12 ± 1 mm).

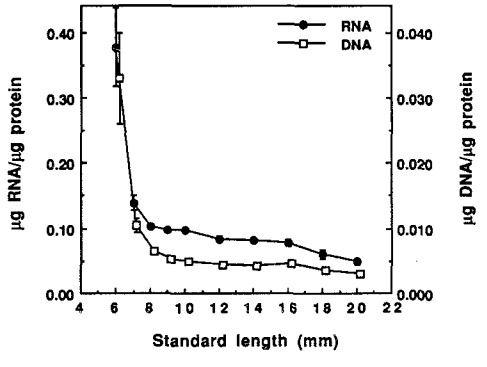

Fig. 2. Relationship between mean nucleic acid concentrations (RNA/protein, DNA/protein) and standard length (mm) for larval and juvenile *Sciaenops ocellatus* ($n = 144$). Values are means of 1–2 mm size class categories ± standard error.

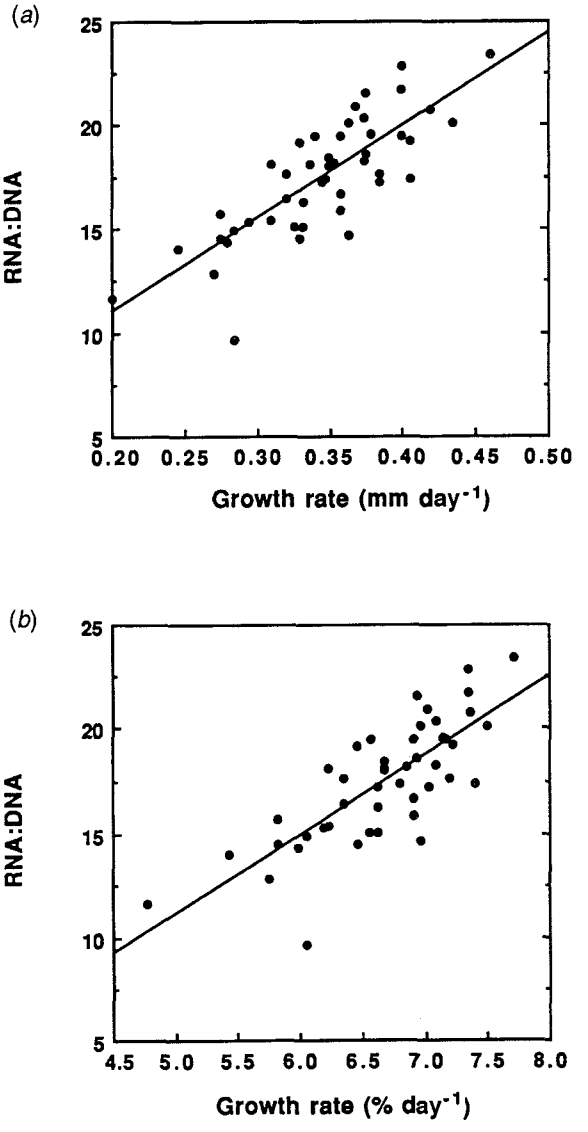

Fig. 3. Linear regressions representing RNA:DNA ratios as a function of (*a*) absolute growth rate (mm day^{-1}) and (*b*) instantaneous (specific) growth rate (% day^{-1}) for 20-day-old *Sciaenops ocellatus* ($n = 48$).

different intervals (16, 18, 20, 25, 30, 35, and 40 days). Regressions of absolute and instantaneous growth rate on RNA:DNA ratio were $y = 5{\cdot}80 + 27{\cdot}60x$ ($r^2 = 0{\cdot}74$) and $y = -1{\cdot}05 + 2{\cdot}93x$ ($r^2 = 0{\cdot}65$), respectively. Although regression equations for mean RNA:DNA ratio and growth rate estimates were significant ($P < 0{\cdot}05$), relationships were associated with markedly higher variability than that observed for individuals at 20 days, as indicated by significance level of regressions.

The effect of starvation was evaluated at three ontogenetic periods for *S. ocellatus*: 20–25, 30–35, and 40–45 days. Data presented here describe the effect of starvation on RNA:DNA ratios over 5 day starvation periods. At each ontogenetic stage, RNA:DNA ratios decreased continuously over the 5 day starvation periods (Fig. 4). Regression analyses indicated that the relative magnitude of the decline in the RNA:DNA ratio was associated with ontogenetic stage (Table 1). As age increased, the relative decline in RNA:DNA ratios was reduced. The slope of the regression equations, indicating relative change in the RNA:DNA ratio, was significantly (ANCOVA, $P < 0{\cdot}05$) higher for younger *S. ocellatus* (T_0 = 20 days; slope = –3·2) than older S. *ocellatus* (T_0 = 40 days; slope = –1·9). The relative reduction in the RNA:DNA ratio was approximately 40% lower for the older stage *S. ocellatus*.

The consequence of starvation on *S. ocellatus* is a significant reduction in RNA:DNA ratios (ANOVA, $P < 0{\cdot}01$). Results from Tukey's HSD test showed that starved larvae were statistically recognizable ($P < 0{\cdot}05$) from fed larvae (control = T_0) within 1 or 2 days of starvation. For starvation trials beginning on 20 and 40 days, RNA:DNA ratios of starved larvae were significantly different from the control (T_0) within 2 days of starvation. Similar results were obtained for trials at 30 days; however, starved larvae could be statistically separated from controls at 1 day. In all cases, significant results distinguishing starved larvae from fed larvae (T_0 controls) were supported by all remaining starvation days within a trial. For two trials, an additional control (*S. ocellatus* fed all 5 days) was incorporated into the experimental design. No significant differences were detected between controls at 0 and 5 days.

The response of RNA:DNA ratios to diel periodicity was examined in both controlled and natural temperature environments over a 48 h period. Highly significant ($P < 0{\cdot}001$) differences in RNA:DNA ratios were observed over diel periods in both environments (Fig. 5). RNA:DNA ratios remained relatively high in both environments during all diurnal periods (0800, 1200, 1600, 2000 hours). Peak values were observed in both treatments at 1200 and 1600 hours. Marked declines in RNA:DNA ratios were observed in both temperature environments during nocturnal periods (0000, 0400 hours). Although temperature changes between controlled and natural tanks were invariably different, diel trends in RNA:DNA ratios were highly similar and no significant differences (ANOVA, $P > 0{\cdot}05$) were detected between temperature treatments.

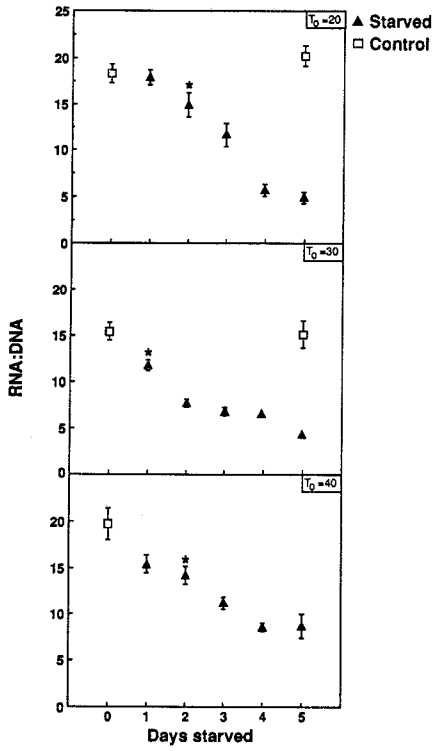

Fig. 4. Variations in RNA:DNA ratios of fed (control) and starved larval and juvenile *Sciaenops ocellatus*. Starvation periods were 5 days in duration and conducted at three ontogenetic stages (T_0 = 20, 30, 40 days); T_0 represents the day before starvation (control). An additional control (fed individuals) is also represented at 5 days. Values are daily means based on 8 individuals ± standard error. Asterisk denotes period when starved larvae differ significantly ($P < 0{\cdot}05$) from T_0 control.

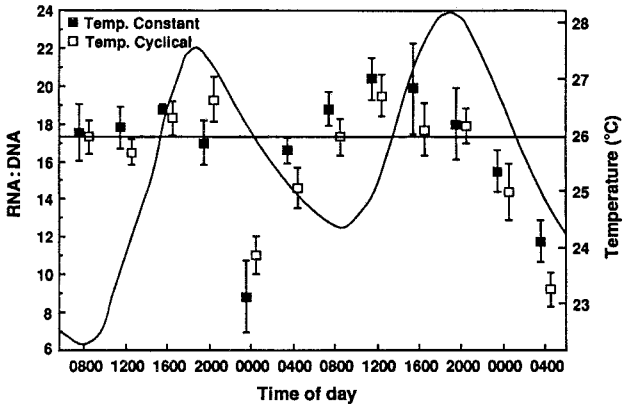

Fig. 5. Diel variations in RNA:DNA ratios of *Sciaenops ocellatus* in controlled (constant) and natural (cyclical) temperature environments. RNA:DNA ratios are examined at 4 h time intervals over a 48 h period. Values are means (6 individuals) ± standard error. Constant and cyclical temperature profiles (solid lines) are superimposed.

Table 1. Regression equations and coefficients of determination (r^2) for starvation-RNA:DNA relationships for *Sciaenops ocellatus*

The RNA:DNA ratio represents the dependent variable (y), and days starved (0, 1, 2, 3, 4, 5 days) is the independent variable (x). T_0 represents the day before the first day starved (T_1)

Independent (x)	Dependent (y)	Regression equation	r^2
Days starved (T_0 = 20)	RNA:DNA	y = 20·238–3·168x	0·958
Days starved (T_0 = 30)	RNA:DNA	y = 14·333–2·160x	0·877
Days starved (T_0 = 40)	RNA:DNA	y = 17·976–1·917x	0·773

Table 2. Diel variation in RNA/protein and DNA/protein (μg/μg) for *Sciaenops ocellatus* at age 20 days

Values are means ± standard error of 24 individuals. Means within each column that do not share a common superscript differ significantly ($P < 0·05$) according to Tukey's HSD results

Time of day (hours)	μg RNA/μg protein		μg DNA/μg protein	
	Day 1	Day 2	Day 1	Day 2
0000	0·060 ± 0·006[a]	0·079 ± 0·006[a]	0·0058 ± 0·0003[a]	0·0052 ± 0·0003[a]
0400	0·089 ± 0·009[b]	0·057 ± 0·003[b]	0·0063 ± 0·0006[a]	0·0057 ± 0·0003[a]
0800	0·099 ± 0·003[b]	0·096 ± 0·004[c]	0·0058 ± 0·0004[a]	0·0052 ± 0·0005[a]
1200	0·106 ± 0·005[b]	0·102 ± 0·001[c]	0·0061 ± 0·0003[a]	0·0053 ± 0·0002[a]
1600	0·096 ± 0·002[b]	0·094 ± 0·003[c]	0·0053 ± 0·0004[a]	0·0052 ± 0·0002[a]
2000	0·108 ± 0·006[b]	0·100 ± 0·002[c]	0·0055 ± 0·0006[a]	0·0058 ± 0·0004[a]

No significant differences were detected among temperature and day treatments; however, a significant diel period·day interaction effect ($P < 0·01$) was detected. RNA:DNA ratios showed a reverse response during consecutive nights: lowest at 0000 hours and 0400 hours on Days 1 and 2, respectively. The profile of diel variation observed for RNA concentrations was highly similar to RNA:DNA ratios (Table 2). In general, RNA concentrations were significantly different ($P < 0·05$) between diurnal (0800, 1200, 1600, 2000 hours) and nocturnal (0000, 0400 hours) periods. Diel variation in DNA concentrations among time periods was relatively constant and statistical differences were not observed ($P > 0·05$).

Discussion

RNA:DNA ratios and RNA and DNA concentrations of *S. ocellatus* showed ontogenetic patterns during early development. Similar to findings by Buckley (1981), biochemical measures were more closely linked to size than to age. The lowest RNA:DNA ratios observed in the present study occurred for the smallest larvae investigated (5–7 mm) and increased with size and age. At approximately 10–14 mm (20–30 days), RNA:DNA ratios reached their highest value and remained relatively constant with increasing size and age. Increased RNA:DNA ratios during early development have been reported (Richard *et al.* 1991; Clemmesen 1994). Westerman and Holt (1994) proposed that increases in RNA:DNA ratios are correlated with rapid cell divisions and increasing cell size (hypertrophy) during early ontogeny. Results from the present study showed that RNA/protein and DNA/protein decreased exponentially during early development; however, after approximately 4 weeks (about 14 mm), both RNA and DNA concentrations remained constant. These findings suggest that hypertrophy is occurring during early development and that, after a certain period, growth at the cellular level becomes relatively uniform.

Because of differences in methodology (e.g., tissue preparation, standards), RNA:DNA ratios determined from this study should not be directly compared with those from other studies; however, general comparisons are warranted and may provide insight for future work. RNA:DNA ratios observed for *S. ocellatus* varied greatly from other reported values for late stage larvae and juveniles (20–40 days). Our value of 18 is markedly higher than ratios reported for other marine species, for which values less than 5 are common (Clemmesen 1987, 1994; Richard *et al.* 1991; Bergeron and Boulhic 1994; Westerman and Holt 1994). However, most studies evaluating biochemical measures are based on cold-water species that generally show lower RNA:DNA ratios (Buckley 1984). In addition, samples used for biochemical analyses in these studies were all based on whole-fish samples rather than white muscle tissue. Preliminary comparisons of whole-fish *v.* white muscle tissue samples performed in our laboratory indicated that the RNA:DNA ratios of whole-fish samples of *S. ocellatus* were approximately 30–35% of values obtained from white muscle tissue samples. Thus, a mean RNA:DNA ratio of 18 for white

muscle samples corresponds to a whole larvae sample of approximately 5–6, which is consistent with whole-fish values of *S. ocellatus* reported by Westerman and Holt (1994)

Westerman and Holt (1994) demonstrated that the RNA:DNA ratio is a useful indicator of growth rate for larval *S. ocellatus*. Moreover, Buckley (1979, 1980) presented similar results for Atlantic cod (*Gadus morhua*) and winter flounder (*Pseudopleuronectes americanus*). In like manner, RNA:DNA ratios in the present study were strongly correlated with both absolute and instantaneous growth rates, explaining over 60% of the variation in growth rates. Results are encouraging, considering that values used for regression analyses were derived from individual estimates rather than mean or pooled samples. A significant correlation also existed between mean population growth rates and RNA:DNA ratios, with over 65% of the variation in absolute and instantaneous growth explained by RNA:DNA ratios. Westerman and Holt (1994) reported that the RNA:DNA ratio loses resolution as a predictor when growth rates are extremely variable. In the present study, individual variability in growth rates was high for sample periods beyond 16 days (e.g. for 30 days, growth ranged from 0·2 mm day^{-1} to 0·8 mm day^{-1}). Consequently, we believe the relationship between growth rate and RNA:DNA ratios may have been compromised, particularly since mean values for certain periods were derived from small samples.

The effect of starvation on RNA:DNA ratios of *S. ocellatus* is in agreement with findings reported for other species (Buckley 1980; Clemmesen 1987; Lowery and Somero 1990; Richard *et al.* 1991). Namely, significant differences in RNA:DNA ratios of fed and starved larvae were observed and changes in RNA:DNA ratios of *S. ocellatus* could be detected statistically within 1 to 2 days of food deprivation. Further extensions of starvation periods resulted in a continued decline in RNA:DNA ratios. Reductions in protein synthesis, related to both a decrease in the muscle quantity of rRNA and the reduced efficiency of amino acid incorporation (Ferron and Leggett 1994), appears responsible for declines in RNA:DNA ratios during starvation trials.

Results from starvation experiments also demonstrated that RNA:DNA ratios were influenced by ontogenetic stage. The decline in RNA:DNA ratios was more rapid in earlier developmental periods. In addition, relative changes in RNA:DNA ratios during starvation were of smaller magnitude and more variable in later periods of development. In the earliest starvation trials (T_0 = 20 days), RNA:DNA ratios decreased approximately 16% per day and 96% of the change in nutritional condition was explained by RNA:DNA ratios. In contrast, RNA:DNA ratios during later stages of development (T_0 = 40 days) were less affected by food deprivations (11% loss per day) and the linear model explained only 77% of the variability. Similar findings were reported by Westerman and Holt (1988) for earlier-stage *S. ocellatus* and, therefore, comparisons of RNA:DNA ratios during starvation must be limited to the same size or developmental stage.

Additional sources of variability, both biotic and abiotic, have been shown to produce strong confounding effects on RNA:DNA ratios (Ferron and Leggett 1994). One factor receiving limited attention in previous studies is diel effects on RNA:DNA ratios. Mugiya and Oka (1991) reported diel variation in RNA:DNA ratios from muscle tissue of immature rainbow trout (*Oncorhynchus mykiss*). They reported that high ratios during daytime periods were followed by a steep decrease at night. The present study on late larval *S. ocellatus* also identified salient diel shifts in RNA:DNA ratios. Similar to the case of *O. mykiss*, high RNA:DNA ratios were present during daytime sampling periods (0800, 1200, 1600, 2000 hours) and marked reductions occurred at night (0000, 0400 hours). The cyclical nature of temperature was originally thought to play a primary role in regulating diel changes in RNA:DNA ratios. However, diel variations in RNA:DNA ratios observed in the present study did not appear to result from temperature fluctuation for two reasons. First, no significant differences in RNA:DNA ratios were detected between constant and cyclical temperature regimes. Similar diel trends were observed in both temperature conditions. Second, no consistent lag period was observed between changes in temperature and RNA:DNA ratios. During certain periods, increases in RNA:DNA ratios were occurring when temperature was decreasing (0400 to 0800 hours) and increasing (1200 to 1600 hours). As a result, it is probably more likely that circadian periodicities in endocrine activity are responsible for observed patterns. Circadian periodicities in growth-regulating hormones have been reported for marine fishes (Bates *et al.* 1989). Since protein and nucleic acid synthesis are influenced by growth-regulating hormones (Weatherley and Gill 1987), cyclic variations in growth hormones, potentially driven by photoperiod, probably play some role in producing diel changes in RNA:DNA ratios.

Although diel fluctuations in temperature failed to produce significant changes in RNA:DNA ratios, temperature appears closely linked to RNA:DNA ratios (Buckley 1982; Buckley *et al.* 1984). Therefore, this effect must be assessed in all field-related studies. From preliminary field investigations we observed large variations in RNA:DNA ratios of similar-sized *S. ocellatus* inhabiting waters of different temperature (Rooker and Holt, unpublished data). RNA:DNA ratios increased approximately 1·0 to 1·2 per 1°C increase in temperature (midday). Therefore, sufficient knowledge of the effect of temperature on RNA:DNA ratios is critical to the application of

biochemical methods in the field, particularly for species such as *S. ocellatus,* whose early development occurs in highly variable environments.

Findings from this study support the use of the RNA:DNA ratio as an indicator of nutritional condition and growth of laboratory-reared *S. ocellatus*. Consequently, we believe RNA:DNA ratios are a potentially suitable measure of condition and growth for some field applications. However, even under controlled conditions, interindividual variability within treatments was moderate, as in other studies (e.g., Clemmesen 1988, 1989; Westerman and Holt 1988; McGurk and Kusser 1992). Furthermore, a number of environmental and physiological effects occur that may potentially bias comparisons. This study has demonstrated that RNA:DNA ratios are length- and age-dependent. As a result, the RNA:DNA ratio of young or small larvae will be lower than values observed for larger or older larvae. Failure to standardize by length and age will bias results and lead to underestimations of the condition of larvae early in ontogeny relative to late larvae and juveniles. Also, the effect of diel periodicity must be accounted for when evaluating the condition of field-caught larvae. Pelagic sampling protocols often occur during both diurnal and nocturnal periods and diel differences in RNA:DNA ratios will also compromise comparisons. In sum, this study has demonstrated the potential usefulness of RNA:DNA ratios for assessing the condition of *S. ocellatus*; however, it has also suggested that the use of the RNA:DNA method for assessing field samples requires certain precautions.

Acknowledgments

We are grateful to C. Arnold and S. Holt for their advice on various aspects of this work. K. Binney, E. Curtis, K. Drescher, P. Pickering, C. Pratt, and A. Soto provided assistance in the collection and analysis of samples. The manuscript benefited from the suggestions made by A. Davis and D. Higgs. Funding was provided, in part, by a grant from the Sid W. Richardson Foundation and a fellowship (E. J. Lund) to J.R.R. This is Contribution 960 of the University of Texas at Austin, Marine Science Institute.

References

Arnold, C. R. (1988). Controlled year-round spawning of the red drum (*Sciaenops ocellatus*) in captivity. *Contributions in Marine Science* **30** (Suppl.), 65–70.

Bates, D. J., Barrett, B. A., and Mckeown, B. A. (1989). Daily variations in plasma growth hormone of juvenile coho salmon, *Oncorhynchus kisutch*. *Canadian Journal of Zoology* **67**, 1246–8.

Bergeron, J. P., and Boulhic, M. (1994). RNA–DNA ratio and evaluation of the nutritional state and growth rate of saltwater fish larvae—experimental analysis of the Dover sole (*Solea solea* L.). *ICES Journal of Marine Science* **51**, 181–90.

Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein using the principal of protein–dye binding. *Analytical Biochemistry* **72**, 248–53.

Buckley, L. J. (1979). Relationship between RNA–DNA ratio, prey density and growth rate in Atlantic cod (*Gadus morhua*) larvae. *Journal of the Fisheries Research Board of Canada* **36**, 1497–502.

Buckley, L. J. (1980). Changes in ribonucleic acid, deoxyribonucleic acid, and protein content, during ontogenesis in winter flounder, *Pseudopleuronectes americanus*, and the effect of starvation. *Fishery Bulletin (US)* **77**, 703–8.

Buckley, L. J. (1981). Biochemical changes during ontogenesis of cod (*Gadus morhua* L.) and winter flounder (*Pseudopleuronectes americanus*) larvae. *Rapports et Procès-verbaux des Réunions, Conseil International pour l'Exploration de la Mer* **178**, 547–52.

Buckley, L. J. (1982). Effects of temperature on growth and biochemical composition of larval winter flounder (*Pseudopleuronectes americanus*). *Marine Ecology Progress Series* **8**, 181–6.

Buckley, L. J. (1984). RNA/DNA ratio: an index of larval fish growth in the sea. *Marine Biology (Berlin)* **80**, 291–8.

Buckley, L. J., Turner, S. I., Halavik, T. A., Smigielski, A. S., Drew, S. M., and Laurence, G. C. (1984). Effects of temperature and food availability on growth, survival, and RNA–DNA ratio of larval sand lance (*Ammodytes americanus*). *Marine Ecology Progress Series* **15**, 91–7.

Clemmesen, C. (1987). Laboratory studies on RNA/DNA ratios of starved and fed herring (*Clupea harengus*) and turbot (*Scophthalmus maximus*) larvae. *Journal du Conseil, Conseil International pour l'Exploration de la Mer* **43**, 122–8.

Clemmesen, C. (1988). A RNA and DNA fluorescence technique to evaluate the nutritional condition of individual marine fish larvae. *Meeresforschung* **32**, 134–43.

Clemmesen, C. (1989). RNA/DNA ratios of laboratory-reared and wild herring larvae determined with a highly sensitive fluorescence method. *Journal of Fish Biology* **35** (Suppl. A), 331–3.

Clemmesen, C. (1994). The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. *Marine Biology (Berlin)* **118**, 377–82.

Cushing, D. H., and Horwood, J. W. (1994). The growth and death of fish larvae. *Journal of Plankton Research* **16**, 291–300.

Ferron, A., and Leggett, W. C. (1994). An appraisal of condition measures for marine fish larvae. *Advances in Marine Biology* **30**, 217–303.

Holt, G. J. (1992). Experimental studies of feeding in larval red drum. *Journal of the World Aquaculture Society* **23**, 265–70.

Holt, G. J. (1993). Feeding larval red drum on microparticulate diets in a closed recirculating water system. *Journal of the World Aquaculture Society* **24**, 225–30.

Houde, E. D. (1987). Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium* **2**, 17–29.

Jones, G. P. (1990). The importance of recruitment to the dynamics of a coral reef fish population. *Ecology* **71**, 1691–8.

Lowery, M. S., and Somero, G. N. (1990). Starvation effects on protein synthesis in red and white muscle of the barred sand bass, *Paralabrax nebulifer*. *Physiological Zoology* **63**, 630–48.

McGurk, M. D., and Kusser, W. C. (1992). Comparison of three methods of measuring RNA and DNA concentrations of individual Pacific herring, *Clupea harengus pallasi*, larvae. *Canadian Journal of Fisheries and Aquatic Sciences* **49**, 967–74.

Mugiya, Y., and Oka, H. (1991). Biochemical relationship between otolith and somatic growth in the rainbow trout *Oncorhynchus mykiss*: consequence of starvation, resumed feeding, and diel variations. *Fishery Bulletin (US)* **89**, 239–45.

Rice, J. A., Crowder, L. B., and Binkowski, F. P. (1987). Evaluating potential sources of mortality for larval bloater (*Coregonus hoyi*): starvation and vulnerability to predation. *Canadian Journal of Fisheries and Aquatic Sciences* **44**, 467–72.

Rice, J. A., Miller, T. J., Rose, K., Crowder, L. B., Marschall, E. A., Trebitz, A. S., and DeAngelis, D. L. (1993). Growth rate variation and larval survival: inferences from an individual-based size-dependent predation model. *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 133–42.

Richard, P., Bergeron, J., Boulhic, M., Galios, R., and Person-Le Ruyet, J. (1991). Effect of starvation on RNA, DNA and protein content of laboratory-reared larvae and juveniles of *Solea solea*. *Marine Ecology Progress Series* **72**, 69–77.

Sinclair, M. (1988). 'Marine Populations: An Essay on Population Regulation and Speciation.' Books in Recruitment Fishery Oceanography. (University Washington Press: Seattle.) 252 pp.

Sokal, R. R., and Rohlf, F. J. (1981). 'Biometry.' 2nd edn. (Freeman: San Francisco.) 859 pp.

Weatherley, A. H., and Gill, H. S. (1987). 'The Biology of Fish Growth.' (Academic Press: New York.) 443 pp.

Westerman, M., and Holt, G. J. (1988). The RNA/DNA ratio: measurement of nucleic acids in larval *Sciaenops ocellatus*. *Contributions in Marine Science* **30**, 117–24.

Westerman, M., and Holt, G. J. (1994). RNA:DNA ratio during the critical period and early larval growth of the red drum *Sciaenops ocellatus*. *Marine Biology (Berlin)* **121**, 1–9.

Wilkinson, L. (1989). 'SYSTAT: The System for Statistics.' (SYSTAT, Inc.: Evanston, Il.)

Wright, D. A., and Martin, F. D. (1985). The effect of starvation on RNA/DNA ratios and growth of larval striped bass, *Morone saxatalis*. *Journal of Fish Biology* **27**, 479–85.

Manuscript received 30 June 1995; revised and accepted 13 November 1995