Morphological development and growth of laboratory-reared larval and juvenile *Thunnus thynnus* (Pisces: Scombridae)

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Abstract–Morphological development and growth of larval and juvenile Pacific bluefin tuna, *Thunnus thynnus*, were studied from laboratory-reared specimens. Average body length (BL) of newly hatched larvae was 2.83 mm and larvae grew on average 5.80 mm by 10 days, 10.62 mm by 20 days, and 35.74 mm by 30 days after hatching. Growth was especially accelerated after 20 days from hatching. Newly hatched larvae had small melanophores scattered over their bodies except for the finfold. On day 1 after hatching (3.35–3.74 mm BL), a characteristic melanophore pattern appeared and it was partially maintained until day 3 after hatching. At approximately 4 mm BL, larvae had developed melanophore patterns similar to those of preflexion *Thunnus* spp. larvae, such as melanophores on the dorsum of the gut, midlateral trunk, and tail, at the dorsal and ventral midlines of trunk and tail, and on the lower jaw. Erythrophores appeared at 4.63 mm BL in the caudal area. Jaw teeth appeared at 5–6 mm BL. The preopercular angle spine, anterior preopercular spine, and posttemporal spine, developed at approximately 7 mm BL, when erythrophores appeared on the trunk and tail. The notochord flexion occurred between 6 and 8 mm BL. At approximately 8 mm BL, erythrophores disappeared and juvenile coloration appeared on the trunk and tail, consisting of dense patches of melanophores at the dorsal and anal-fin bases, embedded melanophores, and melanophores at the periphery of the eye. The adult complement of fin-ray counts was attained at 10 mm BL, when the juvenile melanophore pattern was attained, although the pattern was not fully developed. Specimens larger than 20 mm BL did not have erythrophores. Squamation began at 27 mm BL and head spines disappeared by 38 mm BL.

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The Pacific bluefin tuna is a large scombrid that migrates transoceanically between the western and eastern Pacific Ocean (Orange and Fink, 1963; Fujita, 1998). The Pacific bluefin tuna, *Thunnus thynnus orientalis* (Temminck and Schlegel), is considered a separate subspecies from the Atlantic bluefin tuna, *T. thynnus thynnus* Linnaeus (Gibbs and Collette, 1967; Collette and Nauen, 1983). Recently, Cho and Inoue (1993) showed reproductive isolation between the two subspecies. Most recently, Collette (1999) considered these subspecies to be separate species on the basis of morphology and molecular data.

Our knowledge of the early life history of oceanic species, such as tunas, is incomplete. Morphological characteristics common to scombrid larvae are large head, gape, and eyes; development of head spination; and posterior migration of the anus (Collette et al., 1984). In studies of early-stage bluefin tuna (e.g. Yabe et al., 1966; Matsumoto et al., 1972; Richards and Potthoff, 1974; Kohno et al., 1982), melanophore patterns were used to identify tuna larvae. Erythrophore patterns were proposed as another characteristic by Ueyanagi (1966) and Matsumoto et al. (1972). However, ontogenetic changes of melanophore and erythrophore patterns and morphological characteristics have not sufficiently been investigated because of the difficulty in obtaining a complete series of wild specimens in their early developmental stages.

Morphological, physiological, and behavioral information has recently been collected from laboratory-reared Pacific bluefin tuna specimens (Harada et al., 1971a; Kaji et al., 1996; Takii et al., 1997; Kumai, 1998; Miyashita et al., 1998; Miyashita et al., 2000, in press; Sawada et al., 2000). The ability to produce a complete series of early-stage specimens provides an opportunity to enhance our understanding of the early development of bluefin tuna. In this study, we describe the morphological development and pigment patterns of reared larval and juvenile bluefin tuna.

Materials and methods

Eggs were obtained on 10 July 1994 from bluefin tuna that had been caught off Ohshima when 20–40 cm in total length [0-year class] and that had been raised for seven years naturally in a net cage at the Ohshima Experiment Station of the Kinki University Fisheries Laboratory. The net cage was equipped with a polyvinyl chloride sheet surrounding the top 2 m of the net side wall to prevent eggs from flowing out of the net. Fertilized eggs were collected at the surface with a 330-µm mesh net. Eggs were transferred to a tank in a land-based nursery center and kept in a fine mesh net at 24.5°C for approximately 12 hours. The following morning, eggs were transferred to a 20-m^3 rearing tank and larvae hatched at

approximately 2200 h on 11 July. The first 24 hours after hatching were counted as day 0 (day-0) after hatching. Water temperature ranged from 24.5 to 27.7°C during the rearing period ($\bar{x}$=25.5°C, Fig. 1). The feeding scheme for larvae and juveniles was as follows: rotifers, *Brachionus rotundiformis*, from day 2 to day 22 after hatching, *Artemia* nauplii from day 10 to day 25, and other live fish larvae (*Oplegnathus fasciatus*) from day 12 to day 30.

At 1000 h daily from day 0 (12 July) to day 30 (11 August), 20 tuna were sampled. Before preservation in 5% formalin, individual computer-captured video images were made with a video camera attached to a binocular microscope while all specimens were under MS-222 anesthesia. Ten body parts were measured from these images: total length (TL), body length (BL), preanal length, body height at the base of the first dorsal spine, head length, head height, snout length, eye diameter, caudal peduncle depth, and upper jaw length. Video image measurements to $1/10^2$ mm with an accuracy of ±1% were obtained from NIH Image (version 1.61) image analysis software (NIH, 1997). Before and during notochord flexion, BL was measured from the tip of the upper jaw to the end of the notochord. After notochord flexion, BL was measured from the tip of the upper jaw to the posterior margin of the hypurals. All specimens (*n*=620, 3.49–37.78 mm BL) were used to determine growth estimates and to record pigmentation patterns and spine and fin development.

Sixty-nine specimens (5.26–33.68 mm BL) were stained with alizarin red-S to describe spine development, and squamation. Pigment patterns, especially the position of body melanophores in relation to myomeres, were determined for specimens from 3 to 16 days after hatching (*n*=180, 3.63–9.96 mm BL). At irregular intervals from day 0 to day 22, one to five fish (a total of 53 specimens, 3.38–26.76 mm BL) were examined while anesthetized with MS-222 to determine erythrophore distribution. Additional samples were examined at irregular intervals from hatching to day 2.

A representative series of specimens from this study was deposited in the Aquatic Natural History Museum of the Fisheries Research Station, Kyoto University (FAKU 129041–129075).

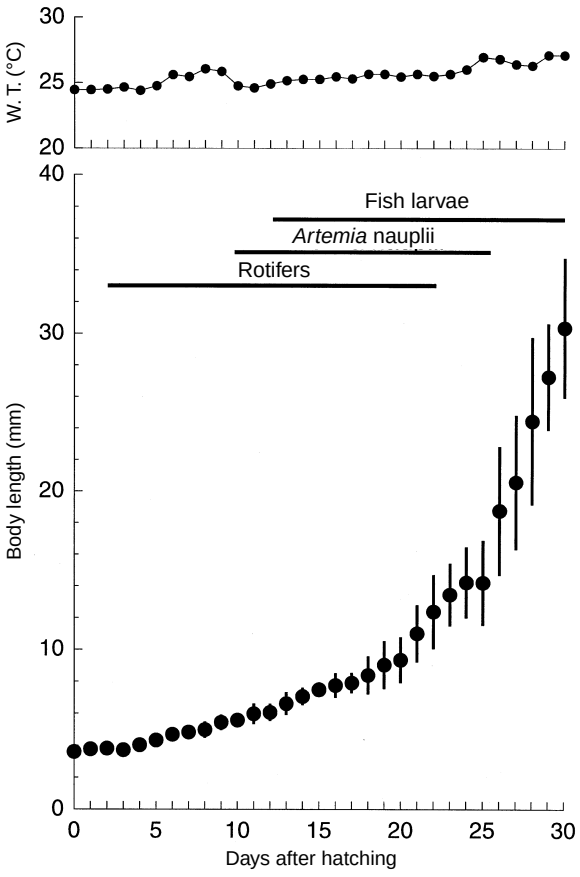

Figure 1

Body length, diet, and water temperature (W.T.) during the rearing of *Thunnus thynnus*. Vertical lines indicate ±SD (*n*=20).

Results

Structural characteristics and growth

Eggs, having a mean diameter of 1.02 mm (SD=0.02; *n*=20), were spherical and pelagic and had a single oil globule of 0.26 ±0.05 (mean ±SD) mm in diameter.

Newly hatched larvae measured 2.83 ±0.16 mm in BL (mean ±SD, *n*=20; Fig. 2A). At 3 days after hatching (3.81 ±0.14 mm BL), the mouth was developed and larvae began feeding. Yolk was present until day 5 (4.32 ±0.13 mm BL). Larvae grew to 5.58 ±0.35 mm by day 10, 9.34 ±1.36 mm by day 20 and 30.36 ±4.34 mm by day 30 after hatching (Fig.1). From hatching to day 20, growth rate was 0.33 mm/day, then increased to 2.10 mm/day from day 20 to day 30.

Notochord flexion began as early as 5.76 mm BL, and the largest preflexion larva was 5.83 mm BL. The smallest postflexion larva was 7.46 mm BL; the largest flexion larva, 8.65 mm BL. Thus, the flexion stage occurred from 6 to 8 mm BL (Fig. 2E).

The relative values of the nine body parts, except preanal length, increased during larval development, then subsequently declined (Fig. 3). The ratio of head length, head height, eye diameter, snout length, and upper jaw length to BL reached a maximum at 11–13 mm BL. Head length increased rapidly from 10% BL at hatching to 40% at 13 mm BL and then decreased slowly to 34 % BL at 40 mm BL. Head height also increased rapidly from 10% BL at hatching to 27% at 11 mm BL and decreased slowly to 23% BL at 40 mm BL. Eye diameter increased from 10% BL at hatching to 15% at 11 mm BL and then decreased slowly to 9% BL at 40 mm BL. Snout length rapidly increased from 2% BL at hatching to 14% at 12 mm BL and then decreased slowly to 9% BL at 40 mm BL. Upper jaw length increased rapidly from 5% BL at hatching to 20–30% at 12 mm BL and then decreased slowly to 14% BL at 40 mm BL. The

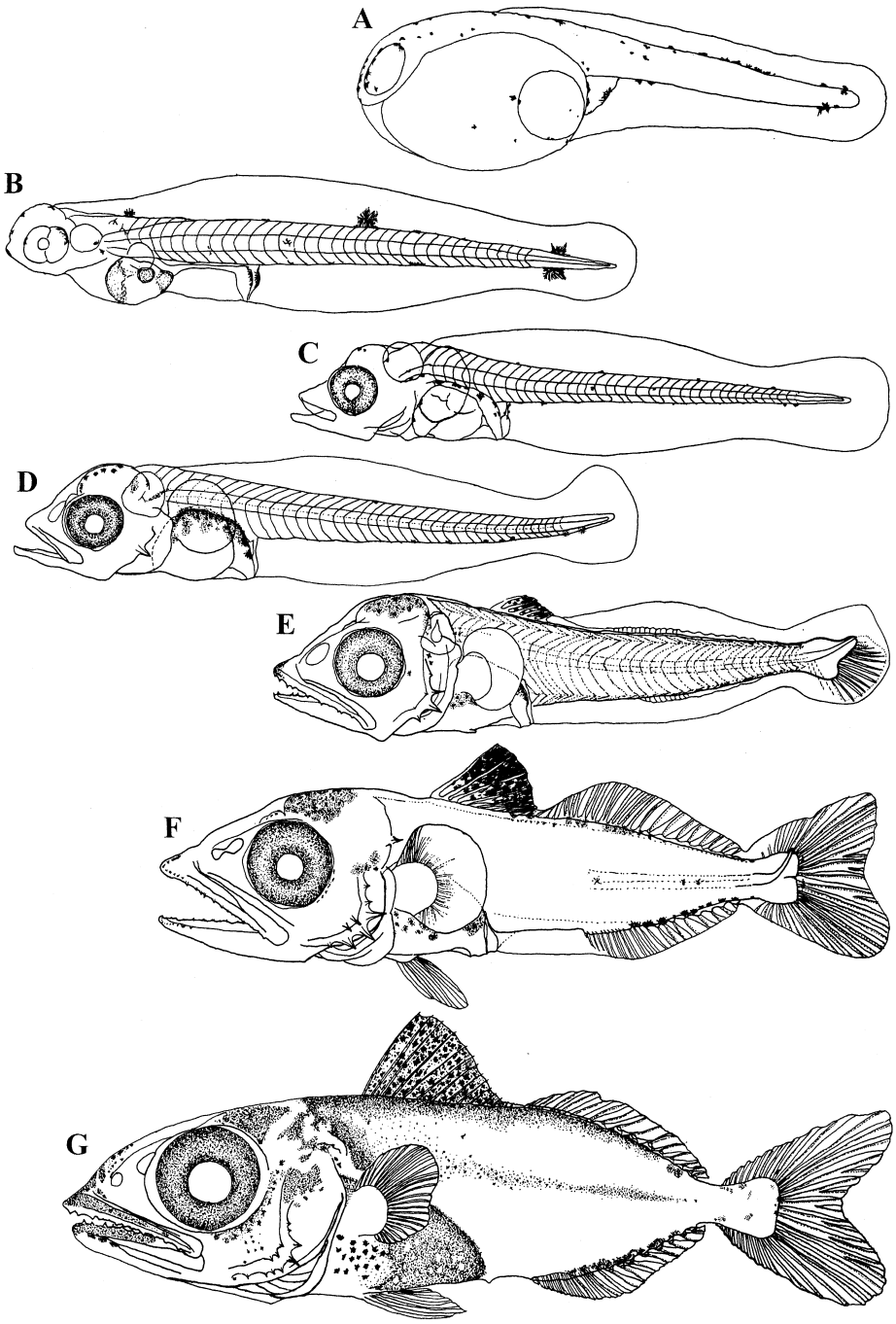

Figure 2

Development of *Thunnus thynnus* (**A**) newly hatched larva, 2.91 mm BL; (**B**) 1-day-old yolksac larva, 3.54 mm BL; (**C**) 4-day-old preflexion larva, 4.02 mm BL; (**D**) 7-day-old flexion larva, 5.34 mm BL; (**E**) 15-day-old postflexion larva, 7.23 mm BL; (**F**) 20-day-old postflexion larva, 8.64 mm BL; (**G**) 25-day-old juvenile, 13.55 mm BL.

relative depth of the caudal peduncle attained a maximum (ca. 7% BL) at 8 mm BL. In contrast, body height and total length were maximum in relation to BL at approximately 16 and 20 mm BL, respectively. Preanal length increased from 35–44% BL at 3–7 mm BL to about 65% BL at approximately 15 mm BL.

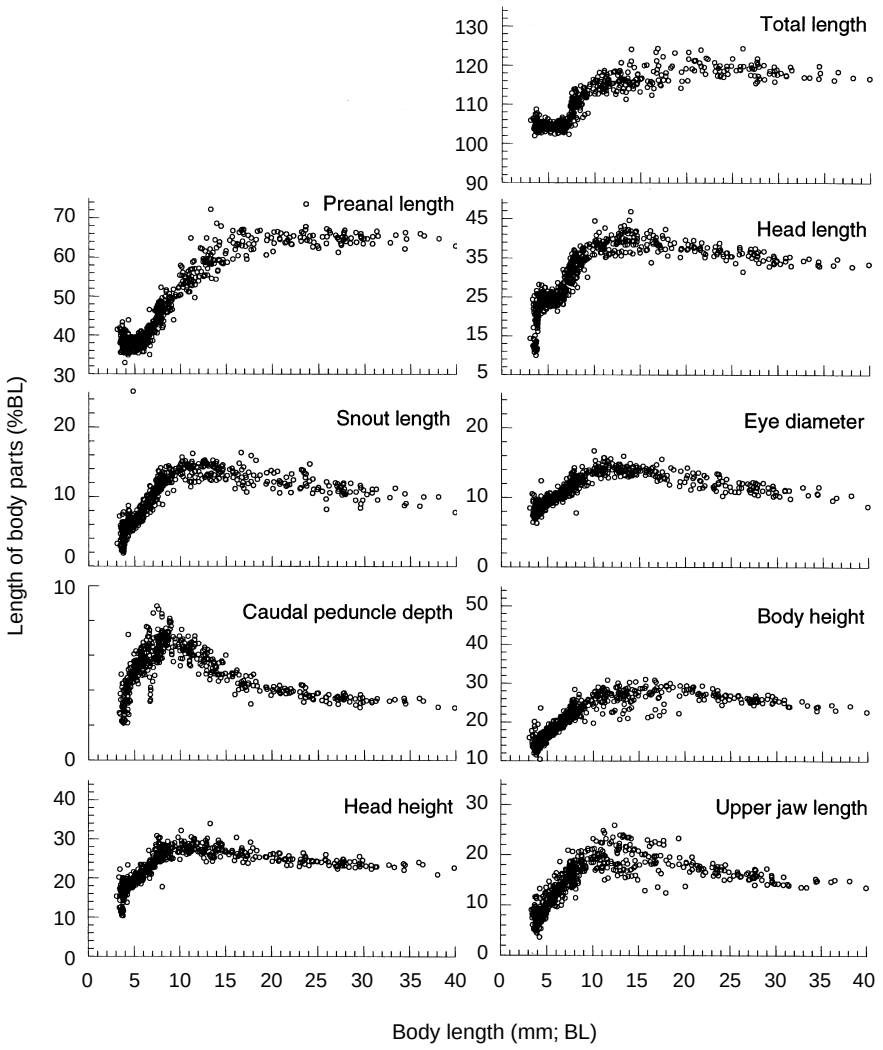

Figure 3

Measurements of *Thunnus thynnus*, shown as a percentage of body length (BL).

Table 1

Numbers of fin spines and rays of *Thunnus thynnus* larvae and juveniles. Roman and Arabic numerals show numbers of spines and soft rays, respectively.

Size range (BL; mm)	First dorsal fin	Second dorsal fin	Dorsal finlet	Anal fin	Pectoral fin	Pelvic fin	Number of specimens examined
BL ≤5.99	0	0	0	0	0	0	10
6.00–6.99	I–X	0	0	0–6	0–13	0–I+1	17
7.00–7.99	0–XIV	0–13	0–8	0–12	0–31	0–I+5	13
8.00–8.99	IX–XIV	12–14	6–9	7–14	11–31	I+4–I+5	11
9.00–9.99	XI–XV	14	8–9	13–14	16–31	I+5	8
10.00–19.99	XIV–XV	14	8–9	14	31–34	I+5	14
20.00–29.99	XV	14	8–9	13–14	31–36	I+5	19
30.00–37.78	XV	14	8–9	14	31–35	I+5	8

Fin development

The number of fin rays increased between 6 and 10 mm BL (Table 1). The smallest specimen possessing dorsal spines was 6.32 mm BL. Caudal-fin rays, second dorsal-, pelvic-, anal-, and pectoral-fin rays appeared at 7.10, 7.76, 8.00, 8.47, and 8.67 mm BL, respectively. The smallest specimen with an adult complement of fin-ray counts (Iwai et al., 1965; Collette and Nauen, 1983) was 9.68 mm BL, whereas the largest specimen with an incomplete fin-ray complement was 10.02 mm BL. The number of pectoral-fin rays for specimens >10 mm BL varied among individuals.

Pigmentation

Newly hatched larvae (2.62–3.05 mm BL) had small melanophores scattered over the body, head, notochord, yolk, and oil globule, but not on the finfold (Fig. 2A).

On day 1 after hatching (3.35–3.74 mm BL) (Fig. 2B), dendritic melanophores were visible from snout to fore- and midbrain and on the dorsum of the gut; three clusters of melanophores occurred in the dorsal midline along with punctate melanophores; melanophores occurred along the ventral midline (punctate melanophores formed a line at the anterior caudal area and a cluster of melanophores occurred in the posterior caudal area); and melanophores appeared along the lateral midline of the trunk. The eyes were pigmented.

On day 2 after hatching (3.40–4.18 mm BL), the pattern of melanophores was transitional between those of 1-day and older preflexion larvae. Melanophores on the snout disappeared, whereas clusters of melanophores on the anteriodorsum of the trunk (second myomere), anteriordorsum of the caudal area (17th to 21st myomere) and posteriodorsum of the caudal area (30th to 39th myomere), as well as on the anterio- and midventral edge (14th to 26th myomere), posteroventral edge (28th to 39th myomere), and lateral midline (posterior to the 13th myomere) of the caudal area began to shrink (although they were still large [Tables 2–5]).

On day 3 after hatching, forebrain melanophores disappeared in all specimens >3.76 mm BL, whereas the smallest specimen lacking these melanophores was 3.63 mm BL. All specimens >3.82 mm BL had melanophores scattered on the dorsum of the gut, which formed a cap of melanophores over the gut in later stages. Clusters of melanophores on the dorsal and ventral midline, and lateral midline of the body now appeared punctate and additional punctate melanophores appeared in these areas (Tables 2–5). Melanophores were present on the midbrain, on the anteriodorsal side and on the base of the hindbrain; and several melanophores appeared on the lower side of the lower jaw. The smallest specimen with melanophores on the lower jaw was 3.41 mm BL, whereas the largest lacking them was 9.46 mm BL (day 16). Most specimens >5.5 mm BL had these lower jaw melanophores. On and after day 4 (3.92–4.43 mm BL), all specimens had the melanophore pattern of preflexion larvae (Fig. 2C).

Melanophores began to develop on several other areas in larvae larger than about 4.5 mm BL (Fig. 2, D–F). Embedded melanophores appeared in the lateral muscle at 4.49 mm BL (Table 6), upper jaw tip at 5.47 mm BL, operculum and preoperculum at 6.18 mm BL, the membrane of the first dorsal fin at 6.32 mm BL, forebrain at 6.83 mm BL, and the cleithral symphysis at 10.79 mm BL. The largest specimens lacking melanophores in these areas were those that were 6.44 mm BL (for upper jaw tip), 6.72 mm BL (for lower jaw tip), 6.94 mm BL (for operculum), 9.69 mm BL (for forebrain), 10.28 mm BL (for cleithral symphysis), and 10.49 mm BL (for preoperculum).

Melanophores forming a dorsal cap of the gut enlarged at about 6.5 mm BL and the rim reached the ventral surface of the gut in some specimens >7.5 mm BL. The body melanophore pattern changed with growth. Dorsal midline melanophores disappeared from the trunk from 4.5 mm BL to 7 mm BL, and ventral and lateral midline melanophores disappeared from the trunk (Tables 2–5).

Larvae showed partial juvenile pigment patterns from about 8 mm BL (Fig. 2F). Dorsolateral and dorsal midline melanophores appeared at the trunk and increased in frequency in the caudal area from 7.5 mm BL (Table 2). Embedded melanophores frequently appeared at the posterior trunk and caudal area (Table 6). Dense patches of melanophores appeared at the fin base of the first and second dorsal, and anal fin bases (Tables 7 and 8; these melanophores occurred on the myosepta), as well as at the periphery of the eye, especially below and behind the eye from about 8.0 mm BL. Embedded melanophores appeared near the notochord and neural and haemal spines, and melanophores at the lateral midline of body extended internally from about 8.2 mm BL. Generally, melanophores on the dorsal and ventral midline, lateral midline, and those embedded increased with BL (Tables 2–6).

The smallest specimen having juvenile pigmentation (i.e. densely pigmented patches appearing on the body) was 13.55 mm BL (Fig. 2G). More distinct body patches appeared and increased in number as juveniles grew.

In many larvae, melanophores were found in the area of the hypural bones and on the caudal finfold or fin rays. The smallest specimens having melanophores in the area of hypural bones and on the caudal finfold were 6.42 mm BL and 6.81 mm BL, respectively.

Erythrophores first appeared at 4.63 mm BL (6 days after hatching) on the tail: thirty-four erythrophores at the posteroventral edge, five at the posterolateral midline, and two at the posterodorsal edge (Table 9). As larvae grew, erythrophores appeared at the caudal finfold at 4.75 mm BL, on the lower jaw at 6.10 mm BL, and at the hypural plate at 6.96 mm BL; at the hypural plate and caudal finfold, erythrophores were observed only on specimens 6.96–7.20 and 4.75–7.20 mm BL, respectively. At the dorsal and ventral edge of the trunk and tail, erythrophores became larger and decreased in number in larvae >7 mm. At the ventral edge, adjacent erythrophores were united. At the lateral midline of the body, the number decreased at 8.5 mm BL. Erythrophores disappeared from the lower jaw at 11.26 mm BL, from the posterolateral midline and dorsal edge of the tail at 15.85 mm BL, and from the ventral edge of the tail at 19.72 mm BL. Erythrophores were not observed on the upper jaw. The number of erythrophores, especially the ventral edge erythrophores, de-

Table 2

Incidence of dorsal midline melanophores at the trunk and tail (%) in *Thunnus thynnus*. Each melanophore is

Size range (BL; mm)	Myomere																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
3.50–3.99		14					7					14	7	7	7	7	36	29	36
4.00–4.49		4	4							4	15		4	4	7	7	15	41	22
4.50–4.99					4	4									4	4	7	15	7
5.00–5.49											8				8	8	8		17
5.50–5.99		10	10											10	10	10			
6.00–6.49																	20	30	30
6.50–6.99																			17
7.00–7.49											10	10	10	10	20	20		30	
7.50–7.99										10	10	10	20	40	20	40	20	10	20
8.00–8.49							8	15	31	23	62	77	77	85	85	85	69	69	62
8.50–8.99	23	54	38	46	46	54	54	54	62	85	85	85	85	92	85	92	92	92	92
9.00–9.49	25	67	17	25	33	33	83	58	50	58	58	67	67	67	58	75	83	67	67
9.50–9.99	30	80	20	30	40	40	100	70	60	70	70	80	80	80	70	90	100	80	80

Table 3

Incidence of the ventral midline and ventrolateral melanophores of the trunk and tail (%) in *Thunnus thynnus*. Each melanophore not observed on the first to sixth myomeres.

Size range (BL; mm)	Myomere																
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
3.50–3.99	7	7				7	7	7	14	14	29	7	7	50	7	21	21
4.00–4.49				4	4				7	7	22	22	11	15		7	19
4.50–4.99						10	5	5	0	10	10	29	24	19	19	24	33
5.00–5.49											8				17	17	17
5.50–5.99											10	10				30	
6.00–6.49													10	10		30	10
6.50–6.99															6	11	6
7.00–7.49														20			10
7.50–7.99																	
8.00–8.49																	
8.50–8.99															8	8	8
9.00–9.49																	
9.50–9.99																	

creased as the fish grew; no erythrophores were observed in specimens >20 mm BL.

Head spination

Major head spines first appeared in specimens at 4–7 mm BL. The posterior preopercular angle spine and anterior preopercular spine appeared at 4.23 mm BL (Fig. 2D), and the posttemporal spine at 6.80 mm BL. The largest specimen lacking anterior and posterior preopercular spines was 4.48 mm BL, and the largest specimen lacking posttemporal spines, 7.21 mm BL (Fig. 2E). No other spines or spinelets of the head appeared in later developmental stages. The number of anterior and posterior preopercular spines increased 2–3 (nine maximum) and 5–7 (nine maximum), respectively, in the size range of 5–16 mm BL (Fig. 2, F

Table 2

recorded for the myomere on which it occurs. *n* indicates the number of specimens examined at each size range.

Myomere																				
20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
29	29	7			7			7	7	14	14	21	21	36	43	14	14	7	14	14
	7	22	7		7	19	15	4	11	19	4	19	19	33	30	26	30	15	19	27
7	11	4	4	4	7	11	7	11	15	4	11	15	19	15	19	22	7	7		21
8	8	17			25	8	17	8	17	8	8	8	17	17	17	25	25		8	12
10					10			10	10	10		30	10	20	10	10				10
	20	10	20		20		30	10	20	10	10	50	40	50	10	10			10	10
22		11		6		6		6	6	6	11	6	22	22	6	11	22	6		18
10					10			10	20		10			20	10	30	70	30	20	10
20	20	20	10			20	10	20	0	10	30	20	20	10	30	40	10	40	10	10
54	38	38	31	23	38	31	23	23	15	31	8	31	15	23	8	15	15	8	38	13
92	77	77	77	69	69	69	69	69	62	54	69	69	69	54	54	62	46	38	23	13
83	67	58	75	58	83	58	58	67	58	75	50	50	33	33	25	58	67	33	17	12
100	80	70	90	70	100	70	70	80	70	90	60	60	40	40	30	70	80	40	20	10

Table 3

is recorded for the myomere on which it occurs. *n* indicates the number of specimens examined at each size range. Melanophores were

Myomere																	
23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
29	14	7	57	7	64	29	14	21	79	50	36	57	50	36	43	36	14
15	15	37	11	26	37	41	41	30	44	44	26	26	48	30	33	52	27
19	33	29	29	24	43	38	38	52	24	57	43	43	19	33	29	57	21
17	8	33	17	17	42	42	17	25	83	50	50	50	33	58	33	25	12
20	30	40	60	10	10	60	60	40	70	60	60	30	20	30	20	20	10
30	10	20	60	30	30	10	30	30	40	50	20	0	10	30	20	0	10
6	11	22	28	39	39	33	56	28	44	28	28	22	50	22	17	39	18
	10	30	50	20	50	60	60	40	60	80	50	40	60	30	30	30	10
10	10	20	30	20	40	40	50	50	50	90	70	60	50	70	40	60	10
		8	15	15	23	54	46	62	54	69	62	62	54	62	46	46	13
8		15	8	23	31	54	46	62	46	85	77	54	77	77	85	31	13
				8	25	33	42	58	33	58	67	67	67	75	50	42	12
				10	40	40	40	60	70	80	70	70	70	70	60	40	10

and G). The number of anterior and posterior preopercular spines decreased at 17 mm BL. At >19.23 mm BL, no anterior preopercular spines were observed, except in one specimen (24.00 mm BL) that had two anterior preopercular spines. Juveniles had three posttemporal spines when these spines were fully developed. The largest specimen with a posttemporal spine was 24.23 mm BL, and the largest specimen examined, 37.78 mm BL, had no spines on its head.

Teeth and squamation

Upper and lower jaw teeth were first observed at 5.35 and 6.32 mm BL, respectively. Palatine teeth appeared at 7.20 mm BL and most specimens >9 mm BL had fully developed palatine teeth. At >18.00 mm BL, specimens did not have these teeth.

Squamation was incomplete in the size range examined in this study. The smallest scaled specimen (27.37 mm BL)

Table 4

Incidence of melanophores on the left lateral side of the trunk and tail (%) in *Thunnus thynnus*. Each melanophore is recorded for the the first to eighth myomeres.

Size range (BL; mm)	Myomere														
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
3.50–3.99			7	7	7	14	7	29	14	21	7	29	14	7	14
4.00–4.49		4			11	4				7	11	11	11	11	11
4.50–4.99					5	5	5		5	10	5	10	5	29	10
5.00–5.49		8				8				8		8		17	8
5.50–5.99					20		20	20	20	10		40			10
6.00–6.49									20			10		20	10
6.50–6.99				6	0	6	6	6		28	11			17	22
7.00–7.49									20				20	20	
7.50–7.99										20	10	20			20
8.00–8.49						15	8			15		8	8	8	15
8.50–8.99										8		15	8	8	8
9.00–9.49										8				17	17
9.50–9.99	10											10		10	20

Table 5

Incidence of melanophores on the right lateral side of the trunk and tail (%) in *Thunnus thynnus*. Each melanophore is recorded for on the first to nineth myomeres.

Size range (BL; mm)	Myomere														
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
3.50–3.99	0	7	7	7	14	7	21	14	21	14	29	14	7	14	
4.00–4.49	4			11	4	4			11	4	15	11	11	11	
4.50–4.99				5				5	10	5	10	5	19	10	
5.00–5.49					17	8			17		8		33	8	
5.50–5.99	10			20		10	20	10		10	40			10	
6.00–6.49								20	10		10		30	10	
6.50–6.99		6	6		6	6	6		28	11			17	22	
7.00–7.49								20	10	20		10	10		
7.50–7.99					10			10		10	20	20		10	
8.00–8.49									31					15	
8.50–8.99					15	15					31	31	31	15	
9.00–9.49					8				8				17	17	
9.50–9.99											10	10		30	

had anterior lateral line scales. Scales near the anterior part of the lateral line appeared at 30.31 mm BL, and postorbital scales at 30.81 mm BL.

Discussion

Development and growth

Thunnus thynnus eggs were reported to have a smooth chorion, narrow perivitelline space, homogeneous yolk, and a single oil globule (Miyashita et al., 2000), and this was reconfirmed in our study. These characteristics are similar to those found in other *Thunnus* eggs: *T. thynnus* from the Mediterranean (Podoa, 1956), *T. obesus* (Kikawa, 1953), *T. albacares* (Harada et al., 1971b; Mori et al., 1971), and *T. alalunga* (Yoshida and Otsu, 1963).

The growth strategy of bluefin tuna apparently is to develop foraging structures before other organs and at an early stage to enable feeding on larger organisms. Parts of the head were large from early development (Fig. 3) as mentioned by Collette et al. (1984) and reached maximum

Table 4

myomere on which it occurs. *n* indicates the number of specimens examined at each size range. Melanophores were not observed on

Myomere																
24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
14	36	7		21	7	7	14	14	7	14	7		14	7		14
	30	11	7	15	4	4	11		11	4	7	11	7	7	7	27
14	10	19	14	5	5		5	5	19	10	14	5	10		5	21
17	8	8	8	33				8	17	25	8	8				12
20		10	10	20		20	40		10	10	30	10				10
	10	10		20	10	20			10							10
11	11	6	11	17	6	6	11	6	11	6	17	11		6		18
		20	10	10			10		10	20	10	10	10		10	10
		20	20	10	20	20		30		10	10	40		20	10	10
8		8	15	8		15	31	31		8		8		8		13
15	8	8	31	15	23	15		23	15	15	8	8	8	8	8	13
8	8	17	17	25	25	42	58	33	50	33	50	33	33	50	42	12
	10	10		20	20	20		50	60	40	50	40	30	20	50	10

Table 5

the myomere on which it occurs. *n* indicates the number of specimens examined at each size range. Melanophores were not observed

Myomere																
24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
14	36	7		21	7	7	14	14	14	7	7		14	7	7	14
4	30	19	7	15	7	4			15	4	7	15	19	7	7	27
14		10	14		5			5	19	5	5				5	21
17	25	17	17	42			25	8	17	8	17	8				12
10			30	20		20	10		10	50	30	10				10
10	10	10		10	20	10		10	10	10						10
6	11	11	11	17	6	6	11	6	11	6	17	11		6		18
		10	10	30	10	10	10	10			20	20	20			10
	10	10	30	20	10			10		10	20	30		30	20	10
31		8	8	8		15	15			8		8		8		13
8	15	15	54	23	31	46	46	31	31	31	8		8	23	15	13
8	17	8	8	25	25	42	50	50	42	25	42	25	25	42	25	12
	30	20		20	20	10	10	30	30	40	50	50	30	20	50	10

ratios in relation to BL at sizes smaller than other body parts, such as total length and body height, except for the caudal peduncle depth (Fig. 4).

Another ontogenetic characteristic of growth in *T. thynnus*, the posterior migration of the anus (Collette et al., 1984), was also confirmed: the anus initially positioned in the anterior part of the body, was located at the center of the body at late flexion, and thereafter in the posterior part of the body (Fig. 3).

Kaji et al. (1996) examined the relative growth of larvae of the Pacific bluefin tuna at 3–14 mm BL and reported that body proportions showed constant relative growth values from 10 mm BL, except for preanal length. Our results showed that at 10 mm BL, constant ratios had not been reached in relation to BL. This difference may be due to an insufficient number of larger specimens in Kaji et al.'s (1996) study. Even at the juvenile stage, ratios in relative BL decreased gradually, except for a few cases (Fig. 3), suggesting that body proportions of juvenile bluefin tuna are different from those of adult fish.

Early growth is rapid in *T. thynnus* compared with other marine fishes cultured in Japan (e.g. red sea bream,

Table 6

Incidence of melanophores embedded in the lateral muscle and on the myosepta (%) in *Thunnus thynnus*. Each melanophore is recorded on the first to nineth myomeres.

Size range (BL; mm)	Myomere													
	10	11	12	13	14	15	16	17	18	19	20	21	22	23
3.50–3.99														
4.00–4.49										4				
4.50–4.99	4	4	4	7	4			4	4	4	4		7	
5.00–5.49						8			8	8				
5.50–5.99			10								10			
6.00–6.49				10		10	10	10	10					
6.50–6.99														
7.00–7.49	10	10		10			10	20	10	10		10		
7.50–7.99			10			10				10		10		
8.00–8.49			8	8	8	8	8	8	8	8	15	23	31	15
8.50–8.99			8	0	8	8	0	0	15	31	38	46	54	62
9.00–9.49			8	8	8	8	25	33	33	42	50	50	50	58
9.50–9.99			10	10	10	10	30	40	40	50	60	60	60	70

Table 7

Incidence of melanophores on the dorsal fin base (%) in *Thunnus thynnus*. Each melanophore is recorded for the nearest myomere. for larvae <7.50 mm BL.

Size range (BL; mm)	Myomere																	
	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
7.50–7.99	10	10	10	10	10	20	20	20	30	20	30	20	30	20	20	20	20	10
8.00–8.49	8	8	8	8	8	15	15	15	23	15	23	15	23	15	15	15	15	8
8.50–8.99		8	15	31	46	46	46	46	46	54	38	54	31	31	31	31	46	46
9.00–9.49	8	17	25	50	58	83	83	83	83	83	83	83	83	83	83	83	83	83
9.50–9.99		20	40	70	80	100	60	60	60	60	100	100	100	100	100	90	100	100

Table 8

Incidence of melanophores on the anal fin base (%) in *Thunnus thynnus*. Each melanophore is recorded for the nearest myomere. *n* indicates the number of specimens examined at each size range. Melanophores were not observed on the first to twenty-first myomeres and for larvae <7.0 mm BL.

Size range (BL; mm)	Myomere																		
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
7.00–7.49			10	10	20	10	20	20	20	10	10	10	10	10					10
7.50–7.99	10	20	30	30	50	40	60	40	40	40	40	20	30	20	20	20	10		10
8.00–8.49		15	8	15	31	38	31	62	69	54	62	62	46	31		15			13
8.50–8.99		8	8	8	8	31	31	38	62	69	69	69	69	69	62	62	38	23	13
9.00–9.49					17	33	42	58	75	83	83	75	67	67	50	42	33	25	12
9.50–9.99					20	40	50	70	90	100	100	90	80	80	60	50	40	30	10

Table 6

for the myomere in which it occurs. *n* indicates the number of specimens examined at each size range. Melanophores were not observed

Myomere																
24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
																14
																27
																21
8																12
																10
		10									10					10
		6				6							11	11		18
10	10	10				10	10						10			10
	10	10	10	10	30	20	30	20		10	30	20	20	20	30	10
23	23	31	23	38	31	54	38	23	38	15	38	38	31	15	15	13
69	62	69	77	77	77	77	77	77	69	62	62	62	62	46	23	13
75	75	83	83	75	83	75	75	75	75	75	75	58	58	67	33	12
90	90	100	100	90	100	90	90	90	90	90	90	70	70	80	40	10

Table 7

n indicates the number of specimens examined at each size range. Melanophore was not observed on the first to fourth myomeres and

Myomere																	
23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	*n*
10	10	20	20	30	20	30	20	20	30	20	10	10					10
8	8	15	15	23	15	23	15	15	23	15	8	8					13
31	46	38	46	46	38	46	46	38	38	31	46	23		8	0	15	13
83	83	83	83	83	83	83	83	83	83	58	58	58	42	42	8		12
90	90	100	100	100	100	80	80	90	80	80	70	60	20	30	10	20	10

Pagrus major, and white trevally, *Pseudocaranx dentex* [Sawada unpubl. data; Fig. 5]). Rapid growth in bluefin tuna is pronounced after day 20 ($\bar{x}$=9.34 mm BL on day 20) when larvae begin to metamorphose into juveniles. This type of growth phase seems common to scombrids, for example, *T. albacares* (Harada et al, 1971b; Kaji et al., 1999), *Scomber japonicus* (Watanabe, 1970), *Auxis tapeinosoma* (=*A. rocheri*, Harada et al., 1973), and *Sarda orientalis* (Harada et al., 1974) and reaches its height by the time of development of external physical features and the digestive system (Kohno et al., 1984; Miyashita et al., 1998), and by the time of development of their selective feeding characteristics (Sawada et al., 2000).

On day 19 ($\bar{x}$=10.17 mm TL), the mouth size of *T. thynnus* averaged 2.54 mm according to Shirota's (1970) calculation (mouth size index=upper jaw length $\times 2^{0.5}$). This size is comparable to that of other scombrids, such as *T. albacares* (3.3 mm) and *Katsuwonus pelamis* (3.0 mm) at 10 mm TL, although much larger than that of other species (see Table 2 of Shirota, 1970). The digestive system of *T. thynnus* develops earlier than that of other fishes (Richards and Dove, 1971; Miyashita et al., 1998) and attains an adultlike structure by the juvenile stage, thus allowing bluefin tuna to use food efficiently early in development.

The development of pigment, spines, jaw teeth, and squamation in *T. thynnus* is summarized in Figure 6. The jaw teeth of *T. thynnus* first appeared at 5.35 mm BL (day 10), the palatine teeth at 7 mm BL (flexion stage), and were fully developed at 9 mm BL (postflexion stage). At 7 mm BL, *T. thynnus* larvae fed on other fish larvae and at 8 mm BL (postflexion stage), they were cannibalistic. Teeth appearance and development corresponded to the piscivorous stage during the fast-growth phase after the postflexion stage. Future improved laboratory-rearing techniques

Table 9

Number of erythrophores on the body of *Thunnus thynnus* larvae and juveniles.

Body length (mm)	Dorsal edge	Ventral edge	Lateral midline	Lower jaw	Hypural plate	Caudal fin finfold
3.38						
3.41						
4.63	2	34	5			
4.75	8	32	4			1
5.02	4	2				
5.09	4	36				
5.11	3	54	4			
5.35		24	4			
5.78	1	2	4			
6.10	1	24	5	4		
6.72	3	1	6			
6.91	1	1	4			
6.96	2	17	6	2	2	
6.96		1	8	3		
7.03	8		6		1	
7.06	8	12	8	1	5	2
7.12		8	10		5	
7.20	6	8	6	1	3	1
7.92	1	8	6	1		
8.16		6	8	3		
8.41	1	11	8	3		
8.74	1	4	5			
8.81	4	7	34	2		
8.92		3	3	1		
9.02				1		
9.35	3	12	5	3		
9.38		3	2	3		
9.56		2	2	1		
9.60		9	3	1		
9.70	5	1	6			
9.93	2	2	2			
10.03		1	23			
10.56	4	1	2	7		
10.97	4	1	3			
11.26	1	15	12	2		
11.71						
12.20			4			
13.71		5				
14.00						
14.79		3	1			
14.83		1				
15.33	2	4	1			
15.49						
15.85	1	3	2			
16.86						
18.73		1				
19.71						
19.72		2				
20.40						
21.87						
24.85						
25.89						

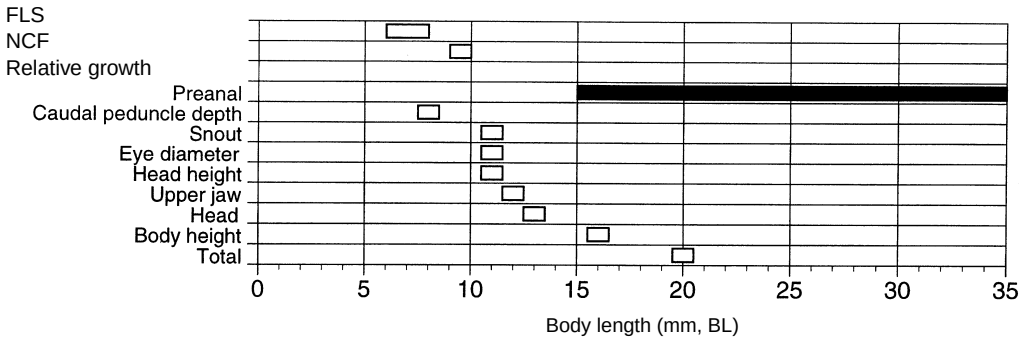

Figure 4

Schematic representation of the relative growth (length of body parts in relation to body length) in hatchery-reared bluefin tuna, *Thunnus thynnus*. Flexion larva subdivision (FLS) and numerical complement of fin rays (NCF) are also shown. In relative growth, □ = the peak value of body part proportion (in relation to BL) and ■ = the attainment of a constant value of body part proportion (in relation to BL).

will allow further study of the structure and development of feeding-related bony elements for *T. thynnus*, as has been the case for *S. japonicus* (Kohno et al., 1984) and *Lates calcarifer* (Kohno et al., 1996).

Pigmentation

Accurate identification of *Thunnus* larvae requires an extensive knowledge of individual, growth-associated, and geographic variations in melanophore and erythrophore patterns. We obtained information on the individual and growth-associated variations of these patterns from laboratory-reared specimens.

The melanophore distribution pattern of *T. thynnus* larvae and juveniles showed four distinct characteristic periods of development: newly hatched, 1–3 days after hatching, preflexion to postflexion, and juvenile.

T. thynnus thynnus larvae <3.99 mm BL from the Mediterranean had embedded melanophores (Kohno et al., 1982); but we did not observe these in our specimens until 4.49 mm BL. This difference may be a subspecific difference between *T. thynnus orientalis* and *T. thynnus thynnus*, or it may be due to insufficient numbers of specimens larger than 6.0 mm BL in Kohno et al.'s study, or to the experimental culture product as mentioned later.

Wild-caught *T. thynnus orientalis* larvae (3.15–8.20 mm BL) most frequently had two melanophores both on the dorsal and ventral edges of the trunk and tail (Nishikawa, 1985). This pattern resembles that of *T. thynnus thynnus* larvae from the Mediterranean (2.53–5.25 mm BL, Kohno et al., 1982). But our cultured specimens in the same size range had greater numbers of these melanophores (Tables 2 and 3). Nishikawa (1985) reported the appearance of melanophores at the dorsal fin somewhat earlier than we observed their appearance, but their appearance on the lower and upper jaws occurred in the same size range in both studies where specimens were examined from the same population. Laboratory-reared fish larvae have been

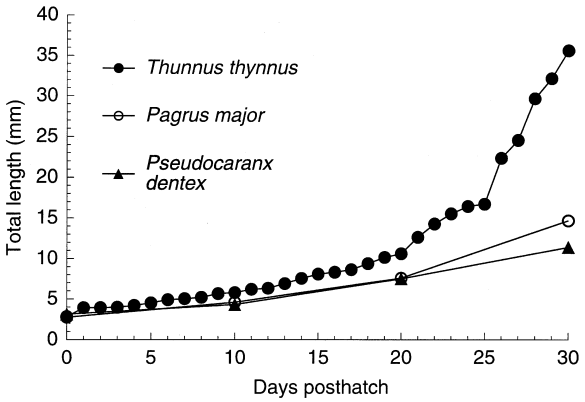

Figure 5

Growth of bluefin tuna (*Thunnus thynnus*), red sea bream (*Pagrus major*), and white trevally (*Pseudocaranx dentex*). Red sea bream and white trevally were reared at the same temperature range (24–27°C) as bluefin tuna (Sawada, unpubl. data).

shown to have greater pigmentation than wild-caught larvae (e.g. *Pagrus major* [Fukuhara and Kuniyuki, 1978], *Sparus sarba* [Kinoshita, 1986], *Parapristipoma trilineatum* [Kimura and Aritaki, 1985], and *Nibea mitsukurii* [Kinoshita and Fujita, 1988]). Thus, the source of specimens, wild-caught or laboratory-reared, may account for observed differences in larval pigmentation between our study and Nishikawa's study (1985).

A slight difference in the occurrence of lower jaw, trunk, and tail melanophores has been reported among *T. thynnus* from the Mediterranean (Kohno et al., 1982), the Atlantic (Richards and Potthoff, 1974), and the Pacific (Matsumoto et al., 1972). Lower jaw melanophores appeared in all specimens larger than 3.0 mm BL from the Atlantic

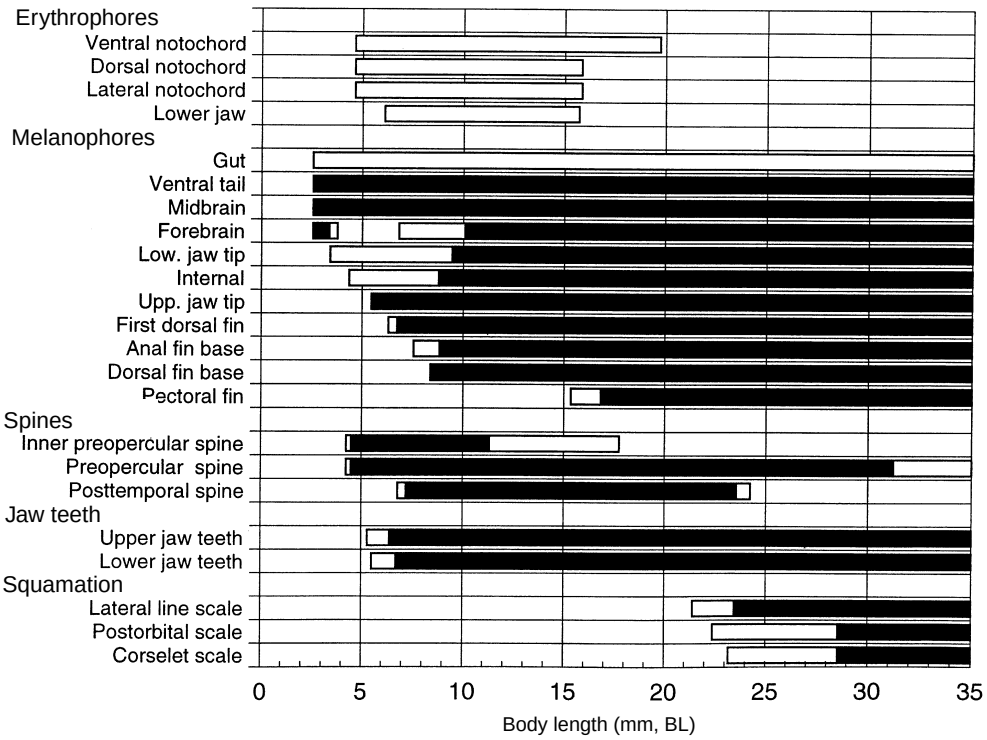

Figure 6

Schematic representation of the development of pigment, spines, jaw teeth, and squamation in hatchery-reared bluefin tuna (*Thunnus thynnus*). □ = appearance of pigment and spines was subject to individual variation; ■ = pigment and spines were present in all specimens examined.

and larger than 4.0 mm BL from the Pacific. In our specimens, the incidence of these melanophores was 7.1% at 3.50–3.99 mm BL, 29.6% at 4.00–4.49 mm BL, 57.1% at 4.50—4.99 mm BL, 83.3% at 5.00–5.49 mm BL, and more than 90% in specimens >5.50 mm BL. The largest specimen we observed lacking these melanophores was 9.46 mm BL. Our data are similar to those for specimens from the Mediterranean. The difference in lower jaw melanophore distribution between our specimens and those of Matsumoto et al. (1972) might be explained by geographic variation of the melanophore pattern in the Pacific bluefin tuna. Further study is needed to confirm this hypothesis.

Ueyanagi (1966) reported species-specific characteristics of the erythrophore distribution pattern of Thunninae larvae from the Pacific Ocean: *T. alalunga* consistently had more erythrophores on the dorsal edge of the trunk and tail in front of the caudal peduncle than did *T. albacares*, which had only one or two erythrophores at the caudal peduncle; *T. thynnus* and *T. obesus* had patterns transitional between those of *T. alalunga* and *T. albacares*. Our data for *T. thynnus* generally agreed with those of Ueyanagi (1966); however, the number of dorsal erythrophores in one individual ranged more (0–8) than in Ueyanagi's study (1–5). In addition, ventral erythrophores appeared in large numbers (more than 20) at the preflexion stage (4.63–6.10 mm BL). From examinations of tuna larvae taken in Hawaiian waters, Matsumoto et al. (1972) considered the erythrophore pattern useful as a morphological characteristic for identifying tuna larvae. At present, however, information is limited on erythrophore patterns of other Thunninae larvae and juveniles and on the difference of these patterns in wild-caught and laboratory-reared specimens. Thus collection of such information is needed to establish the erythrophore pattern as a species-identifying characteristic of Thunninae larvae and juveniles.

Thunnus thynnus did not have xanthophores from hatching to the preflexion stage. However, *T. albacares* (Harada et al., 1971b; Mori et al., 1971) and *T. obesus* (Yasutake et al., 1973) have clusters of xanthophores in the finfolds of both the dorsal and ventral fins, and on the dorsal body, respectively. Thus, the xanthophore pattern can be used for distinguishing these *Thunnus* species at the preflexion stage.

Much additional work on development of tunas under controlled conditions, as well as the study on wild-caught materials, is needed to understand their early life history. We believe recent progress in the technology of rearing tunas will yield important information on the early life history of *Thunnus* spp.

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Literature cited

Cho, S., and S. Inoue.
1970. Intra- and interspecific restriction fragment length polymorphism in mitochondrial genes of *Thunnus* tuna species. Bull. Nat. Res. Inst. Far. Seas Fish. 30:207–225.

Collette, B. B.
1999. Mackerels, molecules, and morphology. Soc. Fr. Ichtyol. 25:149–164.

Collette, B. B., and C. E. Nauen.
1983. FAO species catalogue. Vol. 2: Scombrids of the world: an annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date, 137 p. FAO Fish. Synop. 125, FAO, Rome.

Collette, B. B., T. Potthoff, W. J. Richards, S. Ueyanagi, J. L. Russo, and Y. Nishikawa.
1984. Scombroidei: development and relationships. *In* Ontogeny and systematics of fishes (H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr. and S. L. Richardson, eds.), p. 591–620. Am. Soc. Ichthyol. Herpetol., Spec. Publ. 1.

Fujita, K.
1998. Species and ecology of tunas. *In* From the production to the consumption of tunas (S. Ono, ed.), p. 1–49. Seizando, Tokyo.

Fukuhara, O., and K. Kuniyuki.
1978. Morphological development in the wild larvae of Madai, *Chrysophyrys major*, as compared to that in the laboratory-reared larvae. Bull. Nansei Reg. Fish. Res. Lab. 11:19–25.

Gibbs, R. H., Jr. and B. B. Collette.
1967. Comparative anatomy and systematics of the tunas, genus *Thunnus*. Fish. Bull. 66:65–130.

Harada, T., H. Kumai, K. Mizuno, O. Murata, M. Nakamura, S. Miyashita, and H. Furutani.
1971a. On the rearing of young bluefin tuna. Mem. Fac. Agric. Kinki Univ. 4:153–157. [In Japanese with English summary.]

Harada, T., K. Mizuno, O. Murata, S. Miyashita, and H. Furutani.
1971b. On the artificial rearing of larvae in yellowfin tuna. Mem. Fac. Agri. Kinki Univ., p. 145–152. [In Japanese with English summary.]

Harada, T., O. Murata, and H. Furutani.
1973. On the artificial fertilization and rearing of larvae in marusoda, *Auxis tapeinosoma*. Kinki Univ. Fac. Agric. Bull. 6:113–116. [In Japanese with English summary.]

Harada, T., O. Murata, and S. Miyashita.
1974. On the artificial fertilization and rearing of larvae in bonito. Kinki Univ. Fac. Agric. Bull. 7:1–4. [In Japanese with English summary.]

Iwai, T., I. Nakamura and K. Matsubara.
1965. Taxonomic study of the tunas. Misaki Mar. Biol. Inst. Special Report 2:1–51. [In Japanese with English summary.]

Kaji, T., M. Tanaka, Y. Takahashi, M. Oka, and N. Ishibashi.
1996. Preliminary observations on development of Pacific bluefin tuna *Thunnus thynnus* (Scombridae) larvae reared in the laboratory, with special reference to the digestive system. Mar. Freshwater Res. 47:261–269.

Kaji, T., M. Tanaka, M. Oka, H. Takeuchi, S. Ohsumi, K. Teruya, and J. Hirokawa.
1999. Growth and morphological development of laboratory-reared yellowfin tuna *Thunnus albacares* larvae and juveniles, with special emphasis on the digestive system. Fish. Sci. 65:700–707.

Kikawa, S.
1953. Spawning of bigeye tuna in the sea near the Marshall Islands. Rep. Southern Sea National Research Institute 24:156–158. [In Japanese.]

Kimura, S., and M. Aritaki.
1985. Studies on rearing and development of larval and juvenile threeline grunt (Pisces: Haemulidae). Bull. Fac. Fish. Mie Univ. 12:193–205.

Kinoshita, I.
1986. Postlarve and juveniles of silver sea bream, *Sparus asrba*, occurring in the surf zones of Tosa Bay, Japan. Japan. J. Ichthyol. 33:7–30.

Kinoshita, I., and S. Fujita.
1988. Larvae and juvenile of blue drum, *Nibea mitsukurii*, occurring in the surf zones of Tosa Bay, Japan. Japan. J. Ichthyol. 35:25–30.

Kohno, H., T. Hoshino, F. Yasuda, and Y. Taki.
1982. Larval melanophore patterns of *Thunnus alalunga* and *T. thynnus* from the Mediterranean. Japan. J. Ichthyol. 28:461–465.

Kohno, H., R. Ordonio-Aguilar, A. Ohno, and Y. Taki.
1996. Osteological development of the feeding apparatus in early stage larvae of the seabass, *Lates calcarifer*. Ichthyol. Res. 43:1–9.

Kohno, H., M. Shimizu, and Y. Nose.
1984. Morphological aspects of the development of swimming and feeding functions in larval *Scomber japonicus*. Bull. Japan. Soc. Sci. Fish. 50:1125–1137.

Kumai, H.
1998. Studies on bluefin tuna artificial hatching, rearing and reproduction. Nippon Suisan Gakkaishi 64:601–605.

Matsumoto, W. M., E. H. Ahlstorm, S. Jones, W. L. Klawe, W. J. Richards, and S. Ueyanagi.
1972. On the clarification of larval tuna identification particularly in the genus *Thunnus*. Fish. Bull. 70:1–12.

Miyashita, S., K. Kato, Y. Sawada, O. Murata, Y. Ishitami, K. Shimizu, S. Yamamoto, and H. Kumai.
1998. Development of digestive system and digestive enzyme activities of larval and juvenile bluefin tuna, *Thunnus thynnus*, reared in the laboratory. Suisanzoshoku 46:111–120.

Miyashita, S., Y. Sawada, N. Hattori, H. Nakatsukasa, T. Okada, O. Murata, and H. Kumai.
In press. Mortality of northern bluefin tuna (*Thunnus thynnus*) due to trauma caused by collision during growout culture. J. World Aqua. Soc. 31.

Miyashita, S., Y. Tanaka, Y. Sawada, O. Murata, N. Hattori, K. Takii, Y. Mukai, and H. Kumai.
2000. Embryonic development and effects of water temperature on hatching of the bluefin tuna, *Thunnus thynnus*. Suisanzoshoku 48:199–207.

Mori, K., S. Ueyanagi, and Y. Nishikawa.
1971. The development of artificially fertilized and reared

larvae of yellowfin tuna, *Thunnus albacares*. Bull. Far Seas Fish. Res. Lab. 5:219–232. [In Japanese with English summary.]

NIH (National Institutes of Health).
1997. NIH image analysis software. NIH, Bethesda, MD, 104 p.

Nishikawa, Y.
1985. Identification for larvae of three species of genus *Thunnus* by melanophore patterns. Bull. Far Seas Fish. Res. Lab. 22:119–129. [In Japanese with English summary.]

Orange, C. J., and B. D. Fink.
1963. Migration of a tagged bluefin tuna across the Pacific Ocean. Calif. Fish and Game 49:307–308.

Podoa, E.
1956. Uova, larve e stadi giovanili di Teleostei; Scombriformes. Fauna e Flora del Golfo di Napoli, Monogr. 38:471–507.

Richards, W. J., and G. R. Dove.
1971. Internal development of young tunas of the genera *Katsuwonus*, *Euthynnus*, *Auxis*, and *Thunnus*. Copeia 1:72–78.

Richards, W. J., and T. Potthoff.
1974. Analysis of the taxonomic characters of young scombrid fishes, genus *Thunnus*. *In* The early life history of fish (J. H. S. Blaxter, ed.), p. 623–648. Springer-Verlag, Berlin, Heidelberg, New York.

Sawada, Y., S. Miyashita, M. Aoyama, M. Kurata, Y. Mukai, T. Okada, O. Murata, and H. Kumai.
2000. Rotifer size selectivity and optimal feeding density of bluefin tuna, *Thunnus thunnus*, larvae. Suisanzoshoku 48:169–177.

Shirota, A.
1970. Studies on the mouth size of fish larvae. Bull. Jap. Soc. Sci. Fish. 36:353–368. [In Japanese with English summary.]

Takii, K., S. Miyashita, M. Seoka, Y. Tanaka, Y. Kubo, and H. Kumai.
1997. Changes in chemical contents and enzyme activities during embryonic development of bluefin tuna. Fisheries Sci. 63:1014–1018.

Ueyanagi, S.
1966. On the red pigmentation of larval tuna and its usefulness in species identification. Rep. Nankai. Reg. Fish. Res. Lab. 24:41–48. [In Japanese with English summary.]

Watanabe, T.
1970. Morphology and ecology of early stages of life in Japanese common mackerel, *Scomber japonicus* Houttuyn, with special reference to fluctuation of population. Bull. Tokai Reg. Fish. Res. Lab. 62:1–259. [In Japanese with English summary.]

Yabe, H., S. Ueyanagi, and H. Watanabe.
1966. Studies on the early life history of bluefin tuna *Thunnus thynnus* and on the larva of southern bluefin tuna *T. maccoyii*. Rep. Nankai Reg. Fish. Res. Lab. 23:95–129. [In Japanese with English summary.]

Yasutake H., G. Nishi, and K. Mori.
1973. Artificial fertilization and rearing of bigeye tuna (*Thunnus obesus*) on board, with morphological observations on embryonic through to early post-larval stage. Bull. Far Seas Fish. Res. Lab. 8, June:71–78. [In Japanese with English summary.]

Yoshida, H. O., and T. Otsu.
1963. Synopsis of biological data on albacore *Thunnus germo* (Lacépède) 1880 (Pacific and Indian Oceans). FAO Fish. Rep. 2(6):274–318.