Energetics of larval red drum, *Sciaenops ocellatus*. Part II: Growth and biochemical indicators

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Abstract.—The effects of ration level and temperature on growth were determined for larval red drum, *Sciaenops ocellatus*, during its first two weeks of life. Larvae were raised in the laboratory at 20°C at a ration level of 5.0 prey/mL, at 25°C at ration levels of 0, 0.1, 1.0, and 5.0 prey/mL, and in growout ponds at 25°C and 32°C and at ration levels of 4–6 prey/mL. Growth was measured as standard length, wet mass, and dry mass. Proximate (water, ash, protein, and lipid) and elemental (C, N) composition was determined at larval ages of 0, 2, 4, 6, 10, and 14 d to provide caloric values for the growing larvae and to examine the relative importance of protein and lipid during tissue deposition in the very early life history of these larvae. Biochemical indicators of growth, RNA-DNA ratio, and activity of the metabolic enzyme lactate dehydrogenase (LDH) were examined in larvae reared at all temperature and ration combinations. The effectiveness of the biochemical indicators as proxies for growth was assessed by comparing the directly measured growth rates with RNA:DNA levels and LDH activity. Larvae fed a ration of 1.0 prey/mL or less did not survive past the age of eight days. Growth rate increased with increasing temperature, reaching a maximum of 60% body mass/d in growout ponds at 32°C. Protein level (percent ash free dry mass: %AFDM) increased with increasing age in all treatments where individuals exhibited positive growth, whereas lipid (%AFDM) showed a concomitant decline. Nitrogen (%AFDM) and carbon (%AFDM) varied directly with protein and lipid contents, respectively. Biochemical indicators of growth showed a significant correlation with growth rate. However, the character of the correlation changed with temperature. RNA-DNA ratios and enzymic activities were lower at higher temperatures for equivalent growth rates. Introduction of a temperature term into multiple regression equations improved the relation between growth and the biochemical proxies. LDH activity scaled with the size of larvae, whereas RNA:DNA showed no significant relation with size.

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Red drum, *Sciaenops ocellatus*, is an important species in commercial and recreational fisheries in the southeastern United States, particularly in the Gulf of Mexico. Declines in red drum stocks (Swingle, 1990) have stimulated considerable interest in the early life history of this species, resulting in stock enhancement programs and larval monitoring programs designed both to improve and continually to assess the status of the fish in the field. Studies on red drum and other species indicate clearly that growth during the pretransformation period of development is particularly critical to survival (Buckley, 1980; Holt et al., 1981, a and b; Holt and Arnold, 1983; Holt, 1990). The increase in size and mobility that characterizes development during the early larval period results in an increase in the size range of prey items available to the larvae as forage and a decrease in the size range of potential predators on the larvae.

Two variables with great potential to influence rates of growth are temperature and ration levels. As a subtropical species, red drum develop at temperatures greater than 20°C, grow rapidly, and have a greater energy demand for metabolic processes than do larvae developing in colder systems. For example, red drum eggs at 25°C hatch in 24 h, and larvae begin feeding in 48–72 h, whereas cold water species, such as Atlantic cod, *Gadus morhua*, and winter flounder, *Pleuronectes americanus*, developing at 4–8°C, spend 30 d as developing eggs.[1] High temperatures during early development stimulate rapid growth in red drum but leave them potentially more vulnerable to rapid starvation in absence of sufficient food. The interaction between temperature, ration level, and growth in size and calories, is an important part of the energetics of larval red drum, basic information which is unavailable for red drum and limited for other subtropical teleosts (Houde and Schekter, 1983).

[1] Hempel, G. 1979. Early life history of marine fishes; the egg stage. Univ. Washington Press, Seattle, WA, 70 p.

Objectives of the present study were four-fold. The first was to examine growth in size and energy in laboratory-reared red drum larvae, from egg to onset of transformation, at a single ration level (5 prey/mL) and at two temperatures (20 and 25°C). This examination was achieved by using direct measurements of standard length and mass with age; the biochemical composition and caloric value of the growing larvae were described by analyzing their proximate and elemental composition (water, ash, protein, lipid, carbon, and nitrogen). The second was to examine the relation of growth in size and energy as a function of ration level (0, 0.1, 1.0, and 5.0 prey/mL) at a single temperature (25°C). The third was to compare growth in size and energy in laboratory-reared larvae at 20 and 25°C and in the more heterogeneous conditions encountered by pond-reared larvae at 25 and 32°C. The fourth objective was to describe the relation between growth, temperature, and biochemical indicators of growth and condition: RNA:DNA ratios and activity of the key intermediate metabolic enzyme lactate dehydrogenase (LDH).

Methods and materials

Laboratory maintenance

Fertilized eggs were obtained from the Florida Department of Environmental Protection (FDEP) hatchery, Port Manatee, Florida. Broodstock were maintained at 25°C and 30 ppt. Eggs were obtained from five females and from separate spawnings, from November 1990 to November 1991, for all growth experiments described below. Broodstock females were similar in size, kept in highly controlled conditions, and fed well. Spawning was induced naturally by manipulation of photoperiod. As a consequence, eggs were very uniform in size, 0.9 to 1.0 mm in diameter.

Eggs were transported to the USF Marine Science Laboratory in St. Petersburg and sorted into 26-L experimental aquaria at a concentration of 2,500–3,000 individuals per aquarium. High mortality associated with first feeding resulted in a 30–40% reduction in initial numbers by day 3. Aquaria were placed in a photoperiod- and temperature-controlled incubator and maintained at either 20°C or 25°C and at a salinity of 30 ppt. Eggs were introduced to the 20°C temperature by slow exchange of water over a 60-minute period. A 13-h light and 11-h dark photoperiod was used throughout all experiments. Larvae were fed rotifers (*Brachionus plicatilis*) beginning at day 3 posthatch until flexion (approximately day 14), when experiments were terminated. Aquaria were aerated and a portion of the saltwater in each was changed daily.

Rotifers were obtained from Florida Aqua Farms, Dade City, Florida, and cultured according to the procedure of Hoff and Snell (1987). Rotifers were fed *Chlorella* once a day to avoid any loss in nutritional value. Seawater for culturing was obtained offshore in the Gulf of Mexico. The seawater was coarse-filtered, then treated with bleach (sodium hypochorite, 5.25%) to remove any additional plankton, and neutralized with sodium thiosulphate. Seawater salinity was adjusted with distilled water and Tropic Marine Seasalt to achieve a final salinity of 30 ppt.

Pond maintenance

Pond-reared red drum larvae were obtained from the FDEP growout ponds, Port Manatee, Florida. Larvae from a single spawn were added to the plankton-rich ponds within 24 hours after hatching and allowed to grow. Two ponds, one at 25°C and another at 32°C, were sampled for the first 18 days of life of the red drum larvae. Temperature was monitored twice daily; the average temperature for the two-week sampling period was used to characterize the ponds.

Prey items in the ponds were monitored by sieving water samples into two size categories: 35–220 μm (copepod nauplii, rotifers, and small copepods) and larger than 220 μm (copepods); prey were then counted in 200-mL aliquots of each size range. The concentration of prey between 35 and 220 μm was 3–5 prey/mL, whereas that greater than 220 μm was 0.5–1 prey/mL in both the 25°C and 32°C ponds.

Growth versus prey density

Eggs from a single spawn were divided into four 26-L aquaria for experiments on growth versus prey density at 25°C. Prey were provided at four densities, 0, 0.1, 1.0, and 5.0 prey items per mL, from first feeding (day 3) through the start of flexion (day 14). Prey concentrations were monitored twice daily by removing a 25-mL sample from each aquarium, counting the number of prey in 5-mL aliquots, and taking the average. Prey concentrations were adjusted as necessary. Larvae reared at 20°C were fed prey at a ration level of 5.0 prey items per mL.

Standard length measurements Growth in standard length was monitored according to prey concentration. Aquaria with 0, 0.1, and 1.0 prey/mL were sampled daily. Aquaria with 5.0 prey/mL were sampled every other day, and ponds were sampled every third day. The samples were taken each morning before the larvae began to feed. Standard length

of five individual larvae that had been anesthetized with MS-222 was measured with the aid of a dissecting microscope. Standard length was considered to be the distance from the snout to the tip of the tail in preflexion larvae and from the snout to the tip of the notochord in post-flexion larvae.

Mass measurements Growth in mass was monitored at the same intervals as those used for standard length. At each monitoring interval, 30 individuals were removed for wet, dry, and ash-free dry mass analysis. To determine mass, larvae were first separated into three groups of ten. Each group of larvae was filtered onto a preweighed 0.5-cm Whatman glass fiber filter (made with an office hole-punch) that was placed in a custom-made miniaturized vacuum funnel. Larvae were then rinsed very briefly by introducing distilled water into the funnel with a pasteur pipette and by removing the water immediately with the vacuum filter. To minimize evaporation, samples were immediately placed in preweighed microcentrifuge tubes which were then weighed to the nearest μg on a Mettler electrobalance to determine wet mass. Specimens were dried at 60°C to a constant mass (about 24 h) to determine dry mass.

Average proximate and elemental composition of prey items

Rotifers were collected in bulk from two 28-L culture bags (approximately 50 mg dry mass/bag) for determination of proximate and elemental composition. Proximate composition (water, ash, protein, and lipid content) was determined by using the methods of Stickney and Torres (1989) and Donnelly et al. (1990). Elemental composition was determined by using a C:H:N analyzer.

Average proximate and elemental composition of fish larvae

Methods used to estimate the proximate and elemental composition of fish larvae were the same as those used for prey. Larvae were obtained in bulk (50 mg dry mass) for each day sampled. Each pond was sampled from the hatchery at 0, 2, 6, 10, and 14 days. Laboratory-raised larvae were sampled at prey concentrations of 0, 0.1, 1.0, and 5.0 prey/mL at 0, 2, 6, 10, and 14 days for each of four spawns. Protein and lipid values as percent ash-free dry mass (%AFDM) were multiplied by individual ash-free dry mass to obtain concentrations as mg/individual. The instantaneous protein growth rate (Gpi) was calculated by using the formula from Buckley (1982):

$$Gpi = \frac{\ln M_{t2} - \ln M_{t1}}{t_2 - t_1} \times 100,$$

where M = mass in mg; and
t = age in d.

Caloric content of prey and larvae

Caloric content was calculated from proximate compositional data of the rotifers and larvae by using a value of 0.0048 cal/μg for protein and 0.0095 cal/μg for lipid (Brett and Groves, 1979).

RNA-DNA ratio

Ten to 20 individuals were removed for analysis of RNA:DNA content each time sampling occurred for measurements of mass. Larvae were filtered onto preweighed Whatman glass-fiber filters, rinsed with distilled water, weighed, placed in microcentrifuge tubes, and frozen at –80°C until analysis. RNA:DNA was analyzed by first homogenizing the freshly thawed groups of larvae in 1.2 M NaCl, then by using the sequential enzymatic method of Bentle et al. (1981) to determine RNA:DNA.

Activity of lactate dehydrogenase

Larvae were sampled in bulk (minimum 10–20 mg wet tissue mass) every day at a prey concentration of 0 prey/mL. Samples were taken at 0, 2, 6, 10, and 14 days for larvae fed 5 prey/mL and for those collected in the growout ponds. Tissue was introduced frozen into the homogenizing medium, ice-cold Tris/HCL buffer (10 mM, pH 7.5 at 10°C), and homogenized by hand at 0 to 4°C with conical glass homogenizers having ground-glass contact surfaces (Kontes Glass Co., "Duall" models). Homogenates were centrifuged at 4,500 ×g for 10 minutes and the supernatants saved for enzyme analysis.

L-Lactate dehydrogenase (LDH, EC 1.1.1.27; Lactate: NAD+ Oxidoreductase) activity was assayed in the pyruvate reductase direction by using methods described in Torres and Somero (1988) at a temperature of 25°C. Enzyme activity was expressed as units/gWM (wet mass), where a unit was 1 μmole of substrate converted to product per minute.

Statistical analyses

Simple regressions for each relationship were fitted by using the least-squares method (Statgraphics Plus, Manugistics Corporation). Data from treatment groups were compared by using one-way analysis of variance (ANOVA). Differences between the means

were determined by using the least-significant-differences multiple range test. Multiple regression analysis (Statgraphics Plus, Manugistics Corporation) was used to examine the relation between the observed protein growth rates and the following combinations of factors: the three experimental temperatures (20°C laboratory, 25°C laboratory, 25°C pond, 32°C pond), the three ration levels at 25°C (0, 5 prey/mL, and pond), RNA:DNA, and LDH activity.

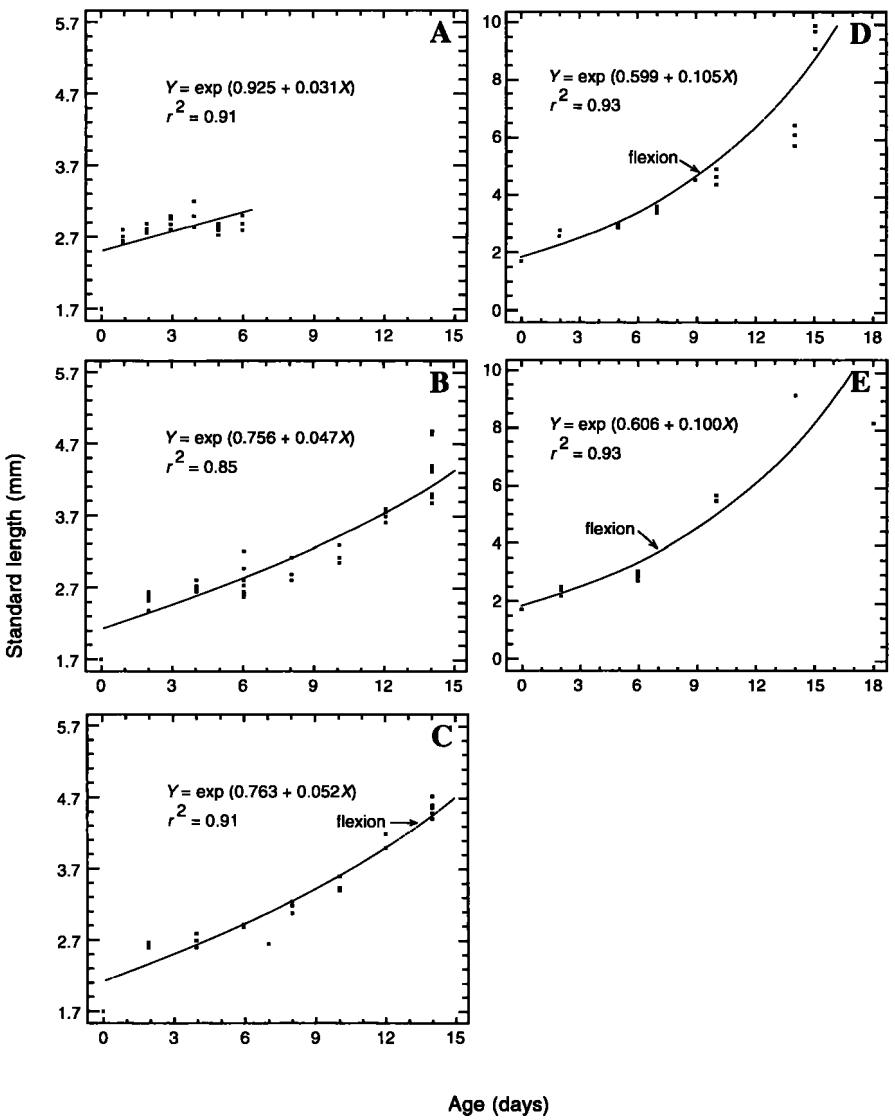

Figure 1

Growth in standard length of red drum larvae raised in the laboratory and in growout ponds: (**A**) laboratory, 25°C and 0 prey/mL; (**B**) laboratory, 20°C and 5 prey/mL; (**C**) laboratory, 25°C and 5 prey/mL; (**D**) pond, 25°C; and (**E**) pond, 32°C.

Results

Growth rate versus prey density

Standard length Starved red drum larvae (0 prey/mL) kept at 25°C increased in standard length even as they were declining in dry mass (Figs. 1 and 2; Table 1). The average size at death on day 6 was 2.89 mm, which corresponds to a daily increase of 0.075 mm/day for days 2–6. Surprisingly, these values were similar to those for larvae fed at 5.0 prey/mL at 25°C, which attained an average length of 2.90 mm by day 6.

Growth in larvae fed ad libitum increased with increasing temperature in both the laboratory and ponds. At 20°C, day-14 larvae averaged 4.13 mm, and very few of the larvae exhibited flexion of the notochord (Fig 1; Table 1). Laboratory-reared individuals at 25°C grew to an average of 4.45 mm by day 14. Mean masses at day 14 were significantly different between the two temperatures (ANOVA: df=1, F=60.3, P<0.001). Flexion of the notochord within the tail region had begun by day 14 at 25°C, indicating the onset of transformation. Larvae raised in ponds at 25°C averaged 6.45 mm at day 14. Notochord flexion in these larvae began on day 9 or 10, when the larvae were at a size between 4.11 and 4.50 mm. Pond-reared larvae at 32°C had reached an average standard length of 7.48 mm at day 14. Notochord flexion occurred on day 7 or 8, when larvae were at a length of 4.53 mm (similar to the size at flexion of the laboratory-raised larvae) and at an earlier chronological age than that of the laboratory-raised individuals.

Mass measurements Growth in mass of laboratory-reared larvae at ration levels less than 5.0 prey/mL (0, 0.1, and 1.0 prey/mL) at 25°C was negative, and no larvae survived more than 8 days (Fig. 2). Slopes

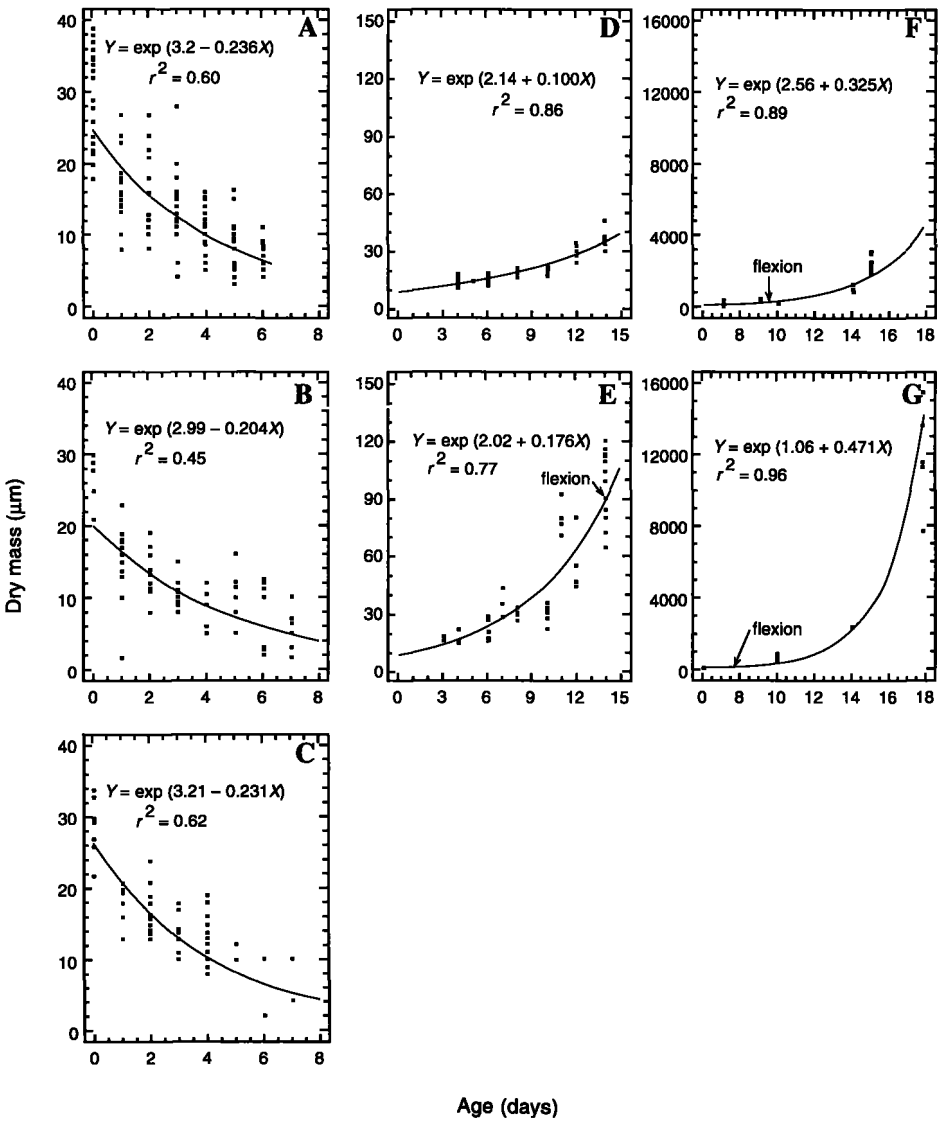

Figure 2

Growth in dry mass of red drum larvae raised in the laboratory and in growout ponds: (**A**) laboratory, 25°C and 0 prey/mL; (**B**) laboratory, 25°C and 0.1 prey/mL; (**C**) laboratory, 25°C and 1 prey/mL; (**D**) laboratory, 20°C and 5 prey/mL; (**E**) laboratory, 25°C and 5 prey/mL; (**F**) pond, 25°C; and (**G**) pond, 32°C.

of the three curves describing the time-dependent decline in individual dry biomass at the three ration levels were not significantly different (Student's *t*-test: $P>0.05$). Growth in dry mass at a ration level of 5.0 prey/mL was significantly higher than at the three lower ration levels (0, 0.1, and 1.0 prey/mL).

In contrast, wet masses in 2–6 day-old larvae were slightly lower at ration levels of 0, 0.1, and 1.0 prey/mL than at 5.0 prey/mL but were not significantly different. This finding indicated that body water content was increasing in larvae maintained at the three lower ration levels.

Larvae held at 25°C and fed 5.0 prey/mL had higher growth rates than those raised at 20°C at the same ration level (Fig. 2; Table 1). Growth averaged 2.86 µg/day for the first two weeks of life in larvae raised at 20°C and 10.09 µg/day for larvae reared at 25°C. Expressed as a percent increase in mass, larvae reared at 20°C increased 10.5 %BM/d and those reared at 25°C increased 19.3 %BM/d.

Table 1

Proximate and elemental composition and energetic density of red drum larvae determined at different levels of ration, temperature, and age. (nd = no data; DM=dry mass; WM = wet mass; AFDM = ash-free dry mass). Parenthetical values (SD, *n*) represent standard deviations and sample sizes below each of the mean values in the table.

Ration (prey/mL)	Temp C°	Day	Standard length (mm)	Wet mass (µg)	Dry mass (µg)	Calories per ind.	% Water	AFDM as %DM	Protein		Lipid		Carbon % AFDM	Nitrogen % AFDM	Calories/ mg DM
									% WM	% AFDM	% WM	% AFDM			
0	25	0	1.70 (0.05, 5)	447.00 (86.10, 18)	29.28 (7.08, 18)	0.1063	94.4 (1.4, 15)	91.3 (4.5, 15)	2.58 (0.80, 15)	43.84 (4.47, 15)	1.22 (0.50, 15)	19.72 (4.16, 15)	51.83 (3.98, 3)	10.31 (0.55, 3)	3.631
0	25	1	2.69 (0.09, 5)	274.80 (176.00, 15)	16.36 (4.52, 15)	0.0698	92.8 (1.2, 5)	94.5 (0.2, 5)	4.01 (0.81, 5)	57.33 (5.53, 5)	1.23 (0.01, 5)	18.45 (2.08, 5)	48.93 (0.93, 3)	10.41 (0.52, 3)	4.266
0	25	2	2.83 (0.06, 5)	140.40 (24.30, 15)	16.66 (5.54, 15)	0.0725	90.8 (0.7, 10)	90.7 (1.8, 10)	4.71 (0.56, 10)	55.68 (3.79, 10)	1.74 (0.47, 10)	22.39 (6.02, 10)	53.99 (1.10, 4)	9.70 (0.48, 4)	4.353
0	25	3	2.91 (0.09, 5)	126.20 (26.30, 15)	13.48 (4.93, 15)	0.0596	92.8 (1.0, 10)	90.6 (0.9, 10)	3.90 (0.47, 10)	59.68 (5.41, 10)	1.34 (0.29, 10)	21.24 (6.06, 10)	49.79 (4.84, 3)	9.80 (0.50, 3)	4.423
0	25	4	3.01 (0.15, 5)	120.60 (21.70, 15)	10.99 (3.31, 15)	0.0438	91.1 (0.8, 10)	90.1 (1.2, 10)	4.69 (0.43, 10)	58.74 (2.48, 10)	1.32 (0.26, 10)	16.86 (4.35, 10)	49.79 (1.92, 3)	10.05 (0.13, 3)	3.983
0	25	5	2.81 (0.07, 5)	103.30 (26.00, 15)	8.13 (3.86, 15)	0.0308	91.8 (0.6, 5)	89.6 (0.7, 5)	4.33 (0.36, 5)	62.89 (1.20, 5)	0.83 (0.17, 5)	12.67 (1.72, 5)	46.06 (5.81, 3)	10.60 (0.23, 3)	3.783
0	25	6	2.89 (0.08, 5)	91.50 (20.90, 15)	7.00 (1.80, 15)	0.0278	91.0 (1.0, 5)	93.1 (1.6, 5)	5.21 (0.26, 5)	62.47 (3.28, 5)	1.14 (0.01, 5)	13.29 (1.28, 5)	nd	nd	3.967
5	20	0	1.70 (0.05, 5)	363.30 (109.90, 0)	29.40 (4.26, 0)	0.1230	95.4 (0.5, 5)	94.8 (7.4, 5)	2.35 (0.44, 5)	43.74 (4.27, 5)	0.89 (0.18, 5)	24.34 (1.28, 5)	nd	nd	4.182
5	20	2	2.54 (0.10, 5)	156.80 (22.30, 10)	15.52 (3.78, 10)	0.0651	90.3 (1.1, 5)	93.2 (2.3, 5)	4.20 (0.49, 5)	53.08 (4.86, 5)	1.70 (0.31, 5)	20.54 (3.12, 5)	nd	nd	4.194
5	20	6	2.79 (0.21, 5)	192.00 (106.50, 10)	15.64 (1.87, 10)	0.0509	90.3	91.0	4.71	53.19	0.95	10.78	nd	nd	3.255
5	20	10	3.14 (0.10, 5)	233.80 (20.30, 10)	20.43 (2.15, 10)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
5	20	14	4.33 (0.12, 5)	384.40 (47.30, 10)	35.91 (4.57, 10)	0.1056	85.7	87.7	6.50	51.73	1.15	9.17	nd	nd	2.943
5	25	0	1.70 (0.09, 5)	385.80 (17.40, 5)	35.25 (1.26, 3)	0.1373	95.7 (0.7, 5)	97.4 (2.4, 5)	1.81 (0.01, 5)	43.39 (0.67, 5)	0.84 (0.01, 5)	20.18 (2.16, 5)	46.66	10.46	3.896
5	25	2	2.64 (0.03, 5)	206.30 (85.20, 5)	18.67 (0.58, 5)	0.0849	93.5 (2.1, 5)	91.8 (1.3, 5)	3.27 (0.01, 5)	54.64 (1.24, 5)	1.49 (0.01, 5)	24.54 (0.02, 5)	49.88	10.29	4.548
5	25	6	2.90 (0.02, 5)	207.80 (74.50, 5)	20.33 (5.47, 5)	0.0775	88.0	91.3	5.79	52.91	1.89	17.23	45.77 (0.41, 3)	12.03 (0.14, 3)	3.813
5	25	10	3.46 (0.10, 5)	273.50 (121.00, 6)	29.71 (4.23, 6)	0.1005	85.8	89.4	7.07	55.59	1.49	11.74	42.92	11.91	3.382
5	25	14	4.53 (0.12, 5)	682.80 (181.40, 15)	98.92 (18.43, 15)	0.3120	87.8 (0.1, 3)	86.7 (0.7, 8)	5.59 (0.08, 8)	55.13	1.22 (0.16, 8)	11.64	45.73 (1.17, 3)	12.27 (0.50, 3)	3.253
Pond	25	0	1.72 (0.04, 5)	352.30 (71.00, 5)	28.91 (4.43, 5)	0.1008	95.0	92.56	1.74	42.70	0.72	18.08	nd	nd	3.450
Pond	25	2	2.70 (0.12, 5)	179.00 (62.60, 5)	15.67 (2.71, 5)	0.0738	91.4	93.1	4.42	56.21	1.72	24.82	nd	nd	4.707
Pond	25	7	2.93 (0.06, 5)	334.05 (34.80, 5)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pond	25	10	4.70 (0.03, 5)	2833.80 (381.50, 5)	137.30 (23.20, 5)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Pond	25	14	6.30 (0.31, 5)	6010.00 (542.50, 5)	983.30 (167.60, 3)	3.1318	91.7	87.6	10.18	56.22	1.99	9.87	nd	nd	3.185
Pond	32	0	1.75 (0.04, 5)	nd	nd	nd	93.9	97.2	nd	45.62	nd	24.98	47.70	9.10	nd
Pond	32	2	2.37 (0.14, 5)	187.80 (72.40, 5)	17.32 (4.78, 5)	0.0806	92.7	91.3	3.60	54.00	1.76	26.36	51.53	10.51	4.652
Pond	32	6	2.89 (0.12, 5)	374.30 (83.50, 5)	136.20 (35.30, 5)	0.5476	88.5	91.6	6.92	65.61	1.38	13.05	nd	nd	4.021
Pond	32	10	5.65 (0.10, 5)	5491.00 (1802.00, 5)	583.00 (168.40, 5)	2.1330	88.0	87.1	6.71	64.16	1.23	11.70	44.35	11.86	3.658
Pond	32	14	7.48 (0.27, 3)	nd	2127.80 (293.00, 3)	6.9808	89.3	81.7	5.00	64.16	nd	9.87	46.79	13.14	3.282

Growth rates for pond-raised red drum larvae (Fig. 2) were far greater than those for larvae fed rotifers in the laboratory, owing almost certainly to the greater prey diversity in the ponds. Larvae raised at 25°C in the ponds increased in size an average of 81.9 µg/day over 14 days: a 47.2 %BM/d increase. Larvae raised at 32°C in the ponds increased an average of 799.22 mg/day: a 60.2 %BM/d increase; an increase of 10°C in the pond environment resulted in a two- to three-fold increase in absolute daily mass gain.

Proximate and elemental composition of prey

Brachionus plicatilis raised on *Chlorella* exhibited a protein level of 32.71% and a lipid level of 9.37% of its ash-free dry mass (AFDM). Carbohydrate level was low, averaging 2.84% (AFDM). The unrecovered mass was assumed to be due to refractory structural molecules that were not assayed for, e.g. chitin. Using a figure of 0.24 µg for average individual biomass (Hoff and Snell, 1987) and the caloric values for protein, lipid, and carbohydrate of Brett and Groves (1979, see methods section), we suggest that each rotifer has an average energetic value of 0.000526 calories.

Elemental composition of the rotifers showed that the percent carbon was 42.02 %AFDM and the percent nitrogen was 10.41 %AFDM. The carbon-nitrogen ratio was 3.56:1.

Proximate and elemental composition of larvae

Proximate composition can be expressed in three ways: as a percent of wet mass (%WM), a percent of ash-free dry mass (%AFDM), and as the total content per larva (mg/individual). Table 1 shows the changes in proximate composition (%WM and %AFDM) as a function of ration level and age of the larvae; total content (mg/individual) is reported below in the text.

Red drum eggs exhibited large water content (94.65 %WM), a high protein content (42.17 %AFDM) and an intermediate lipid content (19.35 %AFDM)(Table 1). Carbohydrate, generally an extremely small fraction of the overall proximate composition of marine species, proved to be so in this case as well (0.47 %AFDM).

Viewed as a fraction of the total body mass of each larva, the protein level (%AFDM) shows an increase through time at zero ration (43.84% to 62.47%) accompanied by a reduction in lipid (19.72% to 13.29%), which indicates that, in starving larvae, lipid was used in preference to protein for energy production. On a µg/individual basis, protein actually decreased in starved larvae from 8.44 µg/individual in newly hatched larvae to 4.07 µg/individual for 6-day-old starved larvae (Table 2). Lipid values declined from 2.59 µg/individual on day 3 to 0.87 µg/individual on day 6. Percent water remained high until death at day 6, averaging 91.0% throughout the survival period.

Table 2

Protein content, protein growth, RNA:DNA, and LDH in red drum larvae. Protein content was calculated from values reported in Table 1. Where values for % protein or % ash-free dry mass were missing, nearest neighbor values were used. Protein growth was calculated as described in text. RNA:DNA values are the mean for all RNA:DNA measurements within the last 2 d of the age interval shown in the table. They are reported as mean ±SD (n). Values for the 25°C pond were taken at day 7 instead of day 6 and calculated accordingly. All (6–14) day RNA:DNA values are significantly different ($P<0.05$, Students t). nd = no data

	Day															
	2	6	10	14	2–6	6–10	10–14	2–14	6–14	6	10	14	6–14	6	10	14
	Protein (ug/indiv)				Protein growth (%/d)					RNA:DNA				LDH (units/gWM)		
Starved	8.44	4.07	nd	nd	−18.20	nd	nd	nd	nd	0.68±0.35 (14)	nd	nd	nd	7.48	nd	nd
5/mL at 2°C	7.66	7.55	9.29	16.34	−0.40	5.20	14.10	63.00	9.50	1.51±0.43 (6)	1.45±0.24 (4)	1.49±0.14 (4)	1.50±0.30 (14)	9.19	14.44	19.69
5/mL at 25°C	9.36	9.77	14.80	47.33	1.10	10.40	29.10	13.50	19.70	1.15±0.20 (2)	1.19±0.32 (3)	1.26±0.19 (7)	1.25±0.21 (12)	15.06	24.33	33.60
Pond 25°C	8.16	15.00	67.70	484.30	12.10	50.20	49.20	34.00	49.60	2.89±0.51 (4)	3.06±0.81 (4)	3.56±0.27 (2)	3.09±0.62 (10)	18.94	30.86	42.78
Pond 32°C	8.51	82.20	324.60	1,116.20	56.70	34.30	30.90	46.00	32.60	1.19	2.21	1.39	1.60±0.54	12.69	19.44	26.19

The counterpoint to 0-ration data is provided by the data at 5.0 prey/mL at 20°C and 25°C (Table 1). The data clearly demonstrate accumulation of energy as protein and little storage of lipids. Larvae raised at 20°C increased in protein level (%AFDM) from 43.74% as eggs to 51.73% as day-14 larvae. Lipid levels decreased (%AFDM) from 24.34% in eggs to 9.17% in day-14 larvae. Protein concentrations increased from 7.66 µg/individual at day 2 to 16.34 µg/individual at day 14. Lipid concentrations increased from 1.53 µg/individual at day 6 to 2.89 µg/individual at day 14. An identical pattern was observed in larvae reared at 25°C (Table 1).

The data set for proximate composition values collected on pond-raised larvae was smaller than ideal owing to problems in obtaining adequate sample sizes from the ponds. However, the data on accumulated protein and lipid concentrations give an excellent indication of maximum growth. Pond-raised larvae at 25°C and 32°C showed faster accumulation of total protein and lipid than larvae raised in the laboratory (Table 1). Protein levels of larvae increased in %AFDM from 42.70% and 45.62%, in eggs, to 56.22% and 64.16% in day-14 larvae, at 25°C and 32°C, respectively. Lipid levels (%AFDM) decreased from 24.98% to 9.87% at 32°C; decreases in lipid percentages were also observed in the 25°C pond and in the laboratory-raised larvae.

Pond-reared larvae at 25°C increased in total protein content from 8.16 µg/individual at day 2 to 484.30 µg/individual at day 14, whereas those kept at 32°C increased from 82.20 µg/individual at day 7 to 1,116.20 µg at day 14 (Table 2). Thus, an increase in 10°C resulted in a three-fold increase in protein (µg/individual) in 2-week-old larvae raised in the ponds. Lipid values for larvae raised at 25°C and 32°C were also much higher than those for larvae raised in the laboratory; day-14 pond larvae on average had lipid contents of 85.06 µg/individual and 171.44 µg/individual, respectively.

Carbon (%AFDM) remained about the same with age in all rearing conditions (Table 1), whereas nitrogen (%AFDM) remained fairly constant or increased with age at all ration levels. Carbon-nitrogen (C:N) ratios were higher in larvae kept at a ration level of 0 prey/mL than in larvae raised either at 5.0 prey/mL or in the ponds, indicating that protein commanded the largest fraction of the starved larva's mass. Values for C:N remained high in starved larvae until death at day 5 (4.35 ± 0.46). Pond-raised larvae had values similar to those observed in larvae reared on 5.0 prey/mL in the laboratory but were slightly lower at day 14 (3.56 vs. 3.73). Caloric contents of the larvae were calculated from protein and lipid content by using the conversion factors in Brett and Groves (1979) and are reported in Table 1.

Protein-specific growth and biochemical indicators

Table 2 summarizes results for growth in protein (absolute and instantaneous) and the two biochemical indicators, RNA:DNA and LDH activity, for easy comparison. Instantaneous growth shows an interesting trend with the age interval chosen for calculation. If growth in protein was calculated from day 2 to day 14, larvae exhibit the trends discussed previously for growth in dry mass (see results) where lowest growth was observed in the 20°C laboratory treatment and highest in the 32°C ponds. If instantaneous growth was calculated instead for the interval from day 6 to day 14, the highest growth was observed in the 25°C ponds (Table 2) and would suggest that the growth spurt during the first 4 d of feeding in the 32°C pond was important in determining growth during the larvae's first 14 d of life.

RNA-DNA ratio RNA:DNA in each treatment showed a decline from a high value typical of the yolk-sac stage (day 1: grand mean for all treatments 4.27 ± 0.83; $\bar{x}$ ± SD) to a plateau at day 4 that characterized the treatment and showed no significant change over the remaining 10 days (Fig 3; Table 2). In starved larvae RNA:DNA reached a plateau at a value of 0.7, indicating that protein synthetic capacity was severely diminished after that time. RNA:DNA in larvae raised at 5.0 prey/mL in the laboratory showed a gradual decline to a plateau of 1.5 at 20°C and 1.3 at 25°C (Fig 3; Table 2); values at the plateau were significantly different between the two temperatures (ANOVA: df=25, F=6.31, P=0.019)

Pond-raised larvae had higher growth rates than laboratory-reared individuals (Figs. 1 and 2; Tables 1 and 2), and values for RNA:DNA were much greater in the 25°C pond than in any of the laboratory treatments (Fig. 3). Larvae raised in the ponds at 25°C had a value of 3.6 at 2 weeks of age, whereas those reared at 32°C averaged 1.5 at day 14. RNA:DNA values were significantly different between the two pond treatments (ANOVA: df=12, F=14.19, P=0.003).

Three treatments took place at a temperature of 25° C: starved, 5 prey/mL, and pond. If protein growth rates and RNA:DNA are compared between laboratory and ponds at 25°C (Table 2), there is an excellent correlation between protein growth and RNA:DNA. Instantaneous protein growth rate in the laboratory was 13.5%/d from day 2 to day 14; in the pond it was 34%/d over the same interval: an increase of 2.5 fold. RNA:DNA showed an increase of 1.3 to 3.0 in laboratory- versus pond-reared larvae over the same interval with a similar 2.3-fold increase. The negative growth observed in starved larvae at 25°C

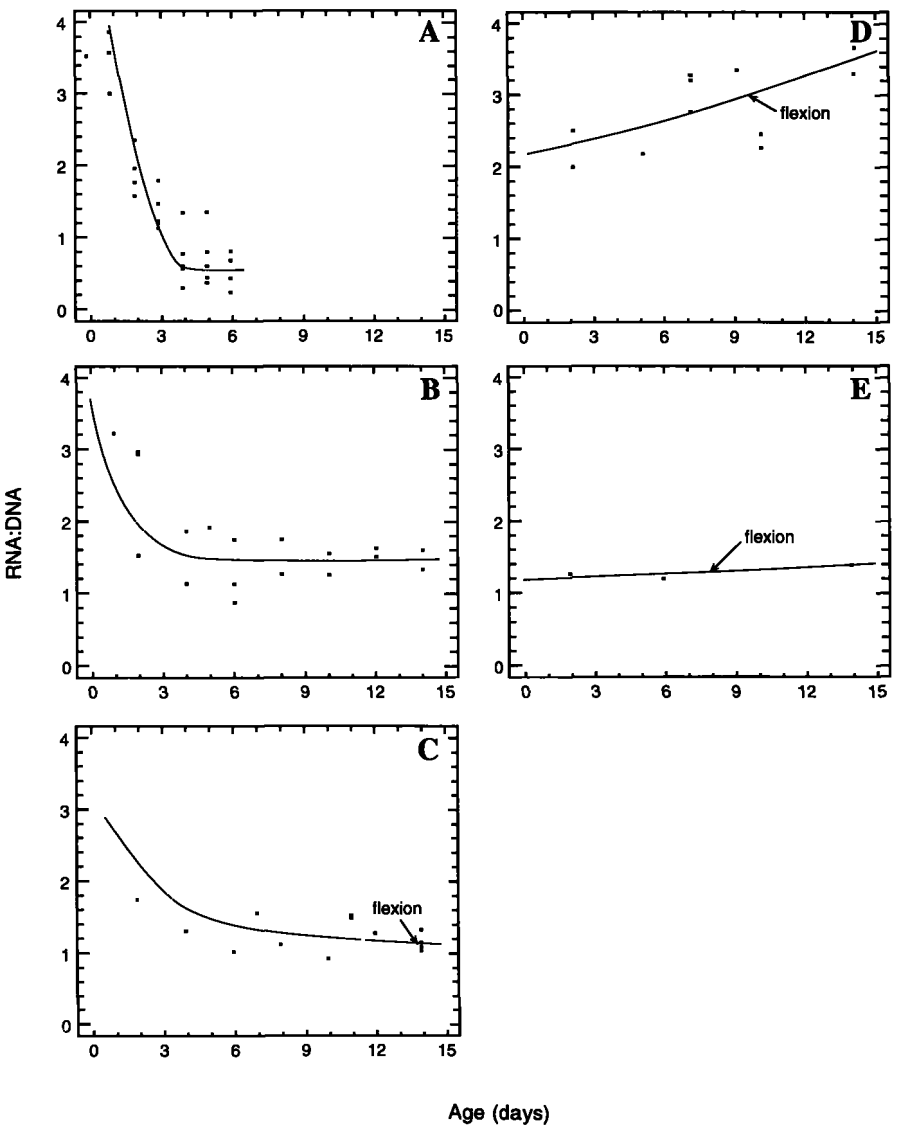

Figure 3

RNA:DNA of red drum larvae raised in the laboratory and in growout ponds: (**A**) laboratory, 25°C and 0 prey/mL; (**B**) laboratory, 20°C and 5 prey/mL; (**C**) laboratory, 25°C and 5 prey/mL; (**D**) pond, 25°C; and (**E**) pond, 32°C.

(–18%/d) also showed a much lower value for RNA-DNA ratio : 0.7:1. Differences in RNA:DNA between the three treatments were highly significant (ANOVA: df=35, F=107.6, P=0.000).

Overall, RNA:DNA was only a modest predictor of protein growth. Regression analysis of protein growth (y, % per d) versus RNA:DNA (x), by using all the values in Table 2, showed a marginal fit (y = –3.64 +14.76x; P=0.01; r^2=0.34). However, as discussed above, within a temperature, RNA:DNA was an excellent predictor of instantaneous protein growth, and this was borne out in a regression using only the data collected at 25°C: y (% per d) = –12.43 + 17.39 (RNA:DNA); P=0.01; r^2=0.64. The performance of RNA:DNA as an overall predictor was improved significantly by using a multiple regression equation with a temperature term (Table 3).

LDH Activity LDH activities of laboratory-raised larvae increased with age at ration levels of 0 and 5.0 prey/mL and with temperatures of 20°C and 25°C (Fig 4). Larvae that were starved continued to pro-

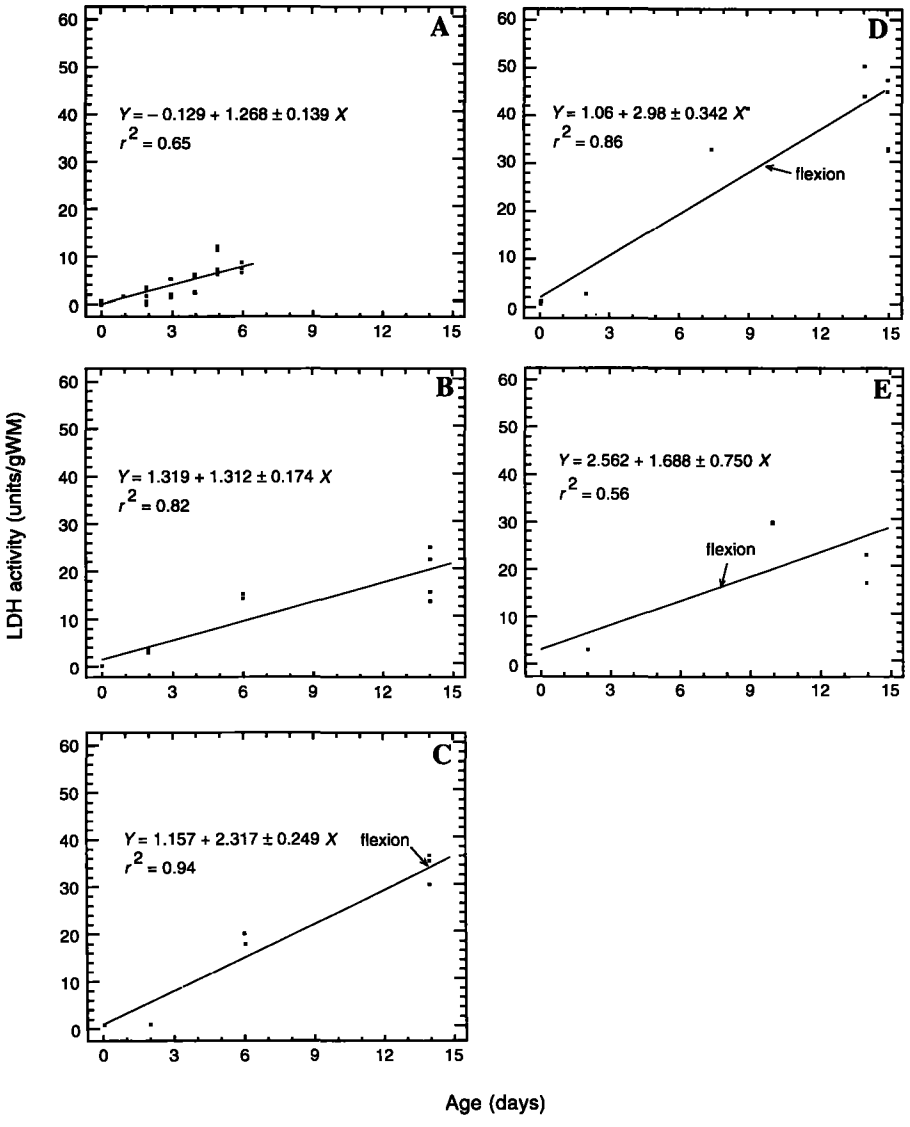

Figure 4

LDH activity (units/gWM) in red drum larvae raised in the laboratory and in growout ponds: (**A**) laboratory, 25°C and 0 prey/mL; (**B**) laboratory, 20°C and 5 prey/mL; (**C**) laboratory, 25°C and 5 prey/mL; (**D**) pond, 25°C; and (**E**) pond, 32°C.

duce LDH, although at lower concentrations than those for fed individuals, until death at day 6. Larvae reared at 20°C had LDH values of 20–25 units/gWM at day 14. These LDH activities were slightly lower than those for larvae raised at 25°C, which had LDH values of between 30 and 35 units/gWM at day 14. Larvae reared at 25°C in the ponds averaged LDH activities of 40–50 units/gWM, higher than the values for larvae raised at 32°C, which averaged 25–30 units/gWM, and higher than the values for larvae reared in the laboratory.

Like RNA:DNA, LDH activity taken overall was only a modest predictor of protein growth rate: y (% per d) = −8.17 + 1.39 (LDH); P=0.02; r^2=0.39. However, within a temperature, its performance as a predictor was much improved. A regression using only the 25°C data yielded an excellent coefficient of determination: y (% per d) = −28.74 + 1.94 (LDH); P=0.003; r^2=0.86. A multiple regression with a temperature term improved its use as an overall predictor (Table 3).

Table 3

Multiple regressions describing instantaneous protein-specific growth (Y; %/d) in red drum larvae versus temperature (T), ration (0/ml, 5/mL, and pond), RNA:DNA, and LDH activity (units/gWM). Only significant regressions are presented ($P < 0.05$).

Equation	X_i	X_{ii}	Y	n	r^2	P
1	T		$Y = 2.71X_i - 49.52$	20	0.21	0.023
2	Ration		$Y = 6.35X_i - 8.53$	20	0.45	0.001
3	T	Ration	$Y = 2.07X_i + 5.67X_{ii} - 58.34$	20	0.57	0.022
4	RNA:DNA		$Y = 14.76X_i - 3.64$	17	0.30	0.013
5	T	RNA:DNA	$Y = 2.59X_i + 14.61X_{ii} - 69.41$	17	0.56	0.007
6	LDH		$Y = 1.39X_i - 8.17$	13	0.34	0.022
7	T	LDH	$Y = 2.55X_i + 1.26X_{ii} - 70.35$	13	0.57	0.034

Discussion

Growth versus prey density

Standard length and mass measurements The basic pattern of growth and development in red drum larvae, e.g. in size at flexion, was similar for larvae under a wide variety of rearing conditions. Within the basic blueprint, growth and development of red drum larvae fed to satiation could be accelerated or retarded according to the rearing temperature.

Thus, larvae raised in the laboratory and the ponds underwent metamorphosis at roughly the same size, independent of the age of the larvae. In the case of the 32°C pond, day-7 larvae were already the size of day-14 larvae reared at 25°C in the laboratory, and were at the same stage of development. Similarly, dry mass at transformation was approximately the same in the laboratory and ponds, despite the differences in chronological age.

Proximate and elemental composition of larvae

Red drum larvae, whether fed to satiation or starved, depleted their lipid level from 40% to 50% by day 6. The increase in protein (%AFDM) reflected the decline in lipid and was most evident in the starved red drum larvae. Larvae that have been starved conserve protein as musculature until the time of death. Conservation of muscular proteins allows the animal to swim as long as possible before complete muscle atrophy, or "point of no return," allowing the larvae to search out prey in other, possibly more productive, areas.

The loss of dry mass in starving larvae, compared to fed larvae of equal age, reflected the catabolism of lipid and protein (Wallace, 1986). A similar, but less severe, drop in lipid was observed in all rearing conditions and has been observed in other species of fish. For example, Fraser et al. (1987) found that larval Atlantic herring had a lipid level of 23% dry mass (176 μg) one day after hatching decreasing to 11% (221 μg) by day 16. Those percentages were similar to those found for red drum larvae in the present study (20.18% to 11.74%) over the first two weeks of life. It is likely that lipid serves as a buffer fuel during the early life history of red drum. It is not accumulated. When high-quality food energy is available in excess, larval red drum larvae grow faster rather than accumulate an energy reserve. This is best exemplified by the differences in larvae growing at 25°C in the laboratory and 25°C in the ponds.

Elemental composition agreed well with other published values for red drum (Lee et al., 1988) and larval herring of similar size (Ehrlich, 1974, a and b; 1975) as well as with our own results on proximate composition (Table 1). Larvae that are growing normally, as in the 5.0 prey/mL experiments and the ponds, show greater increases in protein than in lipid. The increase in %N with age, and the declining %C, mirrored the changes (protein increase, lipid decrease) in proximate composition. This changing elemental composition resulted in a declining C:N in normally growing larvae. Starving individuals had slightly higher C:N than fed individuals as a result of their diminished protein synthesis. Larvae raised in the ponds have the lowest C:N as a result of the high protein levels relative to lipid. Thus, the C:N can be used as an indicator of physiological status in developing fish. It should be noted, however, that this ratio applies in the opposite fashion to adult fish. A declining C:N in older fish indicates starvation where lipid is laid down as an energy reserve and is combusted before protein. The rapidly accumulating musculature of a healthy, growing fish larva results in a declining C:N, giving the appearance of starvation when, instead, this ratio indicates that protein is accumulating at a faster rate than lipid.

Protein-specific growth and biochemical indicators

RNA-DNA ratio Our values for RNA:DNA fall at the low end of the range of ratios reported in the literature for larvae reared under a variety of different conditions (Ferron and Legget, 1994). Wright and Martin (1985) found similar RNA-DNA ratios (1 to 2 at 19–21°C) for starved striped bass, whereas fed striped bass larvae had ratios of 3–3.4 during the first two weeks after hatching. Robinson and Ware (1988) observed a similar trend in RNA-DNA ratios with starvation in the early life of larval Pacific herrings, as we did with red drum; ratios declined up to yolk-sac absorption, where the ratios leveled off. Values for RNA:DNA obtained in the laboratory in this study (1 to 2) were lower than previously reported values (2 to 4) for red drum larvae (Westerman and Holt, 1994).

As has been reported previously (Buckley, 1982; Ferron and Legget, 1994), the relation of growth rate and RNA:DNA changed with temperature. The higher mass-specific and protein-specific growth rates observed in the laboratory at 25°C, in comparison with those at 20°C and in the ponds at 32°C, as well as in comparsion with those at 25°C, were accompanied by lower RNA:DNA values (Tables 1 and 2). The inverse relation between RNA:DNA and temperature holds true in field-caught larvae as well. It was observed by Setzler-Hamilton et al. (1987), who found that in late spring, values for RNA-DNA ratios in striped bass larvae were higher than values measured in hotter, early summer months (spring values were about 3 and summer values were 2 to 2.5).

A high growth rate accompanied by a low RNA-DNA ratio, such as we observed in the 32°C ponds, is probably due to an increase in the efficiency of ribosomes in initiating protein synthesis and to an increase in the rate of chain elongation due to a direct effect of temperature, i.e., an increase in the production of protein per unit of ribosomal RNA due to a Q_{10} effect (cf. Westerman and Holt, 1988). Despite the effect of temperature on the relation of RNA:DNA and growth rate, RNA:DNA is a useful tool for determining nutritional status of fish larvae, particularly if it is understood that temperature contributes substantially to the relationship between RNA:DNA and growth (Buckley, 1982: Buckley et al., 1984; Ferron and Legget, 1994).

LDH Activity LDH, the terminal enzyme in vertebrate anaerobic glycolysis, is an important factor in the ability of some fish to produce sudden bursts of swimming and is found in large quantities in white muscle (Somero and Childress, 1980). The observed increase in LDH activity with age until death of starved larvae seems at first glance to conflict with priorities expected of an energy-deprived individual, in which metabolic processes would be expected to be declining. However, it is to be expected that LDH activity would be conserved, even in starving larvae, so that the muscle would remain functional as long as possible. A larva with no capability for movement would be doomed; thus, a metabolic investment in locomotory capability makes good adaptive sense.

Unlike in RNA:DNA, LDH activity showed a direct correlation with both mass- and protein-specific growth rate in the two fed laboratory treatments despite the increase in temperature from 20°C to 25°C. In the ponds, LDH activities showed an interaction with temperature similar to that seen in RNA:DNA, i.e. a lower specific activity at 32°C despite a higher growth rate. In the case of LDH, the declining activities observed in larvae from the higher temperature pond probably indicate that a lower concentration of enzyme is sufficient to maintain the catalytic efficiency needed by the tissues at the higher temperature (cf. Hochachka and Somero, 1984). The fact that a similar drop was not noted in the laboratory suggests a threshold for the drop in activity between 25°C and 32°C that was not present in the transition between 20°C and 25°C.

Clarke et al. (1992) found similar values for LDH in red drum larvae raised on wild zooplankton. Values for LDH activity in Clarke's study, assuming 87% water content, averaged 19–26 units/gWM for two-week-old larvae, slightly lower than the values we observed in the larvae raised in the laboratory and ponds.

Biochemical parameters as predictive tools

Although similar in their use as biochemical proxies for growth, LDH activity and RNA:DNA are fundamentally different in many other respects. RNA:DNA is a ratio of measured quantities, whereas LDH activity is a determination of a rate: a kinetic measurement. Inherent in the measurement of RNA:DNA is the assumption that the methods for determining the quantities of RNA and DNA are accurate, but there is no direct effect of temperature on the assay itself. For LDH, activities are measured in saturating conditions of substrate, which means that the activities are maximal activities (V_{max} from Michaelis-Menten kinetics; Lehninger, 1982) for each treatment. It is tacitly assumed that if assays are performed in saturating conditions at the same temperature, the differences in activity, or V_{max}, are due to differences in concentration of the enzyme. This assumption is

a reasonable one. It is important, however, to be aware of other potential causes of variability in the relation of both RNA:DNA and LDH activity to growth or condition in fish larvae. It has been demonstrated here and elsewhere (Ferron and Legget, 1994) that rearing temperature alters the relation of growth and biochemical proxies for growth. Another potential source of variability in the relation is the scaling of each of the proxies with individual size.

RNA:DNA increases slightly with individual size (Buckley,1982) but overall is insensitive to the changes in individual mass that would be expected in a study of larval fish growth within a single field sample. This is not the case for LDH activity which scales strongly with mass in fishes (e.g. Somero and Childress, 1980; Torres and Somero, 1988). In this study, a significant relation was observed between LDH activity (y, units/gWM) and protein mass (x, µg protein): $y = 2.25x^{0.187}$; P=0.02; r^2=0.43. RNA:DNA showed no significant change with size. Our study suggests that, for maximum accuracy, direct comparisons of field-caught larvae for LDH activity are best confined to narrow size ranges or the relation between LDH and size is described empirically. On the other hand, it could be argued that since mass-specific LDH activity increases with increasing mass, it is actually incorporating a growth-specific change within its scaling behavior, making it a better proxy. Either way, it shows considerable potential.

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