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Evaluation of RNA concentration as an indicator of growth in young-of-the-year winter flounder *Pseudopleuronectes americanus* and tautog *Tautoga onitis*

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ABSTRACT: White muscle tissue RNA concentration was evaluated as an indirect measure of fish growth. Young-of-the-year winter flounder *Pseudopleuronectes americanus* and tautog *Tautoga onitis* were grown in short-term caging experiments conducted during 1994 and 1995 within 3 geographically distinct estuarine systems in the NE of the USA: the Hammonasset River along the Connecticut coast of Long Island Sound, the Navesink River located in the Hudson-Raritan Bay estuary in northern New Jersey, and Great Bay-Little Egg Harbor estuary in southern New Jersey. Fish were caged in each of 5 habitat types including: eelgrass, macroalgae, unvegetated areas adjacent to eelgrass, unvegetated areas adjacent to macroalgae, and tidal marsh creeks. White muscle tissue RNA concentration (µg per mg wet tissue wt) was measured in winter flounder and tautog recovered from the cages and compared to instantaneous growth rate measurements of these same fish. RNA concentration was significantly correlated with growth rate measured as length ($r = 0.83$) and weight ($r = 0.79$) in winter flounder and length ($r = 0.69$) and weight ($r = 0.73$) in tautog. In most cases, estuary- and habitat-specific differences in growth rate, as determined by RNA concentration, were similar to those determined by measuring instantaneous growth. These results validate the use of RNA concentration as an indirect measure of growth in young-of-the-year winter flounder and tautog.

KEY WORDS: RNA · Winter flounder · Tautog · Estuary · Habitat

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INTRODUCTION

Traditionally, somatic growth measurements obtained through caging studies have been used to assess habitat specific growth patterns in juvenile fish (Sogard 1992, Able 1999, Phelan et al. 2000). Growth observations of caged fish may not accurately represent growth of wild fish in these same habitats. Differences in handling procedures and measuring techniques may introduce variability to growth results. Cage designs, which exclude certain prey types, may reduce growth rates (Able 1999). Caged fish, unable to avoid poor water quality, may experience reduced growth when stressed by unfavorable environmental conditions. Furthermore, caging studies are both time-

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consuming and labor-intensive and require an extensive field effort. These factors suggest that caging may not represent the most efficient and accurate means of assessing growth.

Biochemical techniques such as nucleic acid analyses have proven useful in studying the recent growth of young fish in response to environmental variability (Buckley et al. 1999). Ribonucleic acid (RNA) concentration has been used to indicate and predict protein synthesis rate, relative condition (Bulow 1987), and instantaneous growth rate (Mathers et al. 1992) in many species of juvenile fish (Goolish et al. 1984, Balstrop et al. 1991, Varnavskiy et al. 1991, Mathers et al. 1992, 1993, Foster et al. 1993, Wang et al. 1993, Carter et al. 1998). Nucleic acid analyses offer several advantages. The RNA methodology uses a small sample of white muscle tissue and reflects growth 1 to 3 d prior to sampling. This procedure is not subject to the errors implicit in repeated length and/or weight measurements of slow-growing fish and can be used in studies where no direct measure of growth rate is available. Further, RNA analysis can be used when caging or tagging and recapture of animals is impractical or impossible.

This study uses caging experiments to evaluate white muscle tissue RNA concentration as an indicator of short-term instantaneous growth rate for young-of-the-year winter flounder *Pseudopleuronectes americanus* and tautog *Tautoga onitis*. The suitability of the RNA-growth method and its applications as a relative measure of habitat quality is also addressed. This study is among the first to evaluate field growth of caged fish using both RNA concentration and instantaneous growth measurements.

MATERIALS AND METHODS

Study sites. Caging studies were conducted in 5 different habitat types within 3 geographically distinct estuaries in the NE of the USA during 1994 and 1995. Study sites included the Hammonasset River, along the Connecticut coast, part of the Long Island Sound estuarine system; the Navesink River in northern New Jersey, part of the Hudson-Raritan Bay estuarine system; and the Great Bay/Little Egg Harbor estuary in southern New Jersey. Fish were deployed into replicate areas representing 5 habitat types: eelgrass (EG) beds (*Zostera marina*), bottom colonized by drifting or attached macroalgae (MA) (primarily *Ulva lactuca*), unvegetated areas adjacent to eelgrass (UEG) and unvegetated areas adjacent to macroalgae (UMA), and tidal marsh creeks (MC). Drifting vegetation in the macroalgal habitats was ephemeral, in contrast to the more permanent eelgrass beds, therefore macroalgae was added to the appropriate cages as described in Phelan et al. (2000).

Multiple experimental runs, 9 to 11 d in duration, were conducted during June and July for winter flounder and August to September for tautog over the 2-yr period, corresponding with settlement of young juve-

Table 1. Field experiment characteristics for winter flounder *Pseudopleuronectes americanus* during 1994 and 1995

Estuary	Year	Start of experimental run	Duration of experiment (d)	Fish collection sites	Number of fish analyzed for RNA	Initial size mean and (range) Std. length (mm)	Weight (g)
Hammonasset	1994	June 27 (Expt 1)	10	New Haven Harbor	15	29.5 (24.4–34.6)	0.47 (0.29–0.79)
		July 26 (Expt 3)	10	New Haven Harbor	52	41.5 (31.0–50.0)	1.34 (0.53–3.42)
Navesink	1994	June 7 (Expt 1)	10	Navesink	51	25.0 (19.0–31.2)	0.25 (0.10–0.45)
		July 5 (Expt 3)	10	Navesink	41	32.4 (26.2–41.2)	0.50 (0.21–1.13)
Great Bay/ Little Egg Harbor	1994	June 3 (Expt 1)	10	Great Bay/ Little Egg Harbor	51	16.0 (12.0–21.9)	–
		June 28 (Expt 3)	9	Great Bay/ Little Egg Harbor	60	35.8 (23.7–60.4)	0.99 (0.20–3.84)
Hammonasset	1995	June 26 (Expt 2)	10	New Haven Harbor	76	45.9 (33.3–55.6)	1.62 (0.60–2.67)
Navesink	1995	June 9 (Expt 2)	10	Navesink	52	26.7 (18.7–33.2)	0.32 (0.10–0.56)
Great Bay/ Little Egg Harbor	1995	June 12 (Expt 2)	10	Great Bay/ Little Egg Harbor	50	32.1 (23.1–42.8)	0.58 (0.18–1.33)

Table 2. Field experiment characteristics for tautog *Tautoga onitis* during 1994 and 1995

Estuary	Year	Start of experimental run	Duration of experiment (d)	Fish collection sites	Number of fish analyzed for RNA	Initial size mean and (range) Total length (mm)	Weight (g)
Hammonasset	1994	August 26 (Expt 1)	11	New Haven Harbor	32	37.0 (27.8–51.4)	0.68 (0.27–1.86)
		September 13 (Expt 2)	11	New Haven Harbor	18	43.3 (36.4–66.6)	1.16 (0.55–4.23)
		September 24 (Expt 3)	9	New Haven Harbor	26	48.0 (34.2–64.9)	1.48 (0.48–3.29)
Navesink	1994	July 22 (Expt 1)	10	Navesink	35	32.3 (27.2–38.5)	0.38 (0.21–0.70)
		August 5 (Expt 2)	10	Navesink	59	36.2 (24.6–46.8)	0.60 (0.16–1.28)
		August 19 (Expt 3)	10	Navesink	45	45.3 (30.9–54.2)	1.22 (0.34–2.16)
Hammonasset	1995	August 4 (Expt 1)	10	New Haven Harbor	79	43.1 (26.0–60.3)	1.12 (0.21–3.03)
		August 17 (Expt 2)	11	New Haven Harbor	67	49.3 (30.2–67.1)	1.80 (0.37–4.50)
Navesink	1995	August 11 (Expt 1)	10	New Haven Harbor	46	42.5 (30.7–55.3)	1.02 (0.32–2.19)
		August 25 (Expt 2)	10	New Haven Harbor	50	43.8 (30.4–68.4)	1.12 (0.31–4.21)
Great Bay/ Little Egg Harbor	1995	August 11 (Expt 1)	11	New Haven Harbor	53	40.5 (26.1–60.5)	0.85 (0.13–2.64)
		August 25 (Expt 2)	11	New Haven Harbor	45	42.1 (23.9–59.0)	0.89 (0.14–2.21)

niles in these estuaries (Tables 1 & 2). Additional details concerning each estuary and the experimental sites are provided elsewhere (Goldberg et al. 1993, in press, Phelan et al. 2000).

Collection, maintenance and deployment of experimental animals. Experimental design and fish handling techniques are detailed in Phelan et al. (2000). Briefly, experimental animals, collected by beach seine or beam trawl, were held in the laboratory and fed frozen brine shrimp daily for up to 3 d prior to caging. Each fish was marked for individual identification by subcutaneous injections of a nontoxic fluorescent material (Visible Implant Fluorescent Elastomer[1]). Standard length (SL) in winter flounder and total length (TL) in tautog were measured with calipers to the nearest mm. Fish were blotted, weighed to the nearest 0.01 g, and tagged. Groups of 3 fish were placed in 0.95 l jars for a 6 to 24 h recovery period. Each jar of 3 fish was then randomly assigned to each cage and 3 cages were deployed per replicate site. After each 9 to 11 d experimental run, fish were recovered from the cages and returned to the laboratory, where the blotted wet weight and length of each fish was determined. Fish from selected experimental runs were then frozen whole on dry ice, transported to the Milford Laboratory and stored at −80°C, prior to biochemical analysis.

Laboratory methodology. Frozen fish were dissected on a tray set on ice. Due to small fish size, white muscle tissue samples consisted of the entire dorsal fillet for winter flounder and the entire fillet from 1 side for tautog. Dissecting tools were rinsed with deionized water between dissections to avoid contamination. Each tissue sample was weighed to the nearest 0.001 g and placed in a test tube in an ice slurry bath. The tissue was homogenized in ice-cold distilled water using a Janke and Kunkel Ultra-Turrax[1] tissue homogenizer. Replicate aliquots were frozen for RNA analysis within 48 h.

Muscle tissue samples were analyzed using a modification of the Schmidt-Thannhauser method according to the procedures of Buckley & Bulow (1987), with the following modification based on Buckley & Caldarone (1988): muscle samples were hydrolyzed with 2.24 ml of 0.3N KOH and the hydrolizate acidified with 1.0 ml of 1.32N $HClO_4$. The increased volume of base gave improved recovery of RNA.

[1]Mention of trade names does not imply endorsement by the NMFS

The resulting supernatant containing the RNA was read at a wavelength of 260 nm using a Ciba-Corning Gilford Response Spectrophotometer[1]. RNA concentration, expressed as µg RNA per mg wet tissue, was calculated from the absorbance using the nucleotide extinction coefficient of 0.03. As a quality control measure, a large quantity of muscle tissue was homogenized and frozen in 0.2 g aliquots. One control sample was processed each day along with the tissue samples to verify the accuracy of the run.

Calculations and statistical analyses. Growth of caged winter flounder and tautog was calculated as a daily (d^{-1}) instantaneous growth rate (G) using the formula :

$$G = \ln(m_1/m_0)/t$$

Where m_0 and m_1 are the initial and final standard length (winter flounder) or total length (tautog) in mm or weight in g and t is the duration of the experiment in d. The complete instantaneous growth data set is presented in Phelan et al. (2000). For this analysis, a data subset (Tables 1 & 2) was created consisting of selected experimental runs during which both RNA concentration and instantaneous growth measurements were known for each fish. Mean values for RNA concentration and instantaneous growth rate were determined for each group of fish in a cage and these cage means were used for all computations. A Pearson Product Moment Correlation was used to test the strength of the relationship between RNA concentration and daily instantaneous growth for each species using length and weight. The residuals from regressing RNA concentration on instantaneous growth were also examined.

Two separate analysis of variance models (ANCOVA) were conducted on the cage means of each species to assess the influence of estuary, habitat, and experimental run on each of the 2 growth indices. RNA concentration (µg per mg wet tissue wt) was used as the dependent variable in one model and instantaneous growth rate (G), expressed as standard length in winter flounder (G_{SL}) and as wet wt in tautog (G_{WT}), served as the dependent variable in the other, as these measures correlated most significantly with RNA concentration. Initial length or weight was used as a covariate. The independent variables for each model included year, estuary, habitat nested in estuary, experimental run nested in year and estuary, and replicate as a random effect nested in year, estuary, habitat, and experimental run. A type III sums of squares analysis was used. Independent effects that were not significant ($p > 0.05$) were dropped and the models rerun. Post-hoc tests consisted of multiple pairwise comparisons of least square means (lsmeans) from significant effects ($p < 0.05$). Findings from these analyses were compared to determine how well RNA concentration corresponded to daily instantaneous growth results.

RESULTS

Winter flounder

The correlation between RNA concentration and instantaneous growth rate in winter flounder was highly significant ($p < 0.0001$), as measured by both standard length ($r = 0.83$) and weight ($r = 0.79$) for both study years combined (Fig. 1a,b). Residuals from regressing RNA concentration on growth were randomly distributed when plotted against RNA. When the residuals were plotted against initial standard length, the observed growth of the smallest fish (<20 mm) was higher than predicted by the RNA values.

Differences in experimental design between the 2 years and the interaction of year with other main effects required that a separate ANCOVA be conducted for each year. During 1994, the main effects of estuary, habitat nested in estuary, and experimental run nested in estuary, were significant with either instantaneous growth or RNA concentration as the dependent variable (Table 3). Based on the post-hoc pairwise comparison results, flounder from the Great Bay/Little Egg Harbor estuary had statistically higher growth rates and RNA concentrations than did fish from Hammonasset. Growth rates in Navesink were lower than expected based on the RNA concentration. Habitat differences showed similar patterns for RNA and instantaneous growth in 73% of the 30 post-hoc pairwise comparisons, and agreed statistically in 63% of them. Of the 8 pairwise comparisons which showed dissimilar patterns, 7 can be attributed to marsh creek comparisons in both Great Bay/Little Egg and Navesink, where growth was less than predicted by the RNA values. Overall, few habitats differed in growth rates within an estuary, with the exception of marsh creek. Lsmeans for RNA concentration and growth in Experimental Run 1 were greater than Experimental Run 2 in all of the estuaries and agreed statistically in 2 out of the 3 estuaries.

During 1995, the main effects of estuary and habitat nested in estuary were significant with either RNA concentration or instantaneous growth as the dependent variable (Table 3). Based on the post-hoc pairwise comparison results, fish from the Navesink estuary had statistically higher RNA concentrations and instantaneous growth rates than did fish from the Great Bay/Little Egg Harbor estuary. Growth rates in Hammonasset were lower than expected based on the RNA concentration. Habitat differences showed similar patterns for RNA and instantaneous growth in all 18 post-hoc pairwise comparisons and agreed statistically in 89% of them.

Overall, RNA concentration and instantaneous growth rate showed similar patterns across the habi-

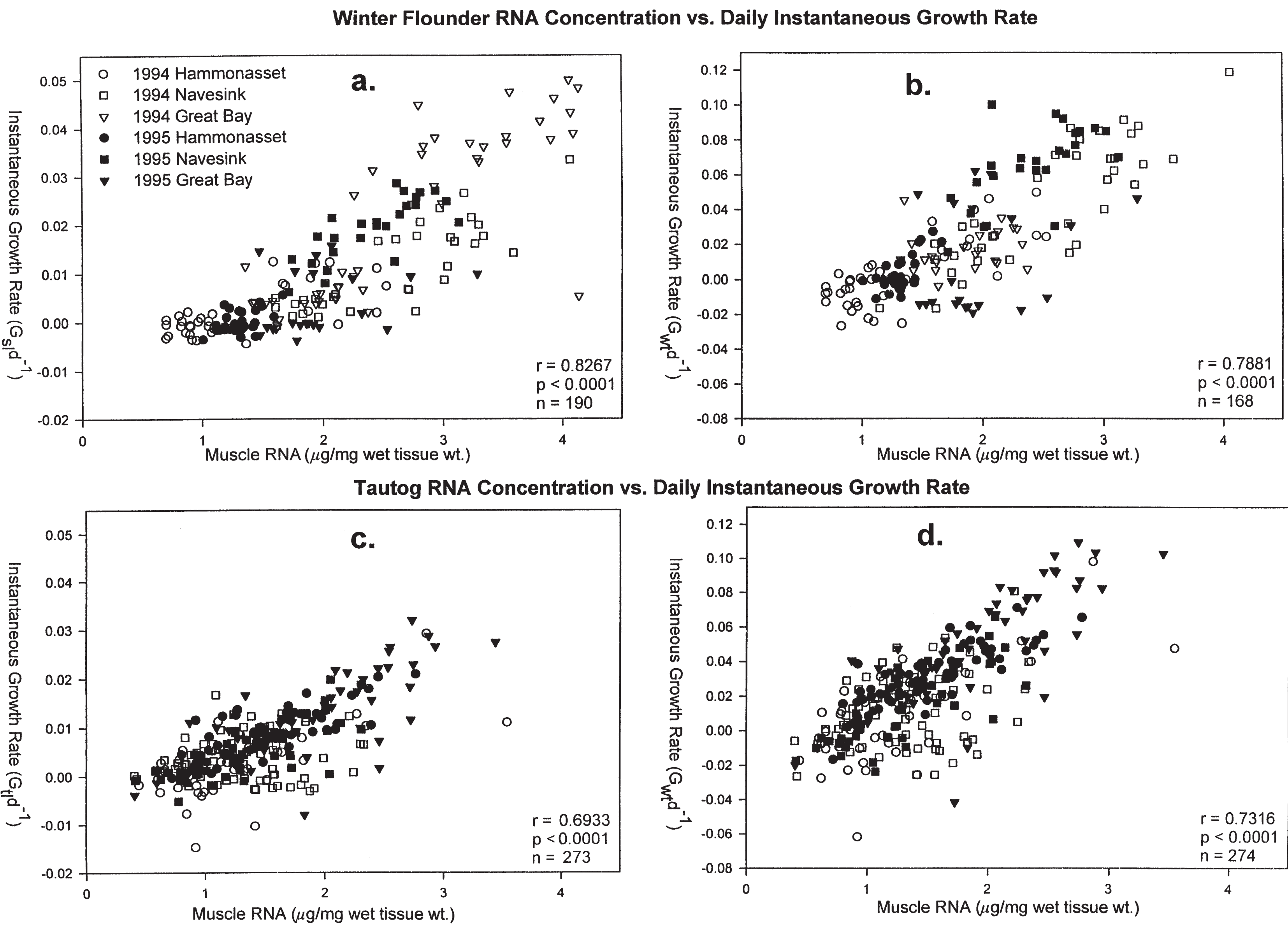


Fig. 1. Daily instantaneous growth rate as G_{SL} and G_{WT} for winter flounder (a,b) and G_{TL} and G_{WT} for tautog (c,d) vs RNA concentrations. Points represent cage means

Table 3. Summary of probabilities ($p > F$) from ANCOVAs, containing significant terms only, for winter flounder and tautog cage means during each study year. Dependent variables were daily instantaneous growth rate as length (G_{SL}) or weight (G_{WT}) and white muscle RNA concentration (µg per mg wet tissue wt)

	1994		1995	
	RNA	G_{SL}	RNA	G_{SL}
Winter flounder				
Estuary	0.001	0.001	0.013	0.001
Habitat (estuary)	0.009	0.018	0.001	0.001
Experimental run (estuary)	0.001	0.001	NA	NA
Replicate (estuary habitat experimental run)		0.009		0.001
Linear covariate (initial standard length)	0.001	0.001	0.001	
Corrected total df	116	116	73	73
r^2	0.94	0.96	0.85	0.94
	RNA	G_{WT}	RNA	G_{WT}
Tautog				
Estuary			0.001	0.001
Habitat (estuary)	0.020	0.001	0.001	0.001
Experimental run (estuary)	0.001	0.001	0.021	0.001
Habitat × Experimental run (estuary)		0.003	0.028	0.001
Linear covariate (initial weight)			0.001	0.001
Corrected total df	116	116	157	157
r^2	0.57	0.57	0.67	0.73

tats within the estuaries over the 2 yr of the study. In general, the smallest winter flounder tended to grow fastest, contain the highest RNA concentrations, and occur earliest in the year. For brevity, 1 example of the relationship between RNA concentration and instantaneous growth in winter flounder is illustrated in Fig. 2 (a,b,c), which depicts the habitat results by estuary from 1994.

Tautog

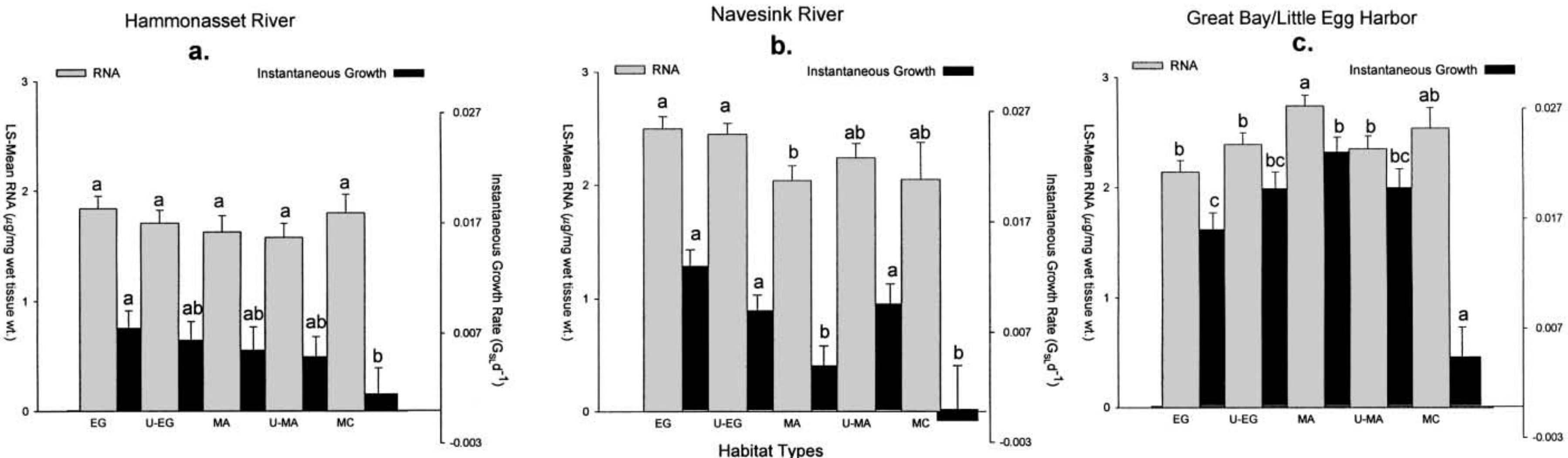
The correlation between RNA concentration and instantaneous growth rate was highly significant ($p < 0.0001$) in tautog, as measured by both total length ($r = 0.69$) and weight ($r = 0.73$), for both study years combined (Fig. 1c,d). The residuals from regressing RNA concentration on growth were randomly distributed when plotted against both RNA and initial weight.

As with winter flounder, separate ANCOVAs were conducted for each year. During 1994, the main effects of habitat nested in estuary, and experimental run nested in estuary, were significant with either RNA concentration or instantaneous growth as the dependent variable (Table 3). A significant interaction between habitat and experimental run indicated the effect of time on growth within habitats. Nevertheless, according to the post-hoc pairwise comparison results, habitat differences for all 3 experimental runs showed similar patterns for RNA and instantaneous growth in 94% of the 52 pairwise comparisons, and agreed statistically in 81% of them. Experimental run differences for all 5 habitats showed similar patterns for RNA and instantaneous growth in 92% of the 26 pairwise comparisons, and agreed statistically in 73% of the cases.

During 1995 the main effects of estuary, habitat nested in estuary, and experimental run nested in estuary were significant with either RNA concentration or instantaneous growth as the dependent variable (Table 3). Based on the post-hoc pairwise comparisons results, tautog from the Great Bay/Little Egg Harbor and Hammonasset estuaries had statistically higher RNA concentrations and instantaneous growth rates than those from Navesink. According to the post-hoc pairwise comparison results, habitat differences for both experimental runs in 1995 showed similar patterns for RNA and instantaneous growth in 97% of the 36 pairwise comparisons, and agreed statistically in 83% of them. Experimental run differences for all 4 habitats showed similar patterns for RNA and instantaneous growth in 92% of the 26 pairwise comparisons, and agreed statistically 67% of the time.

RNA concentrations and instantaneous growth in tautog showed similar patterns across habitats within the estuaries over the 2 yr of the study. In general, the smallest tautog grew fastest, contained the highest RNA concentrations and occurred earliest in the year.

For brevity, 1 example of the relationship between RNA concentration and instantaneous growth in



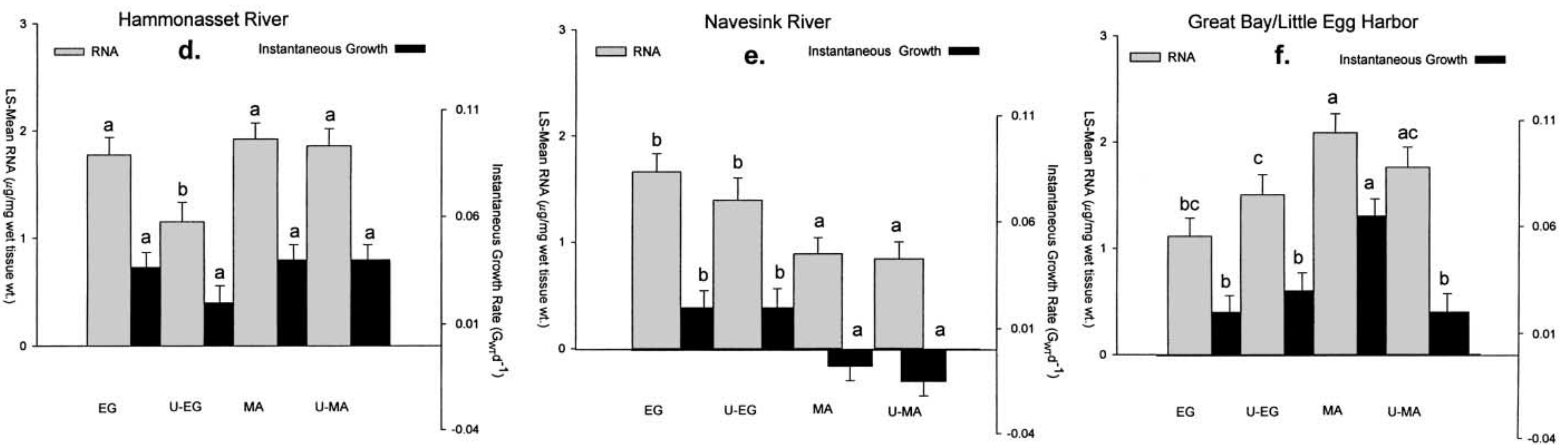


Fig. 2. Least square mean RNA concentrations (µg per mg wet tissue wt) and instantaneous growth rate (G_{SL} d^{-1} or G_{wt} d^{-1}) by habitat within each estuary for winter flounder 1994 (a,b,c) and tautog 1995 Experimental Run 1 (d,e,f). Habitats within an estuary which do not share a letter are statistically different at $p < 0.05$. Bars represent SE. Least square means are cage means

tautog is illustrated in Fig. 2 (d,e,f), which depicts the habitat results by estuary from 1995 Experimental Run 1.

DISCUSSION

Our study found a strong correlation between white muscle tissue RNA concentration and instantaneous growth rate for both young-of-the-year winter flounder and tautog. Other studies have also documented significant relationships between white muscle tissue RNA concentration and specific growth rate (wet wt) in many juvenile marine and freshwater fish species (r^2 range 0.58 to 0.93) (Goolish et al. 1984, Mathers et al. 1992, Foster et al. 1993, Wang et al. 1993, Arndt et al. 1994, Carter et al. 1998). Several of these studies suggest that RNA concentration reflects actual growth rate as well as or better than other nucleic acid measures including RNA/DNA or RNA/protein.

However, not all studies agree that RNA concentration is an accurate growth measure in juvenile fish. McLaughlin (1995a) found no difference in RNA concentration between fed and starved juvenile brook trout and concluded that RNA concentration and other nucleic acid measures are relative, rather than absolute, measures of nutritional status. In a related study, juvenile brook trout exhibited lagged responses in RNA concentration after exposure to fluctuations in food availability (McLaughlin et al. 1995b). In contrast, other studies suggest that nucleic acid measures respond quickly to changes in nutritional condition. For example, RNA concentrations in starved juvenile cod were found to recover within 5 d after the reintroduction of food (Foster et al. 1993). In laboratory experiments, Malloy & Targett (1994a) observed RNA/DNA ratios in juvenile summer flounder to respond rapidly to changes in feeding regime, sometimes within 1 d, and concluded that the high temporal resolution of nucleic acid techniques allow accurate estimates of short-term growth rates.

The overall strength of the relationship between RNA concentration and instantaneous growth as length was slightly better for winter flounder than tautog. This could be a species-specific difference in the RNA/growth relationship or, more likely, a difference in the accuracy of the instantaneous growth measurements among species. The flat morphology of the flounder lends itself better to accurate length measurements than does the fusiform tautog, with its nearly transparent tail.

In the present study, RNA concentration tracked somatic growth well for fish ranging in size from 20 to 46 mm SL for winter flounder and from 32 to 49 mm TL for tautog (Fig. 1). Malloy & Targett (1994a) found that the RNA/DNA ratio-growth rate relationship in summer flounder was unaffected by body weight for fish ranging in size from 18 to 80 mm TL. Stability in the growth relationship over a wide range of sizes is important, since the proportion of components other than RNA-producing muscle tissue begins to increase during the juvenile stage. This can result in large individuals with the same RNA/DNA ratio growing at a slower rate than smaller individuals (Buckley 1984). For this reason, RNA concentration or RNA/protein is thought to be a better indicator of growth than RNA/DNA when comparing late-stage juvenile fish of varying size (Buckley et al. 1999).

The correlation scatter plots, which subdivide growth results by estuary and year, and Table 1, which lists mean size by experimental run, indicates a relationship between growth rate and fish size in winter flounder. The smallest fish, from Great Bay/Little Egg Harbor during the first experimental run of 1994, showed the fastest growth rates and highest RNA values, while the largest flounder, from Hammonasset during both years, experienced the slowest growth rates and lowest RNA values. A similar relationship between RNA concentration, growth rate, and fish size is also present in tautog. These results also show that growth rates of the smallest fish (winter flounder TL < 20 mm) were higher than would be predicted from the RNA values. These positive residuals could be an anomaly due to difficulty in accurately measuring length of small fish, or could indicate a change in the RNA-growth relationship at this size.

Temperature is a significant factor for better defining the relationship between RNA concentration and juvenile fish growth. While RNA represents the cell's mechanism for protein synthesis, temperature controls the rate at which protein is synthesized (Fry 1971). Malloy & Targett (1994a,b) found that the inclusion of a temperature term improved the relationship between RNA/DNA ratio and growth rate in juvenile summer flounder from a Delaware estuary. In his work with carp, Goolish et al. (1984) found acclimation temperature to have a significant effect on the ratios of RNA:tissue and RNA/DNA. Buckley (1984) developed a general equation for larval marine fish that relates RNA/DNA ratios and temperature to growth rates. For a given RNA/DNA ratio, growth increased about 1% d^{-1} for every 1°C increase in water temperature. In a related study, winter flounder larvae were reared at 3 temperatures on a diet of wild plankton. RNA/DNA ratios alone explained 53% of the observed variability in growth, while temperature and RNA/DNA ratio together explained 72% of the variability (Buckley 1982). During the current study, RNA concentration explained 68% and 54% of the observed variability in juvenile winter flounder and tautog growth, respec-

tively. The specific effects of temperature on the relationship between RNA concentration and growth of young-of-the year winter flounder and tautog are not well understood, and larval results may not hold true for older fish (Buckley et al. 1999). Differences in RNA concentration and growth rate between years within an estuary suggest that seasonal effects, such as temperature, may indeed be a confounding factor. Incomplete temperature profiles among the estuaries and habitats during this study preclude a further understanding of the impact of temperature on RNA concentration.

Using RNA concentration as an index of growth, growth rates were found to vary with year, estuary, habitat and experimental run. These conclusions were very similar to the findings of Phelan et al. (2000), although our data set was smaller, consisting of 64% and 42% of the total winter flounder cage means and 64% and 98% of the total tautog cage means for 1994 and 1995 respectively. Winter flounder RNA concentrations closely reflected growth measurements in all of the habitats, except marsh creek, where growth was much lower than predicted by the RNA values. These elevated RNA levels, relative to instantaneous growth, could reflect increased synthesis of proteins unrelated to growth, such as stress proteins. Fish in the marsh creek habitats in all 3 estuaries were exposed to some of the highest temperatures and lowest dissolved oxygen levels recorded and had the lowest recoveries (<20% for winter flounder and <30% for tautog) (Phelan et al. 2000), indicative of a high mortality rate.

RNA concentration closely followed instantaneous growth in young-of-the-year tautog. Results agreed statistically in 80% of the post-hoc tests, while an additional 15% of the comparisons, although not statistically significant, showed similar trends. For a detailed discussion of the ecological implications of the estuary and habitat results, based on the complete data set of instantaneous growth rate measurements, see Phelan et al. (2000).

Laboratory trials using juvenile red drum *Sciaenops ocellatus* have established a strong relationship between RNA/DNA ratio and nutritional condition and have provided a framework for evaluating condition of that species in the wild (Rooker et al. 1996, 1997). The strong relationship between RNA concentrations and growth measurements observed during this study indicate that RNA analyses may be a valuable tool for assessment of field growth in young-of-the-year winter flounder and tautog. Measurement of RNA in field-caught animals from different habitats would provide essential growth information without the need for caging. Nucleic acid measurements are accurate at determining growth over short time intervals and during periods of slow growth, when changes in somatic measurements may be almost undetectable. Laboratory calibration studies of RNA concentrations and growth rates, at a variety of temperatures and feeding regimes, are needed to further define the relationship between temperature and RNA concentration and growth rate. These studies will allow better assessment of nutritional condition in field-caught fish and should allow the assignment of specific growth rates to wild flounder and tautog.

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