Developmental Stage and Morphogenesis of hatchery-raised finfish larvae

- as a fundamental basis of prevention against skeletal deformity -

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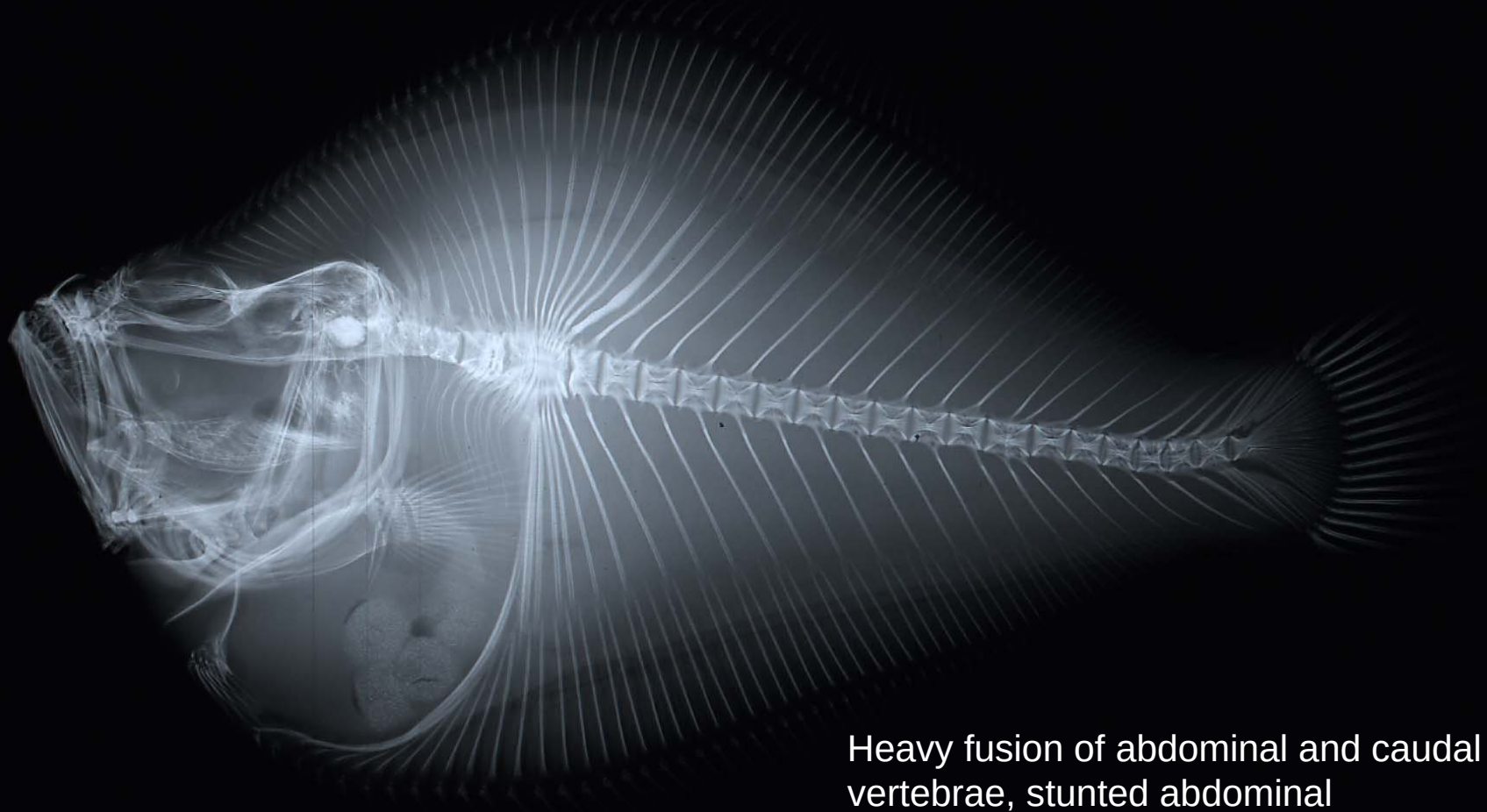
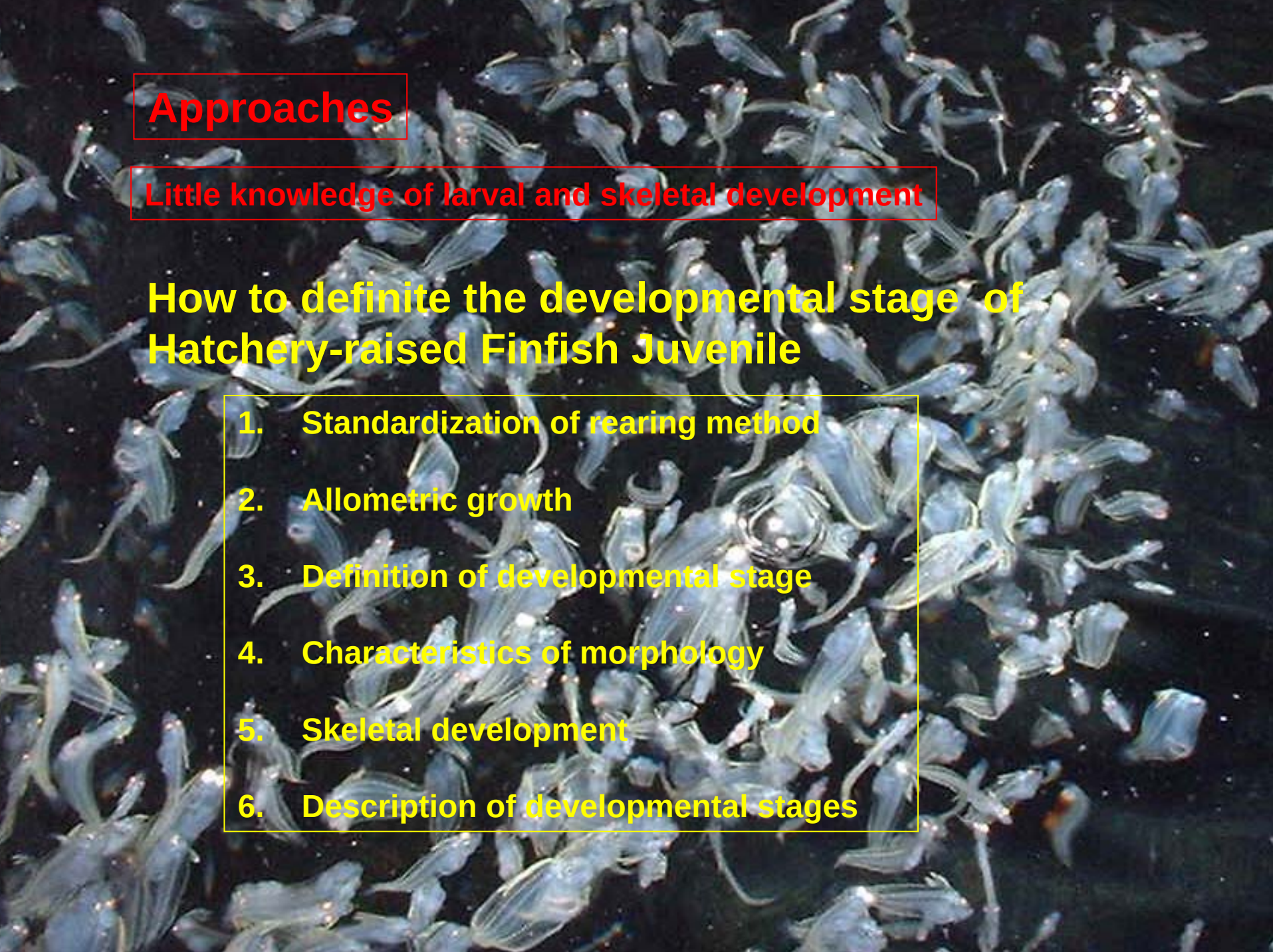
Serious problem occurred in hatchery-raised finfish larvae, skeletal deformity



Heavy fusion of abdominal and caudal vertebrae, stunted abdominal vertebrae, and hypertrophy of caudal vertebrae.


The fundamental objective of our study is to establish the hatchery technology for improvement the health and the quality of hatchery-raised fish.
How to prevent the occurrence of skeletal deformity?



Approaches

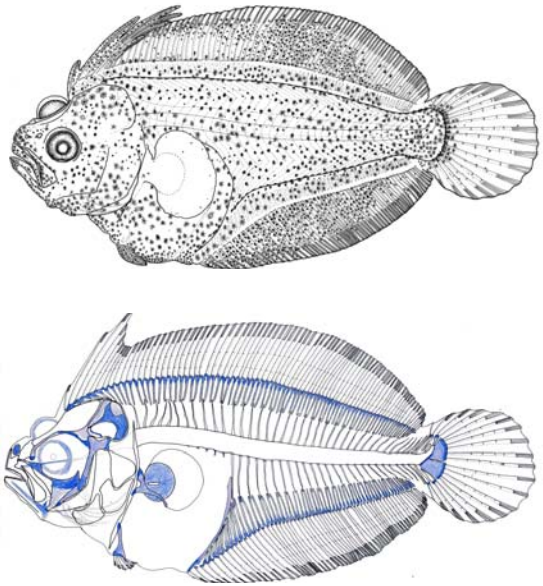
Little knowledge of larval and skeletal development

How to definite the developmental stage of Hatchery-raised Finfish Juvenile

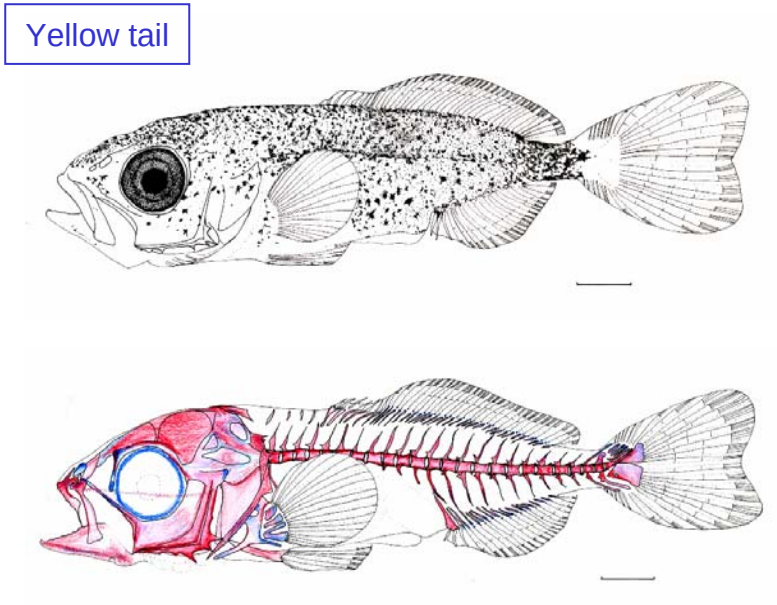
1. Standardization of rearing method
2. Allometric growth
3. Definition of developmental stage
4. Characteristics of morphology
5. Skeletal development
6. Description of developmental stages

We already examined following 4 spicies, and continuing examination of 2 sp.

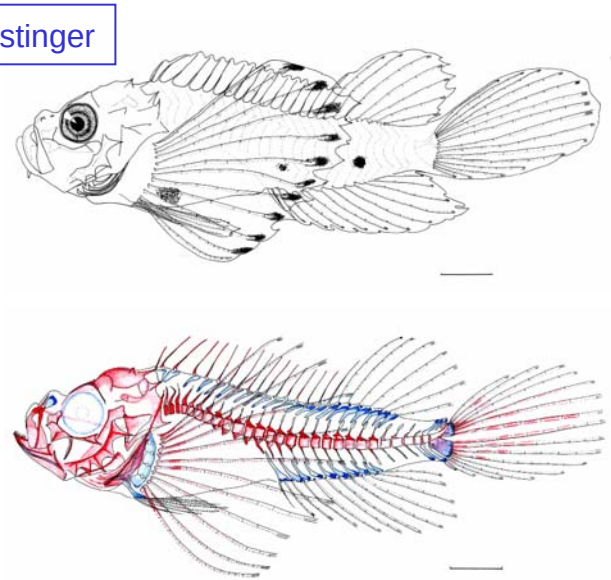
Japanese flounder



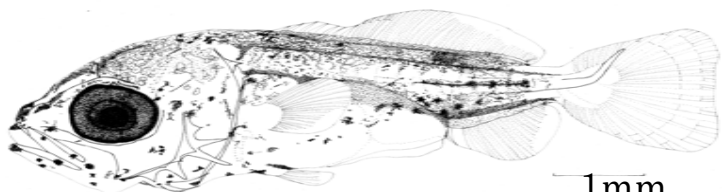
Yellow tail



Devil stinger

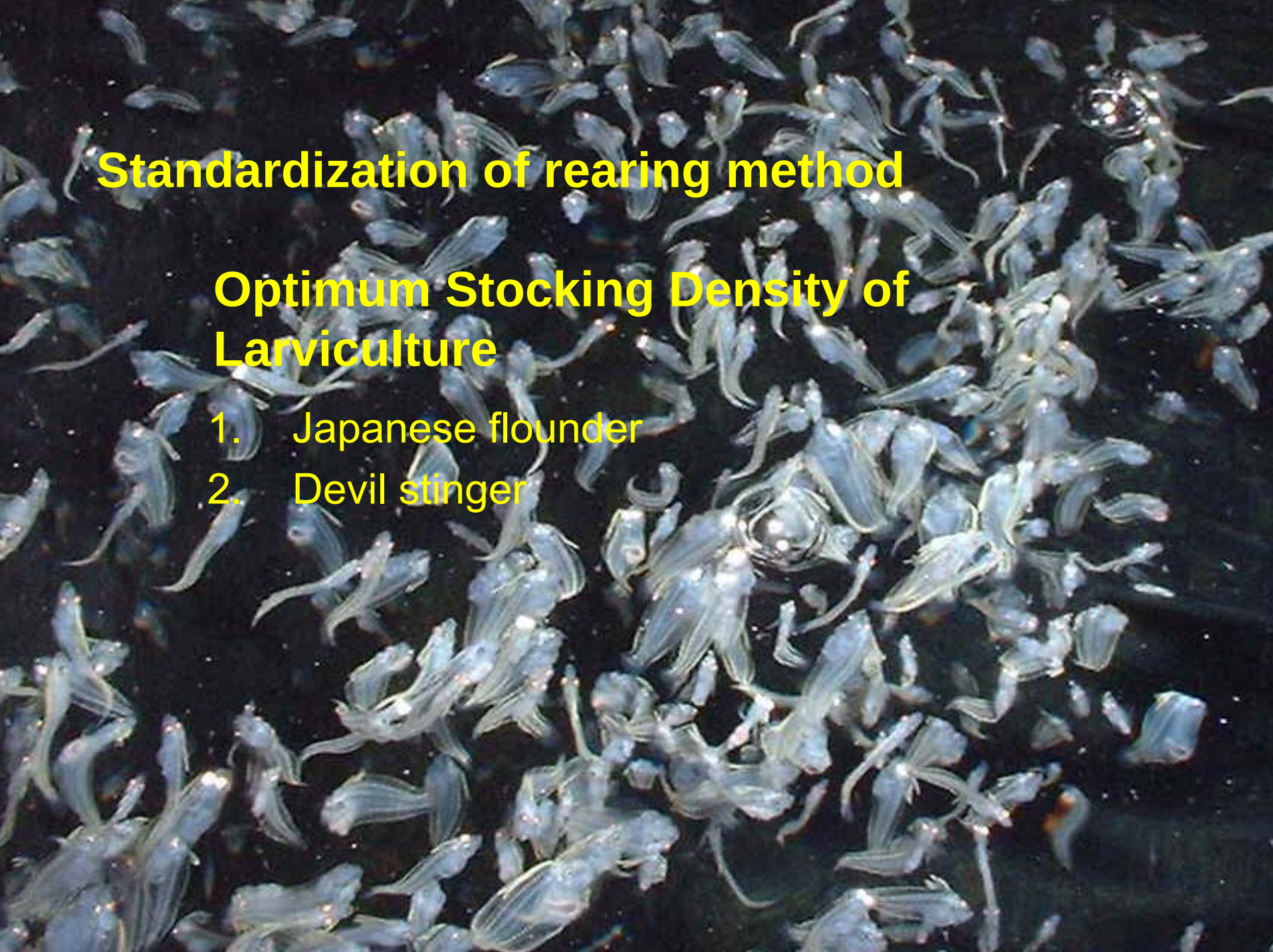


Umber jack



1mm

23dah, TL 7.91mm



Standardization of rearing method

Optimum Stocking Density of Larviculture

1. Japanese flounder
2. Devil stinger

Optimum stocking density – Japanese flounder

We examined the relation between larviculture performance and stocking density at mouth opening.

Maximum growth performance was revealed at stocking density of 20000 inds/m^3.

This fact reveals the fact of the presence of carrying capacity for the larviculture tank. The density of 20000 inds/m^3 should reflect the half of the carrying capacity.

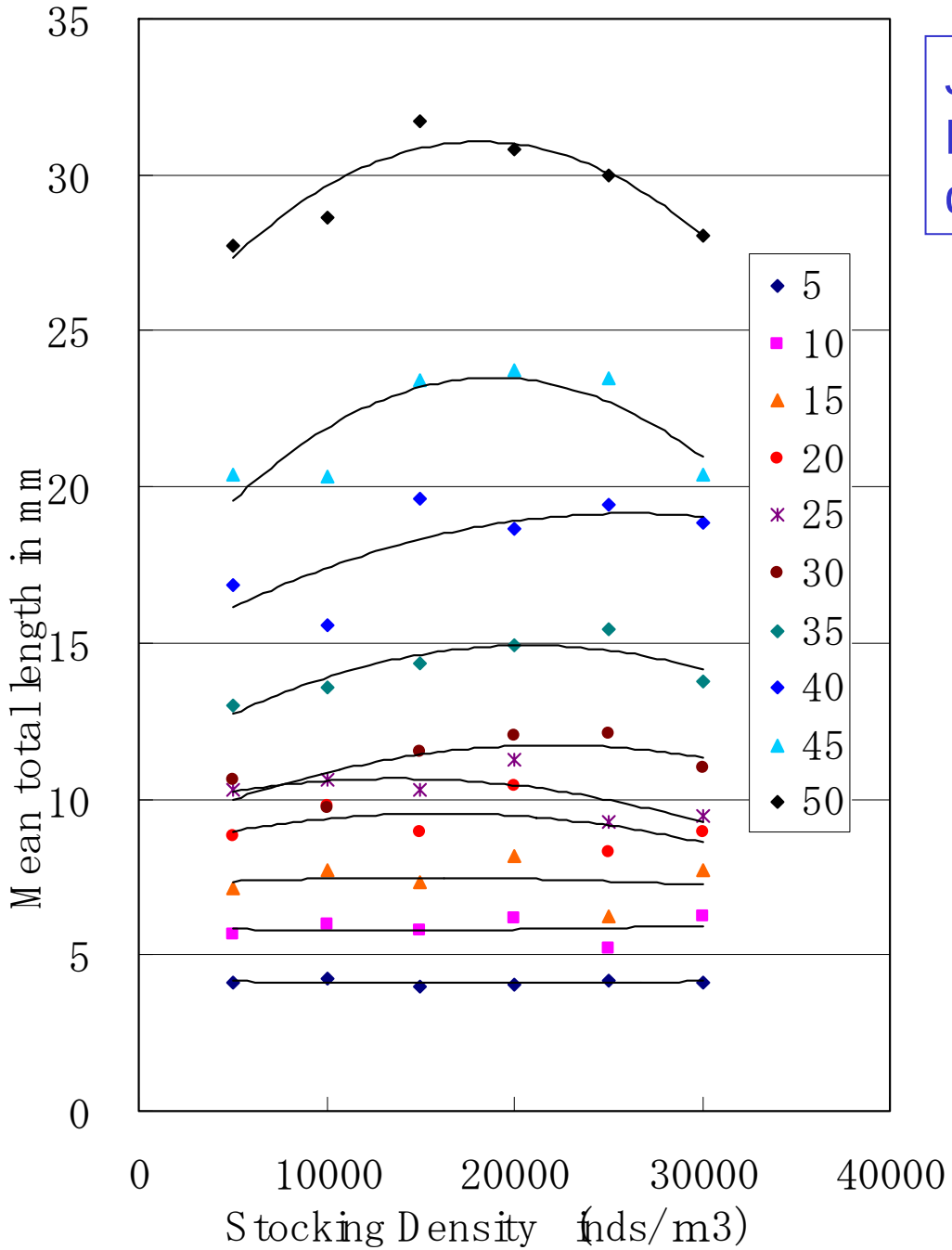
We decided the stocking density of 20000 inds/m^3 as the optimum stocking density at mouth opening of the Japanese flounder larvae.

We should use the density of 20000inds/m^3 as the standard stocking density for the Japanese flounder larviculture.

Optimum stocking density of larviculture – Devil stinger

We conducted experimental larviculture of the devil stinger with gradient of stocking density. We revealed the optimum stocking density of the devil stinger larviculture, 11000-18000 individuals/m3.

We recommended to use the stocking density of 15000 individuals/m3 for future experimental larviculture.



Japanese flounder Relation between stocking density and growth performance

We conducted experimental larval rearing of Japanese flounder using 1m^3 tank. Stocking density at mouth opening were 5000, 10000, 15000, 20000, 25000, and 30000 inds/m3. Each tank was duplicated.

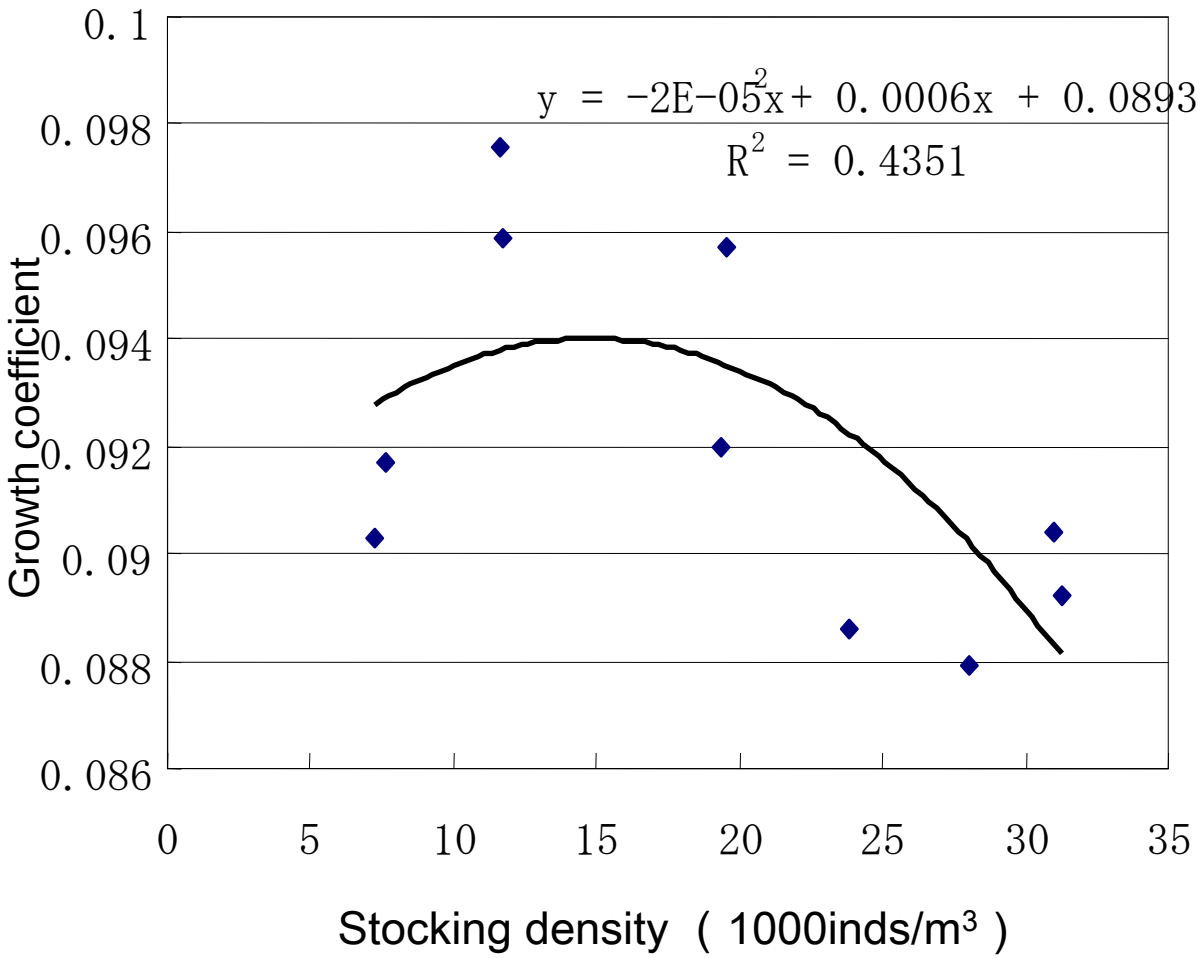
Stocking density was regulated at mouth opening, 3 days after hatching (DAH).

Until 20DAH, we could not definite difference of growth at each tank.

Since 25DAH, TL for density gradation showed dome shaped, fitted by quadratic equation.

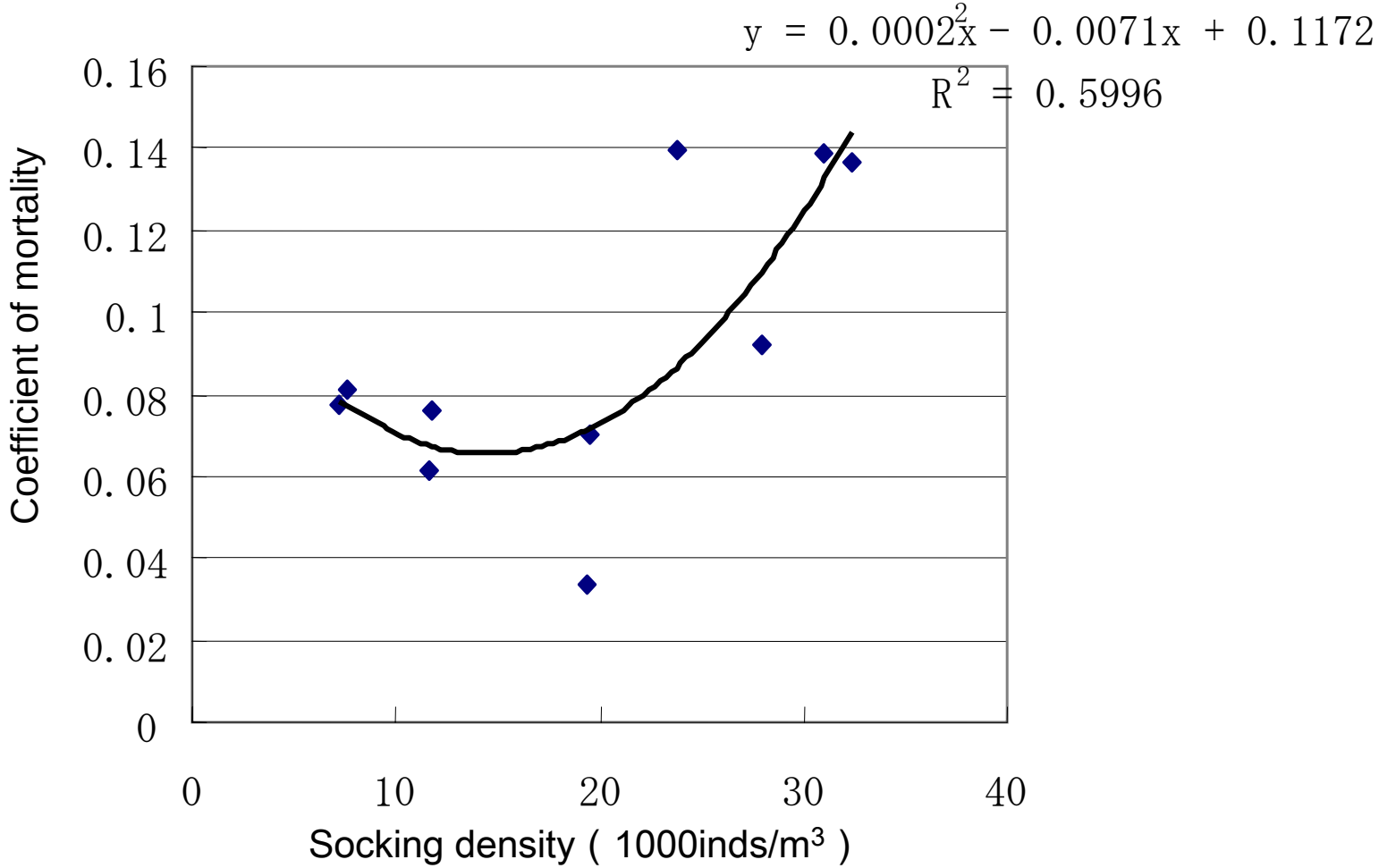
Devil stinger

Relation between stocking density and growth coefficient

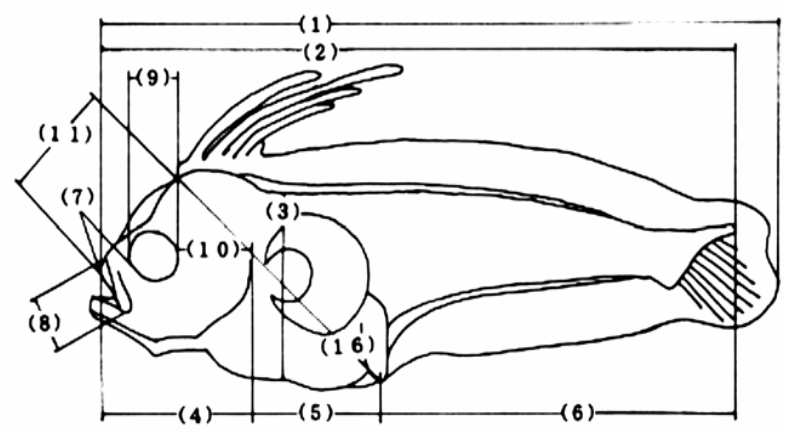


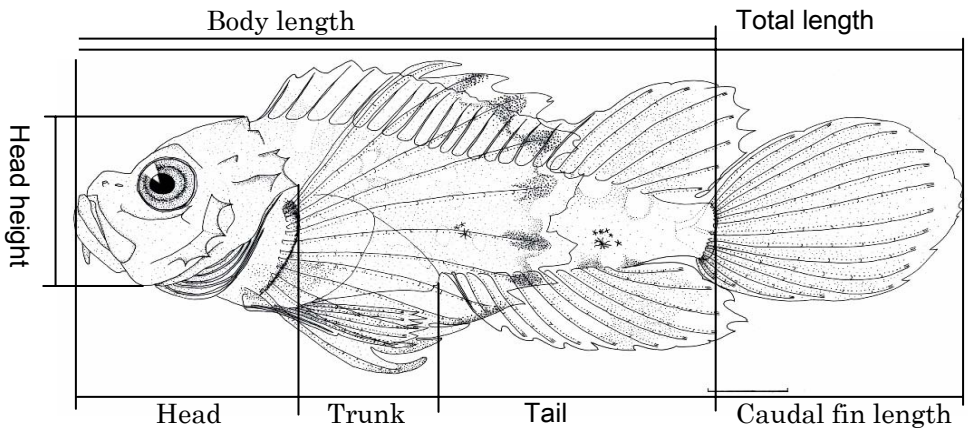
Devil stinger

Relation between stocking density and coefficient of mortality

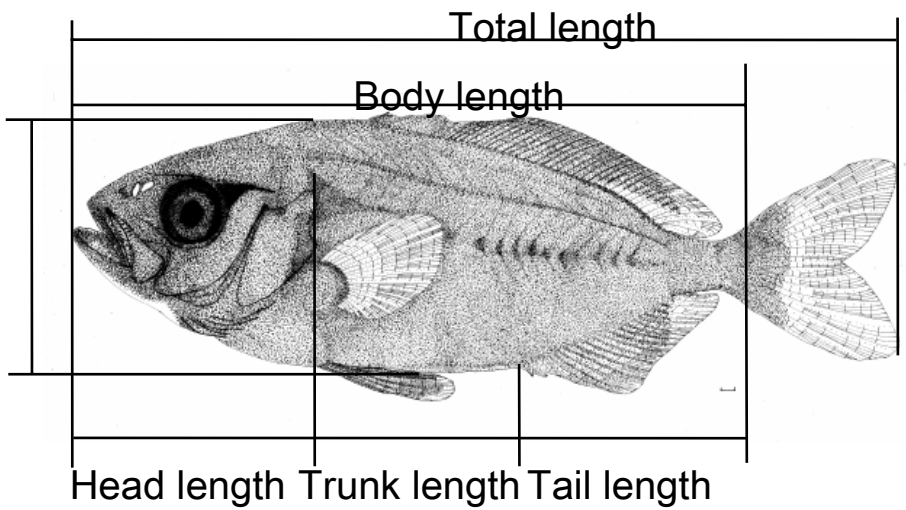


Allometry of finfish larvae





23DAH TL 11.10mm



Allometry equation

$$Y = aX^b$$

$$\log y = \log a + b \log x$$

b > 1 Positive growth

b = 1 Isometric growth

b < 1 Negative growth

Developmental stage and morphogenesis of Japanese flounder

Objective

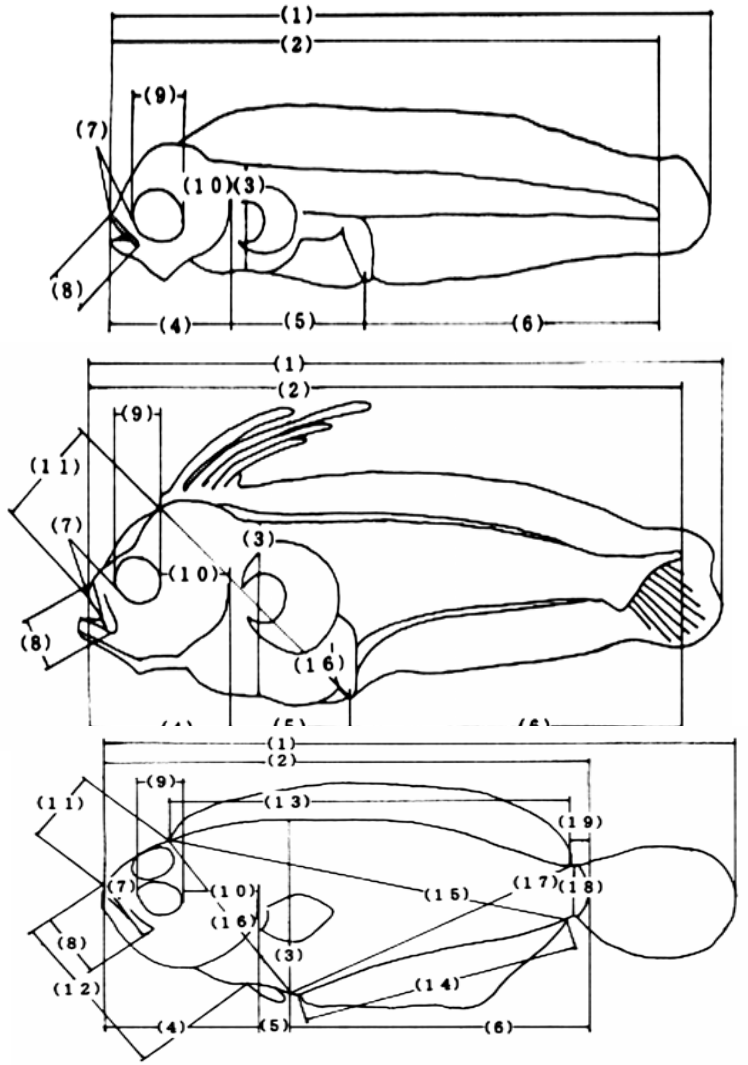
- For the development of hatchery-technology for improvement fish health preventing deformity, we have to reveal the developmental stage in the course of larval period and the characteristics of each developmental stage with special reference to morphogenesis and skeletal development.

- The definition of developmental stage is differ from the morphological classification of development (Okiyama 1974, Minami 1982). It includes morphogenetic, physiological, and ecological meanings in the course of development.

- Relative growth should be one of the fine devices to reveal the developmental stage in the course of larval development.

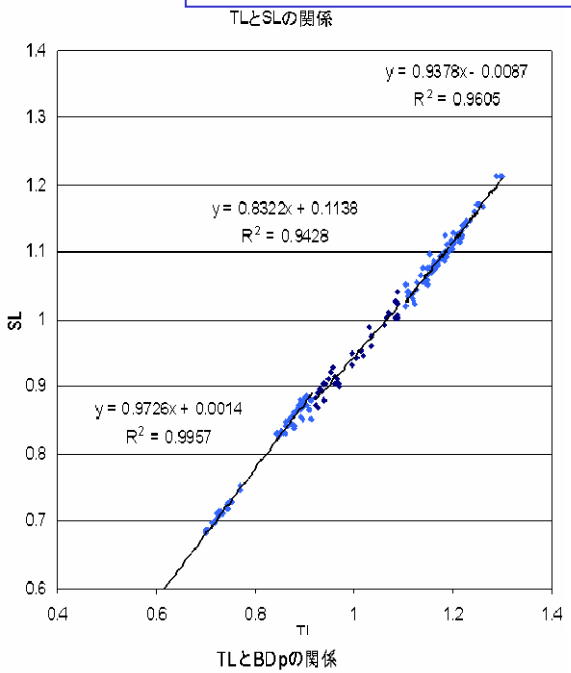
- We should define the developmental stage according to the characteristics of relative growth of juvenile Japanese flounder in the course of larval development, and distinguish the special features of morphological and skeletal development.

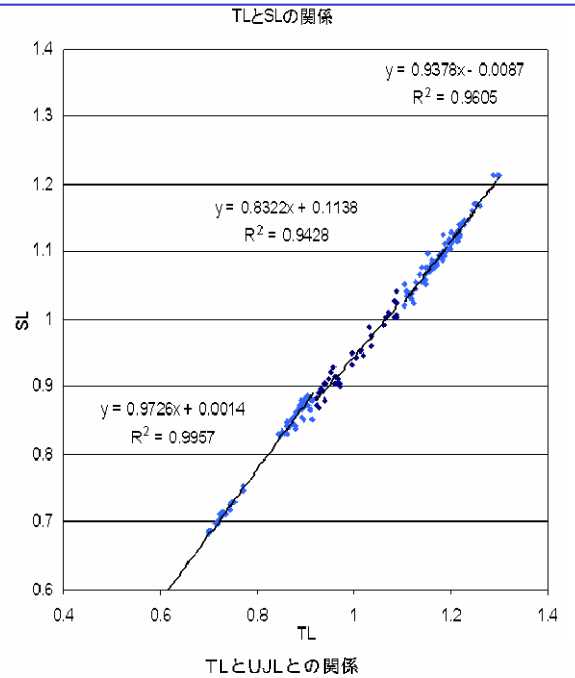
Measurement

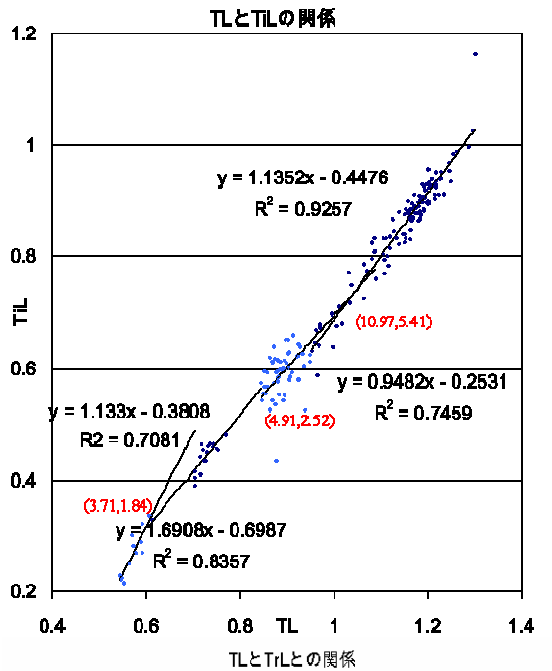


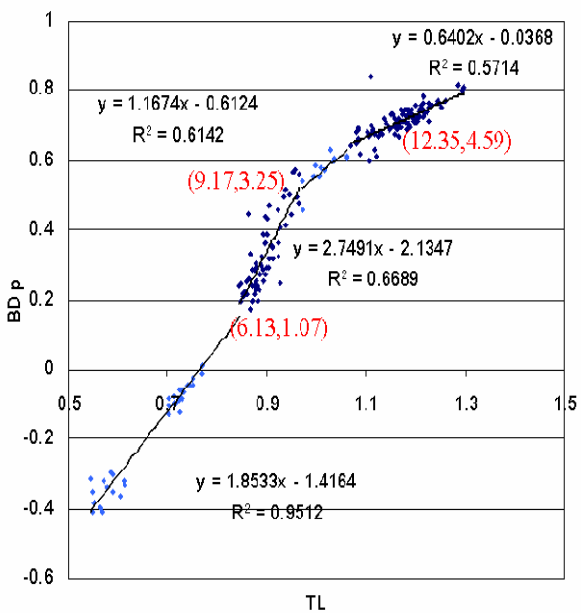
(1)	Total length	TL
(2)	Standard length	SL
(3)	Body depth	BDp
(4)	Head length	HL
(5)	Trunk length	TrL
(6)	Tail length	TiL
(7)	Snout length	SnL
(8)	Upper jaw length	UJL
(9)	Eye diameter	EDm
(10)	眼後長	RHLE
(11)	背鰭前長	FLDrF
(12)	腹鰭前長	FLPlF
(13)	背鰭基底長	DrFBL
(14)	臀鰭基底長、	AFBL
(15)	背鰭前部-臀鰭後部長	LDrAb
(16)	背鰭前部-肛門前部長	LDrA
(17)	肛門前部-背鰭後部長	LADr
(18)	尾柄高	CPDp
(19)	尾柄長	CPL

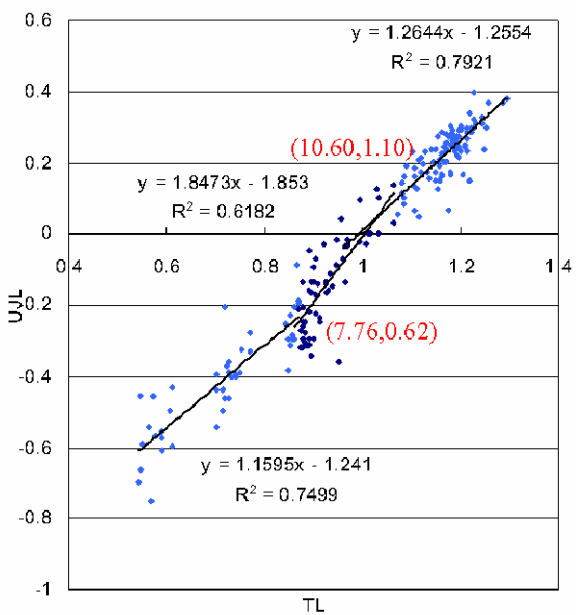
Allometric growth of hatchery-raised Japanese flounder larvae

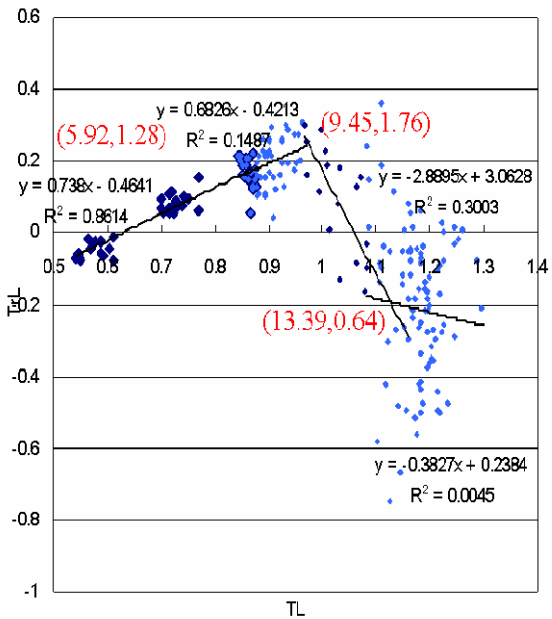








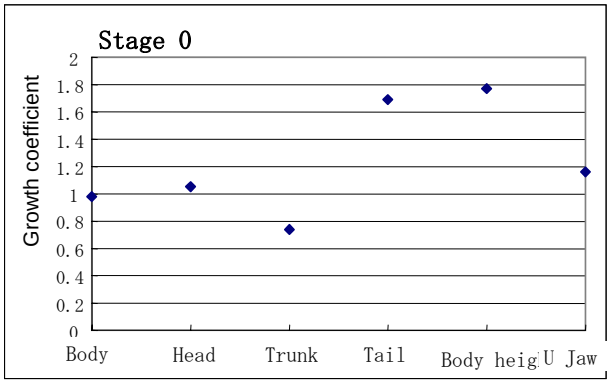


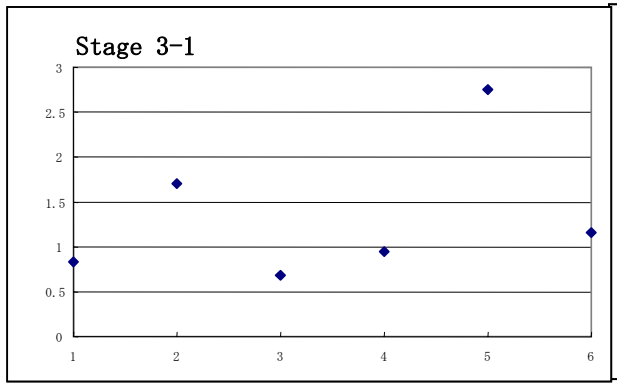


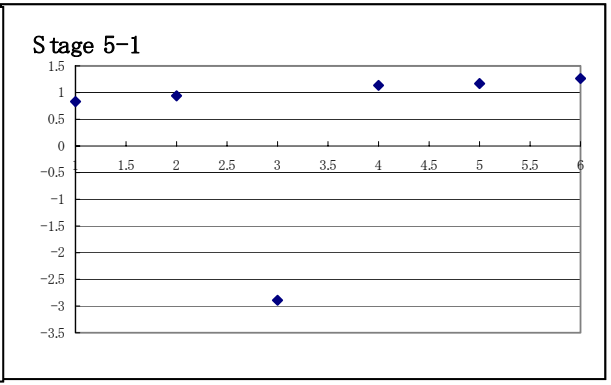
Developmental stage of larval Japanese flounder based on relative growth

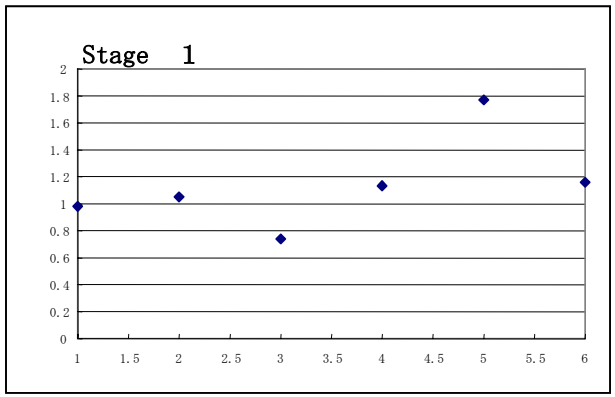
Experimental rearing conducted at march 2002

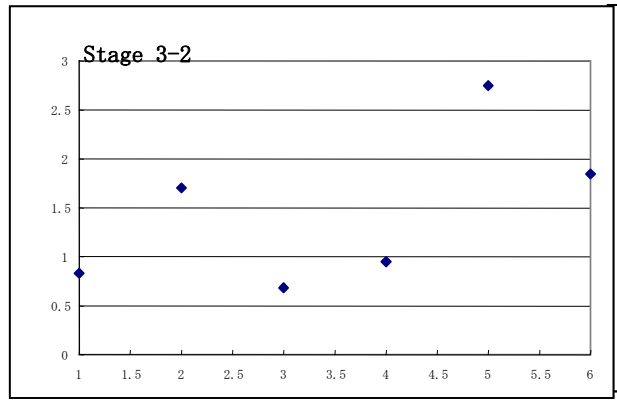
stage	TL (mm)	STL	HL	TrL	TlL	UjL	BDp
0	～3.72	0.973	1.051	0.738	1.691	1.160	1.769
1	3.72～6.32				1.133		
2	6.32～6.70	0.832		0.683	0.948		2.749
3-1	6.70～7.80		1.704				
3-2	7.80～9.17					1.847	
4	9.17～10.70			-2.890			1.167
5-1	10.70～12.35		0.941		1.135	1.264	
5-2	12.35～14.46						0.640
6	14.46～	0.938		-0.383			

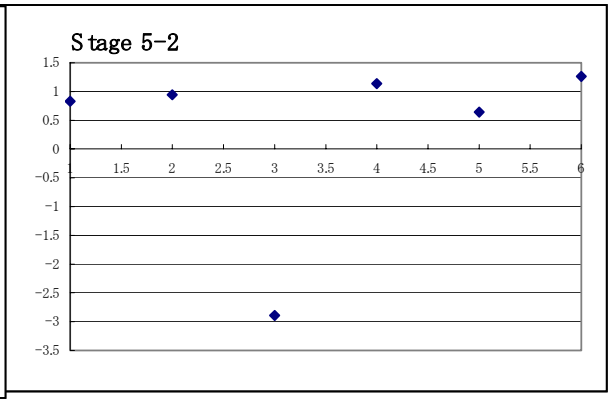


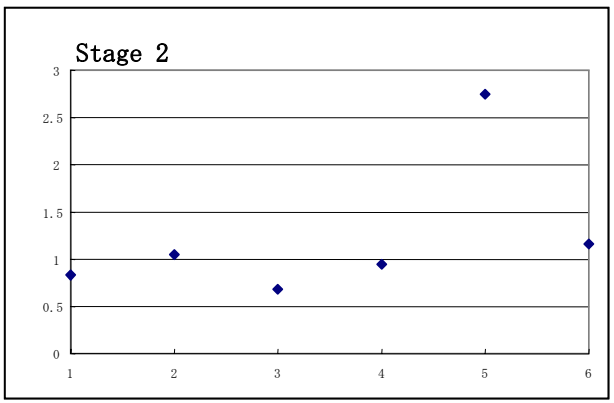


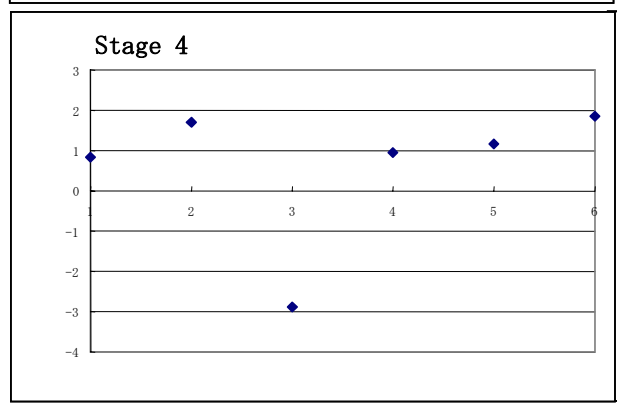


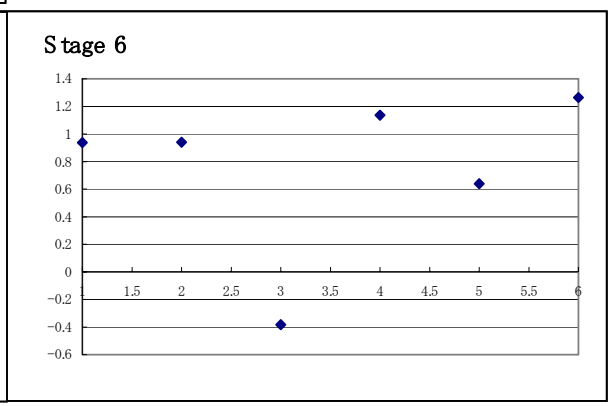






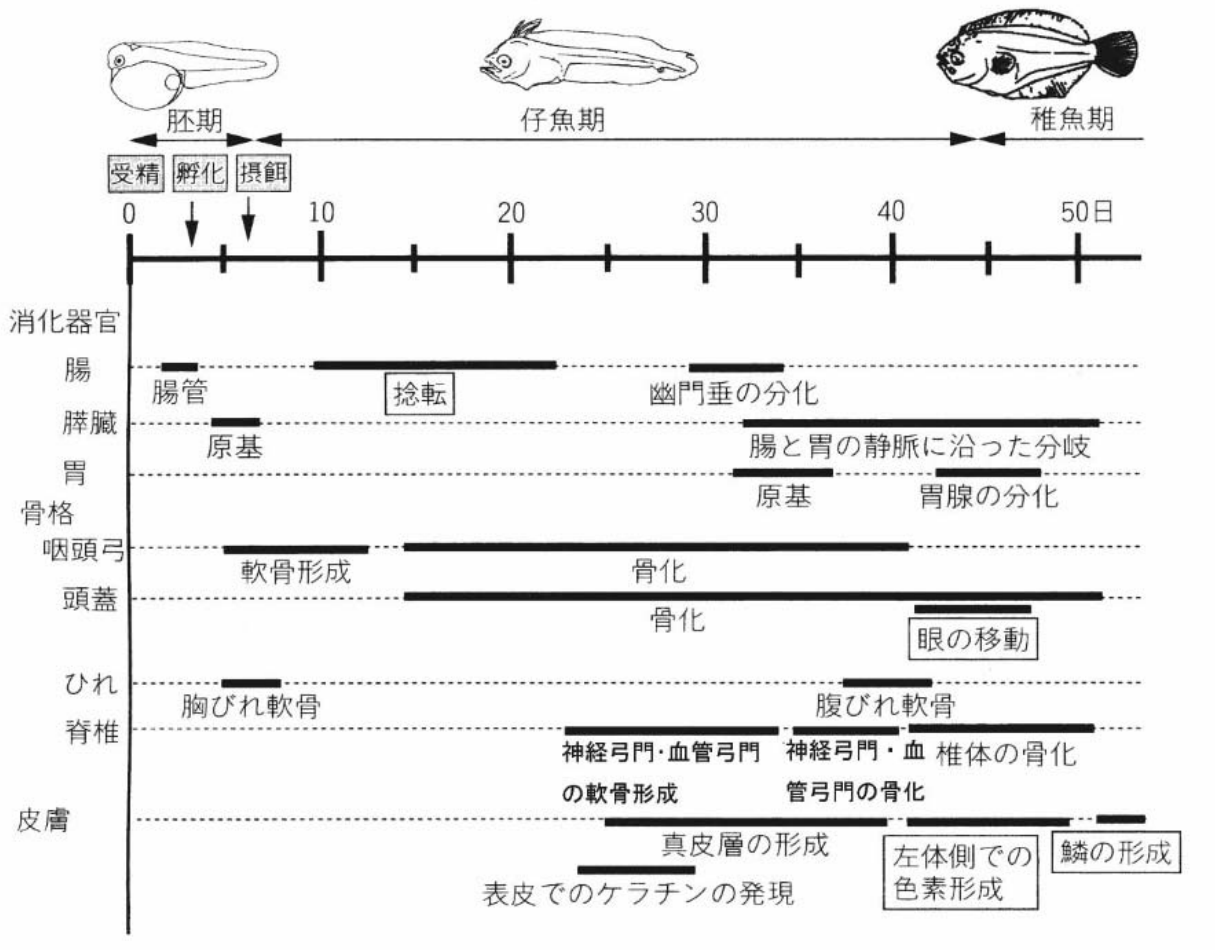




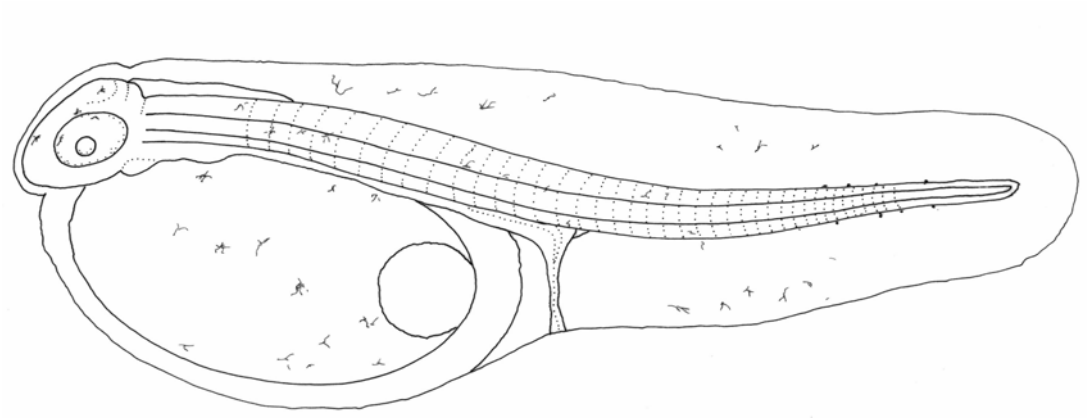


Changes of Growth Coefficients of Larval Japanese Flounder

Morphogenesis in larval period of Japanese flounder.



Developmental Stage 0



Stage 0, T L 1.86mm, 0 DAH

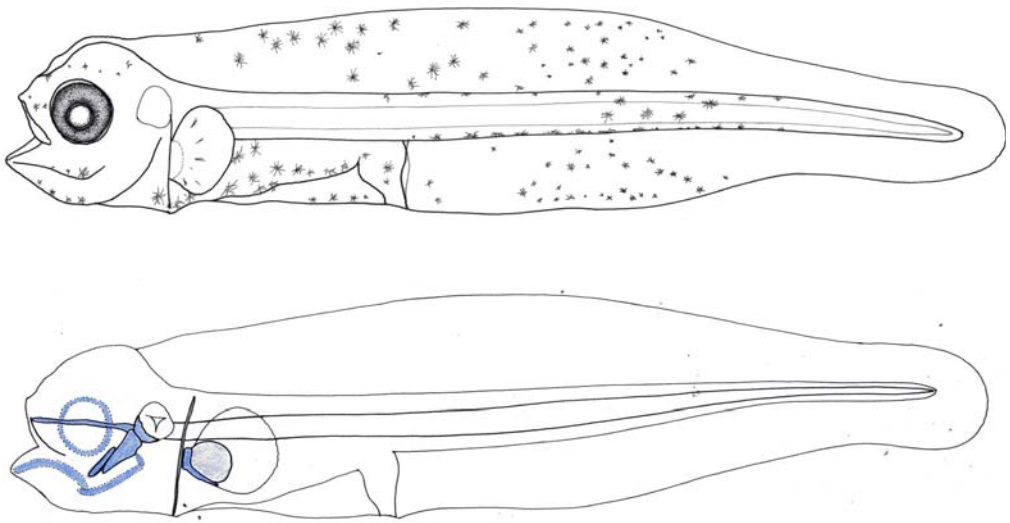
Stage 0
（TL～3.72mm、～3DAH）

• Embryo, Depending on endogenous nutrition

•Positive growth for tail, Body height; Isometric growth for body length; Negative growth for trunk

•Scattered and scarce of melanophore

Developmental Stage 1



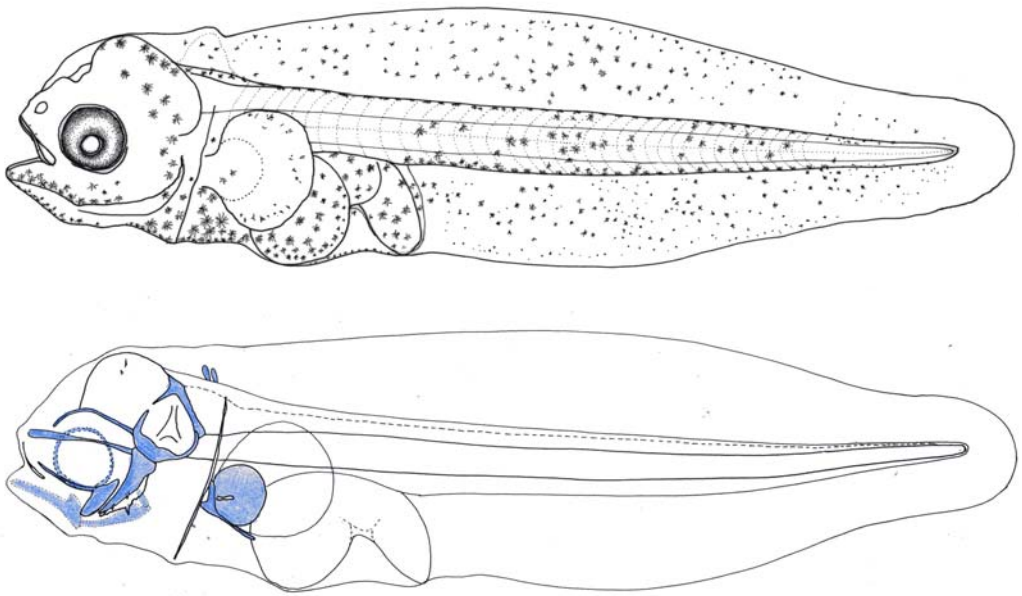
Stage 1, TL 3.84mm, 5 DAH T

Features of external morphology of ocular side and skeleton

Stage 1 (TL3.72 ~ 6.32mm, 5 DAH)

- Mouth opening, Onset of feeding on rotifer, Fry
- Tail: Positive growth to isometric growth
- Appeared: Meckelian cartilage, Hyomandibular, Symplectic, Cleoth, and Coracoid
- Melanophore: developed at dorsal and ventral areas

Developmental Stage 2



Stage 2, TL 5.84mm, 10 DAH

Features of External morphology of ocular side and Skeleton

Stage 2 （ TL6.32～6.70mm, 10 DAH ）

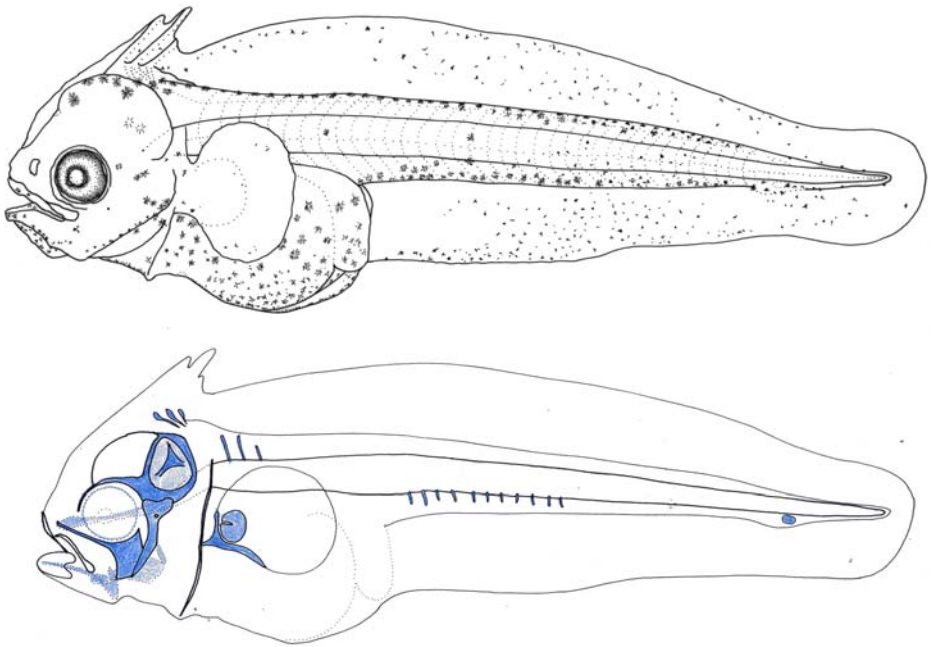
•Body height: Positive growth
•Body length & trunk: Negative growth

•Appeared: Elongated dorsal fin ray, Proximal pterygiophore

•Melanophore: Covered whole body

•Intestine: developed and onset of rotation

Developmental Stage 3-1



Stage 3-1, TL 7.03mm, 15 DAH

Features of external morphology of ocular side and skeleton

Stage 3-1 (TL 6.70 – 7.80mm, 15 – 20 DAH)

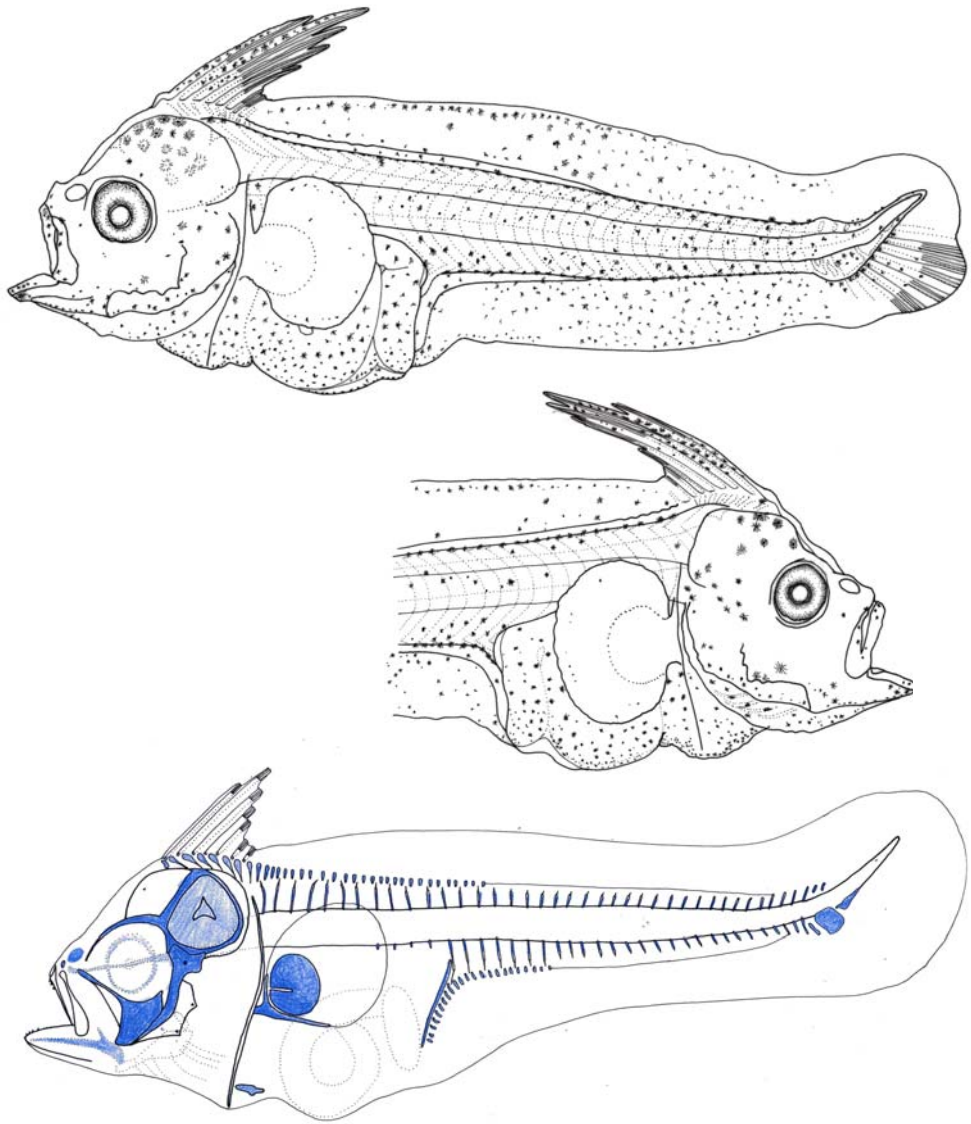
•Head: Isometric growth to positive growth

•Development of neural arch, hemal arch, neural spine, hemal spine, branchiostegals, and dorsal fin rays

•Onset of notochord flexion

•Elongated dorsal fin rays: 3-5rays, elongated

•Onset of feeding on *Artemia*



Stage 3-2, TL 7.88mm, 20DAH

Features of external morphology of ocular and blind side and skeleton

Developmental Stage 3-2

Stage 3-2 (TL 7.80 – 9.17mm, 20 – 25DAH)

•Onset of eye migration: moved to near median plane

•Upper jaw: Isometric growth to positive growth

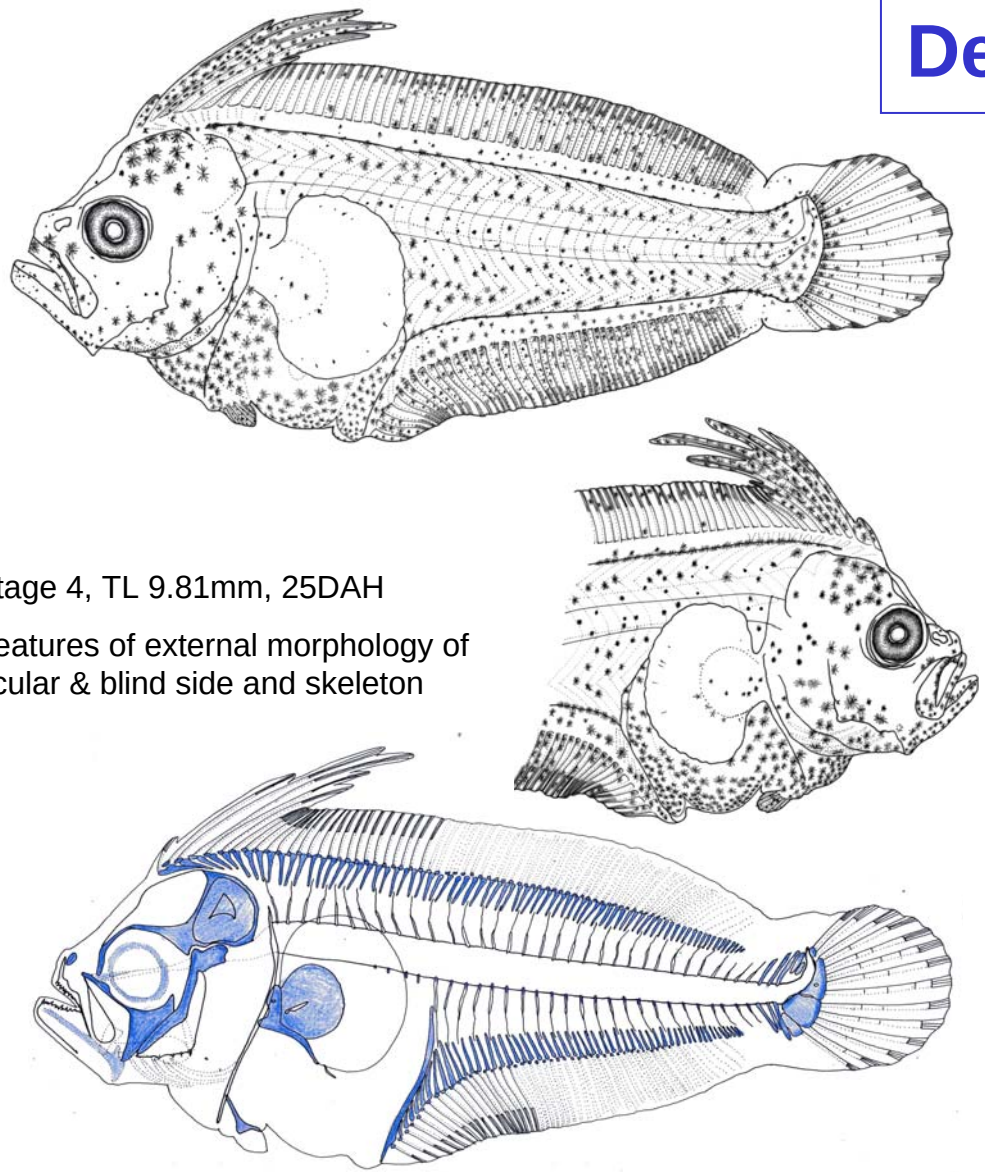
•Myotome: V-shaped to W-shaped

•Onset of compressiform

•Completion of hemal arch development and appearance of neural and hemal spine

•Elongation of elongated dorsal rays and appearance of 6th elongated rays

Developmental Stage 4



Stage 4, TL 9.81mm, 25DAH

Features of external morphology of ocular & blind side and skeleton

Stage 4 (TL 9.17 – 10.70mm, 25 – 30DAH)

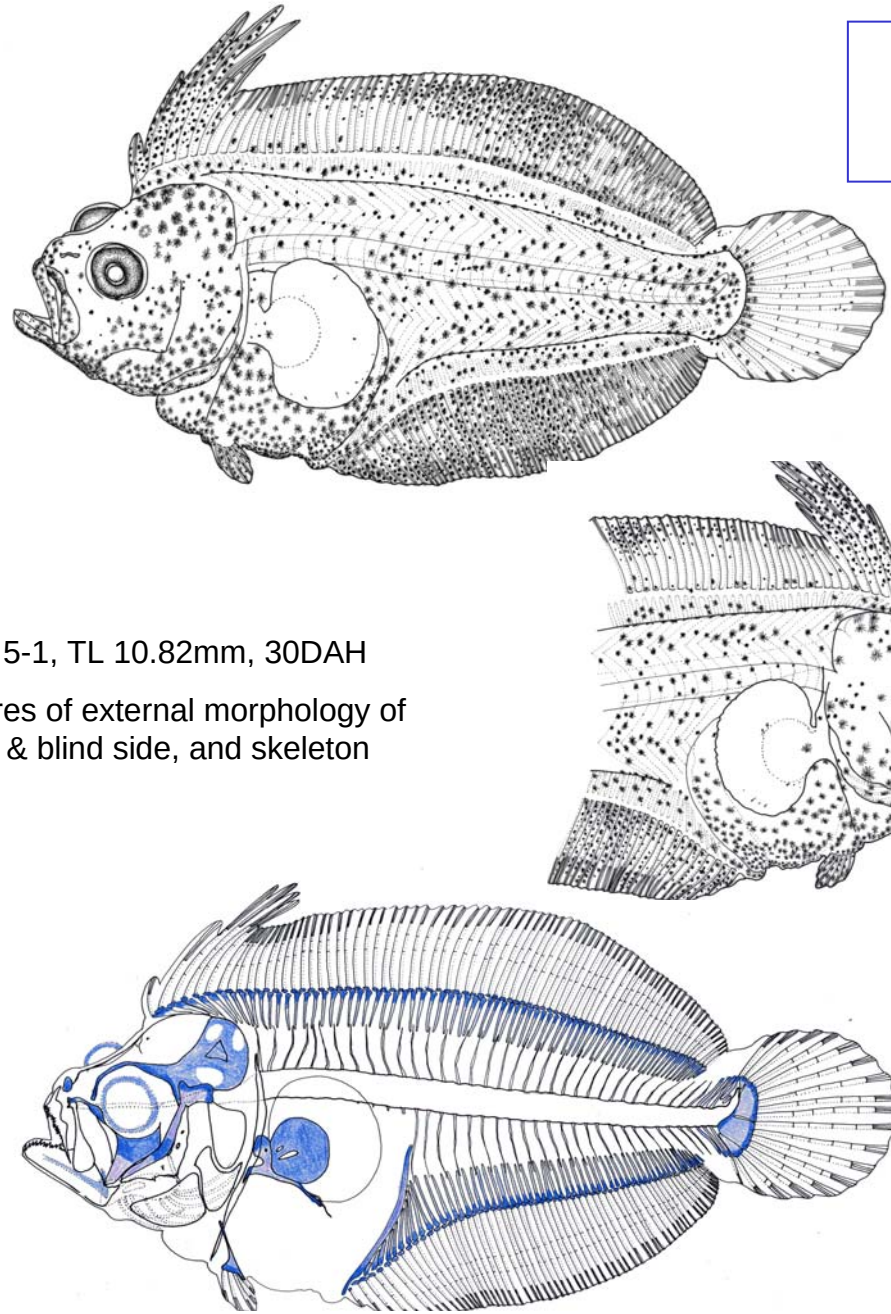
•Left side eye migrated to median plane

•Body height: Positive growth to isometric growth

•Completion of hypural plate, neural spine and hemal spine

•Dorsal and anal fin: reached to caudal

Developmental Stage 5-1



Stage 5-1, TL 10.82mm, 30DAH

Features of external morphology of ocular & blind side, and skeleton

Stage 5-1 (TL 10.70 – 12.35mm, 30DAH)ステージ6

•Head: Positive growth to isometric growth

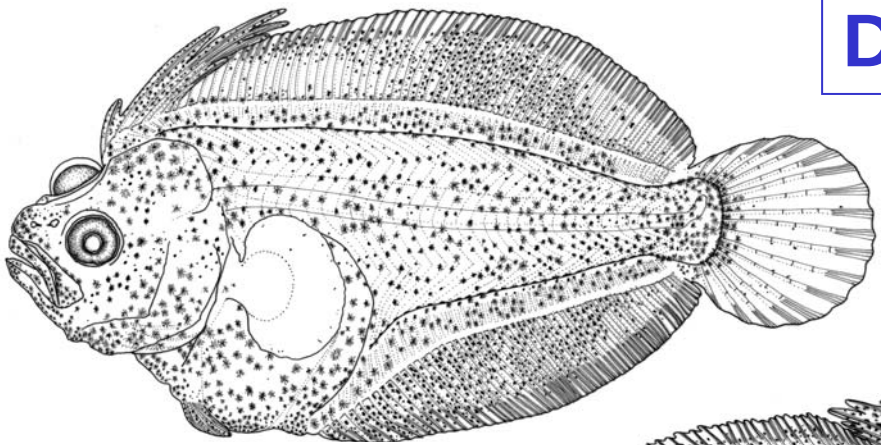
•Hypural: Onset of ossification

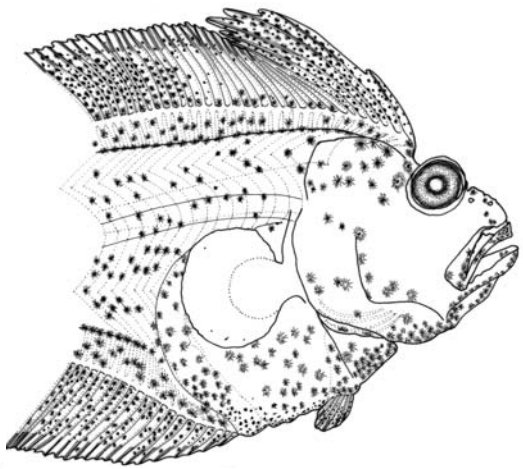
•Elongated dorsal fin: Completion of elongation

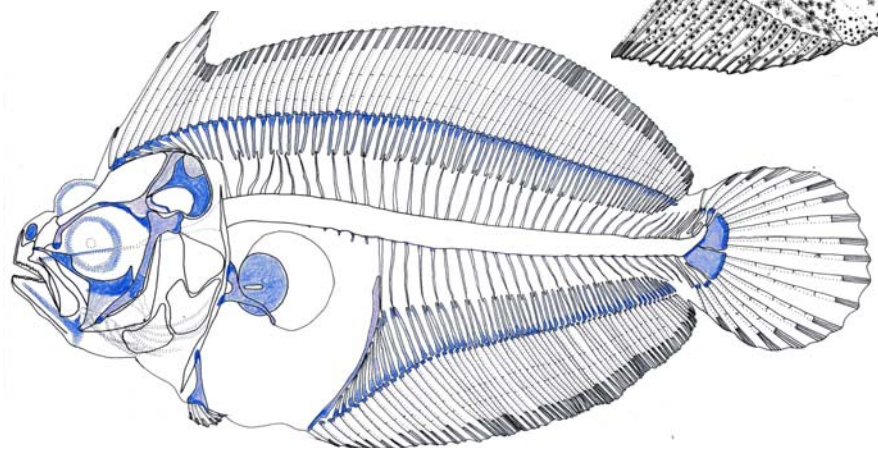
•Melanophore: Changed to adult form

•Eye migration: Appeared to ocular side

Developmental Stage 5-2







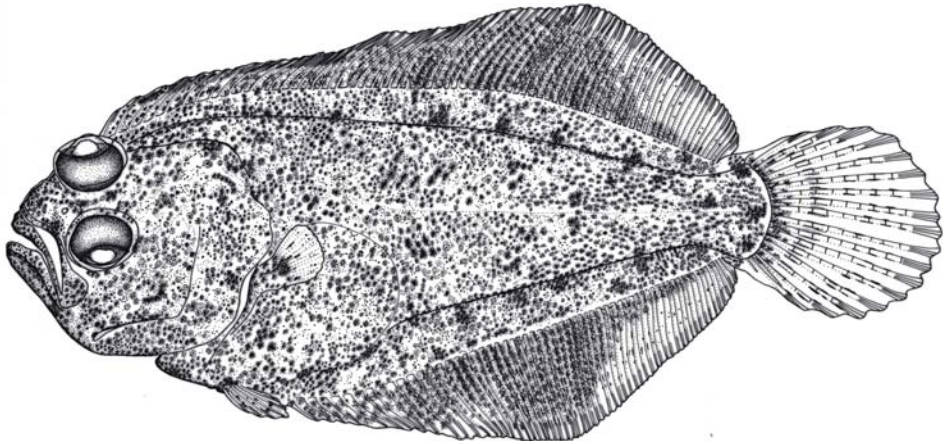
Stage 5-2 TL 12.22mm, 30DAH

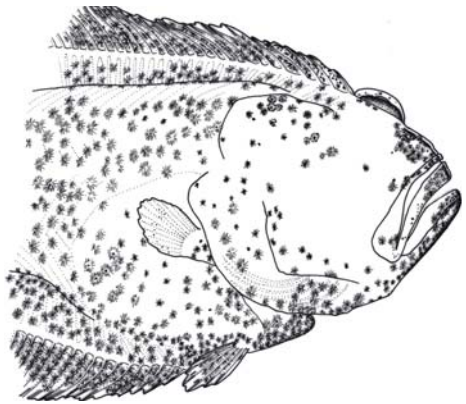
Features of external Morphology of ocular & blind side, and skeleton

Stage 5-2
(TL 12.35 – 14.46mm,30DAH)

- Body height: Negative growth
- Onset of cartilagenous ossification
- Elongated dorsal fin rays: Onset of shrinkage
- Onset of feeding on formulated feed

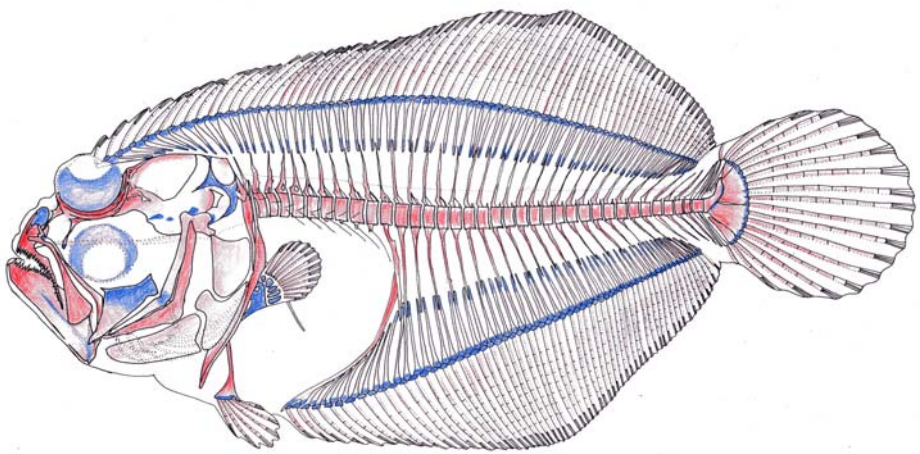
Developmental Stage 6





Stage 6, TL 16.57mm, 40DAH

Features of external morphology of ocular & blind side, and skeleton



Stage 6 (TL 14.46 - , 40DAH)

•Eye migration: Left side eye moved to right side

•Body length: Isometric growth

•Elongated dorsal fin ray: Completion of shrinkage

•Pigmentation: Decreasing of melanophore of blind side

•Completion of vertebral column and fin rays

Developmental process of skull

Syage			0	1	2	3-1	3-2	4	5-1	5-2	6
		DAH	3	5	10	15 ~ 20	20 ~ 25	25 ~ 30	30	30	30 ~ 40
		Eye Migration					Onset	Near median plate	Appear to ocular side	On the median plane	Migrate to ocular side
		Range of TL (mm)	~ 3.72	3.72 ~ 6.32	6.32 ~ 6.70	6.70 ~ 7.80	7.80 ~ 9.17	9.17 ~ 10.70	10.70 ~ 12.35	12.35 ~ 14.46	14.46 ~
Skull	Jaw skelton	Premaxilla		App							
		Maxilla		?	App						
		Dentary		App							
		M cartilage	?	App							
	Hyoid arch	Branchiostegals				App					
		Hyoid arch		App					Ossification		
	Splanchncranium Suspensorium	Symplectic	?	App					Ossification		
		Hyomandibula	?	App					Ossification		
		Quadrate	?	App							
		Ectopterygoid						App			
		Preopercle			App						
		Opercle				App					
		Subopercle				App					
		Interopercle				?					
	Basicranial region	Trabecular c	?	App							
		Parasphenoid									

Appearance of fin rays and axial skeleton of the Japanese flounder in the course of larviculture

Stage			0	1	2	3	4	5	6	7	8
Dayas after hatching			3	5	10	15 ~ 20	20 ~ 25	25 ~ 30	30	30	30 ~ 40
Stage of eye migration							Onset	Near median plate	App ocular side	On the median plate	Migrate to ocular side
Range of TL(mm) in the stage			~ 3.72	3.72 ~ 6.32	6.32 ~ 6.70	6.70 ~ 7.80	7.80 ~ 9.17	9.17 ~ 10.70	10.70 ~ 12.35	12.35 ~ 14.46	14.46 ~
Fin rays	Pec fin	shoulder girdle	?	App							
		Distal pteygiophore									App ~ Ossif
		Fin ray									App
	Dorsal fin					App				Comp	
	Anal fin							App		Comp	
	Caudal fin						App			Comp	
	Pelvic fin							App ~ Comp			
Central skelton		Neural arch				App ~ Comp					
		Hemal arch				App ~ Comp					
		Neural spine				App ~ Comp					
		Hemal spine				App ~ Comp					
		Proximal pteri			App			Comp		Ossification	
		Proximal pteri				App		Comp		Ossification	
		1st anal fin pteri				App				Ossification	
		Vertebara									

Appendicular skeleton and caudal fin skeleton

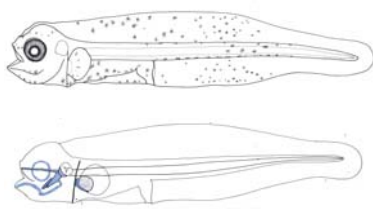
Stage		0	1	2	3-1	3-2	4	5-1	5-2	6
Days after hatching		3	5	10	15 ~ 20	20 ~ 25	25 ~ 30	30	30	30 ~ 40
Eye mygration						Onset	Near median plane	App to ocular side	On the median plane	Migrate to ocular side
Range of TL(mm)		~ 3.72	3.72 ~ 6.32	6.32 ~ 6.70	6.70 ~ 7.80	7.80 ~ 9.17	9.17 ~ 10.70	10.70 ~ 12.35	12.35 ~ 14.46	14.46 ~
Appendicular skeleton	Pelvic girdle				出現					
	Posttemporal									
	Suprracleithrum									
	Cleoth		App							
	Postcleithrum				App					
	Scapular		App							Ossificatio
	Coracoid		App							Ossificatio
Caudal fin skeleton	Epural				App		Comp		Ossification	
	1,2 Hypural				App		Comp		Ossification	
	3,4 Hypural				App		Comp		Ossification	
	5 Hypural						App ~ Comp		Ossification	
	Parhypural				App		Comp		Ossification	
	Urostylar vertebra						App ~ Comp		Ossification	

Conclusion

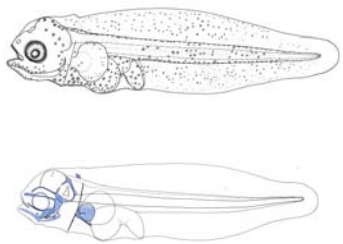
Initial stocking density: 20.000inds/m^3 , WT 18℃

Stage 0（TL～3.72mm、～3dah）
- Mouth closed
- Tail: Positive growth
- Melanophore: Scarce and undeveloped

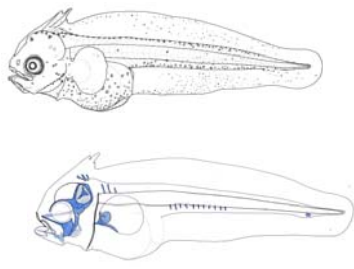
Stage 1,（TL3.72～6.32mm、5dah)
- Mouth opening, onset of feeding on rotifer
- Tail: Positive growth to isometric growth



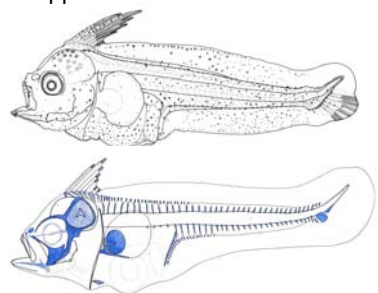
Stage 2,（TL6.32-6.70mm、10dah）
- Body height: High Positive growth
- Elongated dorsal fin ray: Appeared
- Proximal pterygiophore: ^ppeared



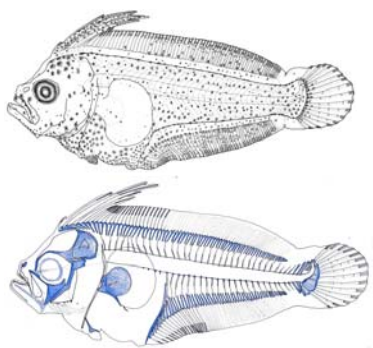
Stage 3-1,(TL6.70-7.80mm, 15～20dah)
- Eye migration: Onset
- Head: Isometric growth to positive growth
- Neural and hemal arch, neural and hemal spine, Branchiostegals, Dorsal fin ray: appeared,
- Onset of notochordal flexion



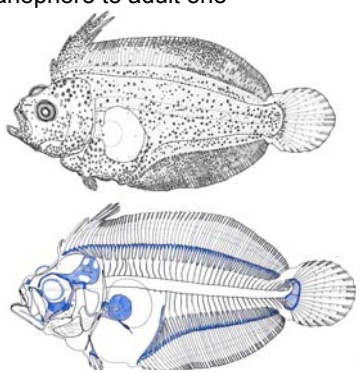
Stage 3-2, (TL7.80～9.17mm, 20～25dah
- Upper jaw: High positive growth
- Neural & hemal arch: Completion
- Neural & hemal spine: Complete of appearance
- 6^{th} elongated dorsal fin ray: Appeared



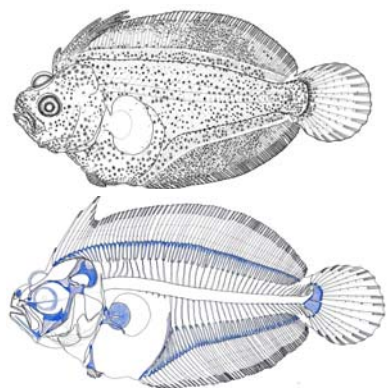
Stage 4,（TL9.17～10.70mm, 25～30dah）
- Eye migration: Near median plane
- Tail: Negative growth to isometric growth
- Hypural plate, Neural & hemal spine: Completion



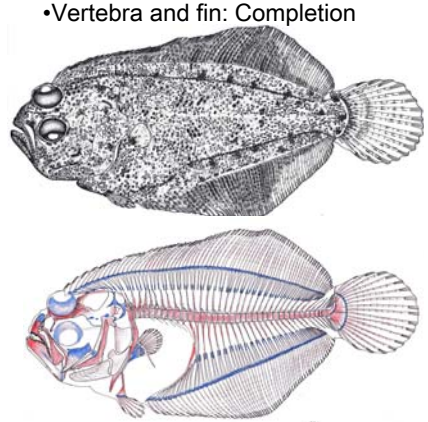
Stage 5-1,（TL10.70～12.35mm, 30dah)
- Head: Positive growth to isometric growth
- Hypural: Onset of ossification
- Elongated dorsal fin: Completion
- Melanophore: Juvenile melanophore to adult one



Stage 5-2, (TL12.35～14.46mm, 30dah）
- Body height: Isometric growth to negative growth
- cartilagenous ossification: Onset

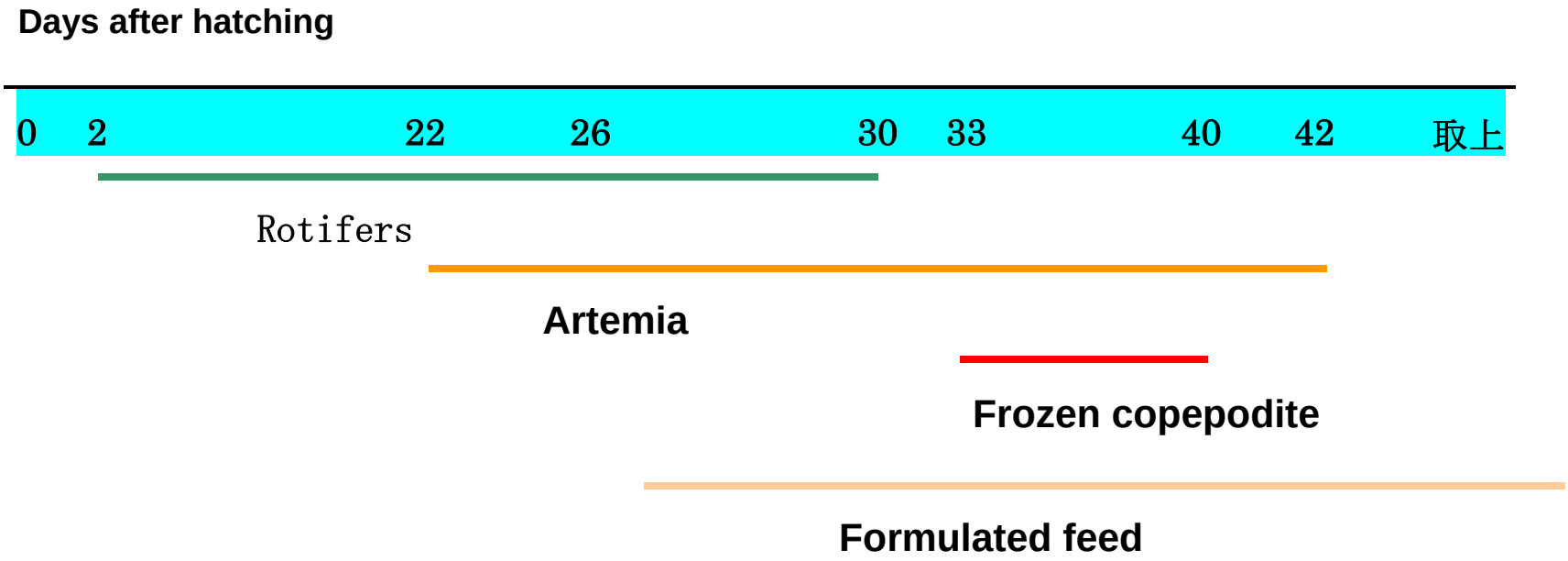


Stage 6,（TL14.46mm～, 40dah）
- Eye migration: Move to ocular side
- Head: Positive growth to isometric growth
- Body length: Negative growth to isometric growth
- Pigmentation: Adult form, decreasing in blind side
- Vertebra and fin: Completion



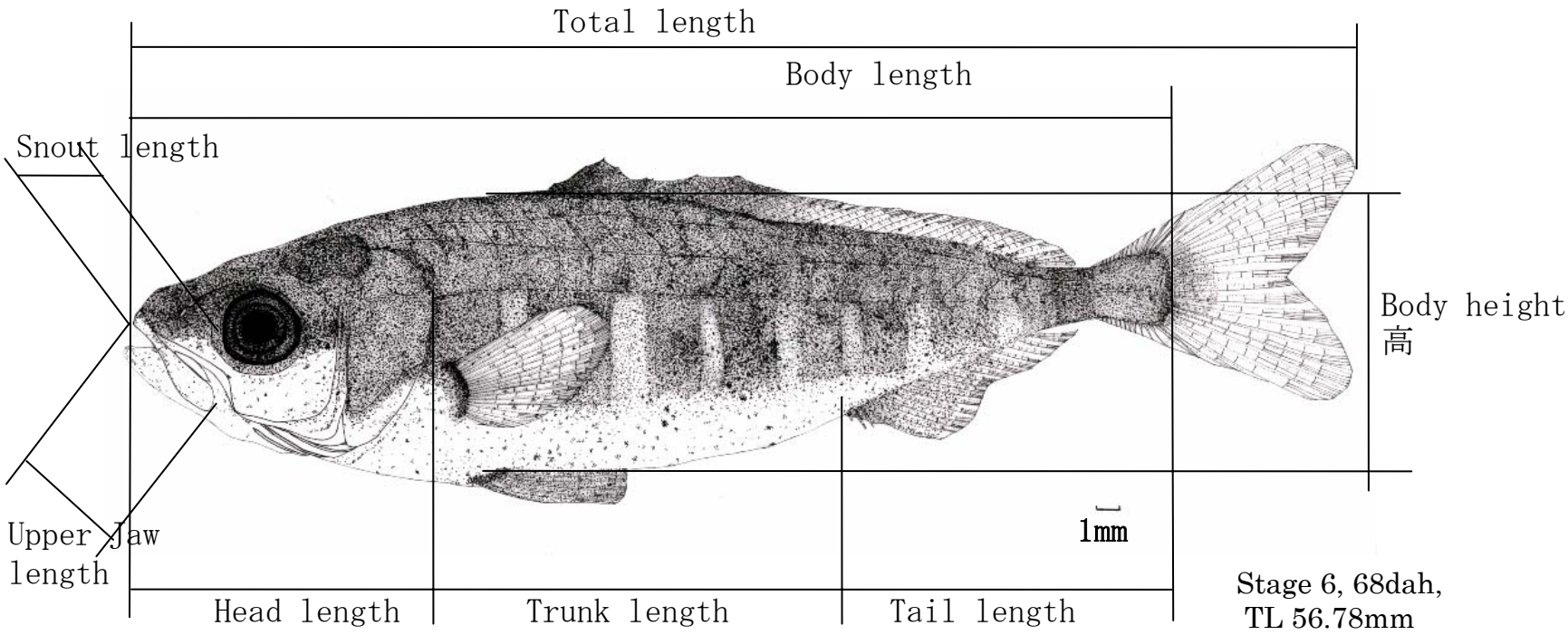
Developmental stages of hatchery-raised yellow tail based on relative growth with their special features of morphogenesis

Feeding regime of yellowtail larviculture applied by JASFA Yashima in 2001



Opening of air bladder：5－10DAH

Measurement



Allometry equation

Positive growth

$Y = aX^b$

1 Isometric growth

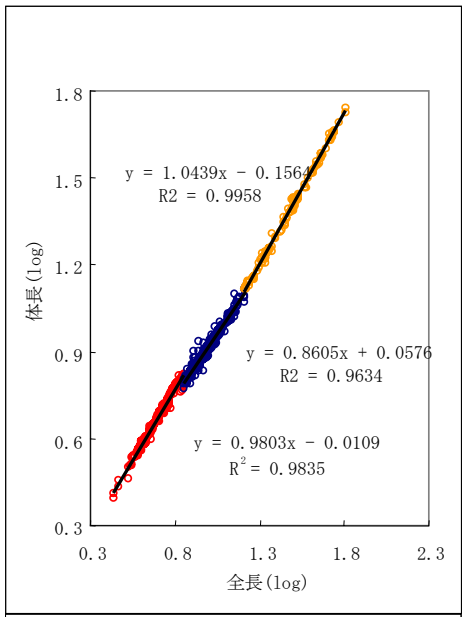
$\log y = \log a + b \log x$

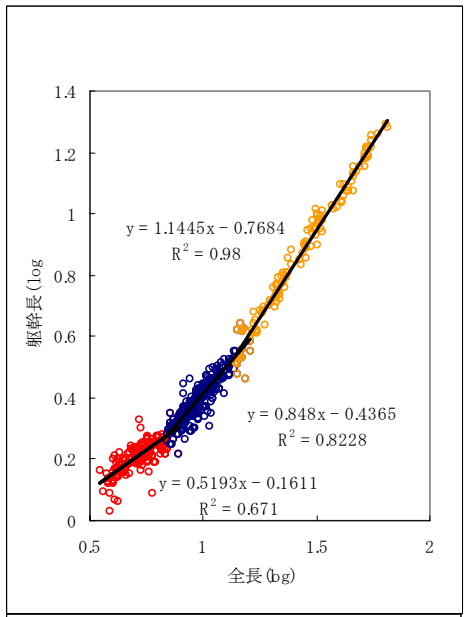
b>1

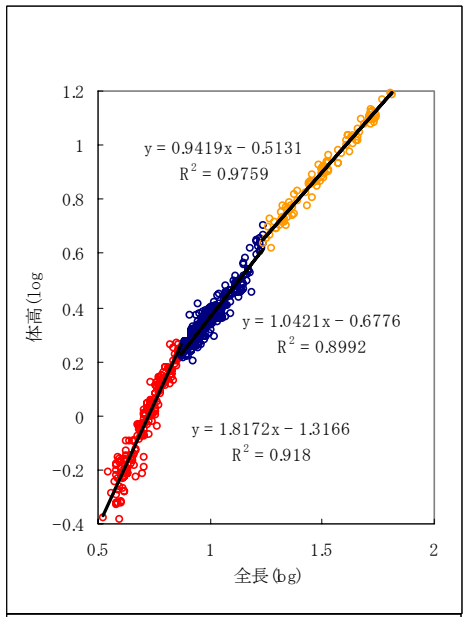
b=

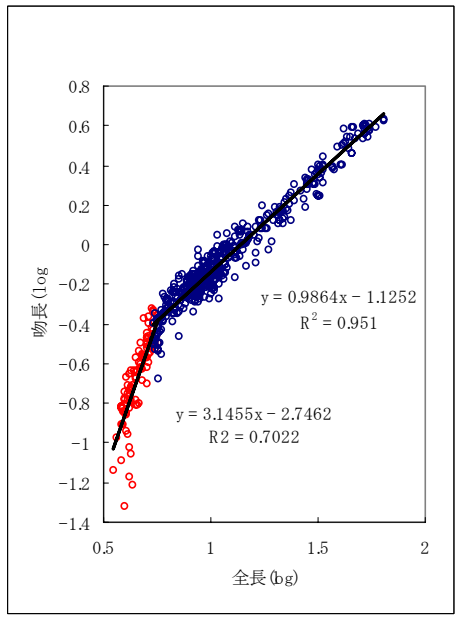
b<1

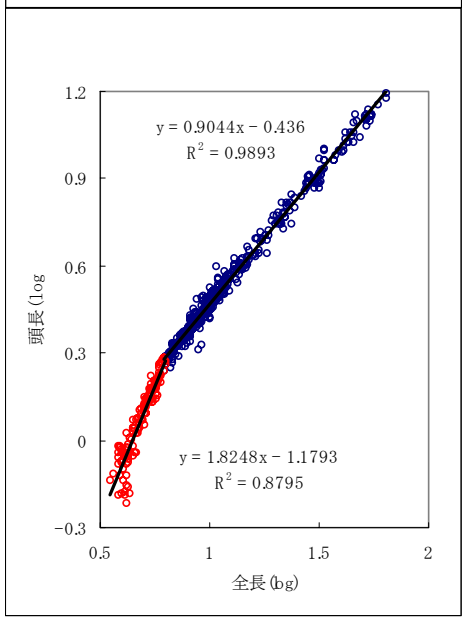
Allometric growth

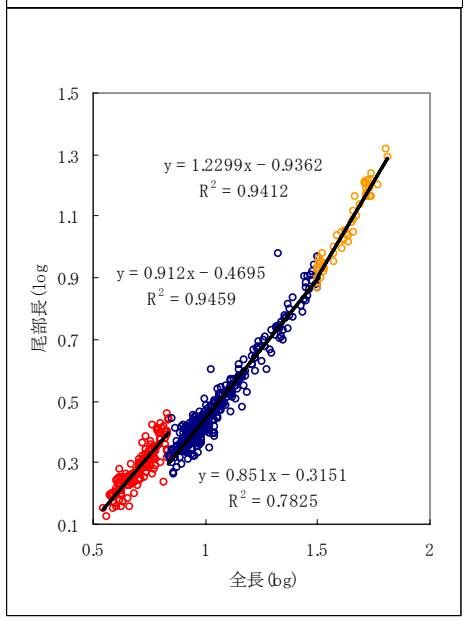


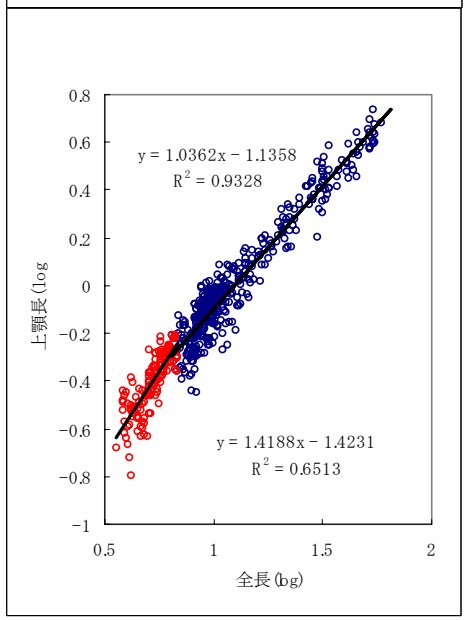










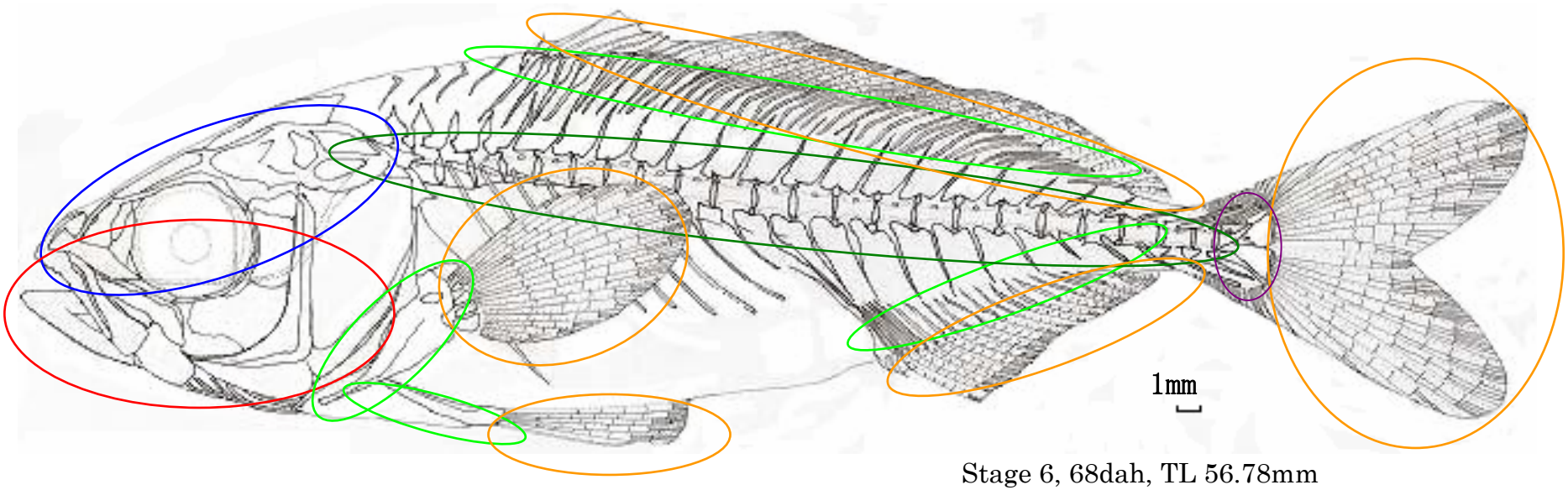


Developmental Stage

Stage		DAH (Range)	TL	BL	Head	Trunk	Tail	BH	U Jaw	Snout
0		1 (0～5)	～3.73	0.9803	1.8248	0.5193	0.851	1.8172	1.4188	3.1455
1	1-1	6 (1～14)	3.73～5.64	0.8605						
	1-2	14 (10～17)	5.64～6.42						1.0362	0.9844
2		15 (13～17)	6.42～6.88		0.9044					
3		23 (16～35)	6.88～13.16			0.8480	0.912	1.0421		
4		31 (26～35)	13.16～14.68			1.1445				
5	5-1	31 (27～35)	14.68～17.31	1.0439						
	5-2	40 (30～50)	17.31～29.38					0.9419		
6		59 (45～68)	29.38～64.52				1.2299			

※Figure shows allometric growth coefficient

Skeleton of yellow tail



Stage 6, 68dah, TL 56.78mm

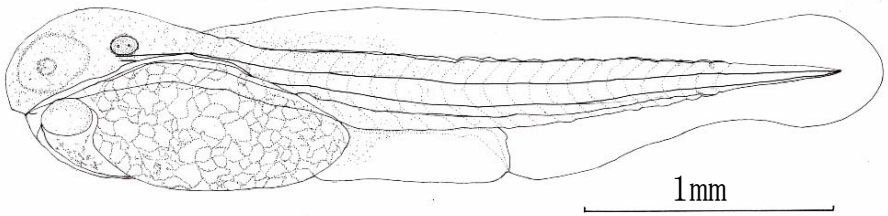
Region	○ : Neurocranium ○ : Vertebral Column ○ : Appendicular skeleton ○ : Splanchnocranium ○ : Caudal fin skeleton ○ : Fin
Ossification	Cartilage Bone : < Cartilagenous ossification Intermembranous ossification

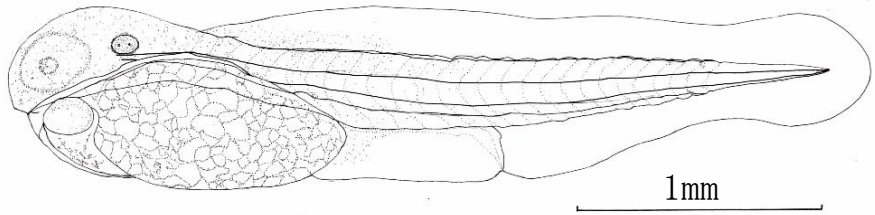
Stage 0, TL 2.72～3.73mm

Blue : Cartilage Red : Bone
Purple : Under ossification,

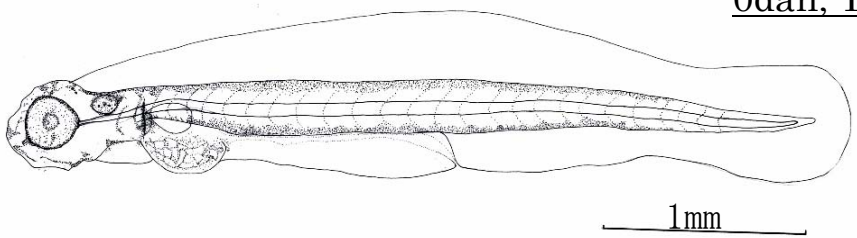
External morphology

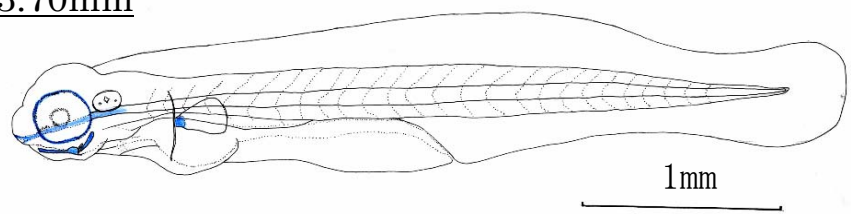
Skeleton





0dah, TL 3.70mm





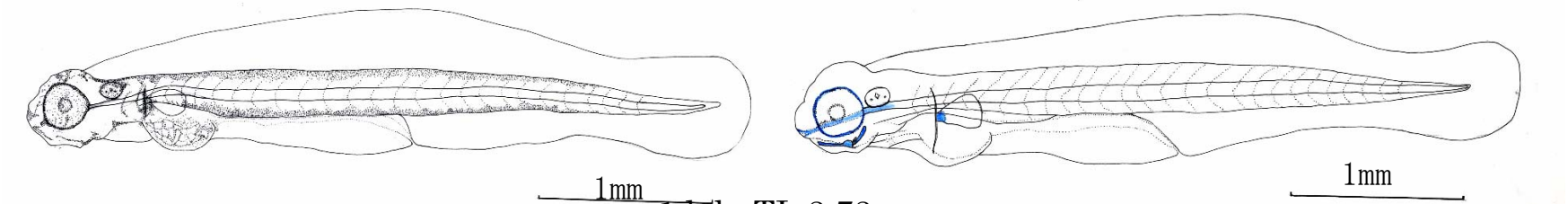
1dah, TL 3.73mm

- Body length: Isometric growth (α = 0.9803), Body height: Positive growth (α = 1.8172) , Head: Positive growth (α = 1.8248) , Trunk: Negative growth (α = 0.51939, Tail: Isometric growth (α = 0.8510), Upper jaw: Positive growth (α = 1.4188), Snout: Positive growth (α = 3.1455)
- Below TL3.4mm, Myotome 14+13=27
- Embryo just hatched out did not have skeletal tissue.
- Melanophore: Dispersed to head, dorso- and vent-lateral areas at TL3.7mm.
- Cartilages of skull appeared at TL 3.7mm.
- Shoulder girdle: Cleoth and radial appeared at TL 3.7mm.

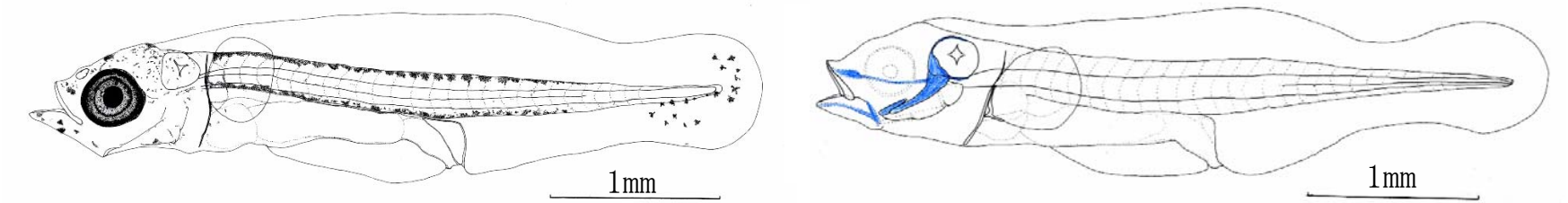
Stage 1-1, TL 3.73～5.64mm

Blue : Cartilage Red : Bone
Purple : Under ossification, Cartilage to bone

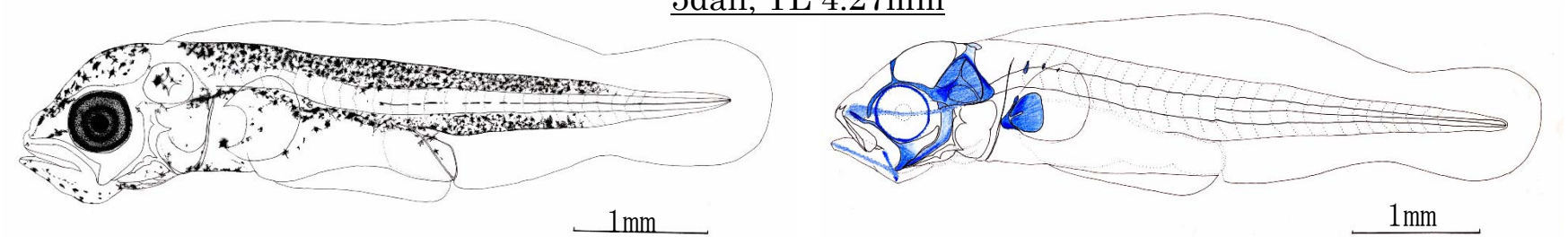
External morphology Skeleton



1dah, TL 3.73mm



5dah, TL 4.27mm

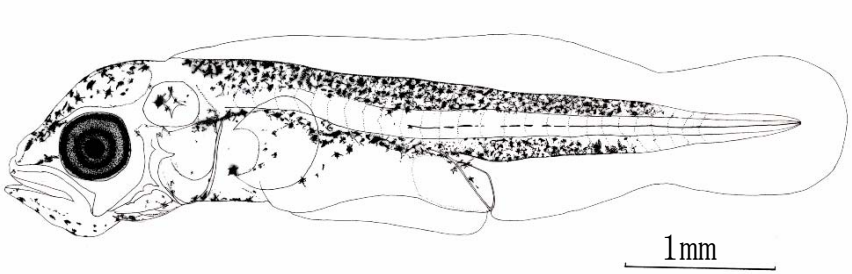


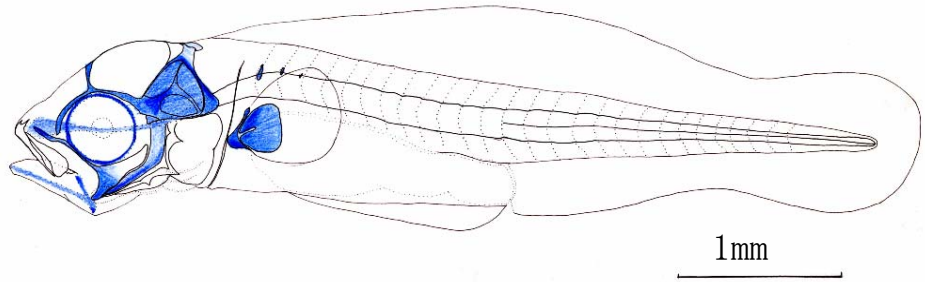
9dah, TL 5.60mm

- Body length: Isometric growth to negative growth
- Mouth opening at TL 3.9mm and onset of feeding on rotifer
- Teeth: Appeared at TL 3.9mm
- Nostril appeared as single pore
- Skull developed
- Neural & hemal arch: appeared
- Parhypural: Appeared

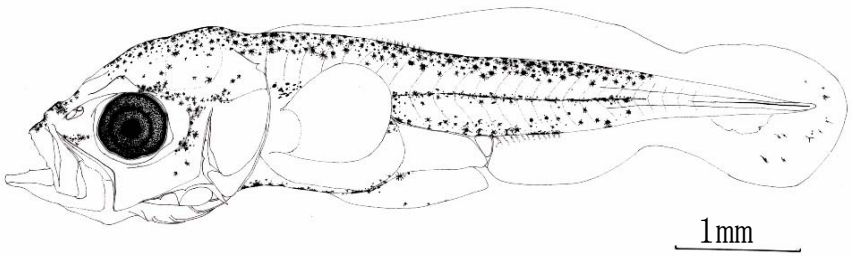
Stage 1-2, TL 5.64～6.42mm

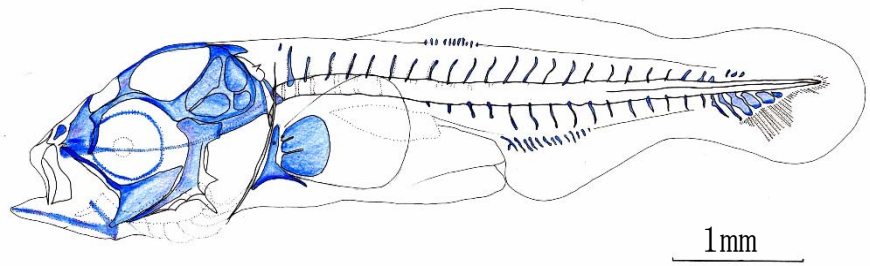
Blue：Cartilage　Red：Bone
Purple：Under ossification, Cartilage to bone





9dah, TL 5.60mm



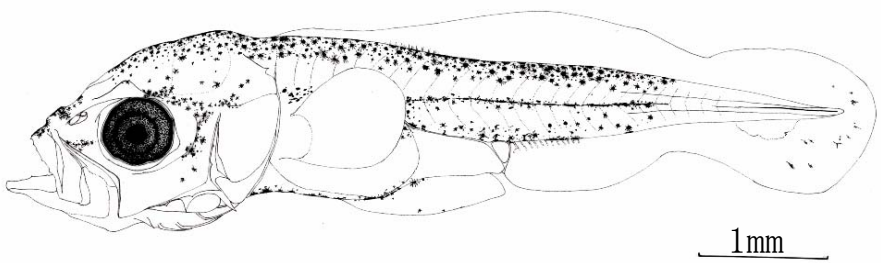


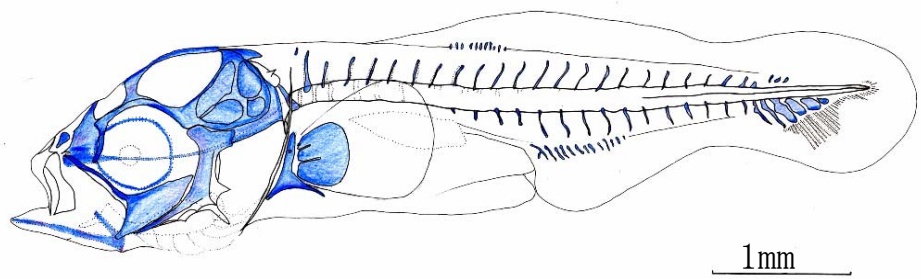
17dah, TL 6.48mm

- Upper jaw: Positive growth to isometric growth, Snout: Positive growth to isometric growth
- Neurocranium: Completion of appearance
- Neural & hemal arch: Completion of appearance
- Vertebral column: Segmentation of notochord
- Fin: Appearance of proximal pterygiophore, development of unpaired fin
- Feeding:: Just rotifer

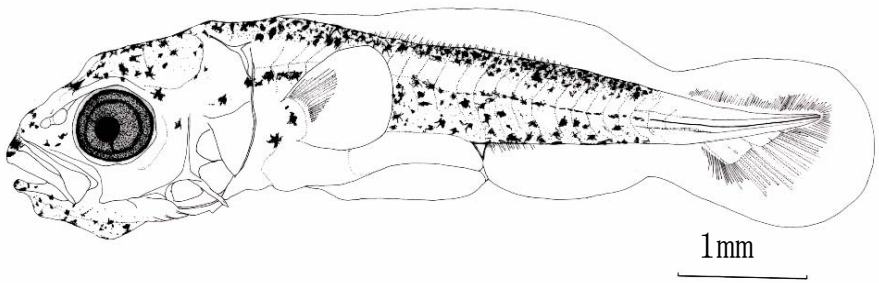
Stage 2, TL 6.42～6.88mm

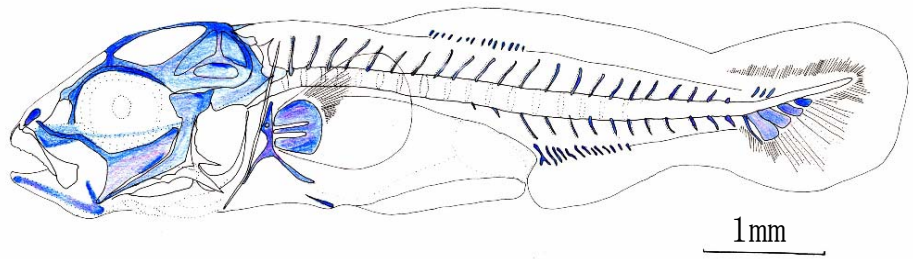
Blue：Cartilage　Red：Bone
Purple：Under ossification, Cartilage to bone





17dah, TL 6.48mm



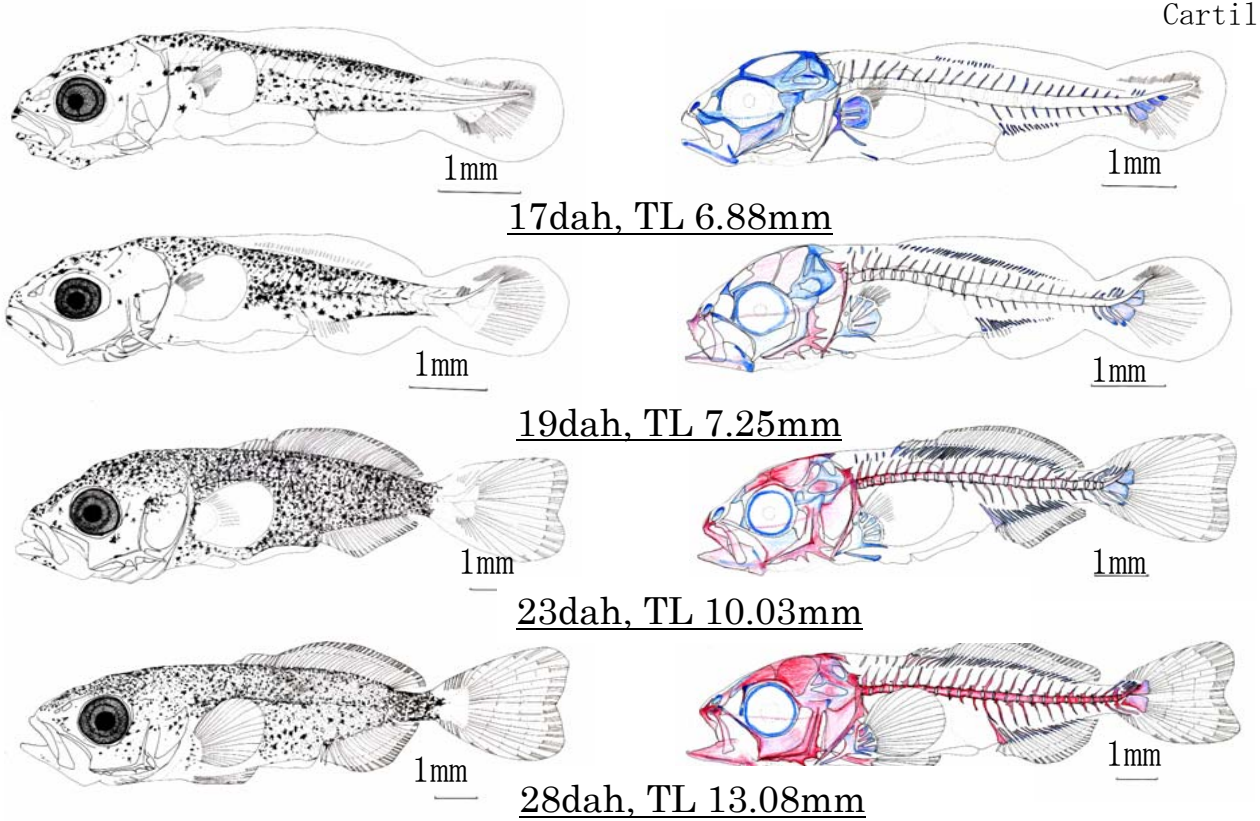


17dah, TL 6.88mm

- Head: Positive growth to isometric growth
- Nostril: Two pores
- Flexion of notochord: Onset
- Shoulder girdle: Cleoth connected to occipital
- Pectoral fin: Fin ray appeared
- Feeding: Just on rotifer

Stage 3, TL 6.88～13.16mm

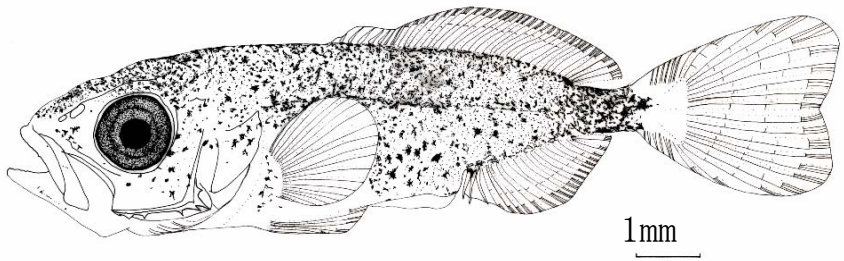
Blue : Cartilage Red : Bone
Purple : Under ossification, Cartilage to bone

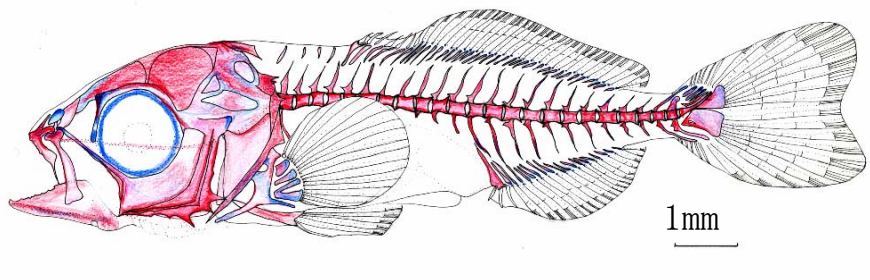


- Body height: Positive growth to isometric growth, Trunk: Negative growth to isometric growth, Tail: isometric growth
- Vertebral column: Neural & hemal arch and hemal spine completely appeared. Onset of ossification of vertebra.
- Caudal skeleton: Completely appeared. Hypural plate appeared.
- Feeding: Mixing feeding period. Rotifer to Rotifer + *Artemia* + formulated feed

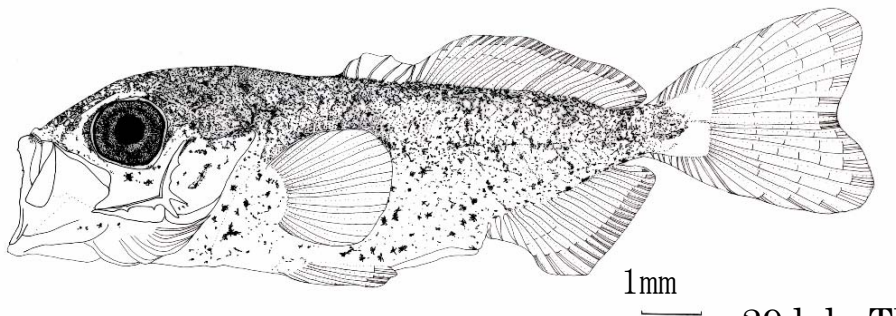
Stage 4, TL 13.16～14.68mm

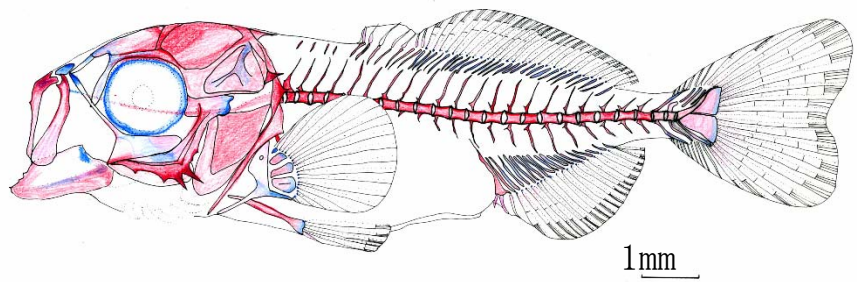
Blue : Cartilage Red : Bone
Purple : Under ossification, Cartilage to bone





28dah, TL 13.08mm



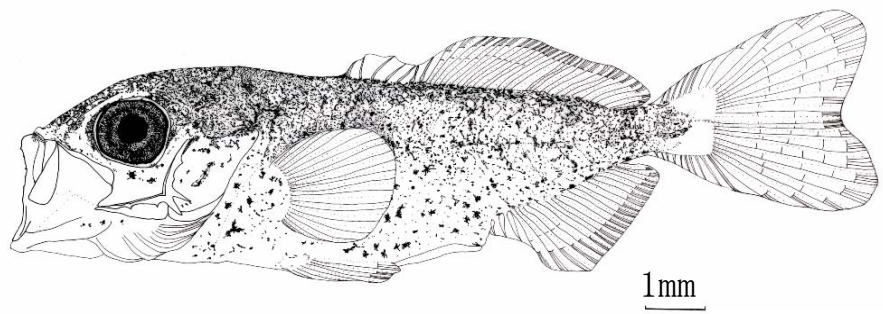


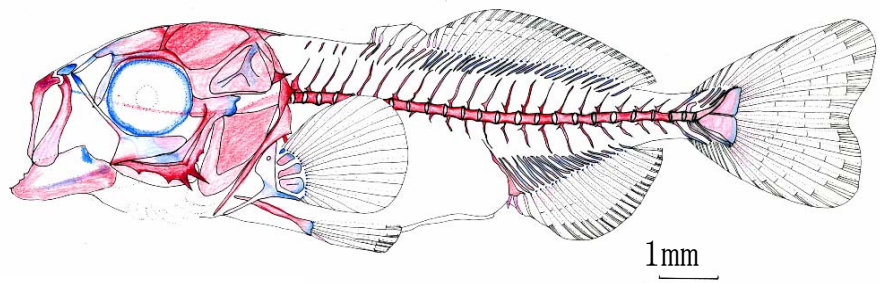
29dah, TL 14.66mm

- Trunk: Negative growth to Positive growth.
- Myotome: V-shaped to W-shaped.
- Ossification: Completion of Vertebral column
- Appencicular skeleton: Completion of appearance.
- Feeding: Mixing feeding period. Rotifer + *Artemia* + formulated feed, *Artemia* + formulated feed.

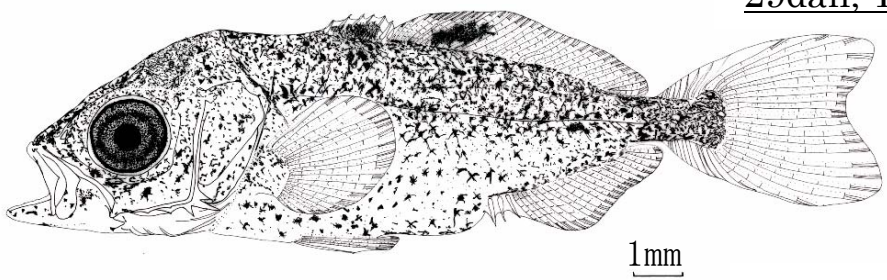
Stage 5-1, TL 14.68～17.31mm

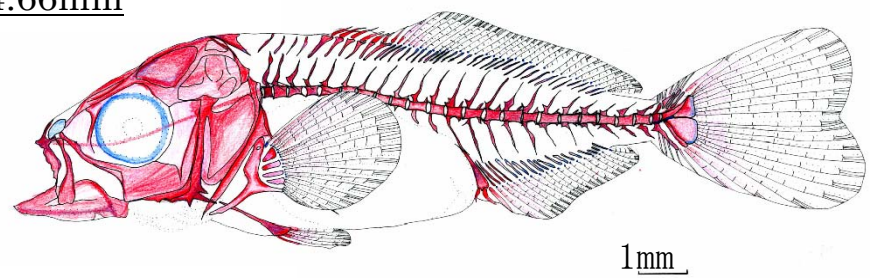
Blue : Cartilage Red : Bone
Purple : Under ossification, Cartilage to bone





29dah, TL 14.66mm



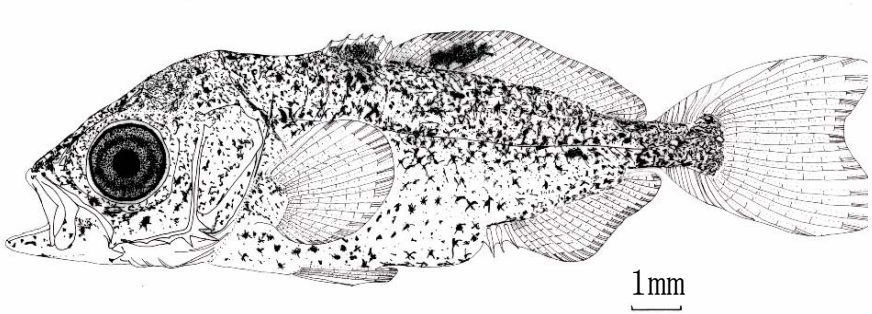


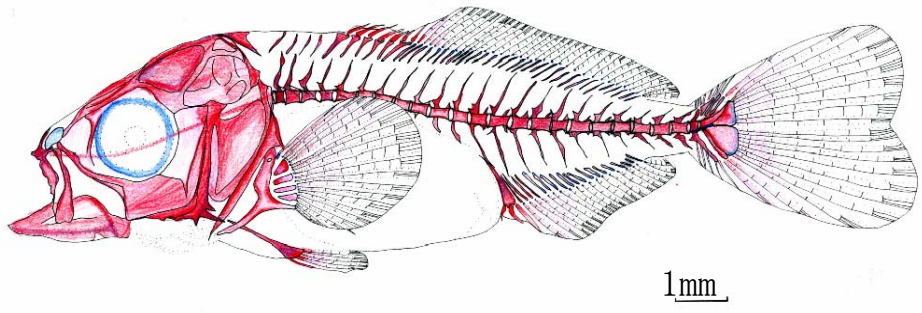
35dah, TL 17.22mm

- Body length: Negative growth to Isometric growth.
- Juvenile: Skeleton and fins completely appeared, larval membrane disappeared.
- Feeding: Mix feeding period. Artemia + Formulated feed, Artemia + Frozen copepod +formulated feed.

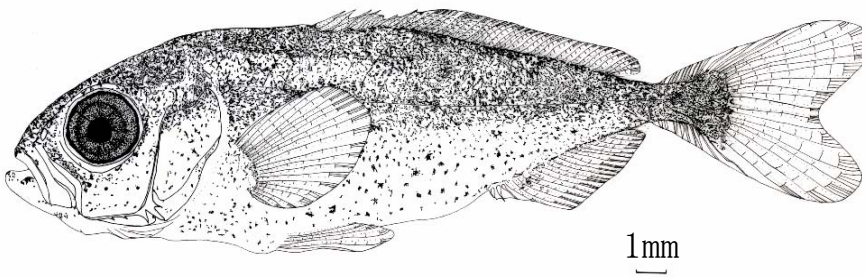
Stage 5-2, TL 17.31～29.38mm

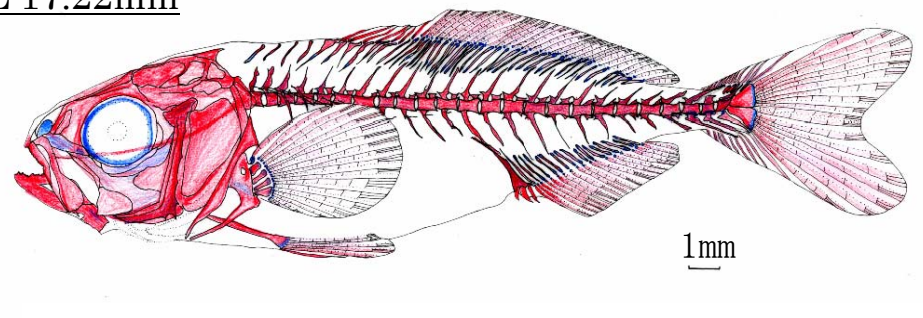
Blue : Cartilage Red : Bone
Purple : Under ossification, Cartilage to bone





35dah, TL 17.22mm



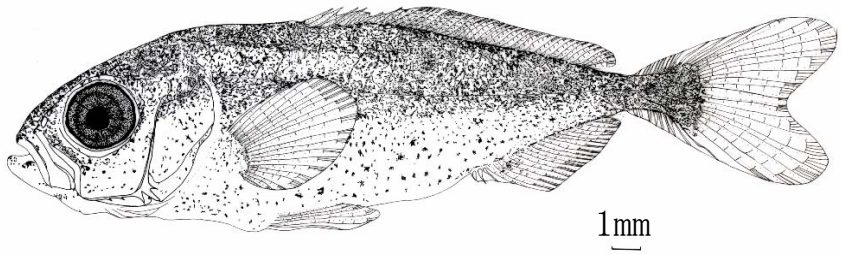


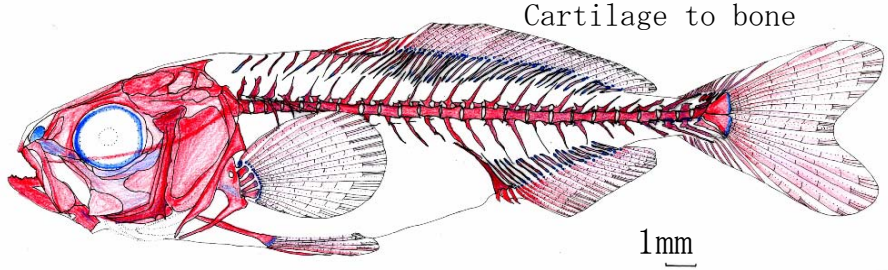
40dah, TL 29.38mm

- Body height: Isometric growth to negative growth.
- Body color: Melanophore spread to whole body. Black colored lateral band appeared at caudal peduncle.
- Ossification of skeleton: Completed except fin rays.
- Feeding: Mix feeding to just formulated feed. Artemia + Frozen copepod + Formulated feed, Artemia + formulated feed, formulated feed only.

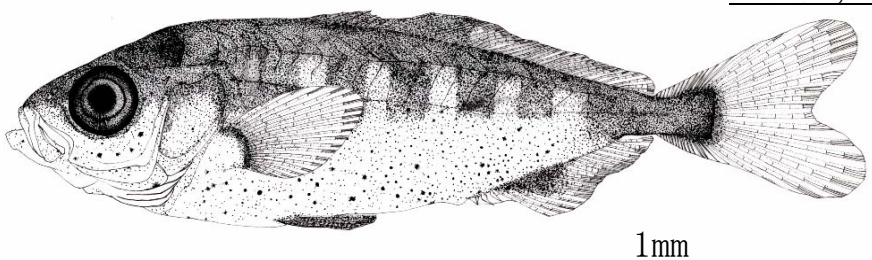
Stage 6, TL 29.38～

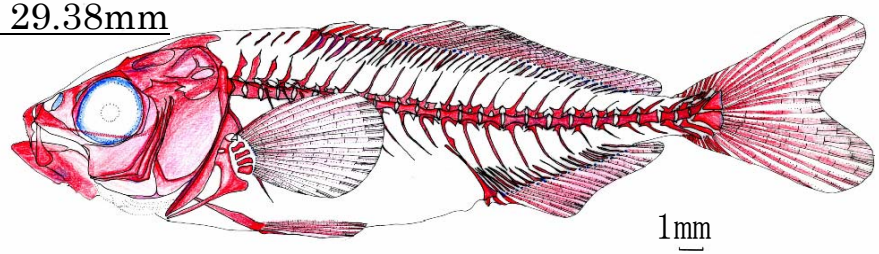
Blue : Cartilage Red : Bone
Purple : Under ossification,
Cartilage to bone



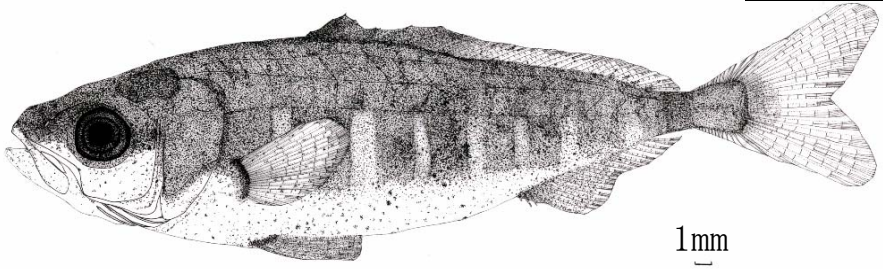


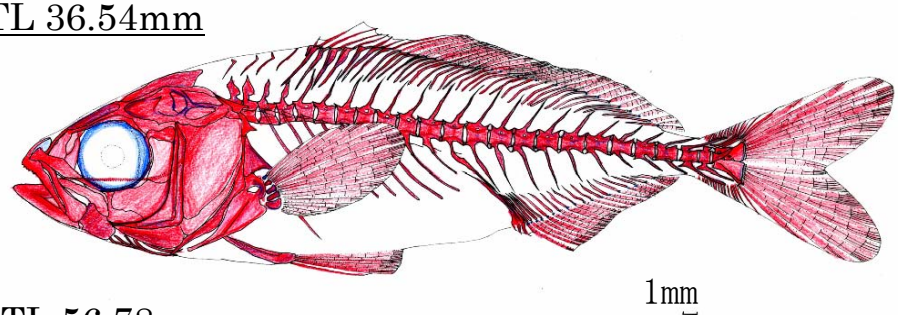
40dah, TL 29.38mm





50dah, TL 36.54mm





68dah, TL 56.78mm

- Tail: Isometric growth to positive growth.
- Young: Completion of ossification.
- Body shape: Increasing body width, shaped.
- Body color: Bluish color and appeared 8 black colored lateral band.
- Feeding: Just formulated feed.

Splanchnocranium

■ : Cartilage

■ : Onset of ossificati

■ : Under ossificatior

■ : Appeared as intermembranous

■ : Bone

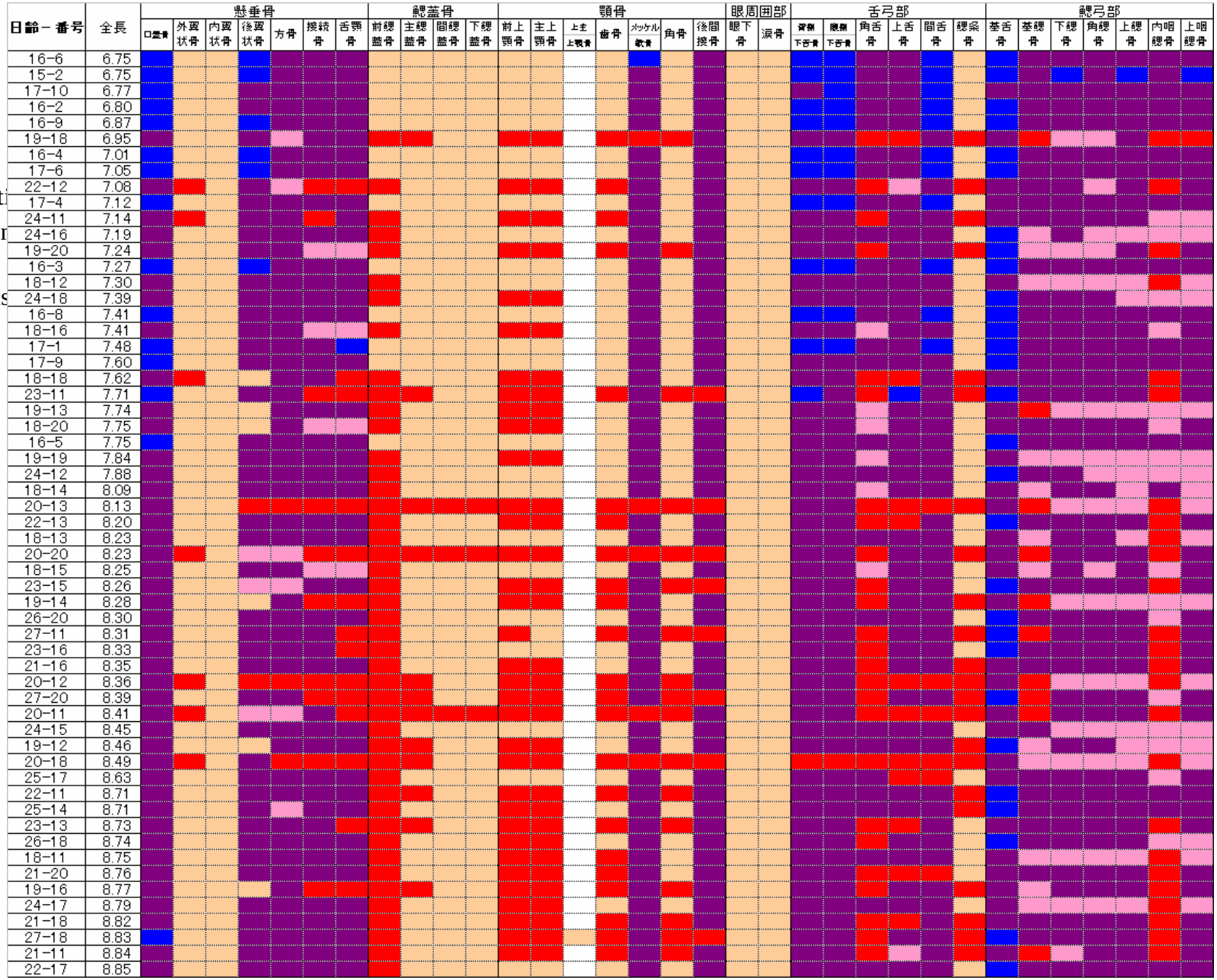
Ossification

Cartilagenous :

■ → ■ → ■ → ■

Intermembronous :

■ → ■



Vertebra (Discus intervertebralis)

: Appearance as intermembranous

: Ossificated

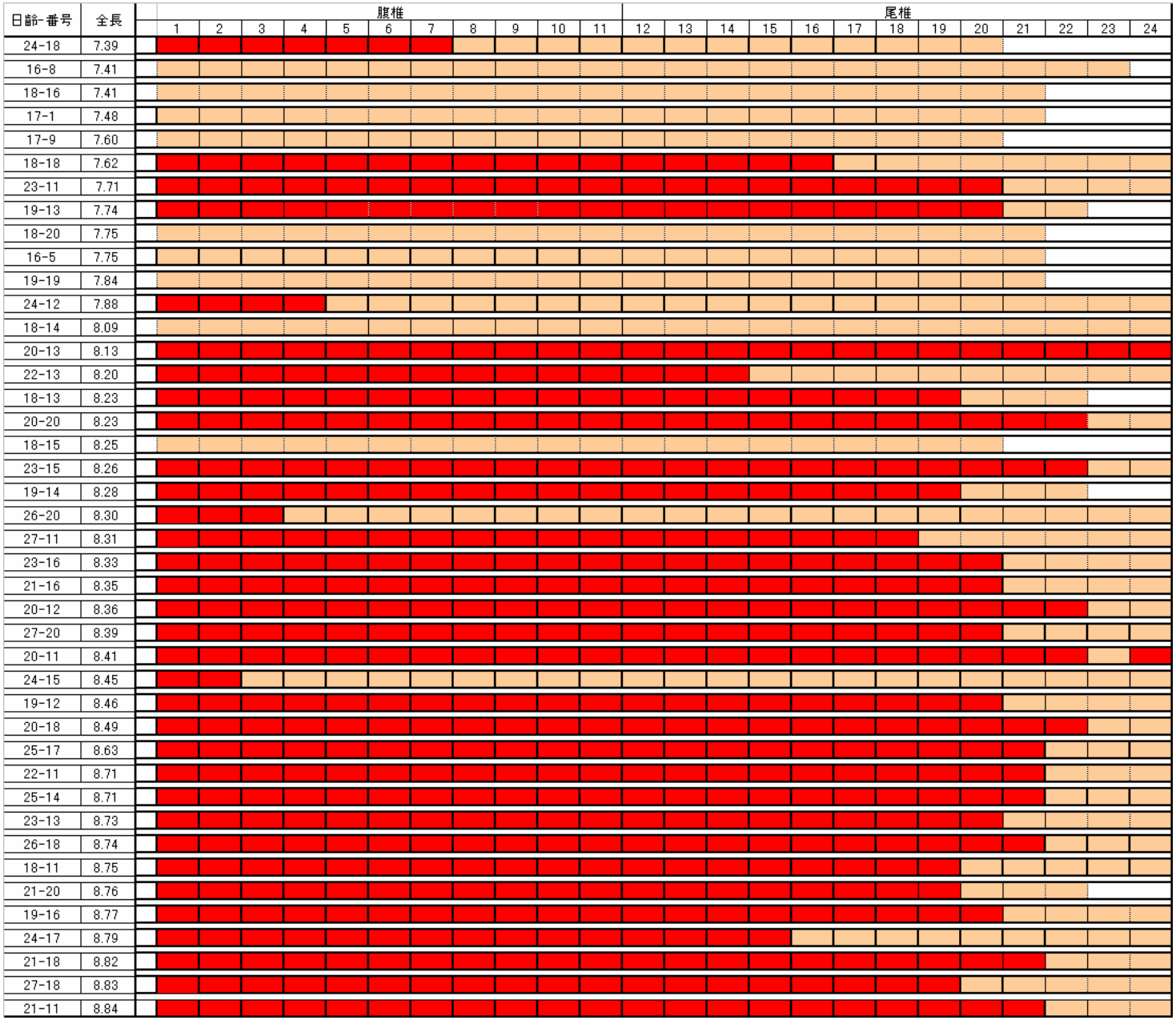
Intermembronous ossification : →

Discus intervertebralis

: Appearance

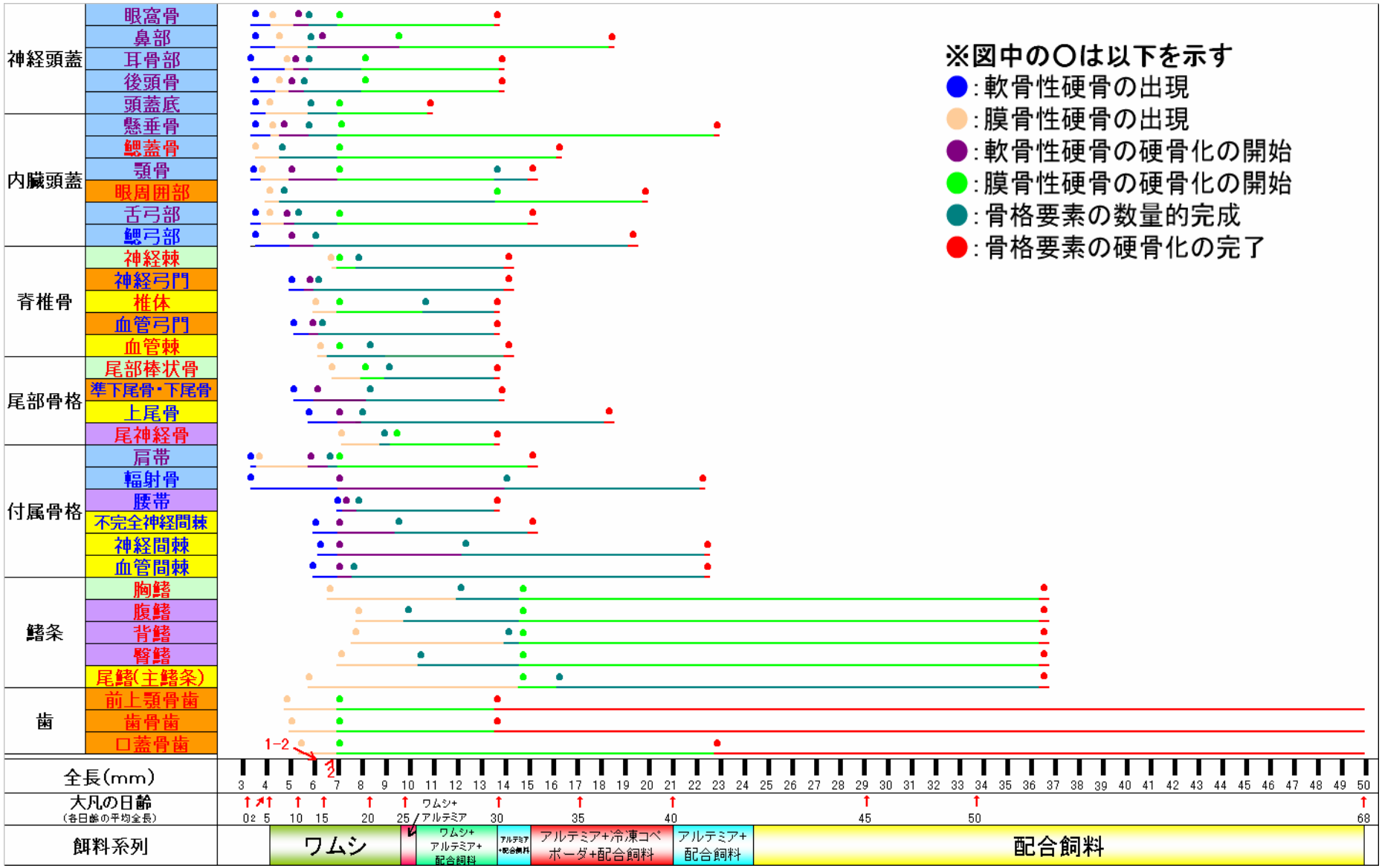
| : Half completion

|| : Completion



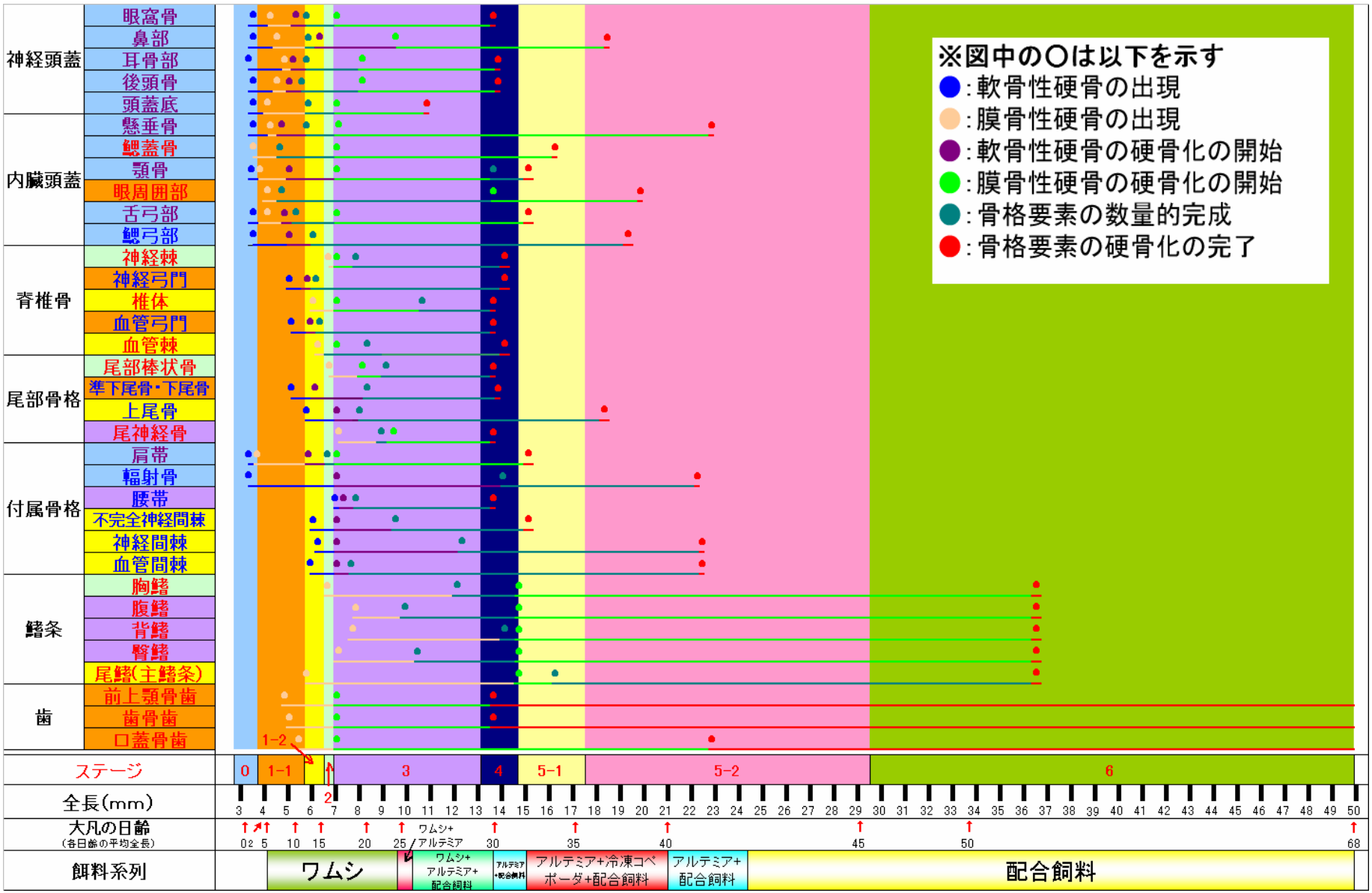
Morphogenesis of skeleton

赤字	:膜骨性硬骨要素
青字	:軟骨性硬骨要素
紫字	:軟骨性硬骨要素＋膜骨性硬骨要素

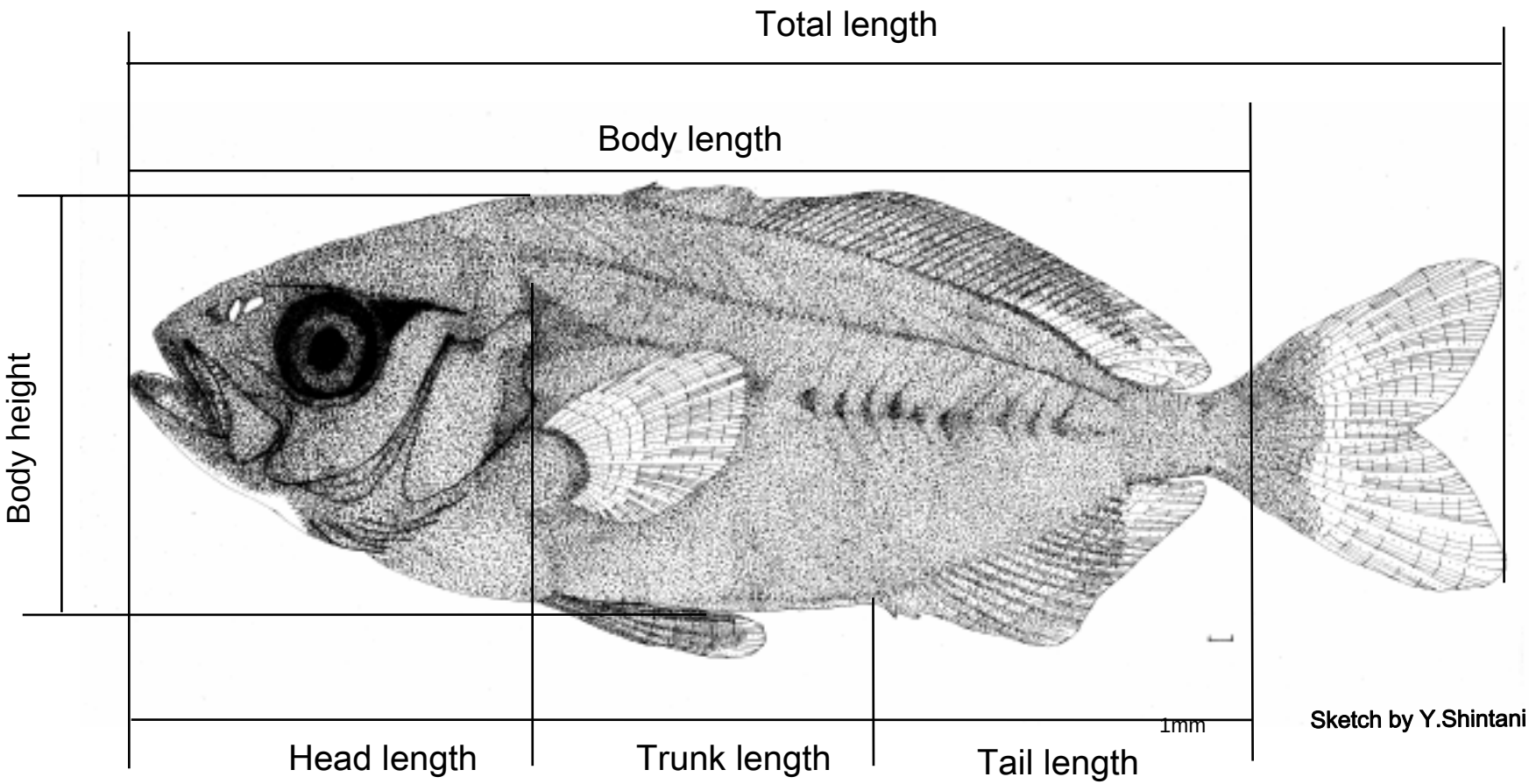


Relation between morphogenesis of skeleton and developmental stage

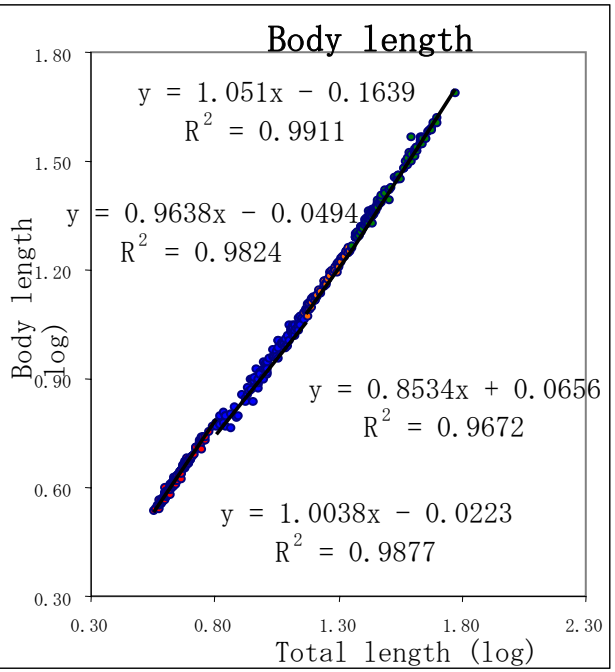
赤字	:膜骨性硬骨要素
青字	:軟骨性硬骨要素
紫字	:軟骨性硬骨要素＋膜骨性硬骨要素

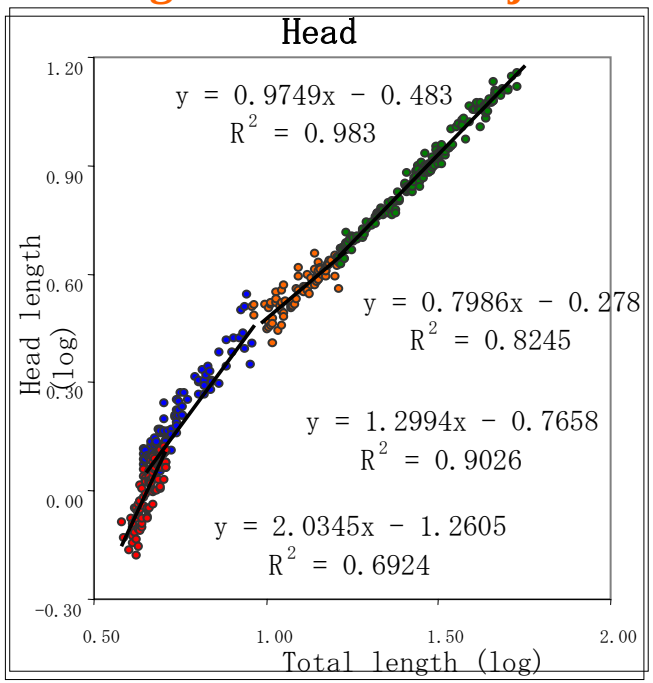


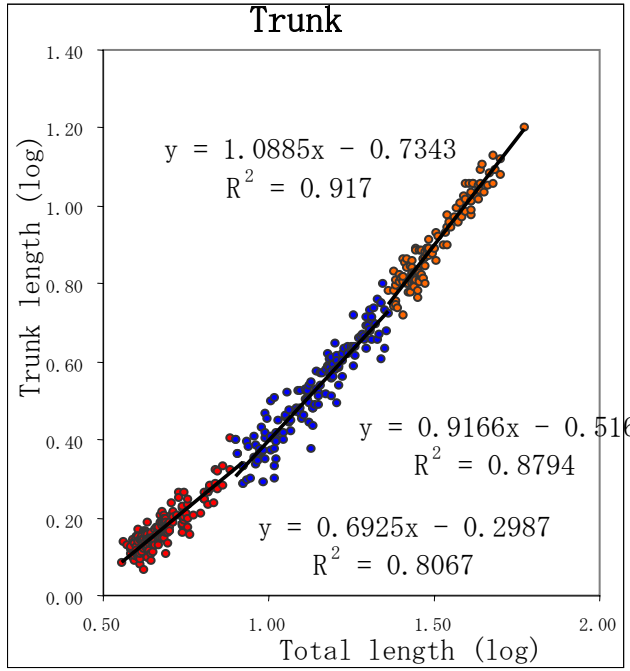
Morphogenesis of umber jack

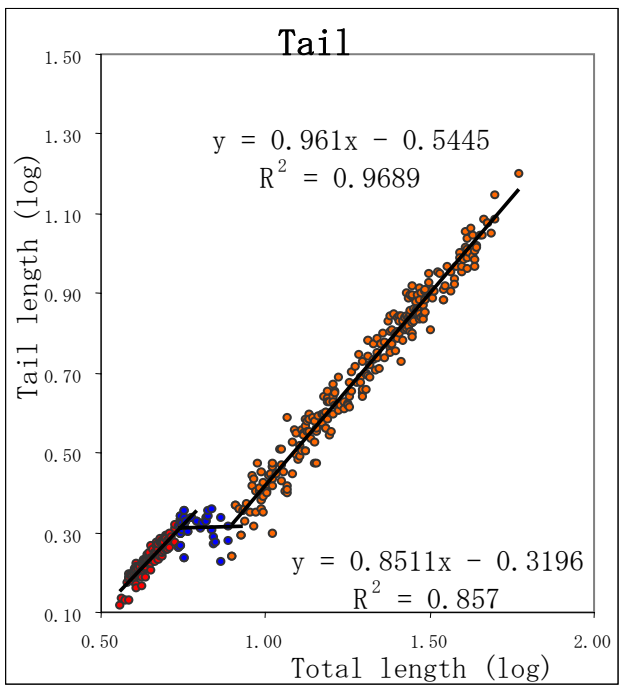


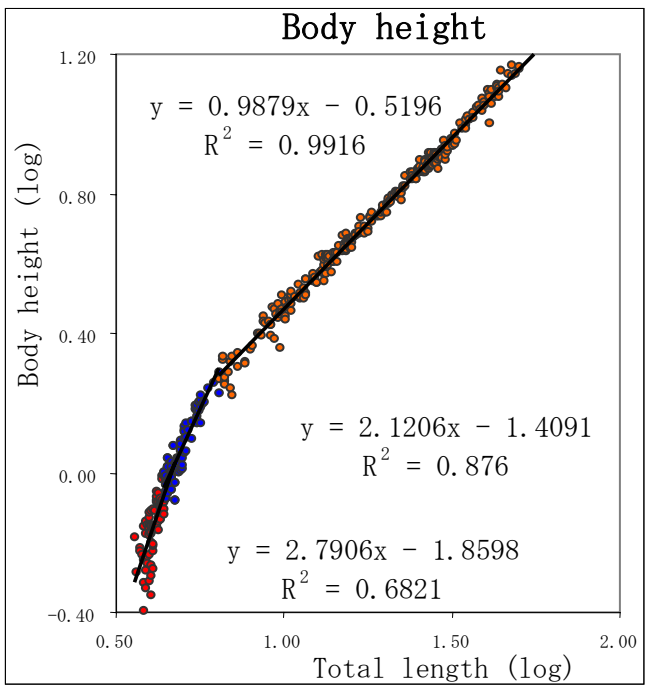
Allometric growth of umber jack larvae







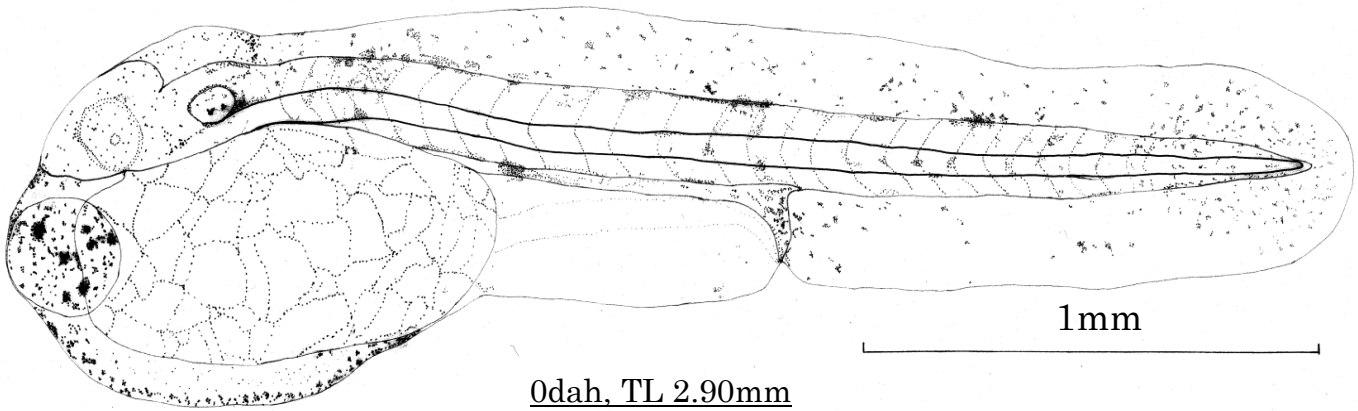




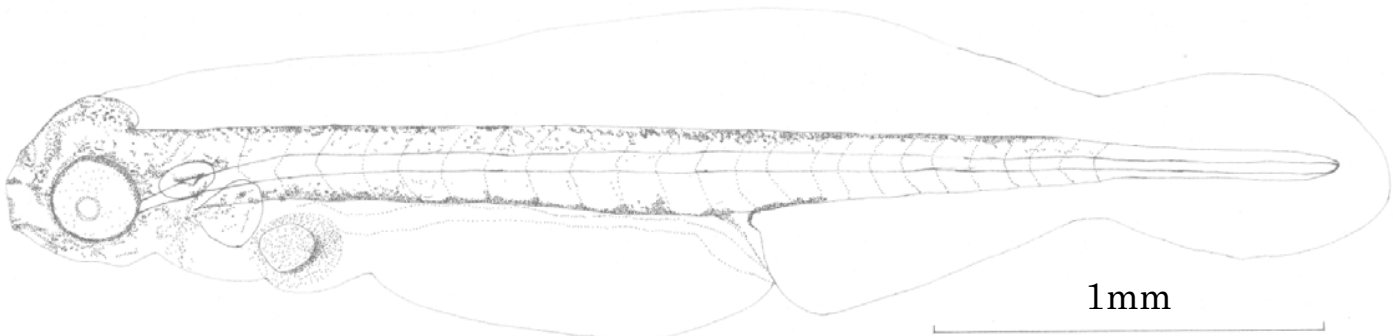
Developmental stage of umber jack larvae

Stage	TL(mm)	Body length	Head	Trunk	Tail	Body height
0	～3.84	1.0038	2.0345	0.6925	0.8511	2.7906
1	3.84～4.71	0.8534				
2	4.71～6.20		1.2994			2.1206
3	6.20～7.91				0.0215	0.9879
4	7.91～9.40				0.9610	
5	9.40～10.59		0.7986	0.9166		
6	10.59～14.55	0.9638				
7	14.55～18.47		0.9749			
8	18.47～23.78			1.0885		
9	23.78～59.28	1.0510				

Stage 0, TL 2.90～3.84mm



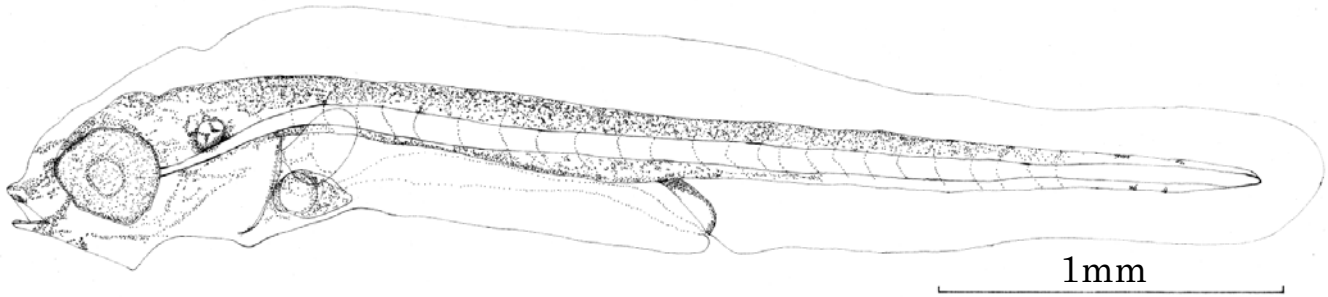
0dah, TL 2.90mm



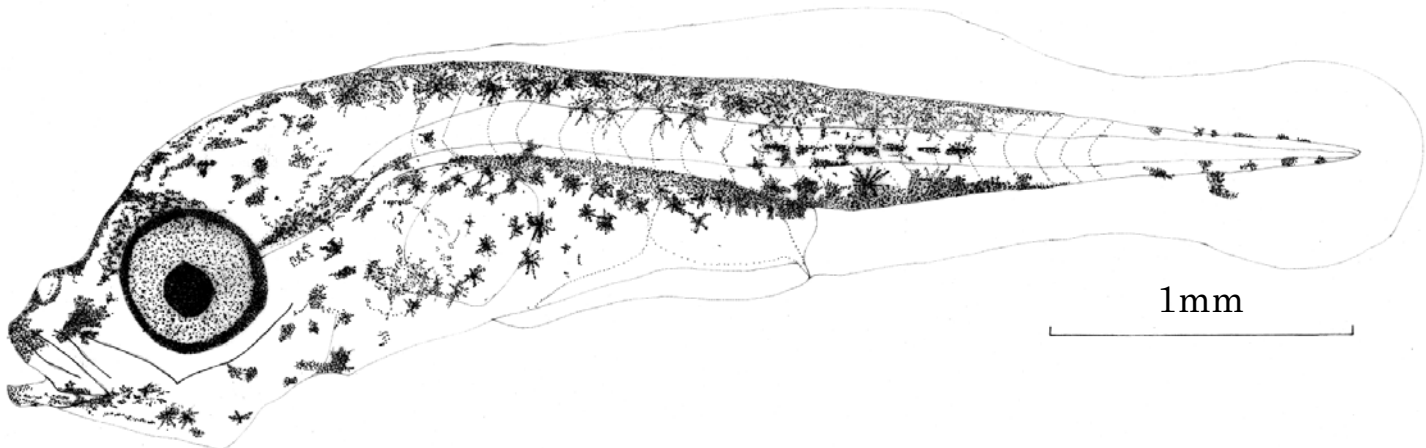
2dah, TL 3.57mm

Stage 1, TL 3.84～4.71mm

Mouth opening
Onset of feeding

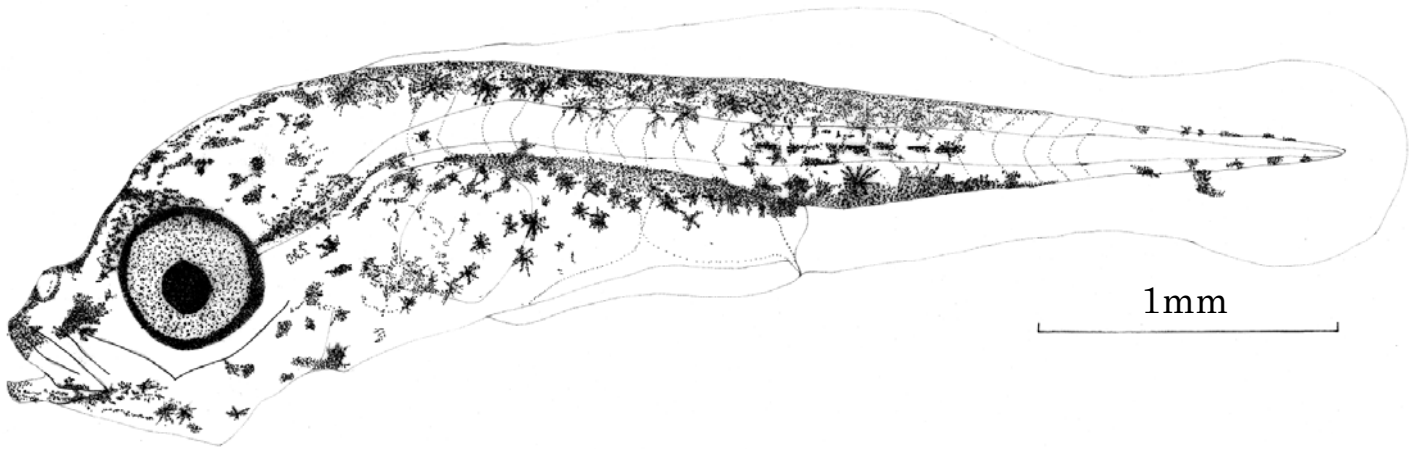


3dah, TL 3.82mm



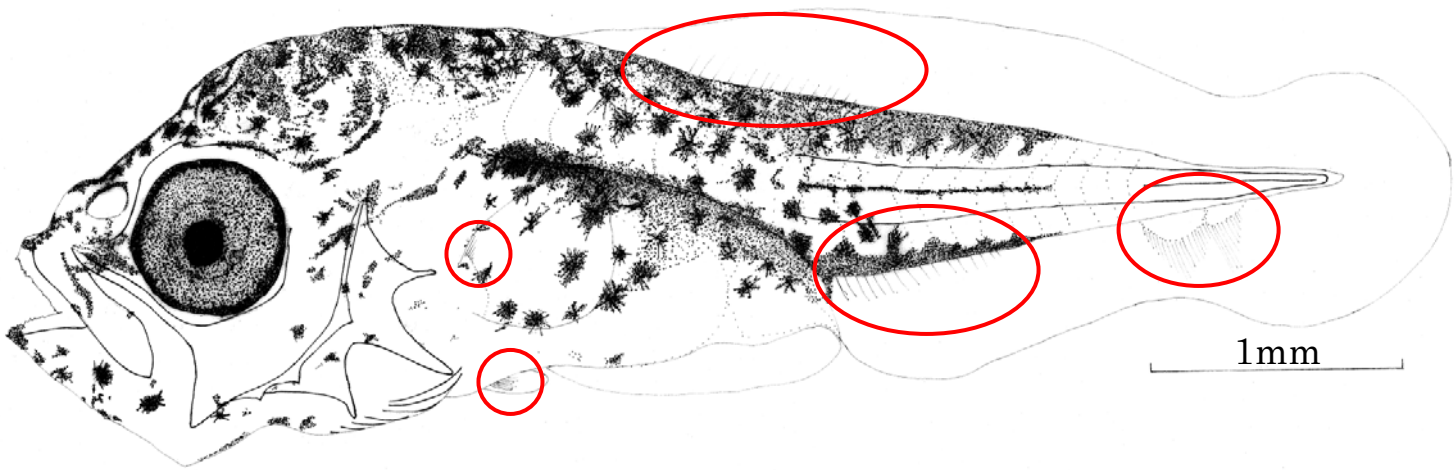
11dah, TL 4.68mm

Stage 2, TL 4.71～6.20mm



11dah, TL 4.68mm

Developed fin ray basement,
Appeared bud of fin ray

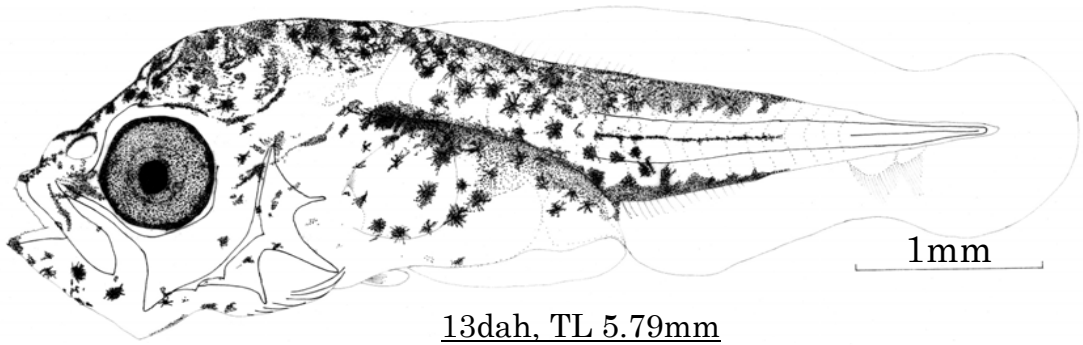


13dah, TL 5.79mm

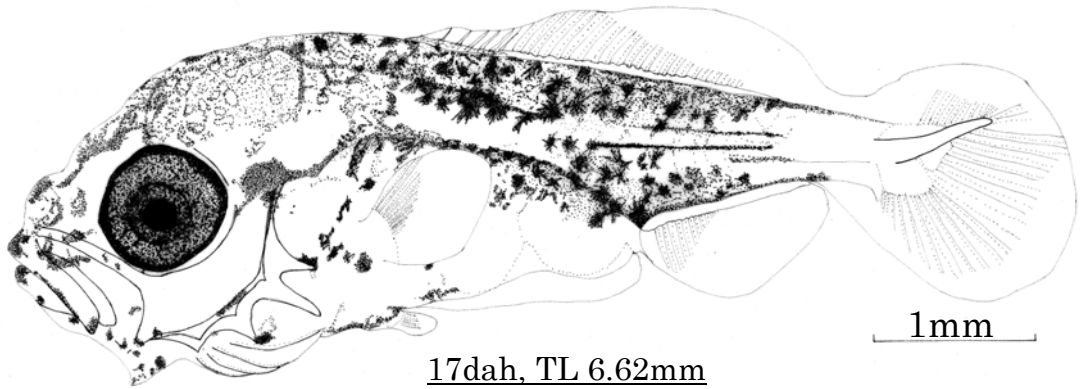
Appeared and developing
Spines of preopercle

Stage 3, TL 6.20～7.91mm

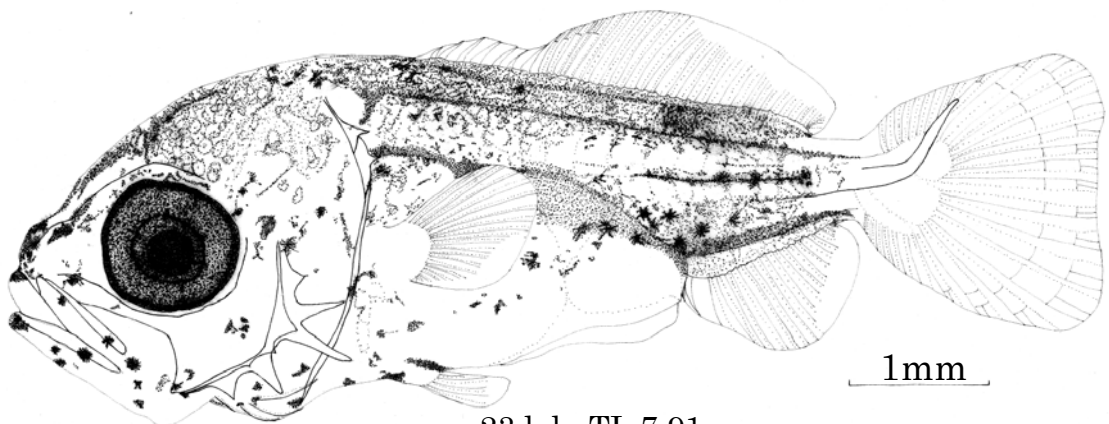
Onset of notochordal flexion
Development fain bases and
Fin rays



13dah, TL 5.79mm



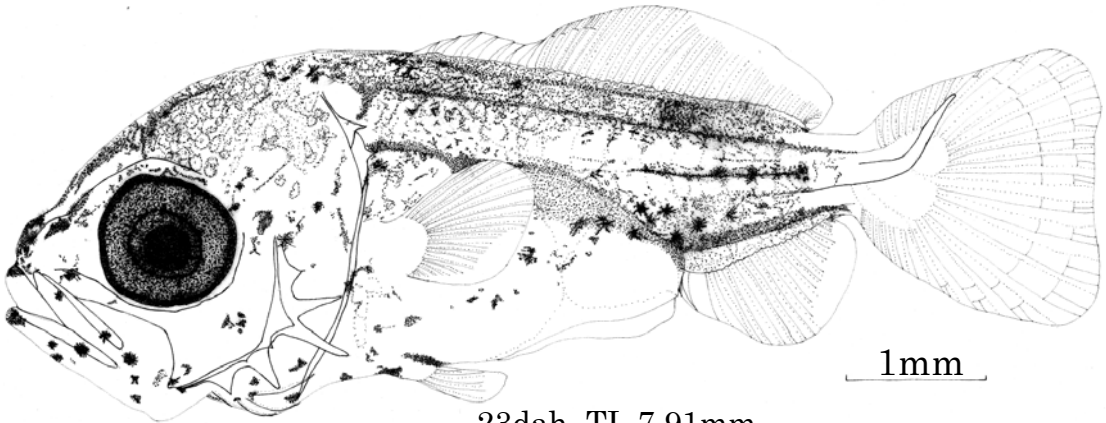
17dah, TL 6.62mm



23dah, TL 7.91mm

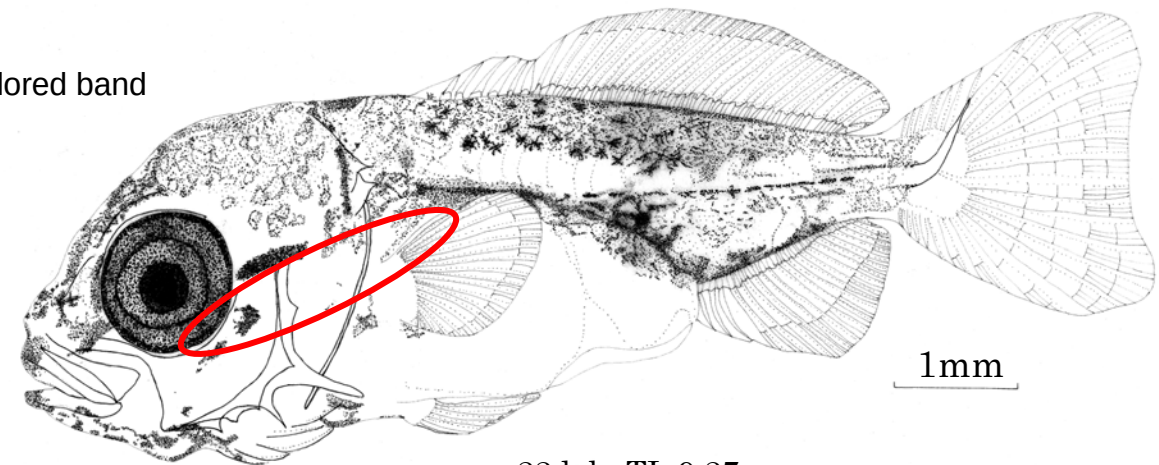
Stage 4, TL 7.91～9.40mm

Continuing flexion
Completion of caudal fin



23dah, TL 7.91mm

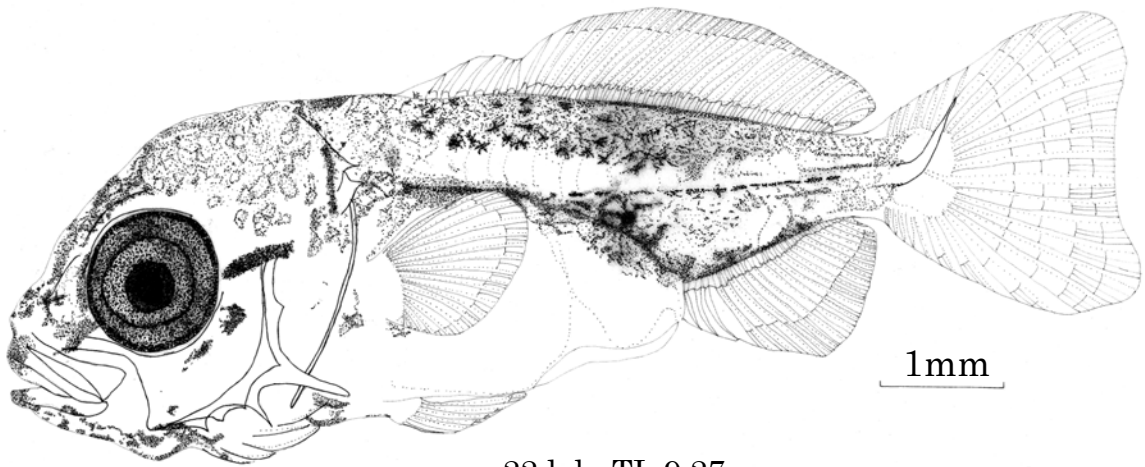
Typical colored band



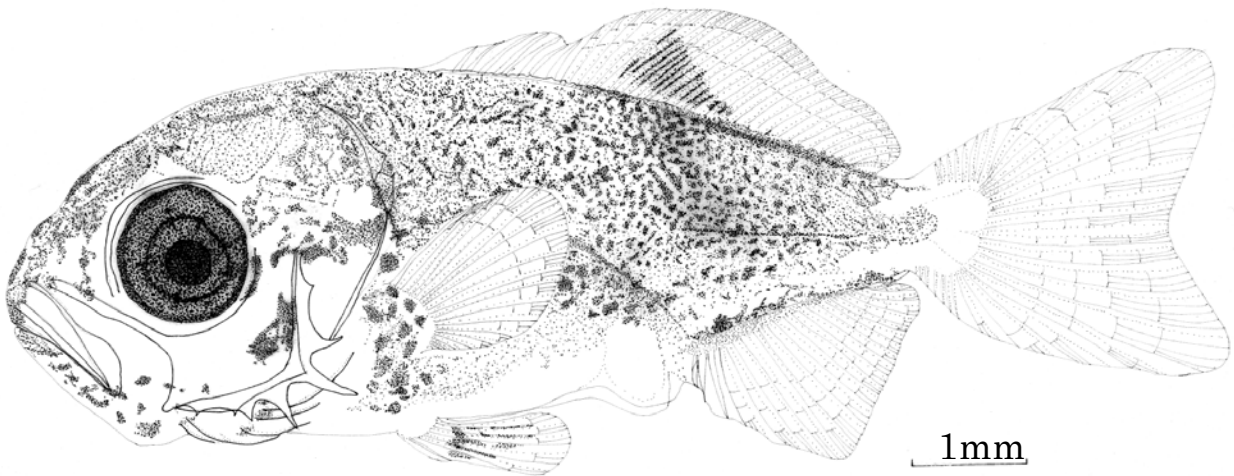
22dah, TL 9.27mm

Stage 5, TL 9.40～10.59mm

Completion of fins
Appeared black colored band
On dorsal and pelvic fin

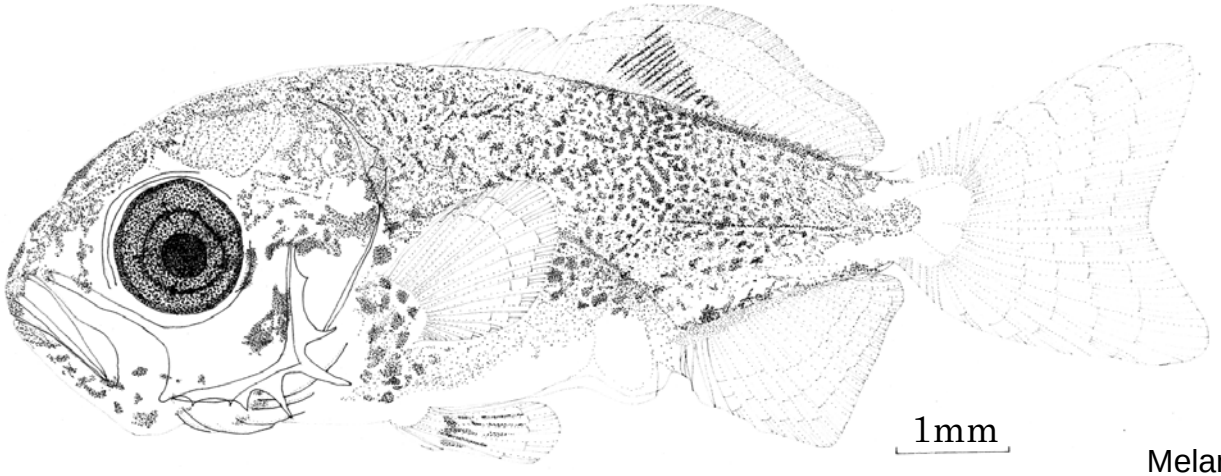


22dah, TL 9.27mm



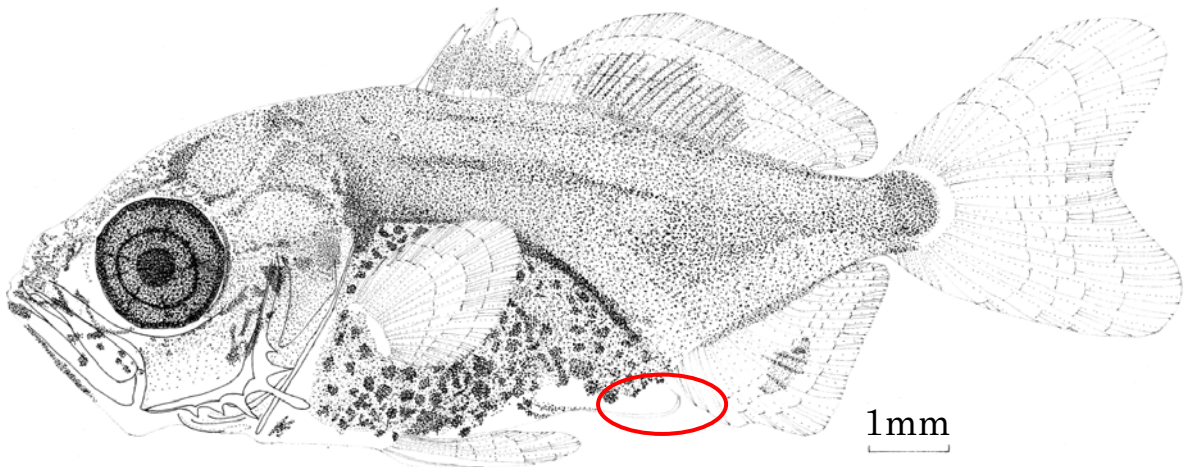
20dah, TL10.51mm

Stage 6, TL 10.59～14.55mm



20dah, TL10.51mm

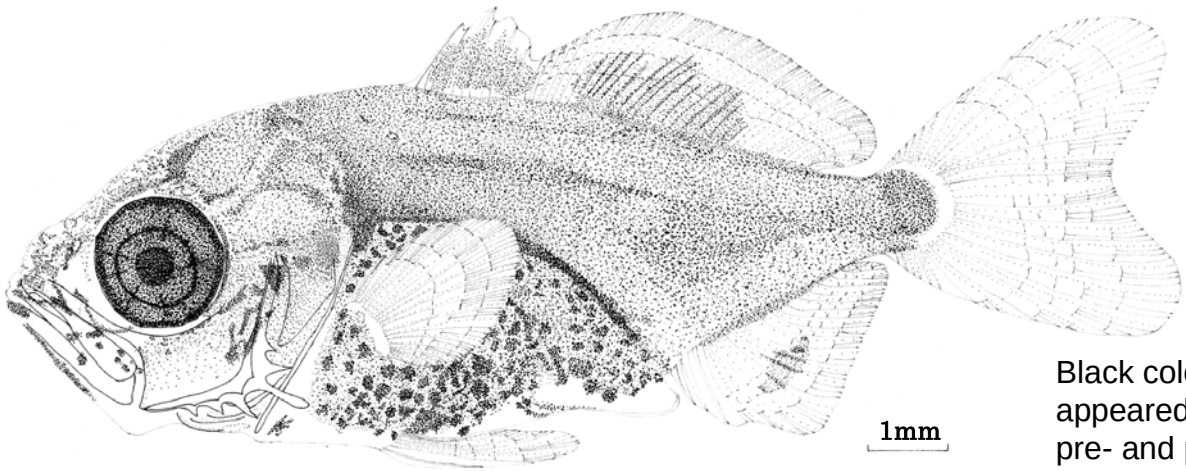
Melanophore spread to whole body.
Larval membrane remained in front of anus.



Larval membrane

21dah, TL14.52mm

Stage 7, TL 14.55～18.47mm



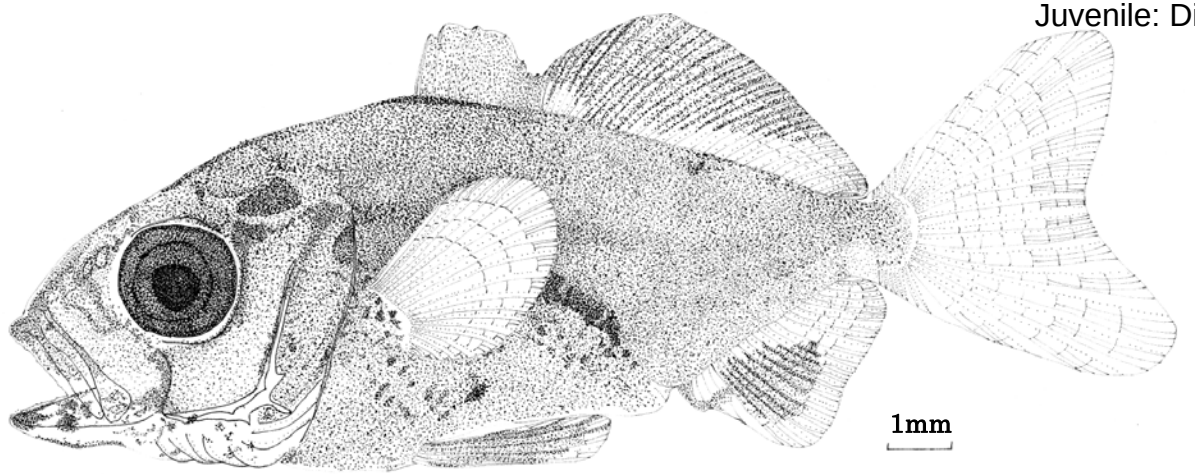
21dah, TL14.52mm

Black colored lateral bands appeared at caudal peduncle, pre- and post-pectoral fin.

Regression of preopercle spines.

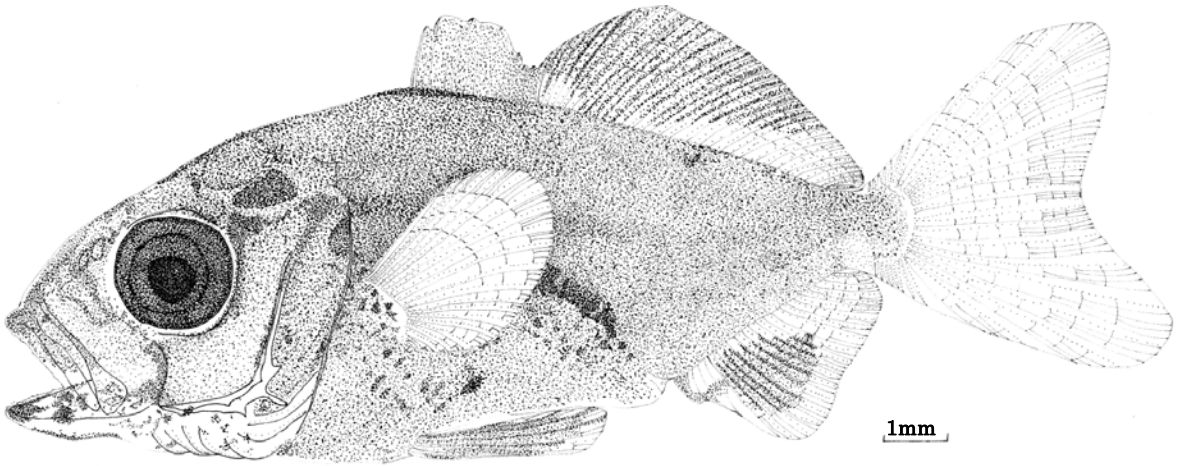
Myotome: V-shaped to W-shaped.

Juvenile: Disappeared larval membrane



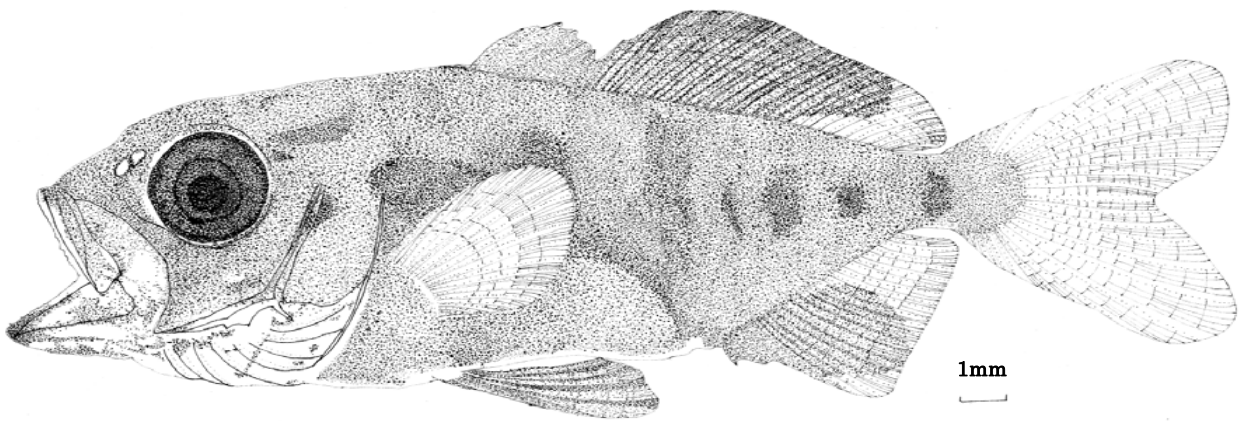
28dah, TL18.32mm

Stage 8, TL 18.47～23.78mm



28dah, TL18.32mm

Increasing black colored lateral band.

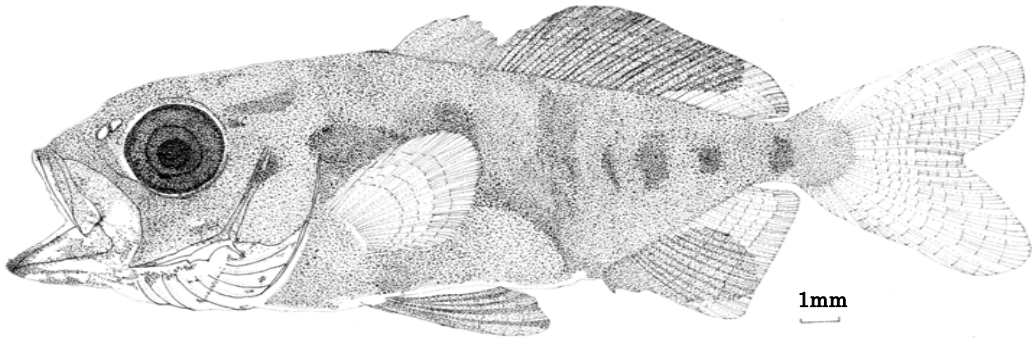


30dah, TL23.73mm

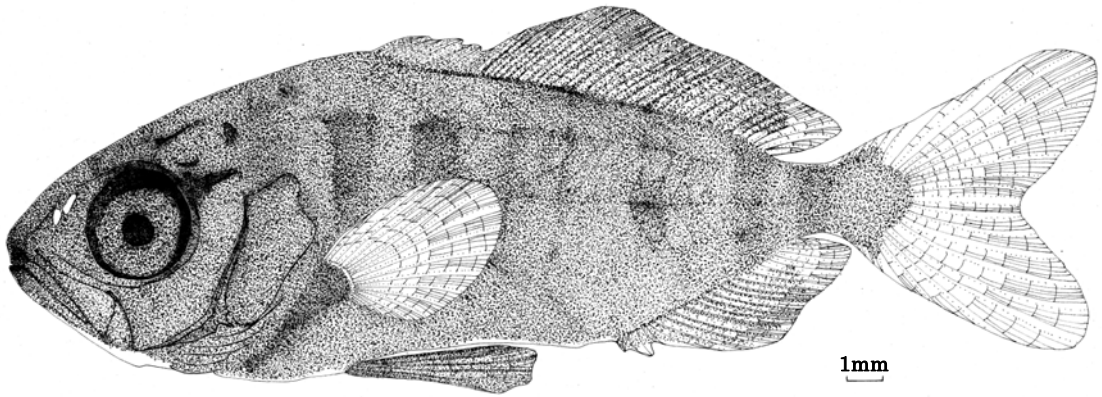
Stage 9, TL 23.78～59.28mm

Black colored lateral band: Increased to 8.
at the middle of stage, disappeared.

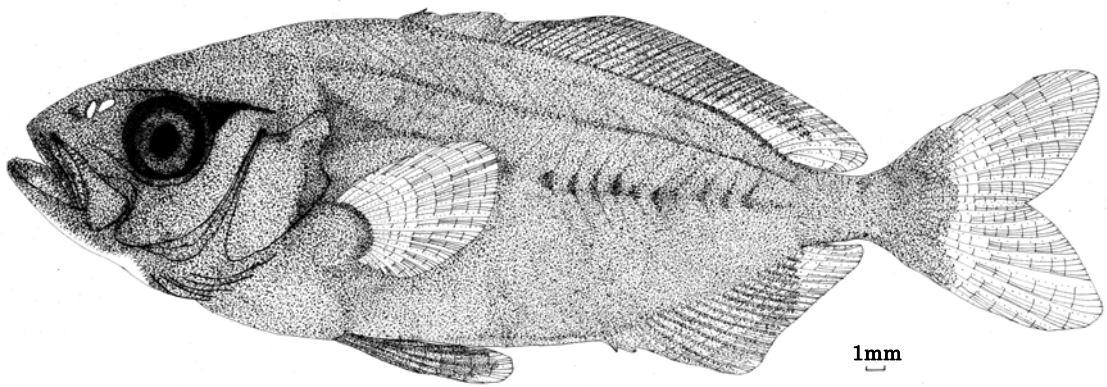
Colored vertical band: Two bands appeared.



30dah, TL23.73mm

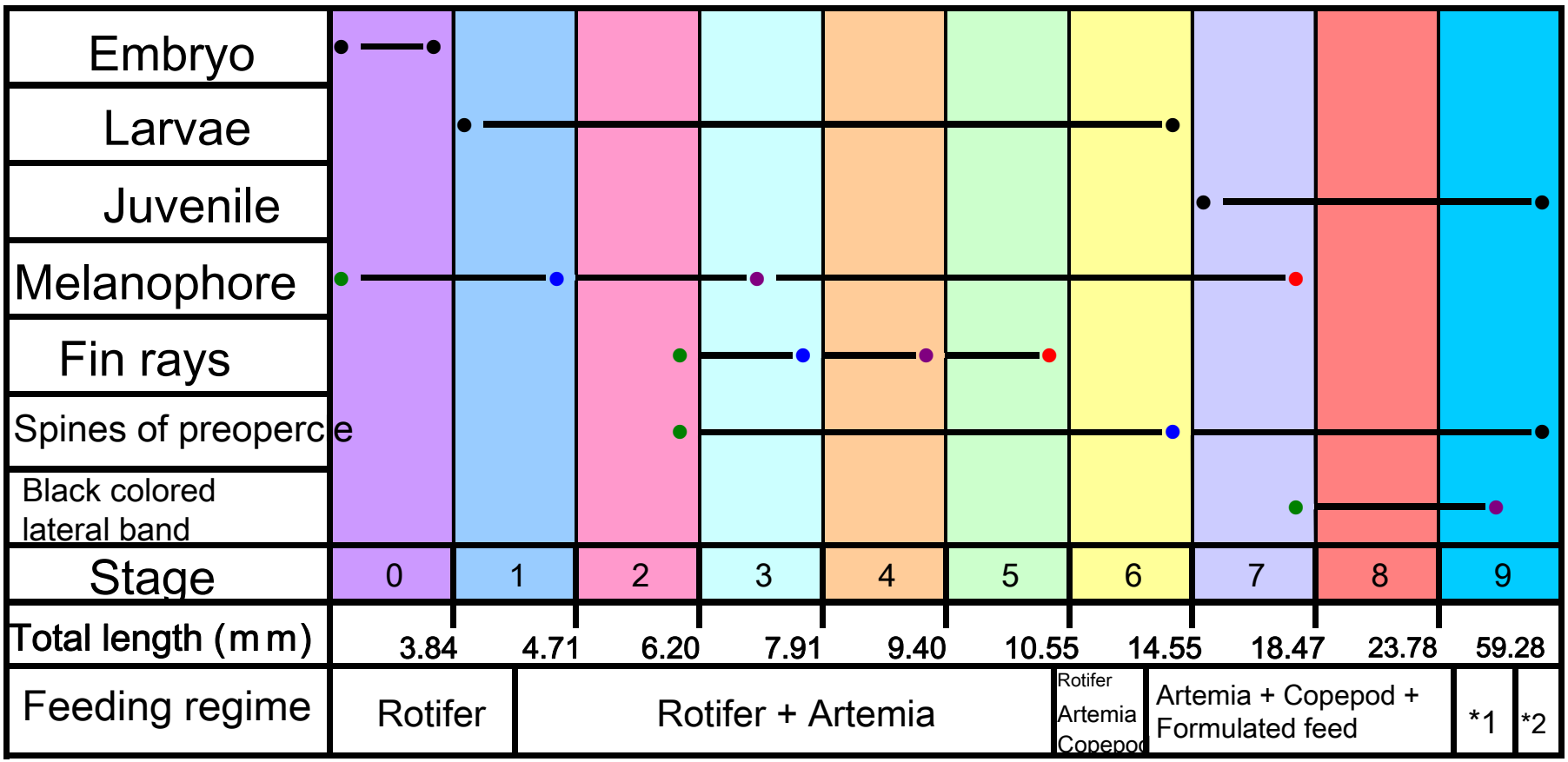


35dah, TL31.21mm



42dah, TL59.28mm

Conclusion



*1 Copepod + formulated feed

*2 Formulated food only

Tentative conclusion

- Course of development of finfish larvae should divide 7-8stages.
- The stage defined the characteristics of body growth, such as body, head, trunk and tail. There is priority of growth depending on stage.
- Skeletal components developed at free embryo/yolk sac larvae have in common.
- Causes of skeletal deformities should classify by endogenous/ maternal effects and exogenous one.
- More study should be necessary to clarify the physiological and ecological characteristics of developmental stage.
- More study should be necessary to clarify the nutritional effect on the skeletal development of hatchery-raised finfish larvae.

Suitable Vitamin A concentration of live food for Japanese flounder

- Excess and deficiency of Vitamin A of live food affected the health of hatchery-raised juveniles of Japanese flounder.
- Excess and deficiency of Vitamin A of live food caused skeletal deformity.
- Vitamin A concentration of rotifer should be most important key issue for skeletal deformity.
- Rotifer with Vitamin A concentration of 2 I2 IU/g (reflected 750IU/g of enrichment agent) was recommended for improvement of the health of hatchery-raised Japanese flounder.
- Vitamin A concentration of Artemia (50 – 2831IU/g) did not cause skeletal deformity of hatchery-raised Japanese flounder juvenile raised by rotifer with Vitamin A of 212 IU/g

Effect of VA concentration on performance of larviculture and morphogenesis of Japanese flounder – I. *Rotifer* feeding period

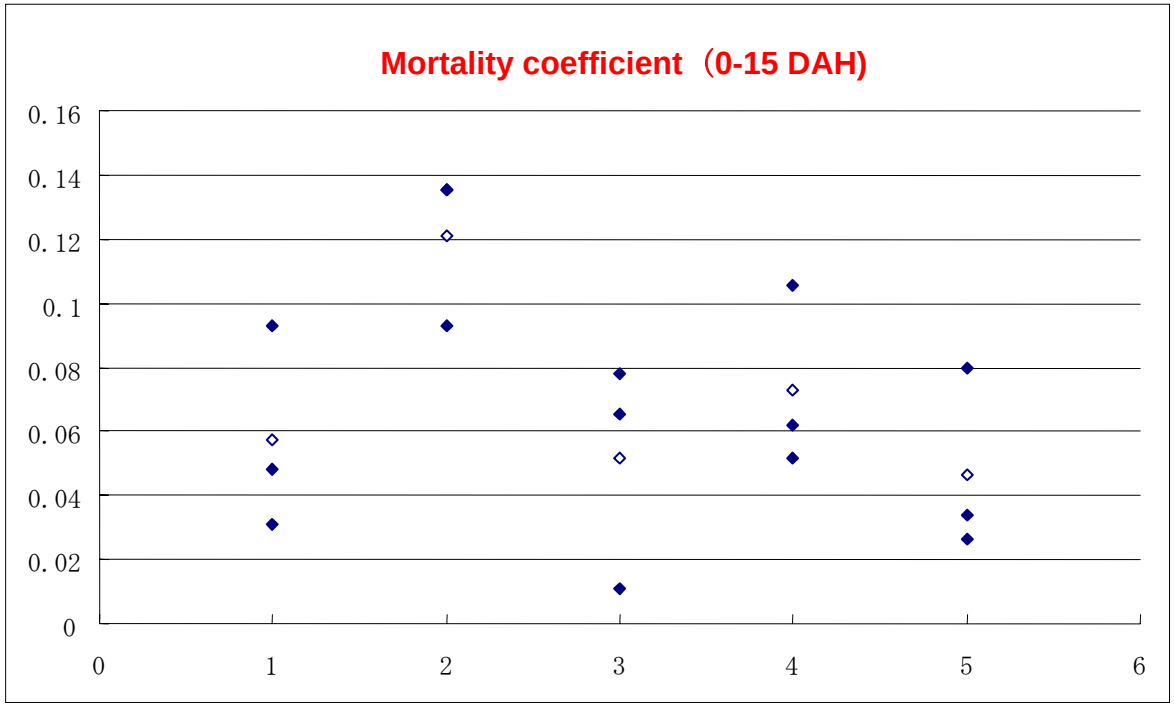
Objective and Goal

- Objective; To clarify the effect of vitamin A concentration on occurrence of deformity in the course of Japanese flounder larviculture.

- Goal; To establish the ideal vitamin A concentration in enrichment of live feed for improvement of quality and health of hatchery-raised Japanese flounder juvenile.

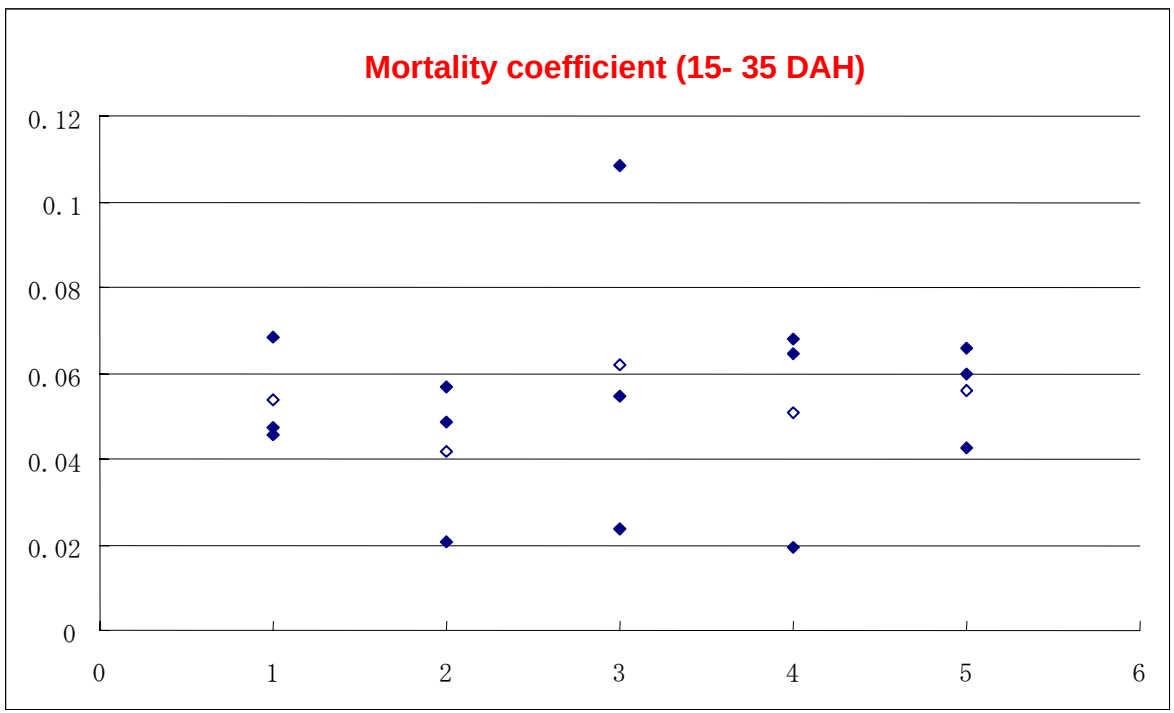
Rotifer period

- As control, we used L-rotifer cultured by *Chlorella* enriched by *Nannochrolopsis* and Artemia enriched by Marine ω R .
- For treatment, L-rotifer enriched by experimental agent with different VA concentrations. Using Artemia enriched by Marine ω R.
- VAa : 150IU/g VAb : 750IU/g VAc : 1500IU/g VAd : 7500IU/g.



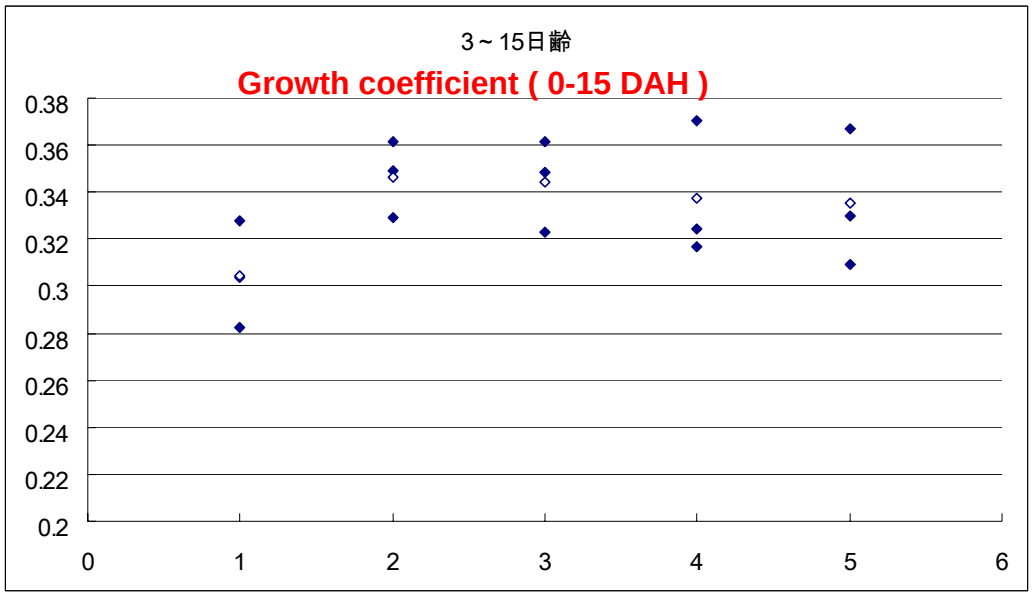
Conclusion of survival performance in Experiment-I

Mortality coefficients in rotifer feeding period for all treatments were higher than control. Treatment Ab (2) was highest, and values of other three treatments were similar.

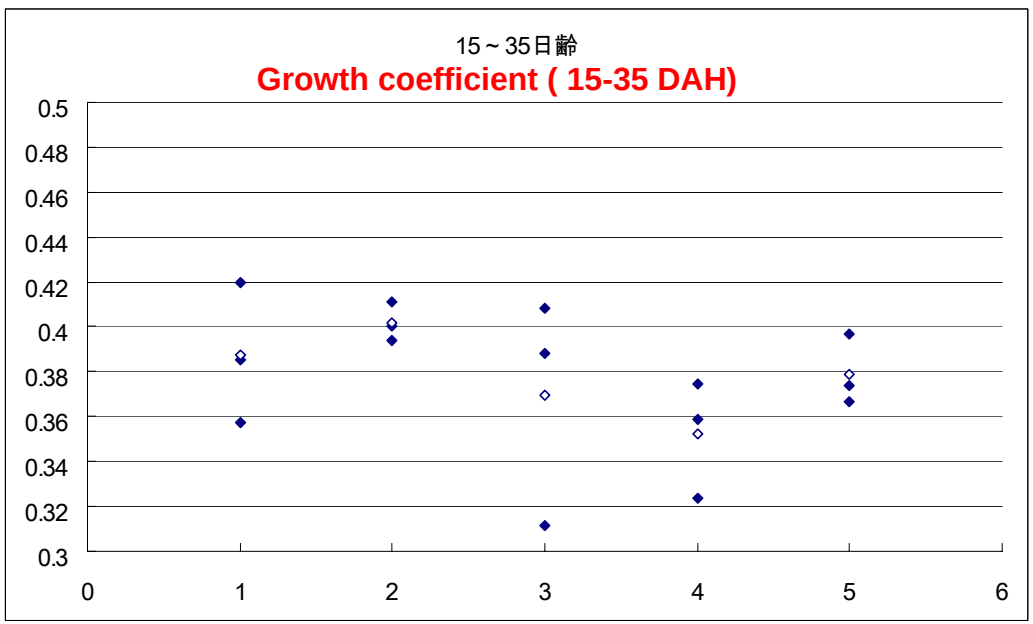


Mortality coefficients in mix feeding period for all treatment and control were almost same, except treatment Ac (3).

Conclusion of growth performance in Experiment-I



Growth coefficients in rotifer feeding period were almost similar, except treatment Aa. Treatment Ab was highest,



Growth coefficients in mix feeding period were highest in treatment Ab. Values of Treatment Aa, Ac, and control were similar, and Ad was lowest.

Experiment for rotifer feeding period

Rearing tank 1000l Polycarbonate

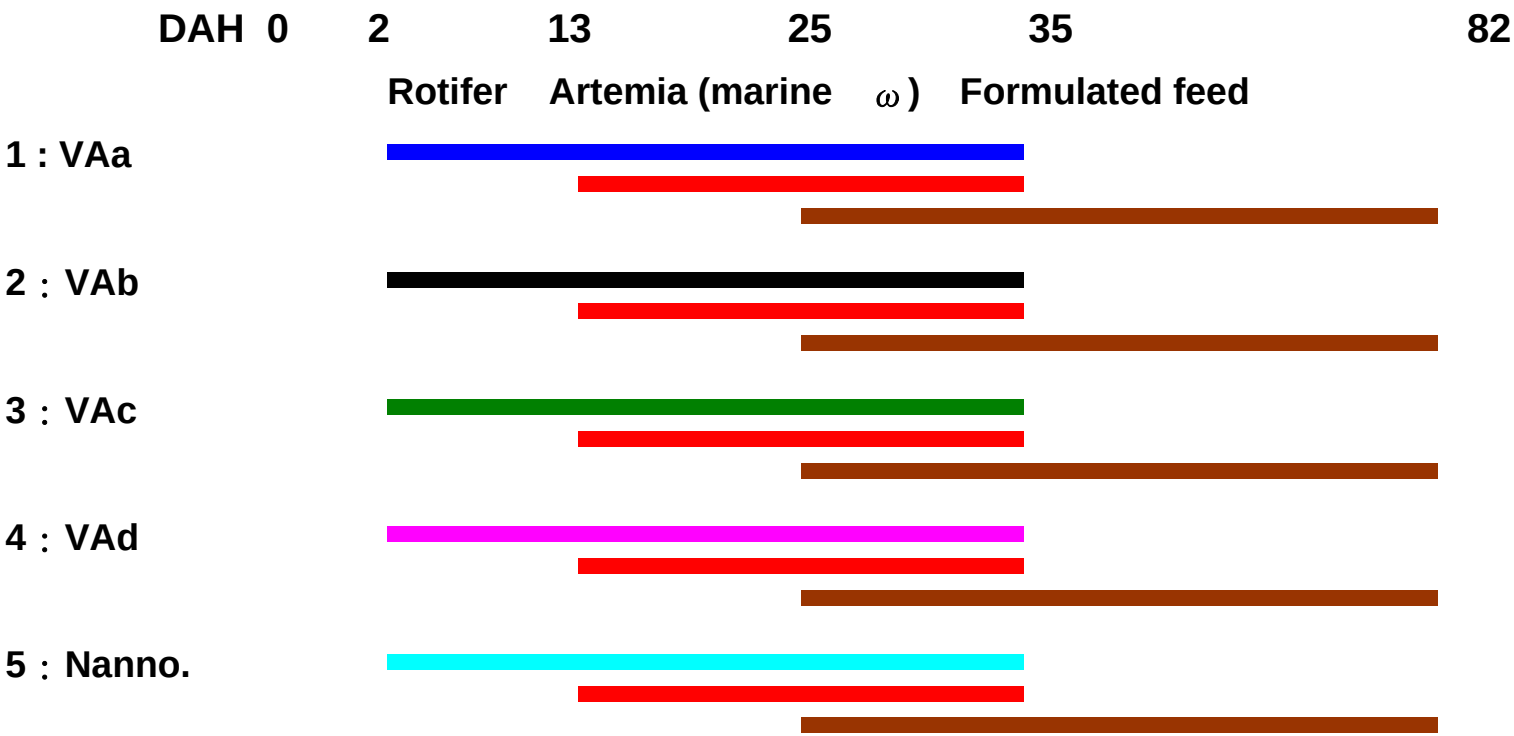
Stocking density 20000 inds/m3 (Triplicate)

Feeding time 2 - 13DAH Rotifer (08:00 and 14:00) 5inds/ml

13 -15DAH Rotifer (09:00 and 15:00) 5inds/m l

Artemia (08:00, 14:00 and 17:00)

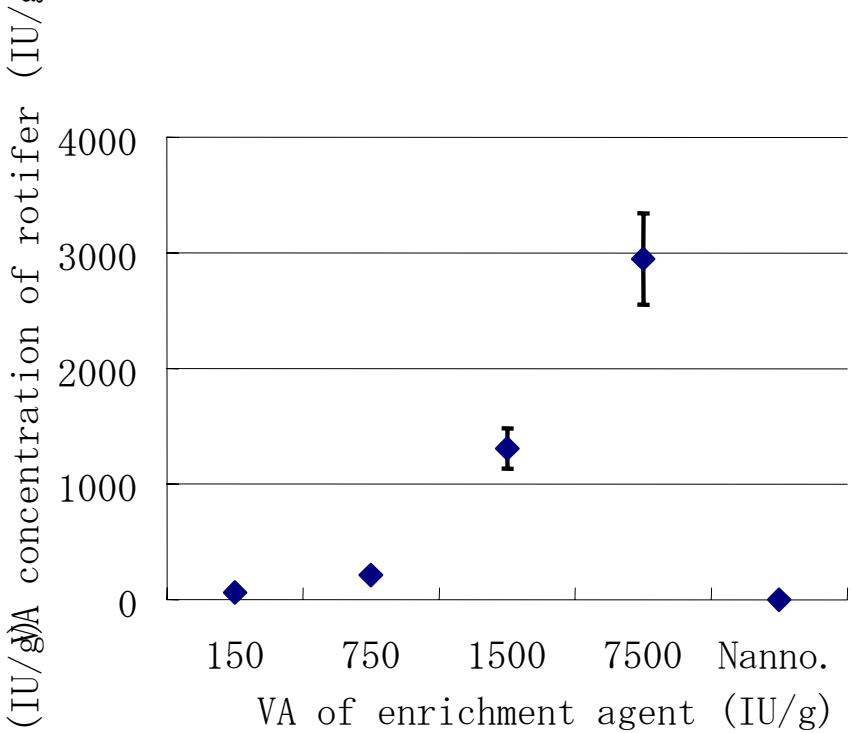
80 -200inds/larva

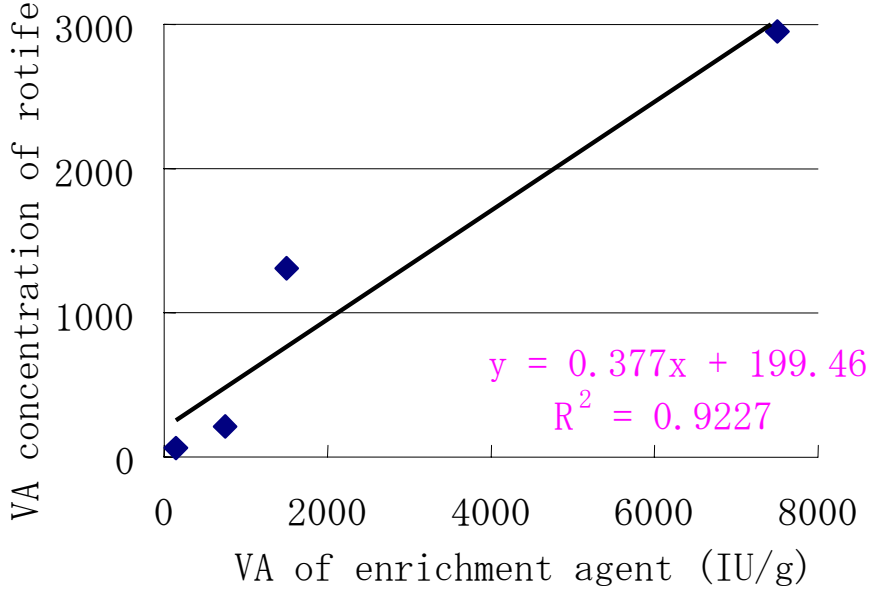


Vitamin A concentration of rotifer

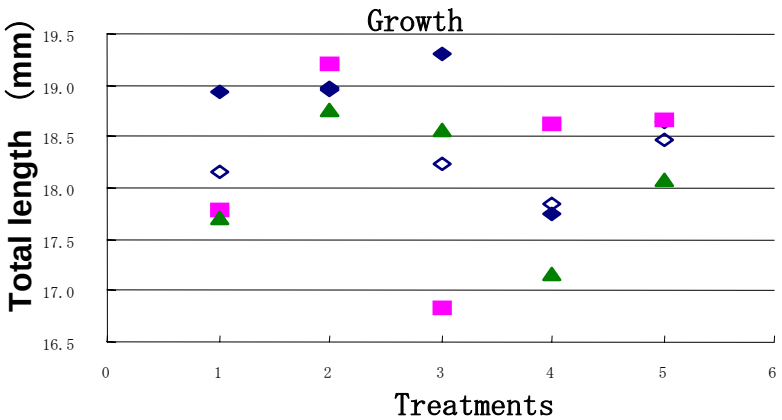
VA of agent (IU/g)	150	750	1500	7500	Nanno.
VA(IU/g)	61.0^{c}	212.1^{c}	1307.9^{b}	2949.5^{a}	0^{c}

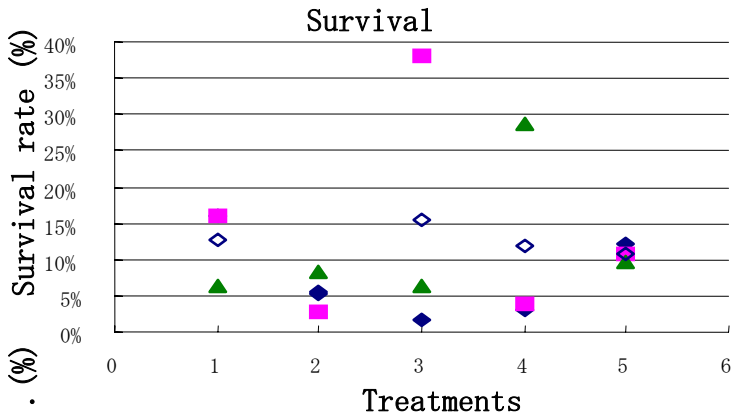
Fisher's PLSD at (α<0.05) n=5 a>b>c

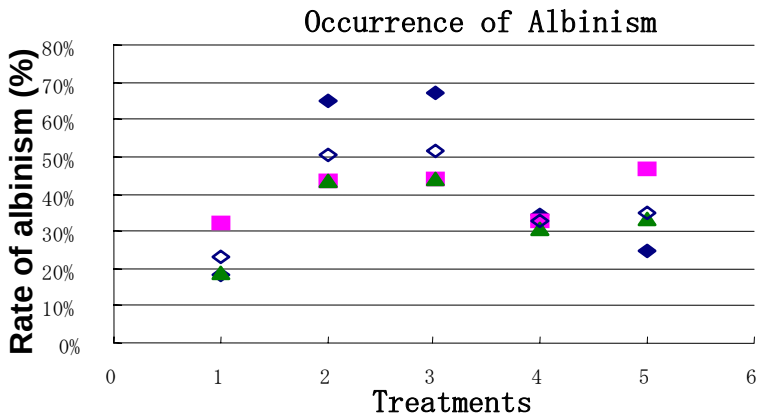


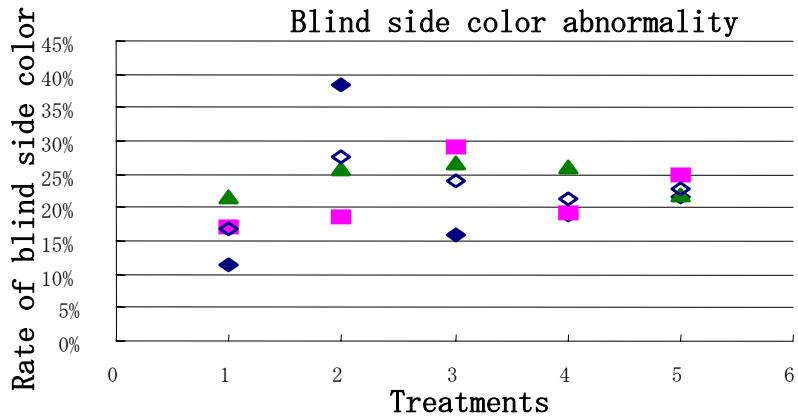


Performance of Experimental Larviculture for rotifer Feeding Period at 38-46 DAH









Treatments 1 2 3 4 5

a b c d

Nannochloropsis

Tank 1

Tank 3

Tank 2

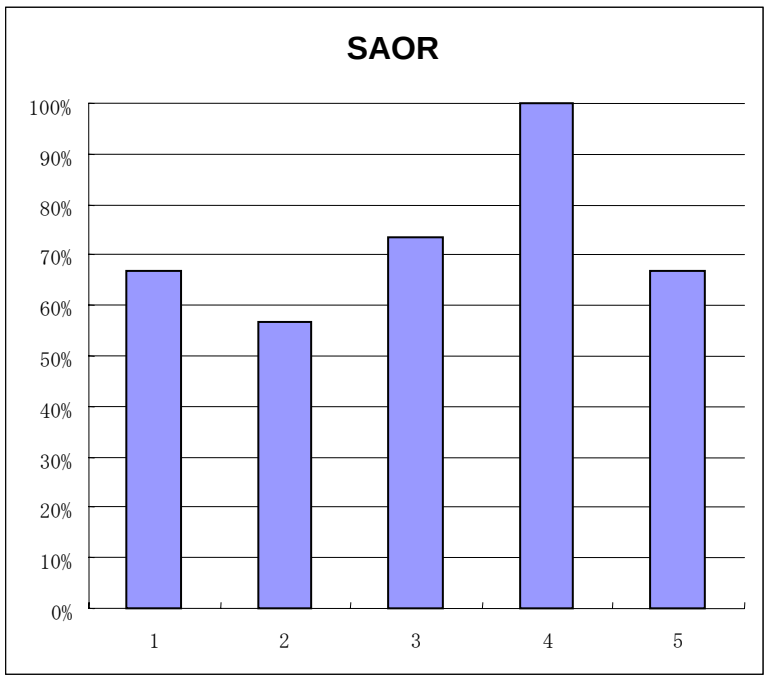
Average of tank 1-3

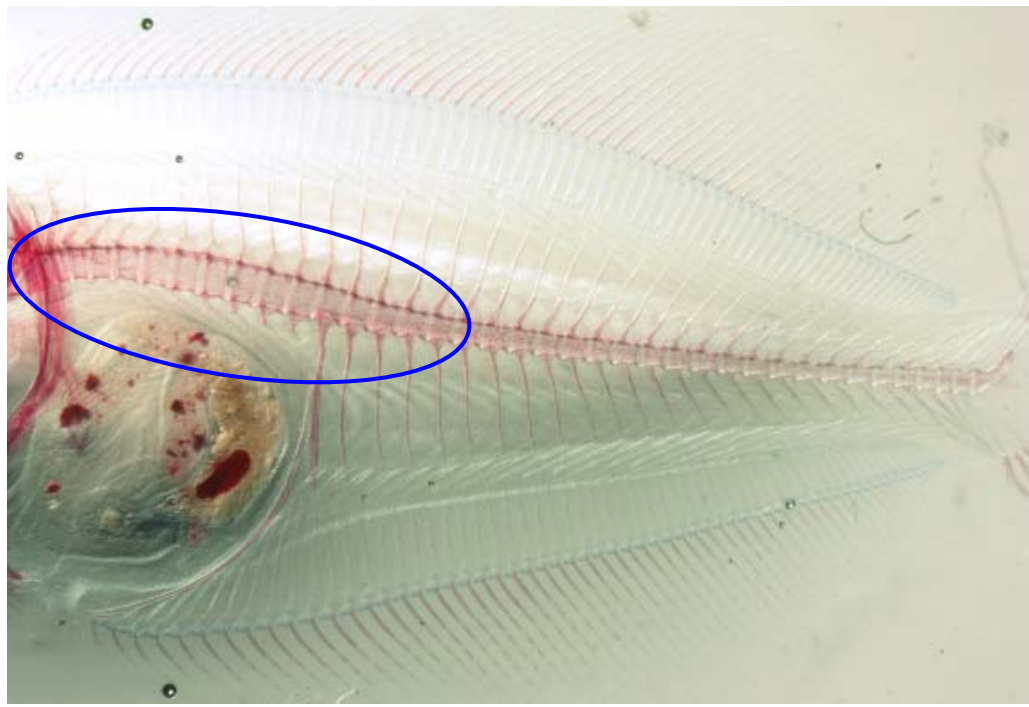
Skeletal abnormality of 38- 46 dah, just after metamorphosis

Skeletal abnormality occurrence rate

d＞c＞a＝Nannochloropsis＞b

Excess formation of vertebrae appeared in Vd treatment.





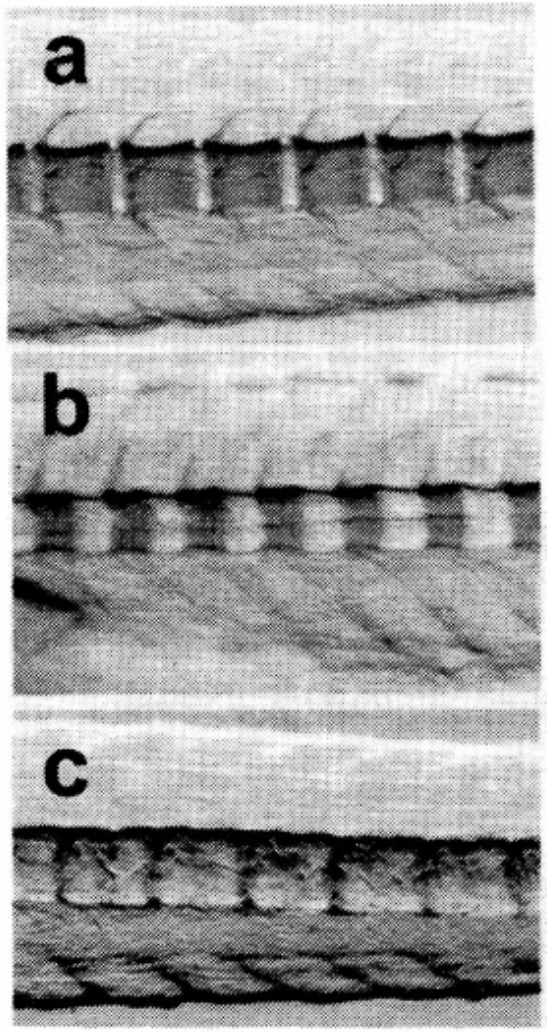
Treatment 1 2 3 4 5

a b c d

Nannochloropsis

Effect of retinoic acid on vertebrae formation

Retinoic acid affected vertebrae formation depending on its concentration.



Normal discus intervertebralis

Normal

Enlargement of discus intervertebralis

Deficiency

Shortening of Discus intervertebralis

Excess

レチノイン酸の欠乏と過剰によって起こる椎体の異常

a：正常
b：レチノイン酸欠乏
c：レチノイン酸過剰

Effect of VA concentration on performance of larviculture and morphogenesis of Japanese flounder – II. *Artemia* feeding period

Conclusion

- We conducted the experimental larviculture using rotifer enriched by *Nannochloropsis* and *Artemia* enriched by experimental enrichment agents with gradient of Vit A concentration.

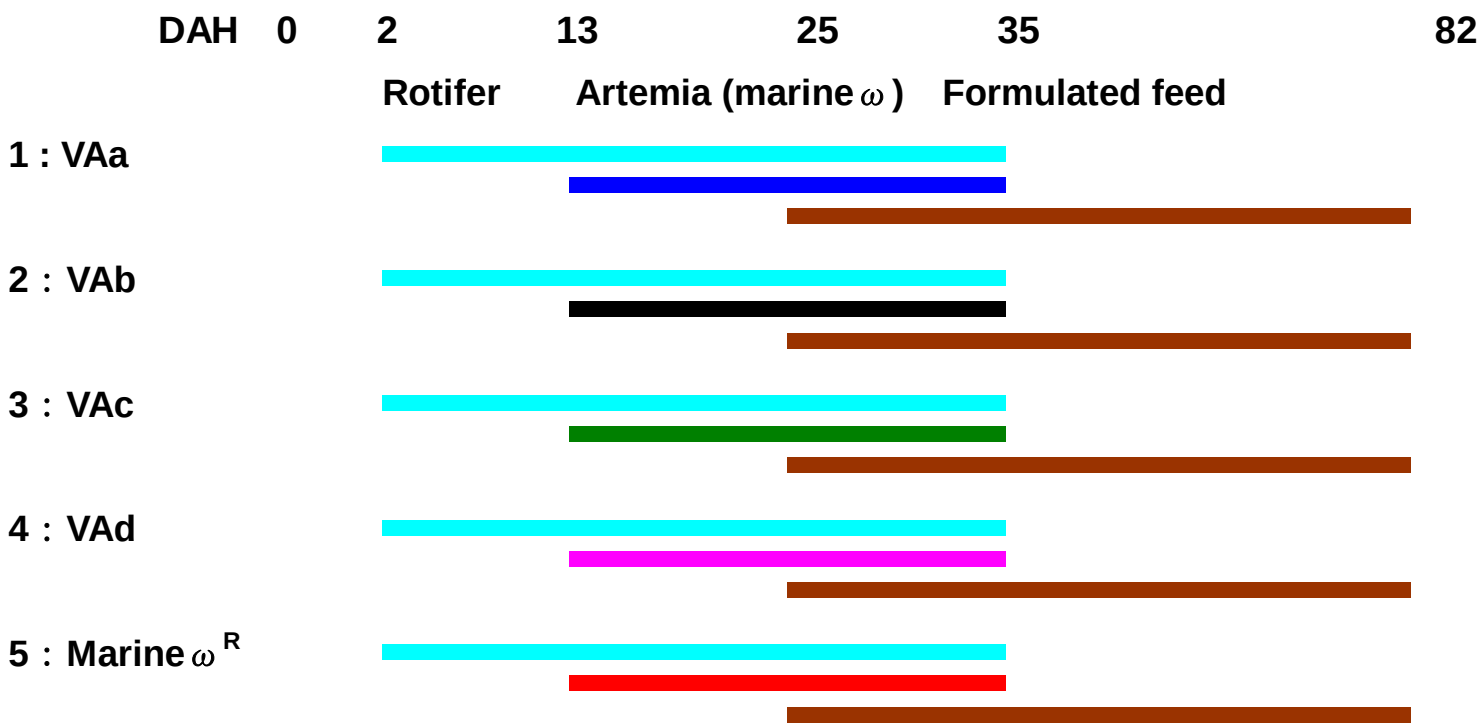
- There were no significant differences of the performance of larviculture in the course of rotifer feeding period.

- In the course of *Artemia* feeding period using experimental enrichment agents with gradient of Vit A concentration, performance of treatment b (325IU/g reflected 750IU/g of enrichment agent) showed highest growth rate and low color abnormality of ocular and blind sides.

- Skeletal abnormality occurrence rate was lowest in the treatment b (325IU/g reflected 750IU/g of enrichment agent) .

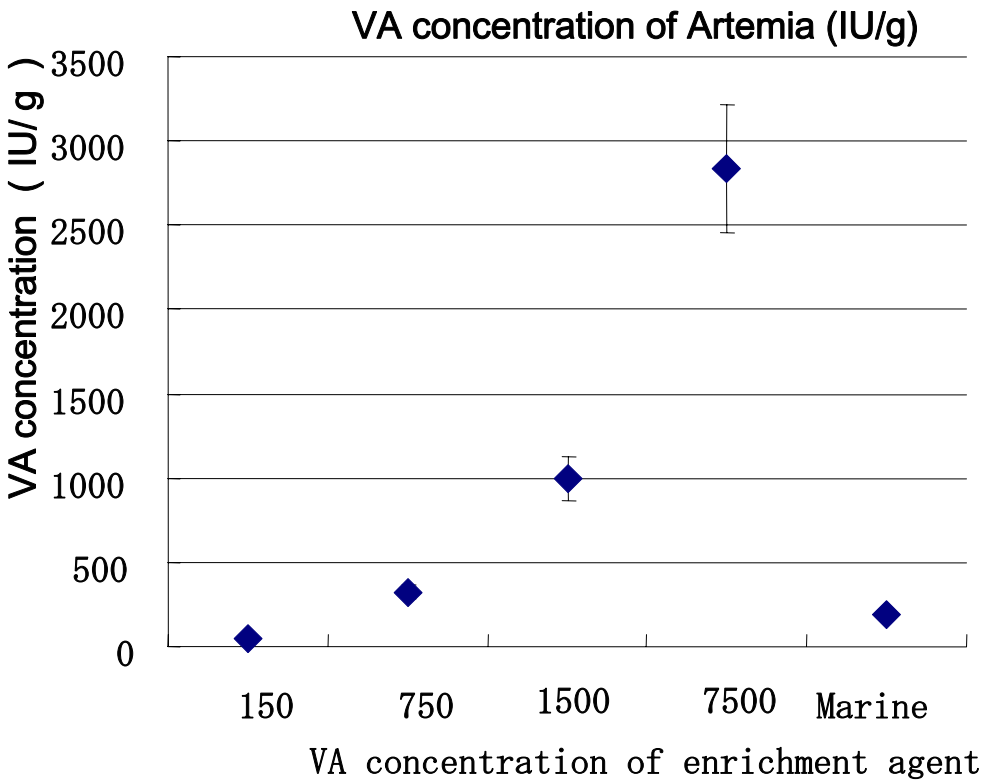
Experiment for *Artemia* feeding period

Rearing tank	1000l Polycarbonate	
Stocking density	20000 inds/m3 (Triplicate)	
Feeding time	2 - 13DAH	Rotifer (08:00 and 14:00) 5inds/ml
	13-15DAH	Rotifer (09:00 and 15:00) 5inds/ml
		Artemia (08:00, 14:00 and 17:00)
		80-200inds/larva



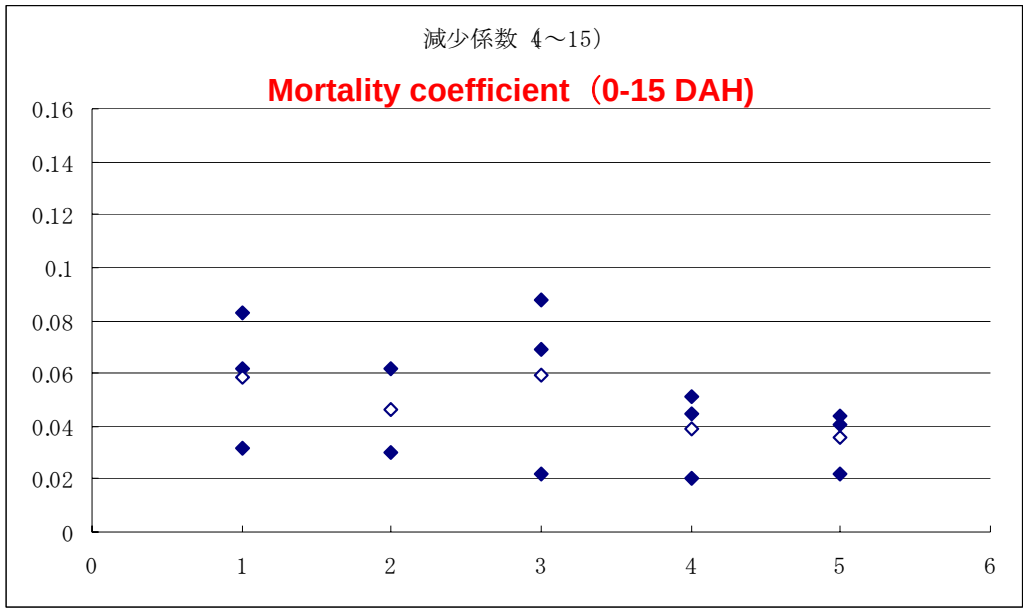
Vitamin A Concentration of *Artemia*

VA of agent (IU/g)	VA of Artemia (IU/g)
a 150	49.7
b 750	325.7
c 1500	994.3
d 7500	2830.8
Marine ω	192.8

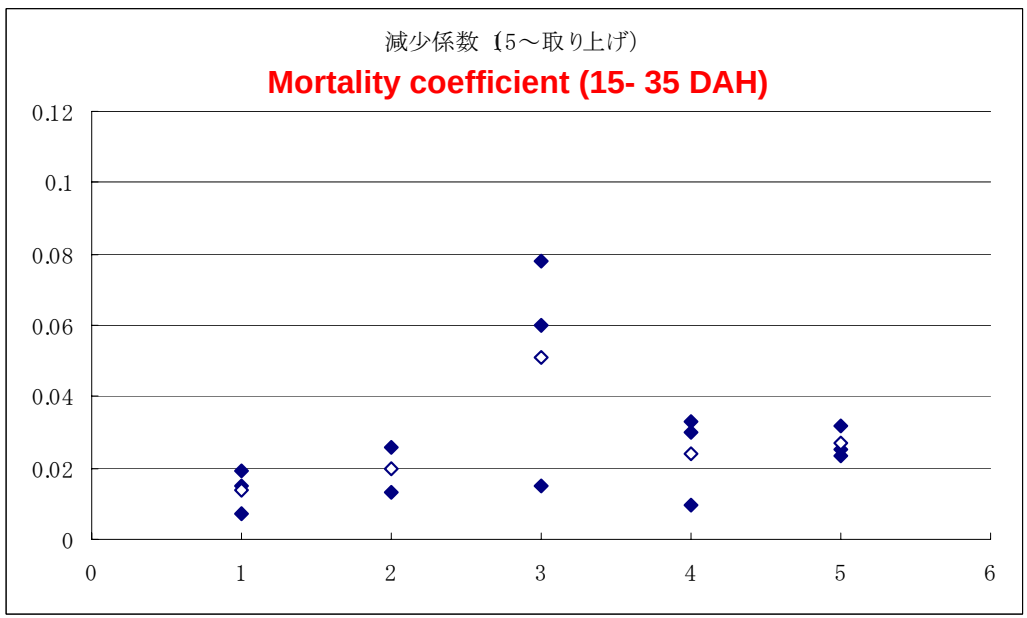


Vitamin A concentration of Artemia reflected VA concentration of enrichment agent.

Conclusion of survival performance in Experiment-II

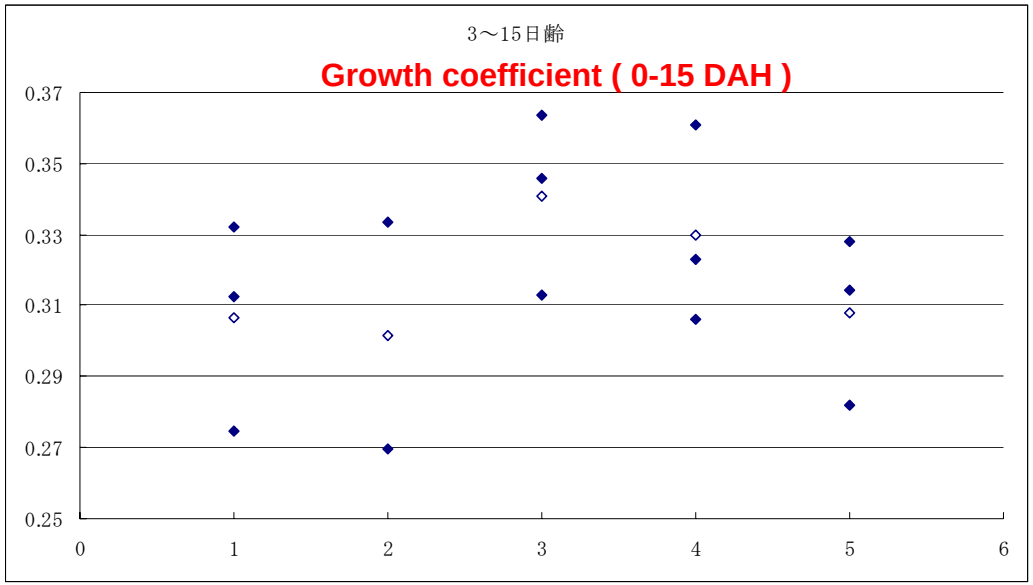


Mortality coefficients in the period of Rotifer feeding period showed no difference.

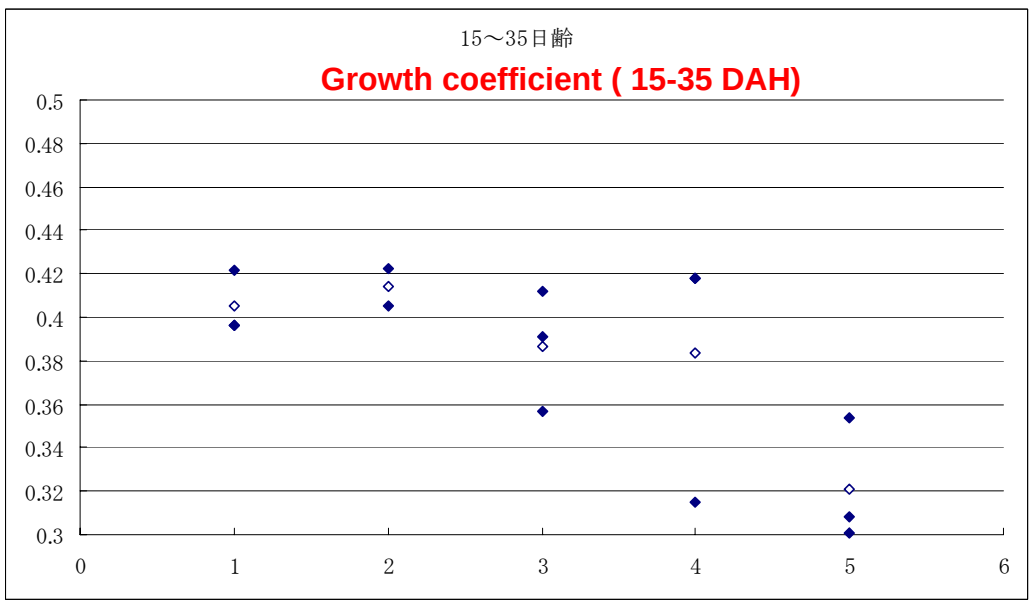


Mortality coefficients in Treatment Ac showed highest value. Treatment Aa , Ab, and Ad showed relatively same value to control.

Conclusion of growth performance in Experiment-II

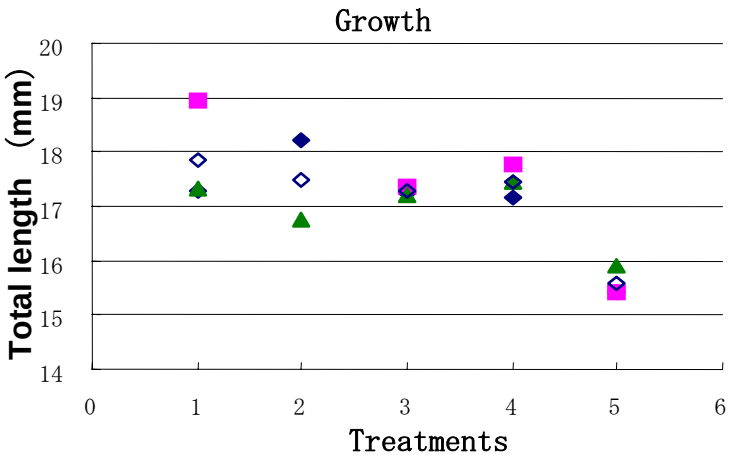


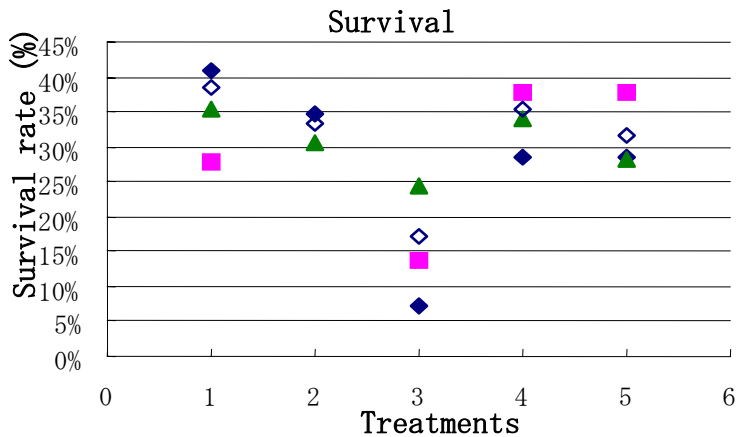
Growth coefficients in rotifer feeding period were almost similar.

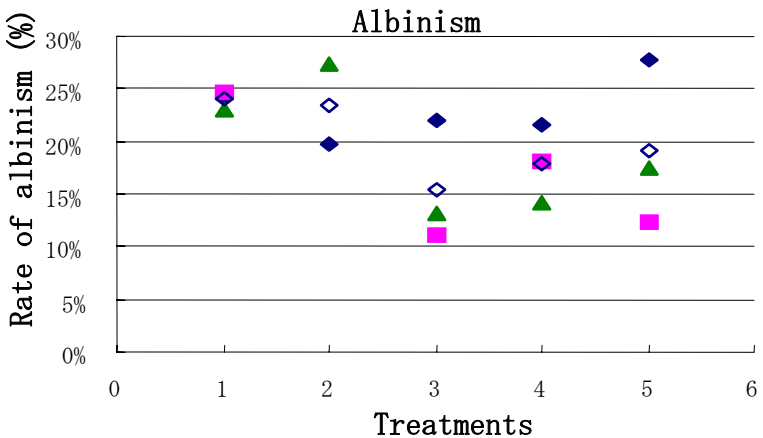


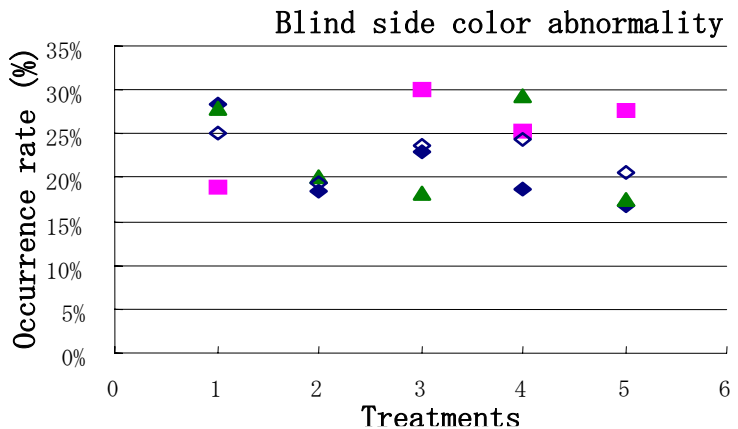
Growth coefficients of in mix feeding period were almost similar, except treatment Aa. Treatment Ab was highest.

Performance of Experimental Larviculture for *Artemia* Feeding Period at **37-41 DAH**









Treatments 1 2 3 4 5

a b c d

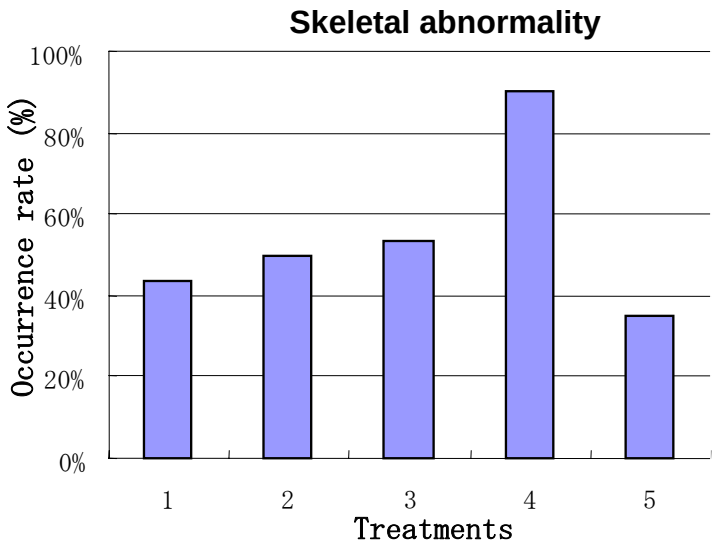
Marine ω

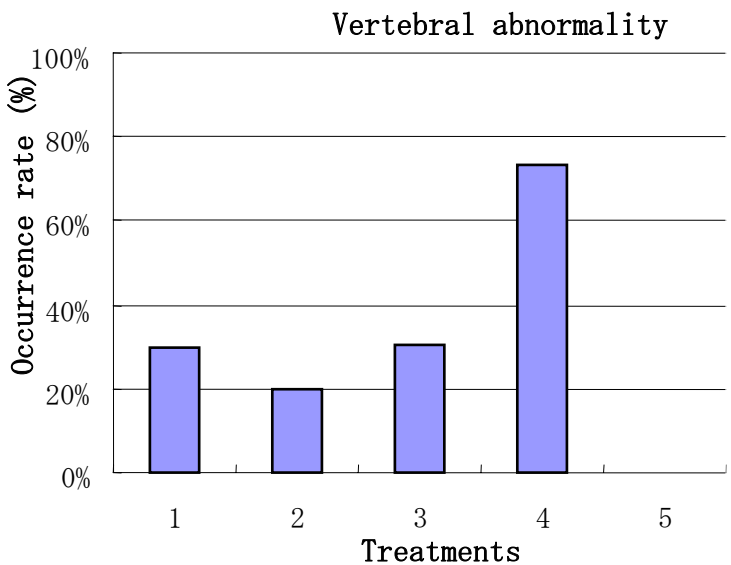
Tank 1 **Tank 3**

Tank 2 **Average of Tank 1-3**

Skeletal abnormality at 37-41 dah, just after metamorphosis

- Skeletal abnormality occurrence rate
  d＞c＞b＞a＞Marine ω
- Skeletal abnormality occurrence rate
  d＞c＝a＞b
  Vertebral abnormality did not occur at Treatment Marine ω.
- The hypertrophy of vertebrae occurred at Treatment d.






Thank you, and see you again