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Biochemical changes during ontogenesis of the winter flounder (Pseudopleuronectes americanus) and the effect of starvation

by

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Abstract

The RNA, DNA and protein content of winter flounder (Pseudopleuronectes americanus) eggs and fed and starved larvae were followed through metamorphosis. The RNA-DNA ratio was found useful for diagnosis of the starving condition.

Introduction

Normal development of most embryonic and prolarval teleosts depends on material stored in the yolk for a source of both energy and biosynthetic precursers. After hatching there is a transition period when larvae shift from dependence on yolk to an exogenous food supply. The availability of sufficient prey of the proper quality and the ability of larvae to capture and assimilate it are critical to survival during the larval stage. Since differential mortality during the larval stage could be important in determining the year-class size of marine fish, a method for determining the nutritional condition of fish larvae in plankton samples could aid in determining larval survival and prediction of subsequent year-class size. In the past, weight-length relationships (Blaxter, 1971), morphometric (Ehrlich, Blaxter and Pemberton, 1976), chemical (Ehrlich, 1974a, 1974b) and histological (O'Connell, 1976) methods have been used with varying degrees of success. All four approaches have limitations and diagnosis of the starving condition in sea caught larvae is difficult.

Bulow (1970) used RNA-DNA ratios as indicators of recent growth rates in adult golden shiners (Notemigonus crysoleucas). He reported that RNA-DNA ratios were very sensitive to changes in feeding levels. The RNA content of a wide variety of organisms has been related to growth rate (Sutcliffe, 1970). Sutcliffe (1965) was able to predict quite accurately the growth rates of laboratory populations of brine shrimp (Artemia salina) and mud snail larvae

(Nassarius obsoletus) using a growth-RNA relation determined on whole amphipods (Orchestia platesis). Dagg and Littlepage (1972), however, concluded that the general positive relationship between growth rate and RNA content lacked sufficient specificity for determination of growth rate. A positive relationship is expected since growth in marine teleosts is primarily accomplished by protein synthesis and certain species of RNA are directly involved in protein synthesis serving as both a template and organizer. The DNA content of an organism has been used as an indication of cell number since the DNA content of somatic cells is generally constant for a given species. Some eggs and early larvae, however, have been shown to contain large amounts of cytoplasmic DNA, greatly exceeding the amount of nuclear DNA (Neyfakh and Abramova, 1974). This study was undertaken to determine the relationships between changes in DNA, RNA and protein content and events in the development of winter flounder (Pseudopleuronectes americanus) and to determine if measurements of these classes of biochemicals could be used to determine the nutritional condition of winter flounder larvae.

Methods

Adult Pseudopleuronectes americanus were caught by trawl net off Rhode Island, U.S.A. and kept in a 1900-l aquaria. Eggs were obtained, fertilized and incubated according to methods previously described (Smigielski, 1975). Larvae were maintained at 8°C in 38-l all black glass aquaria and were fed zooplankton collected in the Narragansett Bay area in excess of 2 organisms per ml according to the methods of Laurence (1975). On days 3 and 28 a portion of the larvae were transferred to an identical aquarium containing sea water filtered through a 0.45 μ Millipore filter.

About 40 eggs or larvae were pooled per sample through day 11 after hatching, thereafter, 10 larvae were pooled per sample except on days 43, 50 and 58 when only 5 larvae were used for the largest size group. All samples through day 36 were run in triplicate, thereafter samples were run in duplicate. Starting on day 28 the standard length of each larva sampled was determined using an ocular micrometer and 10 larvae were taken on each sampling day for dry weight determinations.

Eggs and larvae were homogenized in 2 ml of ice cold distilled water immediately after capture. Protein was determined on duplicate 0.1 or 0.05 ml samples of homogenate using a modification of the Lowrey method (Hartree, 1972). RNA and DNA were extracted and partially purified from 1.7 ml of homogenate using a modification of the Schmidt-Thannhauser method (Munro and Fleck, 1966) adapted for the micro quantities present in larval fish and eggs. RNA was determined from the absorbancy at 260 mμ of the acid-soluble, alkali-hydrolized fraction. The DNA content of larvae was determined from the absorbancy at 260 mμ of the alkali-stable, acid-hydrolized fraction. The DNA content of eggs was determined on the alkali-stable fraction using a modification of the 3,5-diaminobenzoic acid dihydrochloride fluorometric assay described by Holm-Hansen et al. (1968) and Hinegardner (1971). At the beginning of this study RNA was also determined using the orcinol method (Sutcliffe, 1965) and DNA was determined using the indole method (Ceriotti, 1952). These values were in good agreement with the values reported.

Results

DNA content per egg increased rapidly between fertilization and hatching (Figure 1). Upon hatching 10 days after fertilization, larvae contained slightly more RNA and DNA than unhatched eggs and retained 78% of the protein. During the period from hatching to the end of the yolk-sac stage (day 5), DNA content remained essentially constant while a slight increase in RNA and a decrease in protein was observed. Between the end of the yolk-sac stage and initiation of feeding (day 7) DNA content increased sharply while RNA and protein decreased. Of the protein present in the egg just prior to hatching, 45% was lost by the time of feeding initiation. After feeding initiation DNA, RNA and protein content increased steadily (Figure 1, Tables 1 and 2).

The RNA and protein content of winter flounder larvae transferred to filtered sea water prior to feeding initiation continued to decrease until a 100% mortality was observed between day 11 and 14 (Figure 1). Starved larvae did show an increase in DNA content on day 7 similar to that observed in fed larvae. The RNA-DNA ratio of both starved and fed larvae decreased from the end of the yolk-sac stage through day 9. However, the RNA-DNA ratio was significantly higher in fed larvae than starved larvae on day 9.

The RNA content of a second group of larvae transferred to filtered sea water 28 days after hatching decreased within 2 days, while both DNA and protein content increased (Table 1). After 4 days a decrease in all three components was observed. A 50% mortality consisting almost entirely of the smaller individuals in the group occurred 7 days after transfer to filtered sea water, accounting for the high DNA, RNA and protein values observed on the final day of sampling (day 36). The RNA-DNA ratio of larvae transferred to filtered sea water decreased continually until a 100% mortality was observed. No significant change in the RNA-DNA ratio of fed larvae was observed during the same period (Figure 2). The DNA, RNA and protein content of different size groups of larvae through metamorphosis is shown in Table 2.

Discussion

The DNA, RNA and protein content of winter flounder eggs reported in this study are total values for the yolk plus the embryo. Any increase in the amount of a particular component must therefore result from synthesis rather than transfer from the yolk to the embryo. The continual net accumulation of DNA from fertilization to hatching is probably correlated with an increase in cell number (Regnault and Luquet, 1974) although the content of DNA per cell may decrease (Neyfkakh and Abramova, 1974). The small increase in protein content during the same period suggests that protein is not an important energy source during early development in winter flounder. The 46% decrease in protein content between the maximum 3 days prior to hatching and the minimum at initiation of feeding on day 7 suggests that protein is probably an important energy source during this period although this includes a 22% decrease in protein upon hatching, the majority of which may be lost with the chorion. Two periods of decrease in RNA content were observed. One occurred just prior to hatching; the other just prior to feeding initiation. No significant net

decrease in the DNA content of eggs or fed larvae was observed between any sampling periods.

The decrease in protein and RNA content (Figure 1) as well as the decrease in the RNA-DNA ratio (Figure 2) prior to feeding initiation resembles the pattern observed for starved larvae. Even in the presence of excess food the RNA-DNA ratio fell from 4.9 at the end of the yolk-sac stage to 3.0 at initiation of feeding on day 7. The critical importance of food availability at the initiation of feeding capability was demonstrated two days later when fed larvae contain almost 100% more RNA and 55% more protein than larvae held in filtered sea water.

The RNA-DNA ratio was the most reliable and sensitive index of nutritional state evaluated in this study which included relationships between RNA, DNA, protein, standard length and dry weight. RNA content was the most labile decreasing within two days after removal of food. DNA content was generally conserved except in the final stages of starvation prior to death. The protein-DNA ratio, which is an index of the amount of protein per cell, generally decreased as starvation progressed and the protein-RNA ratio generally increased. The RNA-DNA ratio was particularly useful as an indicator of condition since unlike other indices it fell within well defined limits throughout the period studied. Winter flounder larvae established a mean RNA-DNA ratio of between 4.0 and 4.8 shortly after initiation of feeding (Figure 2). This range is similar to the RNA-DNA ratio values reported by Bulow (1970) for golden shiners (N. crysoleucas). The RNA-DNA ratio was not affected by either the age or size of the larvae until metamorphosis when the RNA-DNA ratio increased to between 5.3 and 5.7 and remained at this level until the experiment was terminated on day 58 (Table 2). This is particularly important since the age of sea caught larvae is difficult if not impossible to establish and a large size range is observed in larvae of the same age. This point was demonstrated on day 36 when a large mortality of smaller larvae resulted in an increase in the mean DNA, RNA and protein content of starved larvae. The RNA-DNA ratio, however, was unaffected by the change in size distribution and continued to decrease. Results from larvae transferred to filtered sea water 18 hours prior to sampling and allowed to empty their stomachs (Table 2) indicate that the RNA-DNA ratio is not significantly affected by stomach contents at the time of sampling.

Before measurements of RNA-DNA ratios are useful in the field, the effect of changing environmental conditions such as temperature and salinity as well as low prey concentrations and intermittent feeding should be evaluated. Although adult golden shiners (N. crysoleucas), larval winter flounder and larval cod (Gadus morhua) (Buckley unpublished data) show a similar decline in RNA-DNA ratio when food is withheld, the response of other species should be determined.

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Table 1. RNA, DNA and protein content of starved and fed winter flounder larvae 28 to 36 days after hatching.

Age Days	Starvation Time[a] Days	Standard Length mm		RNA µg/larva		DNA µg/larva		Protein µg/larva		RNA/DNA	
		Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved
28	0	5.69±.2		3.85±.67		0.91±.21		36±7		4.27±.42	
30	2	5.83±.27	5.69±.12	4.74±.73	3.32±.51	0.99±.13	1.04±.11	37±3	44±5	4.81±.56	3.16±.26
32	4	5.99±.15	5.58±.20	4.75±.14	2.59±.61	1.21±.16	0.93±.13	79±4	36±7	3.99±.50	2.78±.29
	C[b]	5.49±.15		3.95±.22		0.99±.05		43±4		3.98±.06	
36	8	6.29±.13	6.31±.19	8.97±.66	3.70±.50	2.17±.41	1.73±.18	94±4	60±8	4.19±.43	2.14±.16
	C[b]	6.20±.42		8.15±2.67		1.92±.46		86±24		4.20±.34	

[a]Number of days starved fish were in filtered seawater.

[b]Fish removed from fed population and transferred to filtered seawater 18 hours prior to sampling to clear stomach contents.

Table 2. RNA, DNA and protein content of various size groups of winter flounder larvae 42 to 58 days after hatching.

Age Days after Hatching	Standard Length Range mm	Mean mm	Standard Deviation mm	RNA µg/larva	DNA µg/larva	Protein µg/larva	RNA/DNA
42	4.98-6.07	5.60	.39	3.55± .23	--	44± 1	--
	6.27-6.89	6.50	.20	9.19± .75	1.99±.06	99±10	4.61±.09
	7.00-8.77	7.77	.57	21.83±1.98	3.91±.70	200±15	5.62±.52
43	5.26-6.35	5.76	.30	3.35± .61	.82±.13	44± 5	4.10±.07
	6.36-7.26	6.82	.26	7.75± .98	1.85±.25	91± 5	4.18±.03
	7.29-8.54	8.19	.33	27.42± .28	4.84±.00	274± 5	5.67±.06
50	4.10-6.54	6.01	.55	5.32± .40	1.27±.08	49± 4	4.19±.03
	6.31-7.69	7.00	.34	11.08± .29	2.52±.02	115± 1	4.40±.08
	7.50-8.67[a]	8.01	.35	32.82±2.83	6.19±.69	370±37	5.31±.14
58	5.79-6.91	6.31	.27	5.70± .03	1.35±.07	64± 1	4.23±.20
	6.67-8.35	7.27	.47	14.33±1.03	3.18±.08	153± 1	4.50±.21
	7.40-8.49[b]	7.95	.31	37.08±1.02	7.05±.10	434± 3	5.26±.07

[a]Six of the 10 fish in this sample had metamorphosed.

[b]All fish in this sample had metamorphosed.

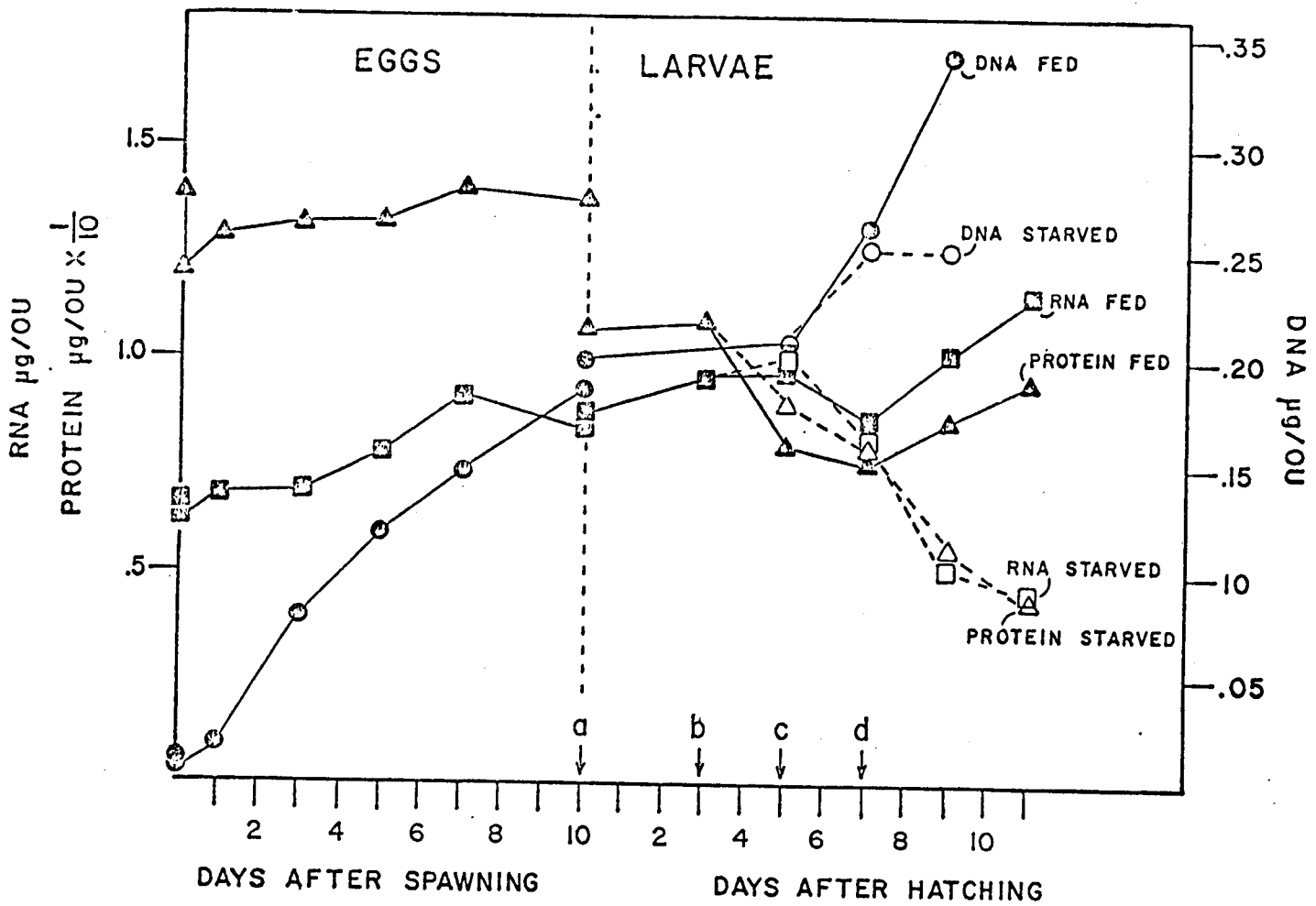

Figure 1. Time course of development of DNA (●), RNA (■), and protein (▲) content per ontogenic unit (OU) of winter flounder.

a - 50% hatch.

b - starved larvae transferred to filtered sea water.

c - most larvae showed no visible yolk.

d - food visible in gut of fed larvae.

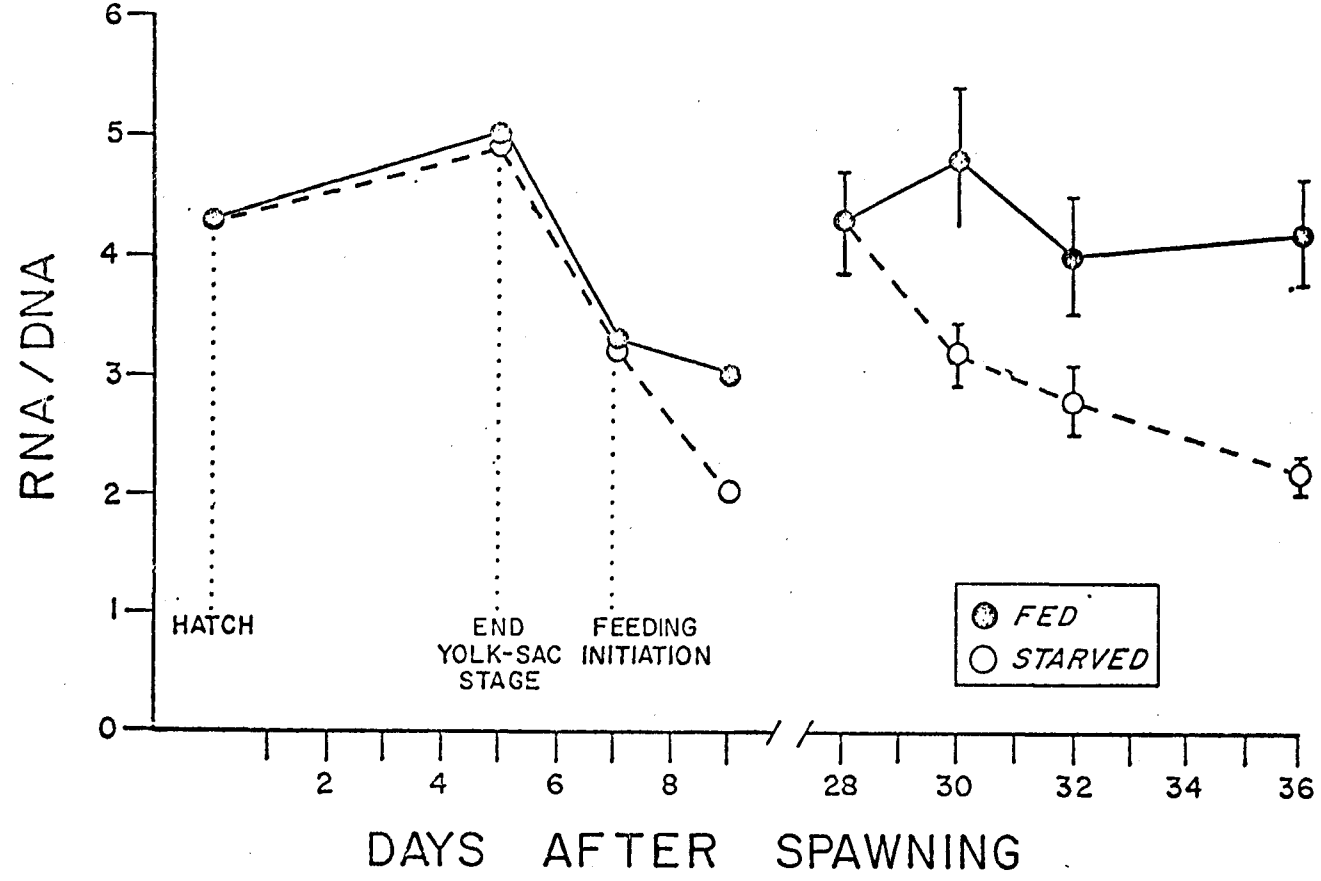

Figure 2. RNA-DNA ratios of starved and fed winter flounder larvae. Open circles indicate values for larvae transferred to filtered sea water on days 3 and 28 respectively. Brackets indicate ±2 standard errors of the mean.