The Effect of Prey Density on Protein, DNA, and RNA Content during the Early Growth of Pacific Herring Larvae, *Clupea Harengus Parasi*

成城大学非常勤講師
福田雅明 FUKUDA Masaaki

Abstract

The effect of prey densities on biochemical accumulation was examined during growth in first feeding and post-first feeding larvae of the Pacific herring, *Clupea harengus parasi*. Newly hatched larvae were reared at prey densities of 0.1, 0.5, 1.0, 5.0 and 10.0 prey/ml in a laboratory for a maximum of 76 days. Changes in protein, DNA, and RNA content per larva were analyzed at these densities and mortality coefficients recorded. The body weight, protein, and RNA contents of first feeding larvae at all densities decreased when the yolk sac was consumed. Consequently, yolk protein appears to be the main energy source for first feeding larvae. The DNA contents, in contrast, increased at all prey densities, implying hyperplasic growth during the first feeding period. Larvae reared with the two lower prey densities（0.1 and 0.5 prey/ml）decreased during yolk-sack consumption, then decreased to less than 10% on days 50 and 66 after hatching, respectively. Food availability is insufficient for the survival of post-first feeding larvae at densities of 0.5 prey/ml or less. Larvae reared at a density of 1.0 prey/ml showed a similar length, weight, and biochemical content to larvae reared at higher densities（5.0 and 10.0 prey/ml）up to day 36（with a standard length of 16 mm), then decreased rapidly. The DNA/protein ratios indicated active hypertrophic growth up to a length of about 16–17 mm for larvae fed with 1.0 prey/ml. These larvae might lack the energy for hypertrophic growth after reaching this size. Our study's RNA/DNA ratios were consistent with protein content, and ratios might be useful indices for feeding conditions and protein synthesis. A comparison of larvae of the same SL size reared with different prey densities indicates that larvae reared at a low prey density accumulated all biochemicals. A food restriction may therefore allow stronger larvae to survive and strengthen the Pacific herring population.

Introduction

Landings of Pacific herring, *Clupea harengus pallasi*, around Hokkaido in Japan reached about one million tons at the end of 1890s,

and then decreased rapidly. Catch has remained low and stable since the 1960s and Pacific herring are important commercial fish in Japan. The causes of the fluctuating abundance of this species have been studied for many years but have only now been understood. Pelagic fish, including populations of both Pacific and Atlantic herring (*Clupea harengus harengus*), are known to fluctuate widely. Hjolt (1914) offered a hypothesis that food availability when larvae convert from yolk nutrition to exogenous feeding is critical to the success of the year class. Most marine fish larvae have a yolk sac just after hatching and initiate feeding before yolk absorption is complete. The period from the end of yolk feeding to exogenous feeding is known as "first feeding." Mass mortality may occur if food supplies are inadequate during this period (Houde 1978). As a result, prey availability for first feeding larvae is an important aspect of larval survival.

Rapid growth has recently been considered to be of special importance for the survival of fish larvae because growth during the larval stage is likely to influence larval mortality. If a longer duration of a larval stage leads to higher predation, then larval mortality may be high. Fast-growing larvae may therefore have a greater chance of survival (Chambers and Leggett 1987; Hare and Cowen 1997; Takasuka *et al*. 2004). Larval growth is primarily influenced by food availability and temperature (Folkvord *et al*. 2000; 2009). Accordingly, the quantity of food for post-first feeding larvae is also important for maintaining the Pacific herring population.

Recently, several biochemical indices have been used for evaluating the nutritional conditions and growth rates of reared and field-collected fish larvae (reviewed by Ferron and Leggett 1994). The RNA concentrations vary with the protein synthesis rate, and the quantity of DNA is proportional to the number of cells. The concentration of DNA per cell remains more constant under conditions of starvation. The RNA content is an index for protein synthesis, and changes in DNA content reflect hyperplasic growth. Consequently, the RNA/DNA ratio is used as an indicator of growth and nutritional condition in laboratory-reared and field-collected larvae (Buckley 1984; Bulow 1987; Mathers *et al*. 1994; Chicharo and Chicharo 2008; DiPane *et al*. 2019). This ratio is especially useful for evaluating the effect of food density on protein, RNA, and DNA changes and for evaluating and understanding fluctuations in the herring population.

The present study investigated the effects of food density at first feeding and post-first feeding on biochemicals in Pacific herring larvae fed at different prey densities. This was in order to better understand their growth and survival. Food densities necessary for supporting significant survival and growth were identified for the stages of first feeding and post-first feeding larvae.

Material and methods

Rearing conditions

Mature parents – 58 males and 45 females – were caught at Lake Fuuren in eastern Hokkaido, Japan. Mixed gametes were artificially fertilized and incubated at 8℃ in an incubation tank at the Akkehi hatchery station at the former Hokkaido Regional Fishery Institute. About 300 newly hatched larvae were removed from this tank and placed in each of five 30-liter experimental aquaria located in a water bath. The larvae were reared

at five nominal prey density levels with rotifers (*Brachinus plicatilis*): 0.1, 0.5, 1.0, 5.0, and 10.0 prey/ml. All aquaria were provided with air-stones, and the temperature was maintained at 8℃ for 40 days, and then gradually raised to 14℃. Sediments in the experimental aquaria were siphoned every day, and the water was replaced every second day. Rotifer concentrations were established on the first day by maintaining a constant volume of seawater in each aquarium and adding the desired number of prey. Counts for concentrations were based on the mean of five to 10 replicate 10 ml water samples from each aquarium. Concentrations were maintained near nominal levels by adding rotifers twice each day: at 06:00 and 18:00.

Sampling procedure

A total of 10 to 73 larvae were removed for chemical analysis on days 8, 17, 36, 50, 66, and 76 after hatching. Fishes were anesthetized in a 1:10,000 solution of MS222 and measured for standard length (SL) to the nearest in 0.1 mm under 10x magnification with a Nikon profile projector. Consequently, all specimens measured for length were still alive. For chemical analysis, these larvae were frozen in a buffer solution (0.25 M sucrose, 1 mM EDTA, 20 mM Tris-HCL pH 7.5) at − 20℃ for chemical analysis. The numbers of survivors were determined by individual counting at days 36, 50, and 66 after hatching. On these days, aquaria were replaced with washed vessels.

Chemical analysis

Wet weights were obtained from frozen samples. Larvae, thawed at room temperature, were rinsed in distilled water, rolled on filter paper, and then weighed on an electrobalance individually or in groups of up to 20 individuals, depending on size. After weighing, larvae were homogenized for 1 min on ice with potter-Elvehjem grass Tefron homogenizers in the buffer solution described above. Nucleic acid content was determined using the Schmidt-Thannhauser method as modified by Nakano (1988). Protein was determined according to Lowry *et al*. (1951).

Calculations

Daily instantaneous mortality coefficient(z) was calculated as:

$$Z = (\ln N_{S2} - \ln N_{S1})/t \quad (1)$$

where N_{S2} and N_{S1} is the number of survivors at the beginning and the end of the time interval, and t is the time interval in days.

The Z of the days 37 to 50, 51 to 66, and 67 to 76 were estimated using formula (1) as survivors were counted on each sampling day. Conversely, estimates of mortality, $\tilde{Z}$, corrected for possible survivors removed in sampling, were calculated with Microsoft Excel Solver using the functional formula (2):

$$N_{t0} = (S_{t36} \times e^{-19\tilde{Z}}) \times (e^{-9} + S_{t17}) \times (e^{-8\tilde{Z}} + St8) \quad (2)$$

where N_{t0} = initial number of each aquarium; S_{t36}, S_{t17}, S_{t8} = number of survivors on days 36, 17, and 8 after hatching, respectively; and exponents of 19, 9, 8 = intervals are from days 17 to 36, 8 to 17, and 0 to 8, respectively. Equation (2) was used as the objective function; the initial number in each aquarium was set as the objective value; and the Microsoft Solver program was used to estimate mortality: $\tilde{Z}$.

Statistical analysis

All experimental data were analyzed using a one-way analysis of variance (ANOVA) to identify significant differences among the

aquaria. Assumptions of normal distributions and homogeneity of variances were checked before analysis. All ANOVA assumed a significance at the 5% level, followed by the Tukey-Kramer' s test.

Results

Survivors

Larval survival steadily declined throughout the experiment. Survivors reared at the lowest prey density（0.1 prey/ml）decreased to less than 10% from day 0 to day 50. On day 66, survivors reared at a density of 0.5 prey/ml also fell to less than 10%. Survival curves calculated from daily instantaneous mortality coefficients were plotted against the larval size（Fig. 1）. At lower prey densities（0.1 and 0.5 prey/ml）, many larvae began to die from day 1 and did not achieve an SL of greater than 15–19 mm. Nutrition was therefore inadequate from the start of the experiment and a suitable number of preys for the number of larvae in the aquaria was not reached. The mortality coefficient for a density of 1.0 prey/ml increased suddenly when larvae attained an SL of approximately 16 mm. Survival continued to decrease until larvae reached about 21 mm. Sufficient food for survival was available from hatching to an SL of roughly 16 mm. Supply then became insufficient, and the mortality rates increased. The survival curves for larvae fed at the two highest densities（5.0 and 10.0 prey/ml）were similar.

Growth in length and weight

No differences were found among mean SL for larvae fed at densities of 0.5, 1.0, 5.0, and 10.0 prey/ml until day 8（Table 1）. Larvae reared at a density of 0.1 prey/ml were significantly shorter. The mean SL of larvae fed 1.0 prey/ml were similar sized larvae fed at the highest density up until day 17. The mean SL then gradually decreased relative to the larvae fed at higher densities. This was the same as larvae of SL fed at 0.5 prey/ml by day 50. Throughout the experiment, the mean larval body weight when feeding at 0.1 prey/ml group was always less than larvae fed at higher densities. In contrast, the body weight of larvae reared at 1.0 prey/ml was relatively high compared with larvae fed at lower densities at day 36; however, the weights later became similar to those fed 0.5 prey/ml. The mean body weight when fed 10.0 prey/ml was similar to the weights of larvae fed 5.0 prey/ml up to day 36; this was the highest among all larvae at the end of the experiment.

Fig 1. Survival curves related to standard length in Pacific herring larvae reared at five prey densities.

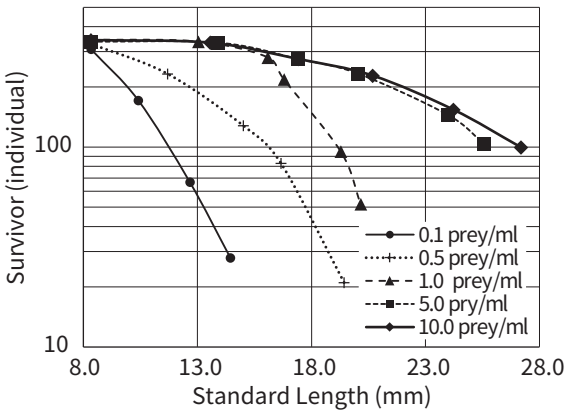


Table 1. Mean (± SD) values of standard length, body weight, protein and nucleic acids at the five prey density levels of Pacific herring larvae.

Days after hatching	Prey densities No/ml	Standard length		Body weight		Protein	DNA	RNA	
		Mean(mm)	No. of sample	Mean(mg/larva)	No. of sample	Mean(mg/larva)	Mean(μ g/larva)	Mean(μ g/larva)	No. of sample
0		8.32 ± 0.395	50	1.31	1(370)	0.086	0.350	7.080	1(370)
8	0.1	8.49 ± 0.661[a]	10	0.70	1(10)	0.037	0.909	3.433	1(10)
	0.5	8.91 ± 0.453[a, b]	10	0.79	1(10)	0.044	0.973	3.690	1(10)
	1.0	9.68 ± 0.747[b]	14	1.00	1(14)	0.051	1.077	4.991	1(14)
	5.0	9.91 ± 0.812[b]	18	1.11	1(18)	0.061	1.260	5.469	1(18)
	10.0	10.58 ± 1.639[b]	25	1.41	1(25)	0.061	1.374	6.381	1(25)
17	0.1	10.4 ± 1.103[a]	30	1.167 ± 0.094[a]	30	0.071 ± 0.004[a]	1.142 ± 0.03[a]	6.062 ± 0.436[a]	3(10)
	0.5	11.68 ± 0.830[b]	32	1.611 ± 0.201[b]	29	0.090 ± 0.008[a]	1.456 ± 0.24[a, b]	8.043 ± 0.983[a, b]	3(10)
	1.0	13.04 ± 0.969[c]	31	2.350 ± 0.308[c]	32	0.128 ± 0.017[a, b]	2.149 ± 0.31[b, c]	11.871 ± 1.175[b, c]	3(12)
	5.0	13.92 ± 0.537[d]	31	2.822 ± 0.137[d]	32	0.169 ± 0.020[b]	2.957 ± 0.37[c]	16.904 ± 1.893[c]	3(12)
	10.0	13.55 ± 0.739[c, d]	32	2.672 ± 0.267[d]	32	0.147 ± 0.011[b]	2.392 ± 0.22[b, c]	13.33 ± 1.094[b, c]	3(12)
36	0.1	12.66 ± 0.991[a]	31	2.448 ± 0.254[a]	31	0.135 ± 0.107[a]	2.569 ± 0.27[a]	8.34 ± 0684[a]	3(11)
	0.5	15.01 ± 1.285[b]	34	5.311 ± 0.793[b]	35	0.309 ± 0.065[a, b]	5.566 ± 0.83[b]	25.236 ± 5.072[b]	3(15)
	1.0	16.09 ± 0.940[c]	39	6.661 ± 0.427[c]	39	0.406 ± 0.035[b]	7.561 ± 0.70[b, c]	31.029 ± 2.095[b]	4(10)
	5.0	17.41 ± 1.116[d]	39	9.575 ± 0.370[d]	40	0.659 ± 0.009[c]	11.42 ± 0.54[d]	60.164 ± 1.201[c]	4(10)
	10.0	17.41 ± 1.012[d]	33	9.631 ± 0.529[d]	34	0.684 ± 0.072[c]	10.201 ± 0.91[d]	67.657 ± 8.069[c]	4(10)
50	0.1	14.44 ± 1.405[a]	27	4.464 ± 0.859[a]	28	0.315 ± 0.108[a]	5.59 ± 1.62[a]	22.287 ± 8.62[a]	4(7)
	0.5	16.66 ± 1.539[b]	50	9.460 ± 0.484[b]	50	0.703 ± 0.049[b]	11.73 ± 0.84[b]	48.808 ± 1.82[b]	5(10)
	1.0	16.79 ± 1.047[b]	54	9.025 ± 0.660[b]	54	0.635 ± 0.055[a, b]	11.62 ± 0.36[b]	44.476 ± 4.85[b]	5(11)
	5.0	20.04 ± 1.416[d]	73	20.82 ± 1.537[c]	50	1.605 ± 0.147[c]	21.08 ± 2.45[c]	124.44 ± 10.9[c]	5(10)
	10.0	20.68 ± 1.324[d]	61	24.26 ± 2.556[d]	49	1.747 ± 0.154[c]	24.99 ± 2.25[d]	156.60 ± 18.6[d]	4(15)
66	0.5	19.43 ± 2.319[a]	21	28.70 ± 1.213[a]	21	2.406 ± 1.302[a]	32.55 ± 14.7[a]	136.28 ± 63.9[a]	4(8)
	1.0	19.28 ± 1.263[a]	31	23.73 ± 5.737[a]	29	1.840 ± 0.471[a]	30.32 ± 6.67[b]	123.96 ± 28.7[b]	5(9)
	5.0	23.97 ± 1.507[c]	34	74.33 ± 10.78[c]	12	6.069 ± 1.073[b]	74.38 ± 13.2[c]	392.62 ± 78.9[c]	4(3)
	10.0	24.22 ± 1.984[c]	38	91.33 ± 27.94[d]	13	7.305 ± 2.242[b]	91.39 ± 29,2[d]	539.7 ± 128.6[d]	5(3)
76	1.0	20.15 ± 1.263[a]	52	29.01 ± 5.05[a]	37	2.361 ± 0.443[a]	38.83 ± 6.1[a]	164.84 ± 42.6[a]	5(12)
	5.0	25.55 ± 1.714[b]	60	103.1 ± 10.10[b]	15	8.292 ± 0.602[b]	126.5 ± 17.4[b]	511.23 ± 16.5[b]	5(3)
	10.0	27.17 ± 2.532[c]	46	177.5 ± 31.66[c]	12	13.91 ± 1.823[c]	206.2 ± 36.5[c]	1111.6 ± 247[c]	4(3)

The figures in parenthesis represent the number of larvae contained in the sample.
Different letters indicated significant differences ($p<0.05$) among the larvae fed on different prey densities.

Protein and nucleic acids

Protein and nucleic acid content per larva at each prey density level increased exponentially with growth throughout the experiment (Table 1). The protein and RNA content decreased at all prey densities during the first eight days, especially in larvae fed at the lowest density. The DNA content, however, increased at all feeding levels during this time. The increased DNA content reflects the cell division required during this period. The DNA/protein ratios increased rapidly to approximately 10–13 mm and gradually decreased with growth at all prey densities (Fig. 2A). Rapid increments in DNA/protein ratios when SL reached about 10–13 mm indicated vigorous cell proliferation, even for larvae reared at the lowest prey density. The RNA/protein ratios also increased for a few days after hatching (Fig. 2 B), although increments were less obvious than for DNA/protein ratios. Subsequently, RNA/protein ratios decreased slightly through the remaining duration. A tendency was noted, suggesting that the higher the prey density, the higher the RNA/protein ratio.

No significant differences in RNA/DNA ratio were seen regardless of prey density at day 17 (Fig. 3). The values of RNA/DNA ratio at day 17 were relatively high, and consistent with the rapid increase in body weight. After day 36, larvae fed 10.0 prey/ml displayed the high-

Fig. 2 Changes in DNA/protein (A) and RNA/protein (B) with growth in Pacific herring larvae reared at five prey densities.

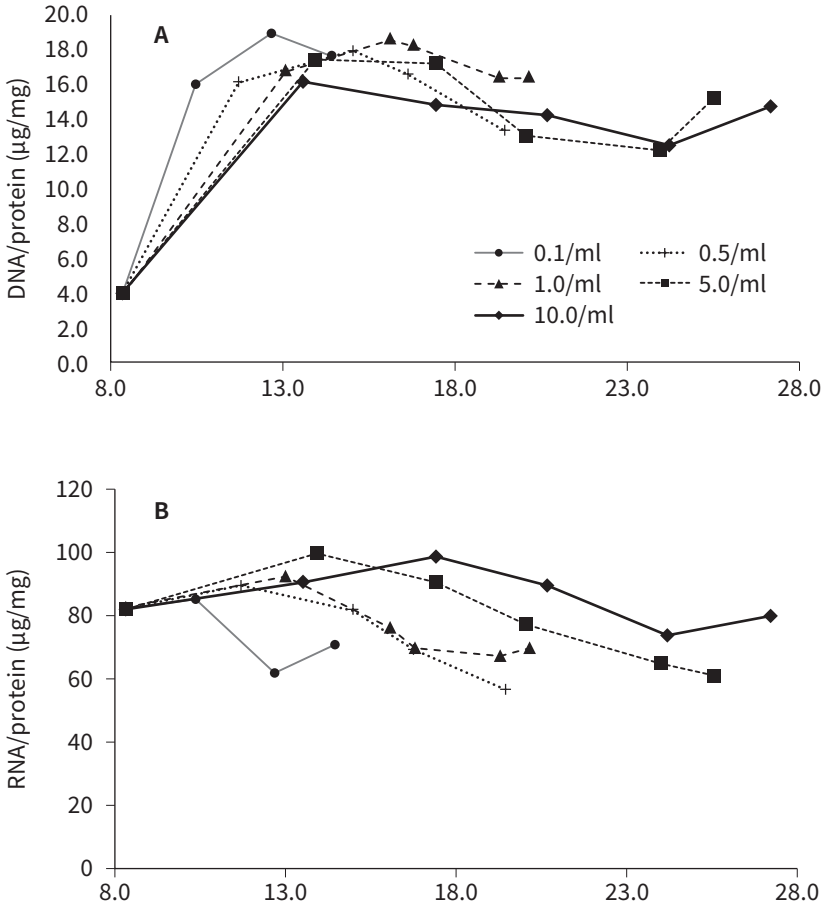
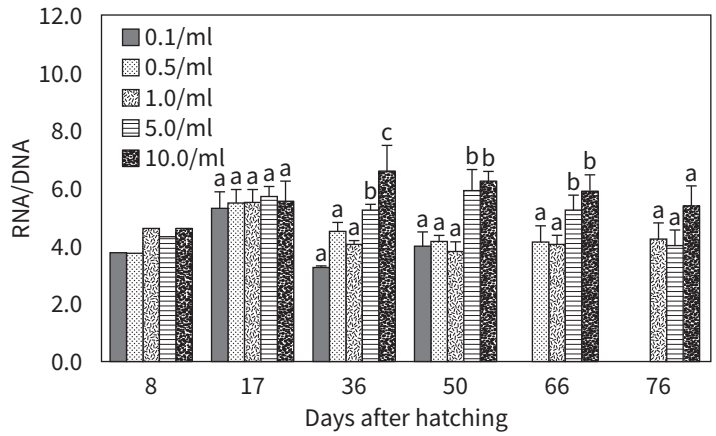


Fig. 3 Changes in mean values and 95% confidence intervals of RNA:DNA ratios with growth in Pacific herring larvae reared at five prey densities. Different letters indicated significant differences ($p<0.05$) among the larvae fed on different prey densities.



est RNA/DNA ratios. The next highest ratios of RNA/DNA were larvae fed 5.0 prey/ml. The RNA/DNA ratios for larvae fed at the lower three densities showed no significant difference during the experiment.

Discussion

The protein content of larvae decreased during the first eight days, regardless of prey density; body weight also decreased, but SL increased during this period. The protein of newly hatched larvae was derived mainly from the yolk and decreased over the first eight days due to the consumption of yolk protein for growth, especially cell proliferation. The increased DNA content supports active hyperplasic growth up to eight days at all prey densities. The yolk sac of Atlantic herring is reported to support larvae for five to 14 days, depending on temperature. The yolk sac usually lasts for six to seven days at 8℃ (e.g. Blaxter and Hempel 1963; Bang et al. 2007). McGurk (1984) reported that the time to exhaustion of the yolk to the age of irreversible starvation for Pacific herring larvae was 8.5, 7.0, and 6.0 days at 6℃, 8℃, and 10℃, respectively. Our experiment temperature was maintained at 8℃ during the yolk sac period; yolk was likely completely absorbed by day seven. The yolk volume was equal in all larvae, indicating the larvae had sufficient energy for growth during the first week. Even so, the growth in length, body weight, and protein content were different depending on prey density. Kiørboe et al. (1985) reported that initiating exogenous feeding in Atlantic herring larvae was delayed by 1–4 days when prey density was low, compared with high prey density. It is obvious that the absence of food in the environment, other than yolk, impairs subsequent growth. The results of our experiment show that less than 0.5 prey/ml food density is insufficient for optimal growth and survival during first feeding.

An increase in the DNA/protein ratio generally reflects hyperplasic growth because a high value signifies less protein content per cell. The DNA/protein ratios of our experiment increased markedly during the first eight days (less than 10 mm in SL), indicating that this phase is dominated by hyperplasic growth. Yolk protein might be consumed

to support the early development of larval organs and tissues; after that time, the DNA/protein ratios of all larvae gradually decreased. No significant differences in RNA/DNA ratios were observed regardless of prey density at day 17; larvae were about 10–13 mm long at this stage. Therefore, the protein synthesis activity of post-first feeding larvae was relatively high, implying that the growth manner changed to hypertrophic growth. Fukuda *et al*. (1986) showed that Pacific herring larvae grow mainly hypertrophically during development from an SL of 10 mm to 20 mm. Fukuda (1990) also investigated muscle development and concluded that the diameter of mean white muscle fiber increased gradually with development up to about 20 mm. Then small new fibers were added from an SL of about 20 mm to 30 mm. A change in the DNA/protein ratios from roughly 10 mm in SL suggests that this is the turning point from hyperplasic growth to hypertrophic growth.

The DNA/protein ratios of larvae fed 1.0 prey/ml were almost the same as larvae fed 5.0 prey/ml up to an SL of about 16–17 mm; subsequent to this, the ratio declined. Larvae fed 1.0 prey/ml might lack energy for hypertrophic growth after reaching an SL of 16–17 mm. Alternatively, these larvae may not switch from hypertrophic to hyperplasic growth once they reach about 16 mm

The RNA/protein ratios did not differ markedly by prey density. These findings are consistent with observations for Atlantic herring, where the RNA/protein ratio was not significantly different between fed and starved larvae (Mathers *et al*. 1994). These authors suggested that the protein content decreased when larvae were starved and then both RNA and protein content decreased, thus maintaining relatively stable ratios.

The RNA/DNA ratios are useful indicators of growth rate and nutritional status under laboratory and field conditions (e.g. Ferron and Leggett 1994). Clemmesen (1994) reported no significant difference between fed and starved larvae in RNA/DNA ratios of Atlantic herring from hatching to about day eight, although values did fluctuate. Prey densities for yolk sac larvae showed a minimal effect on protein synthesis. The RNA/DNA content after day 36 was separated into two groups, except for day 77. Ratios from larvae fed at the three lower densities were less than in larvae fed with higher densities. These trends were also found for protein contents. The RNA/DNA ratios in our experiment might therefore be a direct indication of protein synthesis.

Biochemical indices are often used to assess the health status of field-caught larvae (Ferron and Leggett 1994). The growth history of larvae caught at sea is unknown, with only length and weight as one-time measure-

Table 2. Comparison of body weight, protein, DNA, RNA contents and RNA/DNA ratios between the nearly equal length Pacific herring larvae growing different feeding history.

Standard length (mm)	Nominal prey density (number/ml)	Days after hatchng	Body weight (mg)	Protein (mg/larva)	DNA (μ g/larva)	RNA (μ g/larva)	RNA/DNA
20.0	5.0	50	20.8 >**	1.61 >**	21.1 >**	124.4 >**	5.90
20.1	1.0	76	29.0	2.36	38.8	164.8	4.25

＊＊ : p<0.05

ments. We compared the biochemical content for larvae reared at different prey densities (Table 2). We examined larvae that reached an SL of 20 mm or more. Surviving larvae fed in the 1.0 prey/ml or 5.0 prey/ml group were compared for days 76 and 50, respectively. No significant difference in SL was seen between these larvae ($p<0.001$). Protein, DNA, and RNA contents were higher for larvae reared at the lower prey density. In contrast, there were no significant differences in RNA/DNA ratios, but larvae fed 1.0 prey/ml tended to have lower ratios. Mathers *et al*. (1994) compared reared with field-caught Atlantic herring larvae and reported that field-caught larvae showed higher concentrations of DNA. Mathers et al. stated that wild larvae displayed a higher cell density, possibly associated with a particular developmental stage. The surviving larvae in the current study, which grew during a food shortage, are few and small but with a strong constitution, similar to humans under like conditions. A food restriction may therefore allow stronger larvae to survive and strengthen the population.

Acknowledgment

I would like to thank Dr. Sakurai, a former professor at Seijo University, for allowing me to present old data as a paper.

References

Bang, A., Grønkjær, P. and Folkvord, A. (2007). Possible fitness costs of high and low standard metabolic rates in larval herring *Clupea harengus*, as determined by otolith microstructure. Mar. Ecol. Prog. Ser. 331: 233-242.

Blaxter, J.H.S. and Hempel, G. (1963) The influence of egg size on herring larvae *Clupea harengus*. Rapp. P.-v. Reun. Cons. Int. Explor. Mer. 28: 211-240.

Buckley, L.J. (1984). RNA-DNA ratio: an index of larval fish growth in the sea. Mar. Biol. 80: 291-298.

Bulow, F.J. (1987). RNA:DNA ratios as indicators of growth in fish: a review. In. Summerfelt, R. C., Hall, G. E. (eds.) Age and growth of fish. Iowa State University Press, Ames, pp. 45-64

Chambers, R.C. and Leggett, W.C. (1987) Size and age at metamorphosis in marine fishes: an analysis of laboratory-reared winter flounder (*Pseudopleuronectes americanus*) with a review of variation in other species. Can. J. Fish. Aquat. Sci. 44: 1936-1947.

Chícharo, M.A. and Chícharo, L. (2008) RNA:DNA ratio and other nucleic acid derived indices in marine ecology. Int. J. Mol. Sci. 9: 1453-1471.

Clemmesen, C. (1994) The effect of food availability, age or size on the RNA:DNA of individually measured herring larvae: laboratory calibration. Mar. Biol. 11: 377-382.

DiPane, J., Joly, L., Koubbi, P., Giraldo, C., Tavernier, E., Marchal, P. and Loots, C. (2019) Ontogenetic shift in the energy allocation strategy and physiological condition of larval plaice (*Pleuronectes platessa*). PLoSONE 14: e0222261. https://doi.org/10.1371/journal. pone.0222261

Ferron, A. and Leggett, W.C. (1994) An appraisal of condition measures for marine fish larvae. Adv. Mar. Biol. 30: 217-303.

Folkvord, A., Blom G., Johannessen A. and Moksness E. (2000) Growth-dependent age estimation in herring (*Clupea harengus L.*) larvae. Fish. Res. 46: 91-103.

Folkvord, A., Høie, H., Johannessen, A. and Solbakken, T. (2009) Effects of prey concentration, light regime and parental origin on growth and survival of herring larvae under controlled experimental conditions. ICES J. Mar. Sci. 66: 1702-1709.

Fukuda, M. (1990) Development of the myotomal musculature and changes in swimming speed during early growth in Pacific herring *Clupea pallasi*. Nippon Suisan Gakkaishi 56: 11-17 (in Japanese).

Fukuda, M., Nakano, H. and Yamamoto, K. (1986) Biochemical changes in Pacific herring during early developmental stages. Bull. Fac. Fish. Hokkaido Univ. 37: 30-37 (in Japanese).

Hare, J.A. and Cowen, R.K. (1997) Size, growth, development, and survival of the planktonic larvae of Pomatomus saltatrix (Pisces: Pomatomidae). Ecology 78: 2415-2431.

Hjolt, J. (1914) Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapp. P.-v. Reun. Cons. Int. Explor. Mer. 20: 5-38.

Houde, E.D. (1978) Critical food concentration for larvae of three species of subtropical marine fishes. Bull. Mar. Sci. 28: 395-411.

Kiørboe, T., Munk, P. and Støttrup, J.G. (1985) First feeding by larval herring *Clupea harengus L*. Dana 5: 95-107.

Lowry, O., Rosebrough, N.J., Farr, A.L. and Randal, R.J.（1951）Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265-275.
Mathers, E.M., Houlihan, D.F. and Burren, L.J.（1994）RNA, DNA and protein concentrations in fed and starved herring, *Clupea harengus* larvae. Mar. Ecol. Prog. Ser. 107: 223-231.
McGurk, M. D.（1984）Effects of delayed feeding and temperature on the age of irreversible starvation and on the rates of growth and mortality of Pacific herring larvae. Mar. Biol. 84: 13-26.
Nakano, H.（1988）Techniques for studying on the early life history of fishes. Aquabiology（Tokyo）10: 23-26（in Japanese）.
Takasuka, A., Aoki, I. and Mitani, I.（2004）Three synergistic growth-related mechanisms in the short-term survival of larval Japanese anchovy *Engraulis japonicus* in Sagami Bay. Mar. Ecol. Prog. Ser. 270: 217-228.

和文要旨

太平洋ニシンを含む浮魚類は，資源量の変動が著しいことが知られており，発育初期の生き残り（初期生残）の良否が後の資源量を決定している。稚仔魚の生残については，複数の要因が推定されているが，初期発育期の餌の量が生き残りを大きく左右することは広く知られた事実である。本論文では，ニシンの卵黄吸収期およびその後に続く仔魚期にどの程度の餌が環境中に必要か，また餌の量が成長にどのように影響を与えるか，仔稚魚のタンパク質および核酸（RNA，DNA）の成長に伴う変化から検討を加えた。その結果，卵黄吸収期は，細胞分裂が活発な時期であり，環境中に0.5 個体／ ml の餌量が必要であることが示された。また，卵黄吸収後の仔魚期では，体長 16 ㎜程度を境に細胞分裂を中心とした成長から，細胞肥大を中心とした成長に移行し，1.0 個体／ ml 以上の餌が環境中に無いとスムーズに細胞肥大成長へと移行できないことが示された。