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Growth and developmental patterns based on RNA, DNA and protein content during early ontogeny of laboratory-reared greater amberjack *Seriola dumerili*

Katsuyuki Hamasaki[1]*, Ayako Yoshimura[1], Hiroshi Hashimoto[2], Takashi Iwasaki[3], Shigeki Dan[1]

[1]Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Minato, Tokyo 108-8477, Japan. [2] Yamaguchi Regional Alliance Office, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Shimonoseki, Yamaguchi 759-6595, Japan. [3]Aquaculture Research Department, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Ishigaki, Okinawa 907-0451, Japan.

*Corresponding author, e-mail: hamak@kaiyodai.ac.jp

Abstract

Nucleic acid and protein-based biochemical indices of fish larvae and juveniles have been analysed to evaluate their growth and development. However, little is known about the ontogenetic changes in these biochemical indices in relation to individual fish body sizes. In the present study, larvae and juveniles of laboratory-reared greater amberjack *Seriola dumerili*, from 1 to 25 days post-hatching, were used as a model organism to perform piecewise regression analyses between log-transformed values of total length (TL) and RNA (an index of protein synthesis capacity), DNA (an index of cell number) and protein content (an index of absolute growth), as well as those between TL and RNA/DNA, RNA/protein and protein/DNA ratio of individual fish. The RNA/DNA ratio decreased during the prelarval stage; after that, it linearly increased, suggesting a steady increase in protein synthesis capacity per cell from the preflexion stage onwards. The protein content exhibited positive allometric growth, while the RNA/protein ratio, an index of overall protein synthesis capacity, slowly declined after the transition from the preflexion to flexion stages, indicating that the efficiency of retention of newly synthesized proteins might have improved. Meanwhile, the protein/DNA ratio, which is a measure of cell size, decreased during the prelarval, preflexion and early flexion stages, reflecting hyperplasia with positive allometric growth of DNA content and low protein increment, and hypertrophy occurred after the early flexion stage, reflecting the negative allometric growth of DNA content and positive allometric growth of protein content. The biochemical synthesis appeared to enter a stable growth phase from the mid-flexion stage, accompanying the progress of morphological, physiological and behavioural development in greater amberjack larvae. Therefore, piecewise regression analyses could detect changes in growth and developmental patterns based on nucleic acid and protein content during the early ontogeny of fish species.

Key words: piecewise regression analysis; allometric growth; RNA/DNA; RNA/protein; protein/DNA

Introduction

Numerous fish species have pelagic larvae that hatch at an early developmental stage and depend on endogenous energy sources, such as yolk, for ontogenetic development. After developing eyes and opening their mouths, larvae start exogenous feeding on live prey organisms before fully utilizing their endogenous energy reserves (Yúfera and Darias 2007; Hu et al. 2018). During the preflexion, flexion and postflexion stages, larvae grow while developing external and internal organs, eventually reaching the juvenile stage (Kendall et al. 1984; Finn and Kapoor 2008; Holt 2011).

Allometric growth analysis between body length and the size of specific body parts, such as mouth, eye, head and tail, has been utilized to elucidate the growth and developmental patterns of fish during early ontogeny. The allometric growth of body parts is analysed through log-scaled piecewise linear regression equations with break point(s) (e.g. Gisbert et al. 2002; Peña and Dumas 2009; Martínez-Montaño et al. 2016). The ontogenetic shift in body proportions is associated with changes in ecology, such as feeding habits and behavioural traits of larvae and juveniles.

The analysis of nucleic acid and protein content in larvae and juveniles has been also employed to evaluate their growth and development in different fish species (Buckley et al. 1999). While the amount of DNA in an animal cell is typically stable, the amount of RNA, predominantly linked to ribosomes, is directly correlated with the rate of protein synthesis. Therefore, RNA, DNA and protein content serve as indices of protein synthesis capacity, cell number and absolute growth in fish, respectively, and the ratios of RNA to DNA, RNA to protein and protein to DNA content also serve as indices of the protein synthetic capacity of a cell, overall protein synthesis capacity and cell size, respectively (Richard et al. 1991; Clemmesen 1994; Mathers et al. 1994; Westerman and Holt 1994; Gwak et al. 2003). These biochemical indices are generally presented as the average value for larvae and juveniles in relation to age, that is, days post-hatching (dph), and no study has yet performed piecewise regression analysis to detect ontogenetic shifts in nucleic and protein-based biochemical indices in individual fish larvae and juveniles.

The greater amberjack *Seriola dumerili* (Risso 1810) (Carangiformes, Carangidae) is a widely distributed marine pelagic fish species inhabiting warm and tropical regions across the globe. This species is highly prized for aquaculture, especially in Japan and the Mediterranean region, with larval culture being conducted in hatcheries (Seoka et al. 2000; Hamasaki et al. 2009; Hashimoto et al. 2013, 2014; Roo et al. 2019). Seoka et al. (2000) analysed the RNA, DNA and protein content of greater amberjack larvae and juveniles by pooling fish samples and reported these contents per unit of body weight of the samples. In the present study, we used larvae and juveniles of laboratory-reared greater amberjack as a model organism to elucidate if the growth and developmental patterns of fish during early ontogeny could be evaluated by applying piecewise regression analysis based on the nucleic acid and protein content determined for individual fish.

Materials and Methods

Sample collection

Fish samples were collected from a 70-kl volume larviculture tank of greater amberjack located at the Shibushi Field Station, Japan Fisheries Research and Education Agency, Kagoshima, Japan. The larval culture protocol was outlined in a prior study (Hashimoto et al. 2013). In brief, approximately 440,000 newly hatched larvae were stocked in the tank and raised until 35 dph by being fed rotifers *Brachionus plicatilis* species complex starting from 3 dph (mouth opening), *Artemia* from 19 dph and formula feeds [Ambrose 400 and 600 (crude protein 52%, crude lipid 8%), FEED ONE Co., Ltd., Yokohama, Japan) from 30 dph. The water temperature in the tank was maintained at an average of 25.5ºC, ranging from 24 to 26ºC. Around 69,000 juveniles were harvested at 35 dph, and the larval survival rate was relatively high at 15.6% when compared with other greater amberjack seed production trials (Hashimoto et al. 2013).

Ten fish were sampled at around 2 p.m. every other day, starting from 1 dph to 15 dph, and at 20 and 25 dph. The final sampling time was set when the fish reached the juvenile stage with a TL of over 10 mm (Hashimoto et al. 2014). Fish were anesthetized with ethyl 3-aminobenzoate methanesulfonic acid and checked for swim bladder inflation under a stereomicroscope. Swim bladder inflation success serves as an index of normal growth and the developmental process of greater amberjack larvae (Imai et al. 2011). The TL of the fish specimens was measured using a profile projector. The specimens were then individually rinsed with filtered seawater, placed in 1.5-ml polypropylene tubes and immediately stored in a −80°C freezer.

We did not determine the ontogenetic stages of individual fish specimens, so we inferred the early ontogenetic stages of greater amberjack in relation to body size as follows (Masuma et al. 1990; Tachihara et al. 1993; Hamasaki et al. 2023): prelarva (yolk-sac larva) from 2.8 to 4 mm TL, preflexion larva from 4 to 4.7 mm TL, flexion larva from 4.2 to 6.5 mm TL, postflexion larva from 5.8 to 10.9 mm TL and juveniles from 10.2 mm onwards. The standard-length (SL) values of fish shown in previous studies were converted to TL values using the conversion formulae as shown in Fig. 1a (see the Data analysis subsection for formulating the equations). To this end, we used SL and TL values measured for 301 specimens of greater amberjack larvae and juveniles cultured in tanks at the Kamiura Field Station, Japan Fisheries Research and Education Agency, Oita Prefecture, Japan. The Shibushi and Kamiura Field Stations used almost identical larval culture protocols.

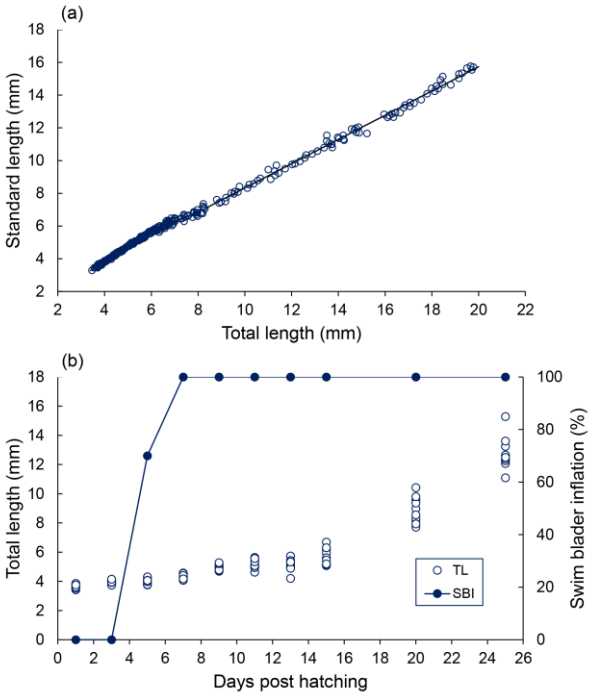

Fig. 1. Relationship between total length (TL) and standard length (SL) of 301 specimens (a) and changes in total length and swim bladder inflation (SBI) rate of greater amberjack *Seriola dumerili* larvae and juveniles (b). Fitted lines are drawn based on three segmented linear equations generated by piecewise regression analysis: (1) SL = 0.1320 + 0.9243TL ($3.40 \leq TL < 6.09$), (2) SL = 2.2408 + 0.5782TL ($6.09 \leq TL < 8.12$), and (3) 0.9209 + 0.7408TL ($8.12 \leq TL$) (adjusted R^2 value, 0.9985; $F_{5,\ 295} = 39660$, $p < 0.0001$). Ten fish specimens were used to observe SBL and measure TL each day. The SBI rate was calculated as (number of fish with an inflated swim blader/number of fish examined) × 100.

Determination of RNA, DNA and protein content

A single whole specimen of the greater amberjack was homogenized with Tris-HCl buffer (0.05 M Tris, 0.01 M EDTA, 0.1 M NaCl, pH 8.0, 0.01% SDS) individually, using either a microtube homogenizer or an ultrasonic homogenizer in ice-cold water. The homogenate was subsequently divided into two halves, with one half being subjected to centrifugation at 5,800×g for 8 min at −2°C to obtain the supernatant for nucleic acid analysis. The other half was centrifuged at 8,000×g for 10 min at 4°C, and the supernatant was collected for crude protein analysis. The RNA and DNA contents of each specimen were assessed with a fluorescent technique using ethidium

bromide, following the ICES (2004) protocol. DNA content was determined after RNA digestion with RNAase (Type I-AS: from bovine pancreas), based on the calibration curve of DNA (Type I: from calf thymus). RNA content was calculated using the difference between DNA fluorescence and total nucleic acid (RNA + DNA) fluorescence, in accordance with the ICES (2004) guidelines. The crude protein content was determined using a commercially available kit (Quick Start Protein Assay, BIO RAD Laboratories, Inc., Hercules, CA, USA) and following the Bradford (1976) method.

Data analysis

The nucleic acid and protein contents of 99 fish specimens were successfully analysed. The RNA and DNA contents were reported in µg per fish, while the protein content was expressed in mg per fish. Statistical analyses were carried out using R statistical software (R4.2.3; R Core Team 2023) at a 5% significance level. The growth of RNA, DNA and protein content relative to TL was assessed using the allometric growth equation: $y = ax^b$, where x represents TL, y denotes RNA, DNA and protein content, b is the allometric growth coefficient and a is the initial growth constant. As the measurement unit of response variables in this model is weight, the relative growth patterns can be defined as follows (Froese 2006): $b > 3$, indicating positive allometric growth; $b = 3$, indicating isometric growth; and $b < 3$, indicating negative allometric growth. The parameters of the allometric growth equation were estimated using a log-transformed equation ($\ln y = \ln a + b \ln x$). Additionally, the ratios of RNA to DNA, RNA to protein and protein to DNA content (all in µg-base) were calculated for individual fish, and a linear regression model ($y = a + bx$) was employed to establish the relationships between TL (x) and these biochemical indices (i.e. RNA/DNA, RNA/protein and protein/DNA ratio) (y).

To deduce changes in growth patterns during early ontogeny, we conducted piecewise regression analysis on the log-transformed allometric growth equation or linear regression equation using the *segmented* function implemented in the segmented package (Muggeo 2017). To avoid model overfitting and based on the scatter plots of the

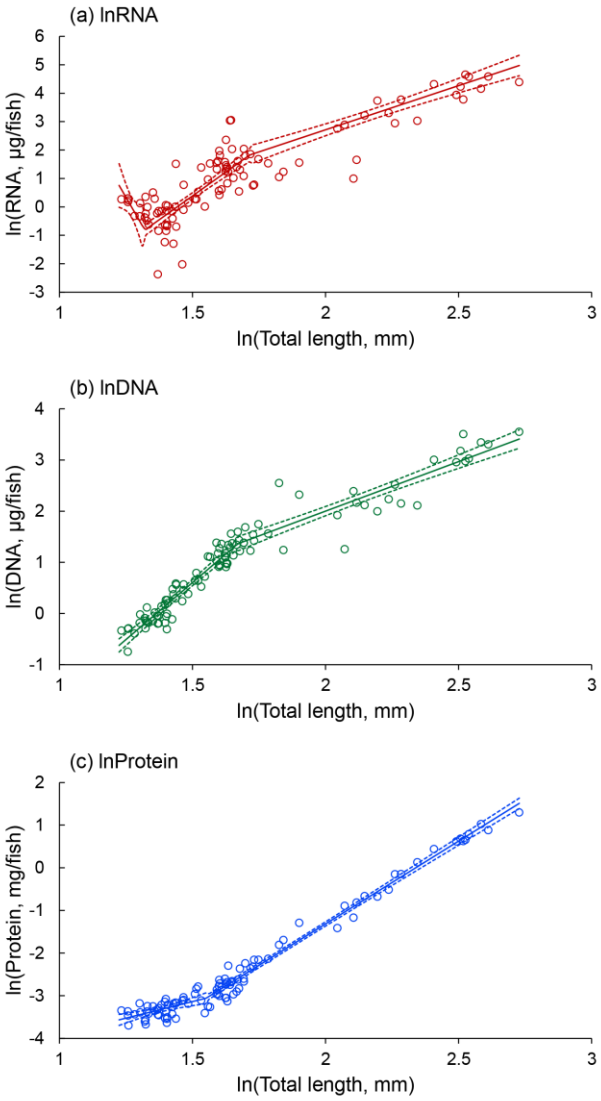

Fig. 2. Relationships between the natural logarithm of total length and natural logarithms of RNA (a), DNA (b) and protein content (c) of greater amberjack *Seriola dumerili* larvae and juveniles. Fitted lines are drawn based on the coefficient estimates of the log-transformed allometric growth equations shown in Table 2. The dotted lines indicate the 95% confidence intervals.

respective response variables to TL, the maximum number of changes in growth patterns was limited to two. Piecewise linear regression models without a break point (BP), with a BP, and with two BPs were evaluated using the Akaike information criterion (AIC) (Akaike 1973) and the Bayesian information criterion (BIC) (Schwarz 1978). The model with the lowest AIC and BIC was considered the best fit. The statistical significance of the regression equations was evaluated using an F-test. Piecewise regression analysis was also applied to formulate the relationship between TL and SL of larval and juvenile greater amberjack and three linear regression equations were obtained as shown in Fig. 1a.

Results

Changes in TL and swim bladder inflation rates

The mean TL of the fish specimens exhibited an exponential increase from 3.63 mm at 1 dph to 12.79 mm at 25 dph (Fig. 1b). Most of the yolk was absorbed by 3 dph, coinciding with the development of eye pigmentation, mouth opening and the initiation of feeding on live prey rotifers. Swim bladder inflation, a hallmark of normal growth and development, occurred after 3 dph, and all larvae successfully inflated their swim bladders (Fig. 1b).

RNA, DNA and protein content

Fig. 2 illustrates the logarithmic associations

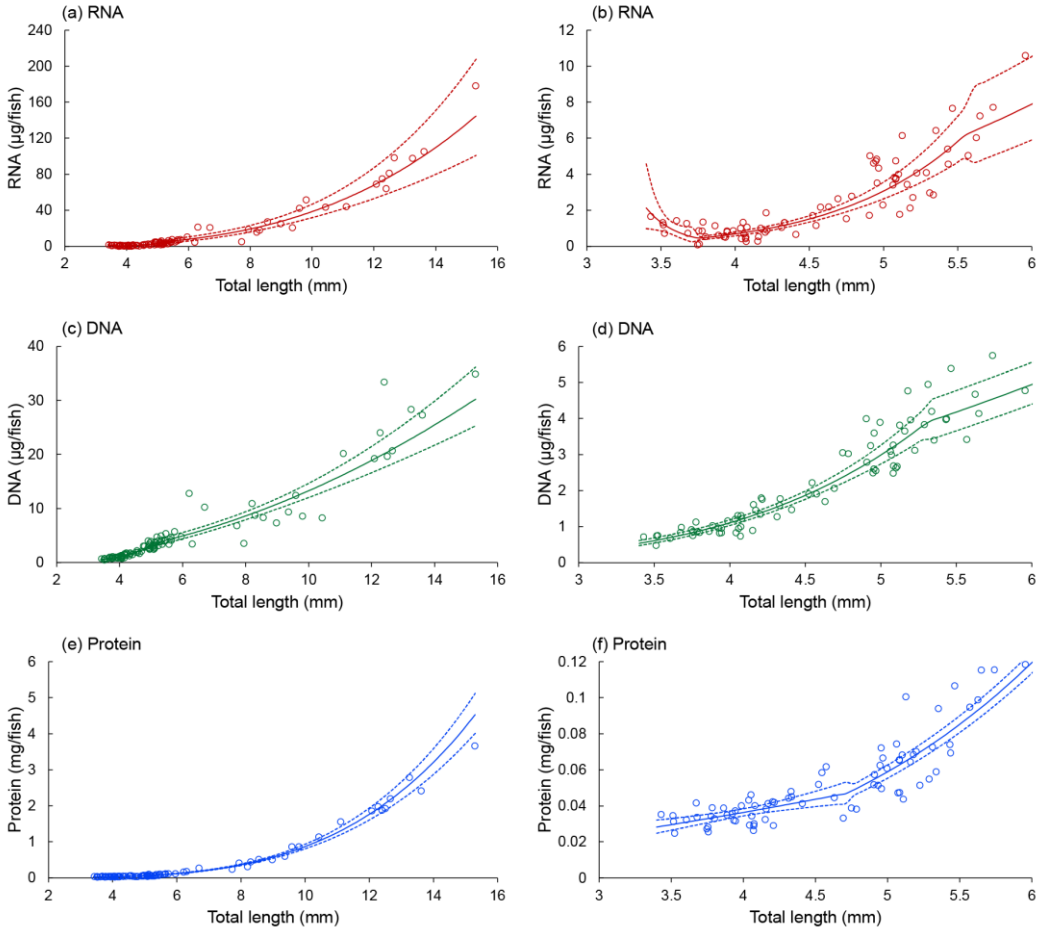

Fig. 3. Relationships between total length and RNA (a, b), DNA (c, d) and protein content (e, f) of greater amberjack *Seriola dumerili* larvae and juveniles. In the right panels (b, d, and f), data are shown for larvae with < 6 mm in total length. Fitted curves are drawn based on the coefficient estimates of the log-transformed allometric growth equations shown in Table 2. Dotted curves indicate the 95% confidence intervals.

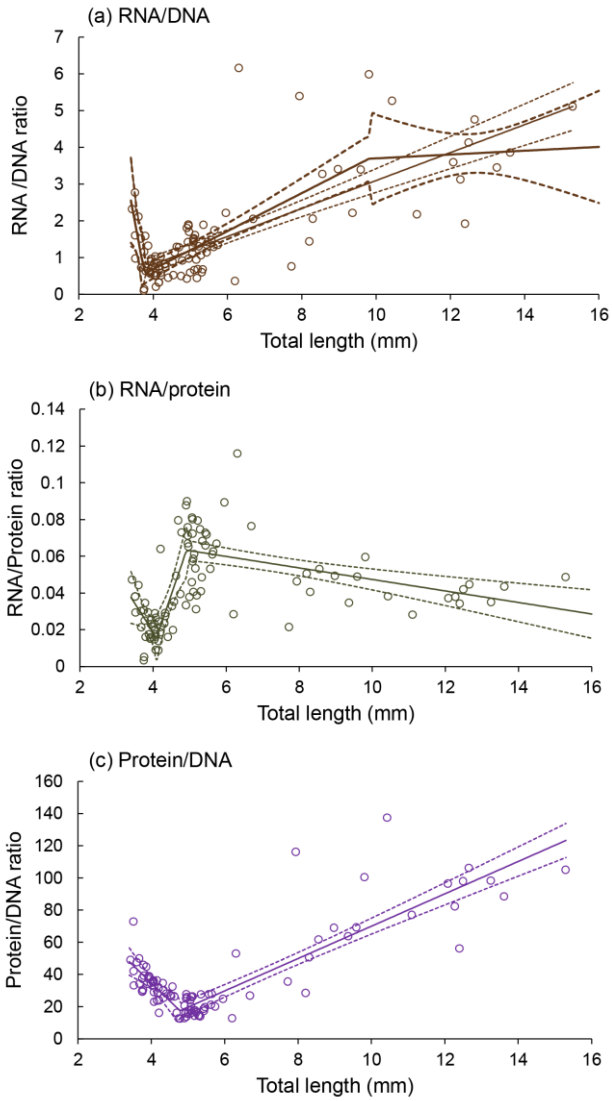

Fig. 4. Relationship between total length and RNA/DNA (a), RNA/protein (b) and protein/DNA ratio (c) of greater amberjack *Seriola dumerili* larvae and juveniles. Fitted lines are drawn based on the coefficient estimates of the linear regression equations shown in Table 2. The dotted lines indicate the 95% confidence intervals. Two kinds of lines based on the models with one BP (thin lines) and two BPs (thick lines) are shown for the RNA/DNA ratio.

amongst TL, RNA, DNA and protein content. Fig. 3 presents these relationships on an antilogarithmic scale. These relationships may have one or two BPs. Based on the piecewise regression analysis, a log-transformed allometric growth model with two BPs was selected for RNA content, and the model with one BP was selected for DNA and protein content, as evidenced by the lowest AIC and BIC values (Table 1). The parameter estimates of the piecewise regression equations for the respective indices are summarized in Table 2. All regression equations were statistically significant ($p < 0.0001$).

RNA content decreased until 3.75 mm TL, then it largely increased, showing positive allometric growth [b = 6.70 (95% confidence interval, CI): 5.73–7.66)] until 5.56 mm TL; after that, it showed isometric growth [b = 3.11 (95% CI: 2.53–3.68)]. DNA content exhibited positive allometric growth [b = 4.45 (95% CI: 3.99–4.92)] until 5.31 mm TL; then, it increased while showing negative allometric growth [b = 1.93 (95% CI: 1.68–2.19)]. Protein content exhibited negative allometric growth [b = 1.55 (95% CI: 0.85–2.25)] until 4.72 mm TL; after that, it largely increased while showing positive allometric growth [b = 3.88 (95% CI: 3.75–4.02)].

RNA/DNA, RNA/protein and protein/DNA ratio

The relationships between TL and biochemical indices including RNA/DNA, RNA/protein and protein/DNA ratio are presented in Fig. 4. It is possible that there are one or two BPs in these relationships. The results of the piecewise regression analysis showed that the linear regression models with two BPs and one BP had the lowest AIC and BIC values, respectively, for the RNA/DNA ratio (Table 1); therefore, these two models were initially selected. Furthermore, for the RNA/protein and protein/DNA ratios, models with two BPs and one BP, respectively, were selected because they had the lowest AIC and BIC values (Table 1). The parameter estimates of the piecewise regression equations for each index are summarized in Table 2. All regression equations were statistically significant ($p < 0.0001$).

In the model with one BP, the RNA/DNA ratio

Table 1. Akaike information criterion (AIC), Bayesian information criterion (BIC) and adjusted R^2 value of the log-transformed allometric growth models ($\ln y = \ln a + b \ln x$) or linear regression models ($y = a + bx$) with no break point (BP) (0), one BP (1) and two BPs (2) for evaluating the larval growth of greater amberjack *Seriola dumerili*, based on the total length (TL) (mm), RNA, DNA (μg/fish) and protein content (mg/fish) as well as RNA/DNA, RNA/protein and protein/DNA ratio.

Response variable	Explanatory variable	Model	BP	AIC	BIC	Adjusted R^2
lnRNA	lnTL	1	0	178.26	186.04	0.8684
		2	1	168.38	181.36	0.8832
		3	2	**139.67**	**157.84**	0.9143
lnDNA	lnTL	1	0	70.87	78.65	0.8935
		2	1	**8.02**	**21.00**	0.9447
		3	2	9.56	27.73	0.9449
lnProtein	lnTL	1	0	7.93	15.72	0.9651
		2	1	**-52.42**	**-39.45**	0.9814
		3	2	-51.53	-33.36	0.9816
RNA/DNA	TL	1	0	264.75	272.53	0.5284
		2	1	257.88	**270.85**	0.5686
		3	2	**253.98**	272.14	0.5931
RNA/protein	TL	1	0	-465.23	-457.44	0.0101
		2	1	-518.25	-505.27	0.4318
		3	2	**-538.67**	**-520.50**	0.5465
Protein/DNA	TL	1	0	852.59	860.38	0.5505
		2	1	**799.07**	**812.04**	0.7433
		3	2	802.47	820.63	0.7394

The bold AIC and BIC values are the lowest among the three models.

significantly decreased until 3.75 mm TL and then increased. In the model with two BPs, the RNA/DNA ratio decreased until 3.77 mm TL, then increased until 9.81 mm TL and finally reached a plateau. The allometric growth coefficient of RNA content from 5.56 mm TL (b = 3.11) was larger than that of DNA content from 5.31 mm TL (b = 1.93), suggesting that the RNA/DNA ratio may increase without a breakpoint. Thus, the model with one BP was ultimately chosen as the best for the RNA/DNA ratio. The RNA/protein ratio decreased until 4.10 mm TL, then significantly increased until 4.92 mm and finally declined slowly. The protein/DNA ratio decreased until 4.78 mm TL and then increased.

Discussion

The present study successfully applied the allometric growth model to establish the relationship between TL and the content of RNA, DNA and protein during the early ontogeny of greater amberjack. The piecewise regression analysis effectively identified variations in the growth and developmental patterns of nucleic acid and protein content, as well as the RNA/DNA, RNA/protein and protein/DNA ratio (Figs. 2–4; Table 2). The RNA/DNA and protein/DNA ratio values obtained from individual specimens in this study were consistent with those reported by Seoka et al. (2000), who analysed the RNA, DNA and protein content per unit body weight of pooled fish

Table 2. Coefficient estimates with 95% confidence intervals (CI) of the log-transformed allometric growth models ($\ln y = \ln a + b \ln x$) or linear regression models ($y = a + bx$) with two segments having one break point (BP) (model 2) or three segments having two BPs (model 3) for evaluating the larval growth of greater amberjack *Seriola dumerili*, based on the total length (TL) (mm), RNA, DNA (μg/fish) and protein (mg/fish) content as well as RNA/DNA, RNA/protein and protein/DNA ratio. The model with the lowest Akaike information criterion and/or Bayesian information criterion was selected from among three models having no BP (model 1), one BP (model 2) and two BPs (model 3) (see Table 1). BPs with asterisks represent the antilogarithmic values in mm.

Response variable	Explanatory variable	Model	Intercept	Estimate	Slope	Estimate	95% CI		BP	Estimate	95% CI		Adjusted R^2	F	df	p
lnRNA	lnTL	3	1	20.585	1	-16.205	-30.899	-1.5109	1	1.3206	1.2835	1.3576	0.9143	210.06	5, 93	< 0.0001
			2	-9.657	2	6.6962	5.7340	7.6583	2	1.7150	1.6042	1.8259				
			3	-3.502	3	3.1073	2.5313	3.6833	1*	3.75	3.61	3.89				
									2*	5.56	4.97	6.21				
lnDNA	lnTL	2	1	-6.071	1	4.4517	3.9874	4.9160	1	1.6698	1.5991	1.7404	0.9447	558.66	3, 95	< 0.0001
			2	-1.867	2	1.9343	1.6812	2.1873	1*	5.31	4.95	5.70				
lnProtein	lnTL	2	1	-5.467	1	1.5518	0.8513	2.2523	1	1.5508	1.4896	1.6120	0.9814	1722.5	3, 95	< 0.0001
			2	-9.083	2	3.8831	3.7476	4.0187	1*	4.72	4.44	5.01				
RNA/DNA	TL	2	1	19.873	1	-5.1047	-10.3720	0.1625	1	3.75	3.57	3.93	0.5686	44.05	3, 95	< 0.0001
			2	-0.706	2	0.3802	0.3136	0.4468								
		3	1	20.878	1	-5.3902	-10.0450	-0.7355	1	3.77	3.61	3.92	0.5931	29.56	5, 93	< 0.0001
			2	-1.385	2	0.5172	0.3862	0.6482	2	9.81	6.79	12.83				
			3	3.179	3	0.0520	-0.3638	0.4677								
RNA/protein	TL	3	1	0.1431	1	-0.0310	-0.0603	-0.0017	1	4.10	3.92	4.27	0.5465	24.62	5, 93	< 0.0001
			2	-0.2207	2	0.0577	0.0301	0.0854	2	4.92	4.67	5.18				
			3	0.0787	3	-0.0031	-0.0046	-0.0017								
Protein/DNA	TL	2	1	122.99	1	-22.066	-33.538	-10.595	1	4.78	4.46	5.10	0.7433	95.58	3, 95	< 0.0001
			2	-30.55	2	10.055	8.821	11.289								

samples in relation to the age (days post-hatch) of greater amberjack larvae and juveniles.

To elucidate the growth and developmental patterns during the early ontogeny of greater amberjack, we have presented a schematic illustration of changes in biochemical indices based on nucleic acid and protein content that accompany the increase in TL (Fig. 5). During the prelarval stage, RNA content exhibited a decreasing trend up to 3.8 mm TL (Figs. 2a, 3a, 3b and 5). Larvae at this stage develop into the preflexion stage while consuming yolk protein. Once the eye pigmentation and mouth are formed, preflexion larvae begin to feed on live prey organisms (Masuma et al. 1990; Tachihara et al. 1993; Hamasaki et al. 2009; present study). Uji et al. (2014) conducted a study on the muscle development of greater amberjack larvae, with a primary focus on the cranial muscles, from 0 dph (3.2 mm TL) to 12 dph (5.8 mm TL). Their findings suggested that the muscles required for feeding develop at the beginning of the preflexion stage at 3 dph. Therefore, during the prelarval stage, larval growth and development are accompanied by the turnover of RNA (ribosome) in the yolk. It is worth noting that a net turnover (loss) of cellular ribosome content during the yolk-sac stage has also been reported for the larvae of the red drum *Sciaenops ocellatus* (Linnaeus, 1766) (Westeman and Holt 1994).

The DNA content of greater amberjack exhibited positive allometric growth from hatching until 5.3 mm TL, after which it displayed negative allometric growth (Figs. 2b, 3c, 3d and 5). This suggests that rapid cell division or hyperplasia occurs in larvae during the prelarval through the

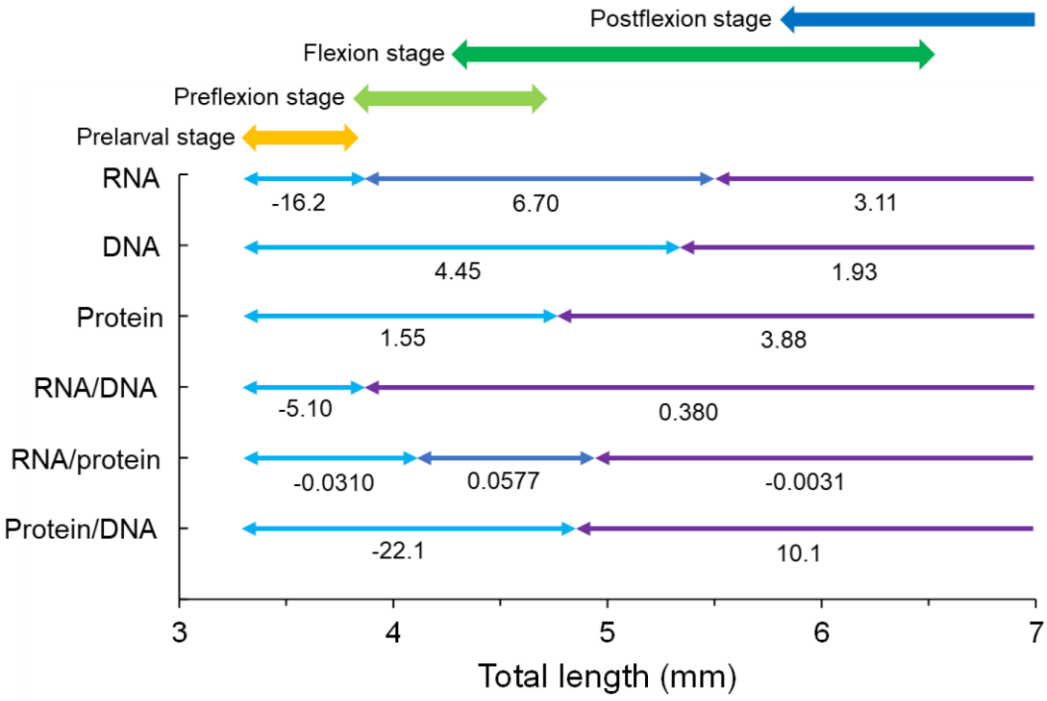

Fig. 5. Schematic model of the growth and developmental patterns based on nucleic and protein content accompanied with the increase in total length (TL) during the early ontogeny of greater amberjack *Seriola dumerili*. Ontogenetic stages and different growth and developmental phases are shown by arrows. Numerical values indicate the slopes of two or three segmented log-transformed allometric growth equations for RNA, DNA, and protein content and those of two or three segmented linear regression equations for RNA/DNA, RNA/protein, and protein/DNA ratio.

preflexion to mid-flexion stages. Similarly, RNA content demonstrated positive allometric growth from 3.8 mm to 5.6 mm TL during the preflexion and mid-flexion stages, followed by isometric growth (Figs. 2a, 3a, 3b and 5). During the preflexion and flexion stages, larval organogenesis progresses. For example, the swim bladder forms and inflates during the preflexion stage (Imai et al. 2011). Additionally, the proportions of body height, head height and upper jaw length to TL largely increase until 5 mm TL during the preflexion and flexion stages, after which they decrease or reach a plateau (Masuma et al. 1990). The positive allometric growth of RNA and DNA content likely contributes to the progression of larval organogenesis.

In accordance with the RNA and DNA content profiles, the RNA/DNA ratio decreased up to 3.8 mm TL, followed by a linear increase (Figs. 4a and 5), implying a continuous rise in protein synthesis capacity per cell from the preflexion stage onwards. The RNA/DNA ratio has been established as a dependable and informative indicator of the nutritional state and growth rate of fish larvae, with starved larvae exhibiting reduced ratios (e.g. Richard et al. 1991; Clemmesen 1994; Mathers et al. 1994; Gwak and Tanaka 2001; Tanaka et al. 2008). The RNA/DNA ratio of greater amberjack larvae showed considerable variability after 6 mm TL (Fig. 4a), suggesting that cultured larvae and juveniles exhibit significant variation in nutritional conditions. A similar phenomenon has been observed in laboratory-reared Pacific bluefin tuna *Thunnus orientalis* (Temminck et Schlegel 1844): individuals with a body length of > 16 mm displayed a large range of RNA/DNA ratios (Tanaka et al. 2007). Tanaka et al. (2007) hypothesized that the heterogeneous distribution of yolk-sac larvae and minced fish provided to piscivorous bluefin tuna larvae in the rearing tank led to differences in feeding success and nutritional conditions, resulting in individual variability in RNA/DNA ratios. Greater amberjack larvae shift their preference to larger prey items, such as larger rotifers with eggs after reaching 5.3 mm TL and *Artemia* after 5.8 mm TL (Hamasaki et al. 2023). Hence, successfully feeding on larger prey items may have affected the nutritional condition of the larvae.

The protein content displayed negative allometric growth until 4.7 mm TL, after which it showed positive allometric growth (Figs. 2c, 3e, 3f and 5). The RNA/protein ratio decreased linearly during the prelarval stage until 4.1 mm TL, similar to the RNA/DNA ratio and then increased significantly during the preflexion and early flexion stages until 4.9 mm TL (Figs. 4b and 5). This increased RNA/protein ratio suggests a high overall protein synthesis capacity; however, the protein content exhibited negative allometric growth until 4.7 mm TL (Figs. 2c, 3e, 3f and 5). Uji et al. (2014) reported that muscle composition in the dorsal branchial arches assumes the adult form between 5 and 8 dph (4.1–4.5 mm TL), and all the muscles involved in feeding and respiration develop in the preflexion larvae of greater amberjack. These muscles are vital for subsequent steady larval growth and development. Thus, the increased RNA/protein ratio from 4.1 mm to 4.7 mm TL may have led to the development of such critical muscles involved in feeding and respiration. Meanwhile, during the flexion stage, protein content exhibited positive allometric growth from 4.7 mm TL (Figs. 2c, 3e, 3f and 5), under the isometric growth condition of RNA content from 5.6 mm TL (Figs. 2a, 3a, 3b and 5), which is responsible for the slow decline of the RNA/protein ratio from 4.9 mm TL (Figs. 4b and 5). These observations suggest that the efficiency of the retention of newly synthesized proteins

might have changed during the transition from the preflexion to the flexion stages and improved thereafter.

The ratio of protein to DNA, which is an indicator of cell size, exhibited a decreasing trend until 4.8 mm TL, after which it demonstrated a linear increase (Figs. 4c and 5). This suggests that during the prelarval, preflexion and early flexion stages, cell size decreased due to hyperplasia, which was characterized by positive allometric growth of DNA content and low protein increment. After the early flexion stage, however, hypertrophy occurred, which was reflected by the negative allometric growth of DNA content and the positive allometric growth of protein content. The high deposition of protein in larvae after the early flexion stage may be associated with the development of their morphological, physiological and behavioural characteristics, including the improvement of their feeding and respiration systems during the preflexion stage (Uji et al. 2014), the enhancement of their feeding performance through the preference for large prey items from the flexion stage (Hamasaki et al. 2023), and the development of dorsal, anal, pelvic and caudal fin muscles for swimming after the flexion stage (Uji et al. 2014).

Conclusion

The current investigation has emphasized the ontogenetic modifications of growth and developmental patterns based on nucleic acid and protein content in laboratory-reared greater amberjack larvae and juveniles. Rapid cell division was observed in larvae during the prelarval through the mid-flexion stages. The protein synthesis capacity per cell showed a steady increase from the preflexion stage onwards, while the efficiency of retaining newly synthesized proteins improved after the transition from the preflexion to the flexion stages. The cell size decreased during the prelarval, preflexion, and early flexion stages and increased steadily after the early flexion stage. Consequently, the overall biochemical synthesis appeared to have entered a stable growth phase from the mid-flexion stage, along with the progression of morphological, physiological and behavioural development in greater amberjack larvae. In conclusion, utilizing piecewise regression analysis for RNA, DNA and protein content and biochemical indices, such as RNA/DNA, RNA/protein and protein/DNA ratio in relation to individual body lengths, would be an advantageous approach for comprehending the growth and developmental processes of fish during early ontogeny.

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